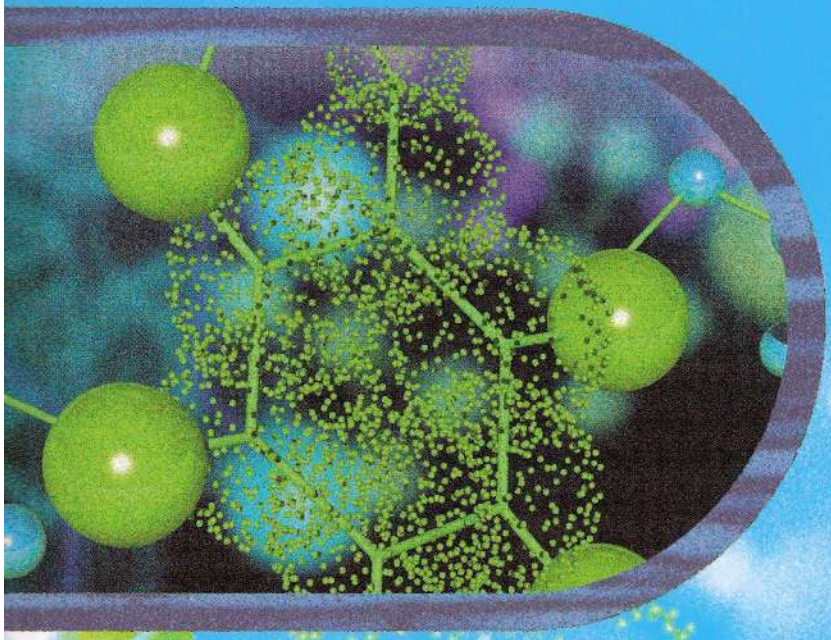
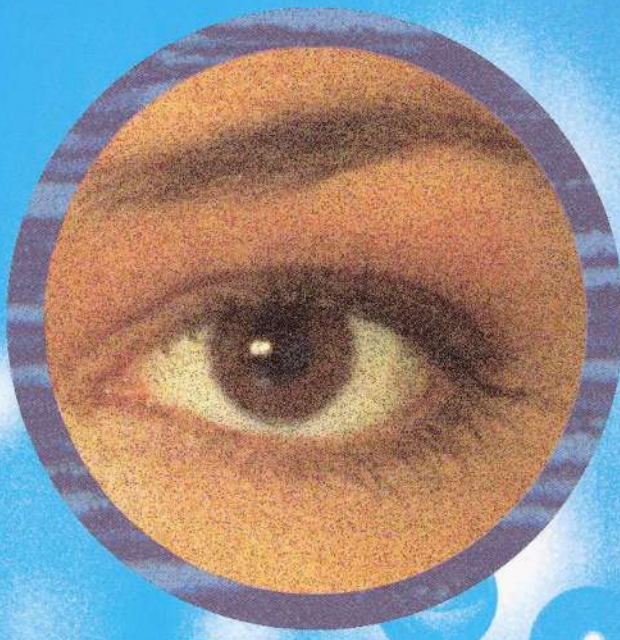
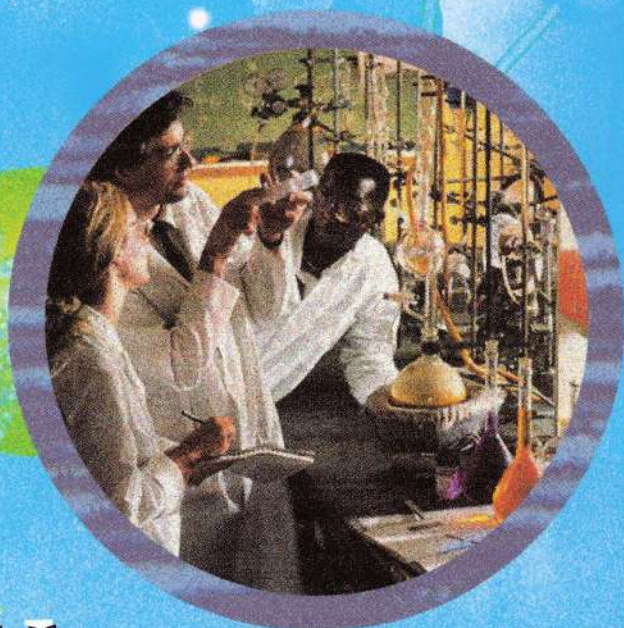




Practical Skills in Chemistry



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Practical Skills in Chemistry

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Contents

Boxes

Preface

Practical skills form the cornerstone of chemistry. However, the diversity of skills required in the laboratory means that a student's experience may be limited. While some techniques do require specific skills, many of them are transferable, generic skills that are required throughout the subject area. Limited time constraints of the modern curriculum often preclude or minimize laboratory time. It is the aim of this book to provide general guidance for use in and outside practical sessions and also to cover a range of techniques from the basic to the more advanced.

The book is primarily aimed at the undergraduate level (principally first and second year), although some information is relevant to final year undergraduates and postgraduate courses. The diversity of the book means that recipe-like solutions are not provided for every potential problem, but guidance on a range of topics is provided.

As the book focuses on practical aspects it is important not to apportion 'labels' to the areas covered. However, for the traditionalist the areas covered are primarily focused on synthetic, physical and analytical aspects of chemistry.

It is intended that the book will be useful in the laboratory, during practical classes, and during project work. It is not intended to replace recipe-like laboratory scripts but to enable students to become familiar with the practical aspects of the subject both at the time of performing the experiment and during the writing-up process. In addition, lecturers should find that the text provides an effective means of supplementing the information given in practical classes, where constraints on time and resources can lead to the under-performance of students.

We have selected material for inclusion in *Practical Skills in Chemistry* based on our own teaching experience, highlighting those areas where our students have needed further guidance. As a result of our comprehensive cover of practical skills, some techniques such as microscale methods and specialized vacuum techniques have been omitted, but specific references are provided. Instead, we have attempted to provide sufficient detail so that students will have the skills to carry out experiments successfully and not produce poor data as a result of poor technique.

Most students will have access to specialist textbooks giving in-depth coverage of the theoretical principles and knowledge covered in lectures. This book aims to supplement – rather than replace – such textbooks, and covers the skills required in laboratory classes, together with practical advice, tips, hints, worked examples, definitions, key points, 'how to' boxes and checklists which are useful in the field of chemistry. The text provides an outline on the underlying theoretical principles where necessary, but emphasis throughout is on the practical applications of this information.

To students who buy this book, we hope that you will find it useful in the laboratory, during your practical classes and in your project work – this is not a book to be left on the bookshelf. Lecturers should find that the text provides an effective means of supplementing the information given in practical classes.

We would like to acknowledge the support of our families and the help provided by colleagues who read early drafts of the material. In particular, special thanks are due to Gary Askwith, Dave Bannister (Manchester

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For the student

This text is designed to help you in your Chemistry studies, before and during laboratory classes, in project work, in tutorials and even in examinations.

Chapters 1–36 cover general and specific skills for laboratory work.

These are based on the authors' experience of questions that students often ask and difficulties they have in performing laboratory work. They include tips, hints, worked examples, definitions and "how to" boxes that set out procedures for you step by step.

Chapters 37–44 explain data analysis and presentation.

This will be an important element of your course and you will find that this section guides you through the key skills that you will need to develop, from presenting graphs and tables to drawing chemical structures and using statistical tests.

Chapters 45–49 cover information technology and library resources.

These chapters will help you get the most from chemistry resources available on the World Wide Web.

Chapters 50–56 deal with the vital subject of communicating information.

Designed to help you report practical and project work and give oral presentations effectively, and to succeed in examinations.

We hope you will find this book a helpful guide throughout your course, and beyond.

Abbreviations

A_r	relative atomic mass
AAS	atomic absorption spectrometry
AC	affinity chromatography
ACS	American Chemical Society
AES	atomic emission spectrometry
ANOVA	analysis of variance
AO	atomic orbital
ATP	adenosine triphosphate
BIDS	Bath Information and Data Services
b.pt.	boiling point
CCD	central composit design
CCP	cubic close packed
CE	capillary electrophoresis
CEC	capillary electrochromatography
CGE	capillary gel electrophoresis
COSHH	Control of Substances Hazardous to Health
CoV	coefficient of variance
CRm	certified reference material
CZE	capillary zone electrophoresis
DAD	diode array detection
DCM	dichloromethane
DNA	deoxyribonucleic acid
DSC	differential scanning colorimetry
DTA	differential thermal analysis
ECD	electron capture detector
EDTA	ethylenediaminetetraacetic acid
EI	electron impact ionization
EIE	easily ionizable element
EMR	electromagnetic radiation
en	ethylenediamine
EOF	electro-osmotic flow
F	Faraday constant
FAAS	flame atomic absorption spectrometer
FID	flame ionization detector
FT	Fourier transformation
FT–IR	Fourier transform – infrared (spectroscopy)
GC	gas chromatography
GFC	gel filtration chromatography
GPC	gel permeation chromatography
h	Planck constant
HASAW	Health and Safety at Work
HCB	hexachloro-1,3-butadiene
HCL	hollow-cathode lamp
HCP	hexagonal close packed
HIC	hydrophobic interaction chromatography
HPLC	high-performance liquid chromatography
ICP	inductively coupled plasma
IEC	ion-exchange chromatography
IEF	isoelectric focusing

IR	infrared (radiation)
ISE	ion selective electrode
IUPAC	International Union of Pure and Applied Chemistry
K_a	acid dissociation constant
K_s	solubility product
K_w	ion product of water
KHP	potassium hydrogen phthalate
LGC	Laboratory of the Government Chemist
M_r	relative molecular mass
MDL	minimum detectable level
MEKC	micellar electrokinetic chromatography
MEL	maximum exposure limit
MO	molecular orbital
m.pt.	melting point
MS	mass spectrometry
NH	null hypothesis
NIST	National Institute of Standards and Technology
NMR	nuclear magnetic resonance
NP–HPLC	normal phase high-performance liquid chromatography
ODA	octadecylsilane
OEL	occupational exposure standard
PCA	principal component analysis
PFA	perfluoroalkoxyvinylether
PLOT	porous layer open tubular (column)
PMT	photomultiplier tube
PTFE	polytetrafluoroethylene
R	universal gas constant
R_f	relative frontal mobility
RA	relative abundance
RNA	ribonucleic acid
RP–HPLC	reverse phase high-performance liquid chromatography
rpm	revolutions per minute
RSC	Royal Society of Chemistry
SAX	strong anion exchange
SCOT	support-coated open tubular (capillary column)
SCX	strong cation exchange
SDS	sodium dodecyl sulphate
SE	standard error (of the sample mean)
SEM	scanning electron microscopy
SI	Système Internationale d'Unités
STP	standard temperature and pressure
TCA	trichloracetic acid
TCD	thermal conductivity detector
TG	thermogravimetry
TLC	thin-layer chromatography
TMS	tetramethylsilane
TRIS	tris(hydroxymethyl)aminomethane *or* 2-amino-2-hydroxymethyl-1,3-propanediol
URL	uniform resource locator
USEPA	United States Environmental Protection Agency
UV	ultraviolet
WCOT	wall-coated open tubular (column)
WWW	World Wide Web

Fundamental laboratory techniques

1 Basic principles

Developing practical skills – these will include:

- observing and measuring
- recording data
- designing experiments
- analysing and interpreting data
- reporting/presenting.

All knowledge and theory in science has originated from practical observation and experimentation: this is equally true for disciplines as diverse as analysis and synthesis. Laboratory work is an essential part of all chemistry courses and often accounts for a significant proportion of the assessment marks. This book aims to provide an easy-to-use reference source dealing with basic practical techniques and information. The skills developed in practical classes will continue to be useful throughout your course and beyond, some within science and others in any career you choose.

Being prepared

KEY POINT **You will get the most out of laboratory work if you prepare well. Do not go into a practical session assuming that everything will be provided, without any input on your part.**

The main points to remember are:

Using textbook in the lab – take this book along to the relevant classes, so that you can make full use of the information during the practical sessions.

If in doubt over any part of the practical procedure – ASK! There is no such thing as a silly question in the laboratory.

- Read any handouts in advance: make sure you understand the purpose of the practical and the particular skills involved. Does the practical relate to, or expand upon, a current topic in your lectures? Is there any additional preparatory reading that will help?
- Take along appropriate textbooks, to explain aspects in the practical.
- Consider what safety hazards might be involved, and any precautions you might need to take, before you begin (p. 7).
- Listen carefully to any instructions and note any important points: adjust your schedule/handout, as necessary.
- During the practical session, organize your bench space – make sure your lab book is adjacent to, but not within, your working area. You will often find it easiest to keep clean items of glassware etc. on one side of your working space, with used equipment on the other side.
- All chemical waste (solid or liquid) should be disposed of in the appropriate containers provided (consult the demonstrator or lecturer-in-charge).
- Write up your work as soon as possible, and submit it on time, or you may lose marks.
- Catch up on any work you have missed as soon as possible – preferably before the next practical session.

Basic requirements

Recording practical results

Presenting results – while you don't need to be a graphic designer to produce work of a satisfactory standard, presentation and layout are important and you will lose marks for poorly presented work.

An A4 loose-leaf ring binder offers flexibility, since you can insert laboratory handouts, and lined and graph paper, at appropriate points. The danger of losing one or more pages from a loose-leaf system is the main drawback. Bound books avoid this problem, although those containing alternating lined/graph or lined/blank pages tend to be wasteful – it is often better to paste sheets of graph paper into a bound book, as required.

All experimental observations and data should be recorded in a notebook in ink at the time they are made because it is easy to forget when you are busy.

A good-quality HB pencil or propelling pencil is recommended for making diagrams etc. as mistakes are easily corrected with a vinyl eraser. Buy a black, spirit-based (permanent) marker to label experimental glassware, sample tubes, etc. Fibre-tipped fine line drawing/lettering pens are useful for preparing final versions of graphs and diagrams for assessment purposes. Use a clear ruler (with an undamaged edge) for graph drawing, so that you can see data points/information below the ruler as you draw.

Calculators

These range from basic machines with no pre-programmed functions and only one memory, to sophisticated programmable minicomputers with many memories. The following may be helpful when using a calculator:

- Power sources. Choose a battery-powered machine, rather than a mains-operated or solar-powered type. You will need one with basic mathematical/scientific operations including powers, logarithms (p. 262), roots and parentheses (brackets), together with statistical functions such as sample means and standard deviations (Chapter 40).
- Mode of operation. Calculators fall into two distinct groups. The older system used by, for example, Hewlett Packard calculators is known as the reverse Polish notation: to calculate the sum of two numbers, the sequence is 2 [enter] 4 + and the answer 6 is displayed. The more usual method of calculating this equation is as $2 + 4 =$, which is the system used by the majority of modern calculators. Most newcomers find the latter approach to be more straightforward. Spend some time finding out how a calculator operates, e.g. does it have true algebraic logic ($\sqrt{}$ then number, rather than number then $\sqrt{}$)? How does it deal with scientific notation (p. 262)?
- Display. Some calculators will display an entire mathematical operation (e.g. '$2 + 4 = 6$'), while others simply display the last number/operation. The former type may offer advantages in tracing errors.
- Complexity. In the early stages, it is usually better to avoid the more complex machines, full of impressive-looking, but often unused pre-programmed functions – go for more memory, parentheses, or statistical functions rather than engineering or mathematical constants. Programmable calculators may be worth considering for more advanced studies. However, it is important to note that such calculators are often unacceptable for exams.

Using calculators – take particular care when using the exponential key 'EXP' or 'EE'. Pressing this key produces $10^{\text{something}}$. For example if you want to enter 2×10^{-4}, the order entry is 2, EXP, −, 4 not 2, ×, 10, EXP, −, 4.

Presenting more advanced practical work

In some practical reports and in project work, you may need to use more sophisticated presentation equipment. Word processing may be essential and computer-based graphics packages can be useful. Choose easily-read fonts such as Arial or Times New Roman for project work and posters and consider the layout and content carefully (p. 341). Alternatively, you could use fine line drawing pens plus dry-transfer lettering and symbols, such as those made by Letraset®, although this approach is usually more time consuming and less flexible than computer-based systems.

Presenting graphs and diagrams – ensure these are large enough to be easily read: a common error is to present graphs or diagrams that are too small, with poorly chosen scales.

Printing on acetates – standard overhead transparencies are not suitable for use in laser printers or photocopiers: you need to make sure that you use the correct type.

To prepare overhead transparencies for oral presentations, you can use spirit-based markers and acetate sheets. An alternative approach is to print directly from a computer-based package, using a laser printer and special acetates, or directly to 35 mm slides. You can also photocopy on to special acetates. The use of Microsoft PowerPoint® as a presentation package has become more important in recent years. It is not uncommon to find a computer and presenter available for student use. Advice on content and presentation is given on p. 344.

2 Health and safety

HASAW – in the UK, the **Health and Safety at Work etc. Act 1974** provides the main legal framework for health and safety. The **Control of Substances Hazardous to Health (COSHH) Regulations 1994 and 1996** impose specific legal requirements for risk assessment wherever hazardous chemicals or biological agents are used, with Approved Codes of Practice for the control of hazardous substances, carcinogens and biological agents, including pathogenic microbes.

Definitions

Hazard – the ability of a substance to cause harm.

Risk – the likelihood that a substance might be harmful under specific circumstances.

Risk – is often associated with the quantity of chemical to be used, e.g. there is a much greater risk when using a large volume of flammable solvent than a few millilitres, even though the hazard is the same.

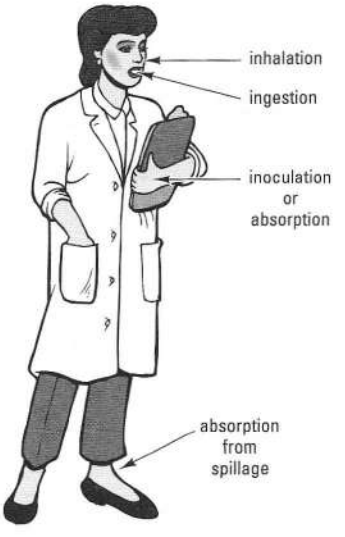


Fig. 2.1 Major routes of entry of harmful substances into the body.

Health and safety law requires institutions to provide a working environment that is safe and without risk to health. Where appropriate, training and information on safe working practices must be provided. Students and staff must take reasonable care to ensure the health and safety of themselves and of others, and must not misuse any safety equipment.

KEY POINT **All practical work must be carried out with safety in mind, to minimize the risk of harm to yourself and to others – safety is everyone's responsibility by law.**

Risk assessment

The most widespread approach to safe working practice involves the use of risk assessment, which aims to establish:

1. The intrinsic chemical and physical hazards, together with any maximum exposure limits (MELs) or occupational exposure standards (OESs), where appropriate. All chemical manufacturers provide data sheets listing the hazards associated with particular chemical compounds.
2. The risks involved, by taking into account the amount of substance to be used, the way in which it will be used and the possible routes of entry into the body (Fig. 2.1). In this regard, it is important to distinguish between the intrinsic hazards of a particular substance and the risks involved in its use in a particular exercise.
3. The persons at risk and the ways in which they might be exposed to hazardous substances, including accidental exposure (spillage).
4. The steps required to prevent or control exposure. Ideally, a non-hazardous or less hazardous alternative should be used. If this is not feasible, adequate control measures must be used, e.g. a fume cupboard or other containment system. Personal protective equipment (e.g. lab coats, safety glasses) must continue to be used in addition to such containment measures. A safe means of disposal will be required.

The outcome of the risk assessment process must be recorded and appropriate safety information must be passed on to those at risk. For most practical classes, risk assessments will have been carried out in advance by the person in charge and the information necessary to minimize the risks to students may be given in the practical schedule. You will be asked to carry out risk assessments to familiarize yourself with the process and sources of information. Make sure you know how your department provides such information and that you have read the appropriate material before you begin your practical work. You should also pay close attention to the person in charge at the beginning of the practical session, as they may emphasize the major hazards and risks. In project work, you will need to be involved in the risk assessment process along with your supervisor, before you carry out any laboratory work. Any new materials synthesized during the project should be treated with the utmost respect. An example of a risk assessment is shown in Fig. 2.2.

In addition to specific risk assessments, most institutions will have a safety handbook, giving general details of safe working practices, together with the

LABORATORY RISK ASSESSMENT

This sheet **must** be completed **before** commencing each experiment

Name:

Experiment: Synthesis of *N*-phenylethanamide **Date:**

1. Hazard and Risk Codes Enter below the correct Hazard Code and Risk Code for each reagent, solvent, product and by product (letter and number in the relevant boxes)

REAGENT AND INSTRUMENTS	HAZARD (H) and RISK (R) CODES															
													Others (insert)			
	A		C		T		F		W		M					
	H	R	H	R	H	R	H	R	H	R	H	R	H	R	H	R
Aminobenzene (aniline)	2	2	2	2	2	2	2	1	1	1	1	1				
Ethanoic anhydride	2	2	1	1	2	2	2	1	2	1	1	1				
N-phenylethanamide	1	1	1	1	2	1	1	1	1	1	1	1				
Ethanoic acid (dilute aqueous solution)	1	1	1	1	1	1	1	1	1	1	1	1				

A = Corrosive/irritant
C = Carcinogenic
T = Toxic
F = Flammable
W = Violent reaction with water/acid/bases
M = Microbiological hazard
R = Radioactive
I = Instrument hazard
X = Explosive
O = Oxidizing agents
1 = Low/no hazard/risk
2 = Moderate hazard/risk
3 = High hazard/risk
4 = Special high category

2. Precautions: containment and protection

Stage of Experiment (including disposal)	Containment Code	Personal Protection	Additional Information
Weighing out chemicals	3	Gloves	

Containment code 1 = Open Lab 2 = Restricted Lab (no naked flames) 3 = Fume Cupboard 4 = Glove Box/Safety Cabinet

Personal Protection Insert all requirements additional to coat and glasses (e.g. gloves, visor, safety screen, etc.)

Additional Information For example, disposal/specified waste container, sink and water, etc.

3. Signatures

Student: _____ **Supervisor:** _____ **Date:** _____

Fig. 2.2 An example of a laboratory hazard assessment form.

Protective clothing is worn as a first barrier to spillage of chemicals on to your body.

Lab coats are for protection of you and your clothing.

Eye protection special spectacles with side pieces to protect you from your own mistakes and those of your colleagues. If you wear spectacles, eye protection with prescription lenses and side pieces is available from your optician, an expensive but worthwhile investment. Otherwise goggles can be worn over spectacles.

Contact lenses should not be worn in the laboratory. Chemicals can get under the lens and damage the eye before the lens can be removed. It is often very difficult to remove the contact lens from the eye after a chemical splash.

Shoes should cover the feet: no open-toed sandals, for example.

Long hair should be tied back and hats (e.g. baseball caps) should not be worn.

names and telephone numbers of safety personnel, first aiders, hospitals, etc. Make sure you read this and abide by any instructions.

Basic rules for laboratory work

- Wear appropriate protective clothing at all times – a clean lab coat (buttoned up), eye protection, appropriate footwear and ensure your hair does not constitute a hazard.
- Never smoke, eat or drink in any laboratory, because of the risks of contamination by inhalation or ingestion (Fig. 2.1).
- Never work alone in a laboratory.
- Make sure that you know what to do in case of fire, including exit routes, how to raise the alarm, and where to gather on leaving the building. Remember that the most important consideration is human safety: do not attempt to fight a fire unless it is safe to do so.

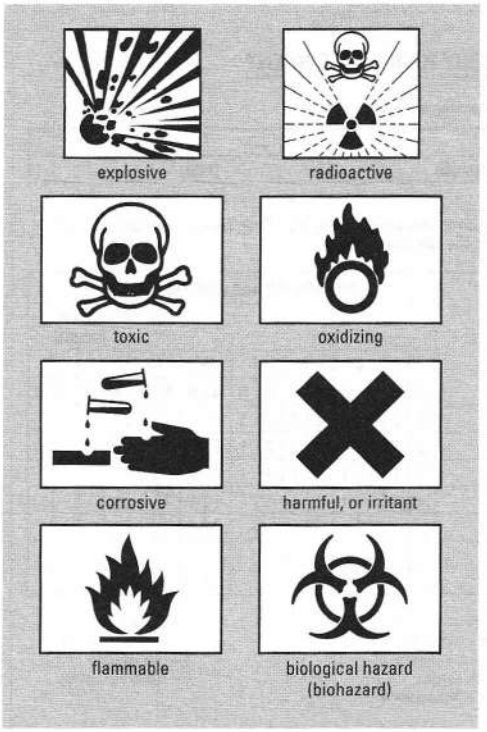


Fig. 2.3 Warning labels for specific chemical hazards.

- All laboratories display notices telling you where to find the first aid kit and who to contact in case of accident/emergency. Report all accidents, even those appearing insignificant – your department will have a reporting procedure to comply with safety legislation.
- Know the warning symbols for specific chemical hazards (Fig. 2.3).
- Never touch chemicals unless they are known to have minimal hazard: use a spatula to transfer and manipulate solids, and pipettes for liquids – see p. 9.
- Never mouth pipette any liquid. Use a pipette filler (see p. 10).
- Take care when handling glassware – see p. 13 for details.
- Use a fume cupboard for hazardous chemicals. Make sure that it is working and then open the front only as far as necessary: many fume cupboards are marked with a maximum opening.
- Always use the minimum quantity of any hazardous materials.
- Work in a logical, tidy manner and minimize risks by thinking ahead.
- Alway clear up spillages, especially around balances, infrared sample preparation areas, etc., for the next worker.
- Always clear up at the end of each session. This is an important aspect of safety, encouraging a responsible attitude towards laboratory work.

3 Working with liquids

Measuring and dispensing liquids

The equipment you should choose to measure out liquids depends upon the volume to be dispensed, the accuracy required and the number of times the job must be repeated (Table 3.1).

Table 3.1 Criteria for choosing a method for measuring out a liquid

Method	Best volume range	Accuracy	Usefulness for repetitive measurement
Pasteur pipette	1–5 mL	Low	Convenient
Conical flask/beaker	25–5000 mL	Very low	Convenient
Measuring cylinder	5–2000 mL	Medium	Convenient
Volumetric flask	5–2000 mL	High	Convenient
Burette	1–100 mL	High	Convenient
Glass pipette	1–100 mL	High	Convenient
Mechanical pipettor	5–1000 μL	High*	Convenient
Syringe	0.5–20 μL	Medium**	Convenient
Microsyringe	0.5–50 μL	High	Convenient
Weighing	Any (depends on accuracy of balance	Very high	Inconvenient

*If calibrated correctly and used properly (see p. 10).
**Accuracy depends on width of barrel: large volumes less accurate.

Reading any volumetric scale – make sure your eye is level with the bottom of the liquid's meniscus and take the reading from this point.

Conical flasks, beakers, measuring cylinders and volumetric flasks measure the volume of liquid contained in them, while burettes, pipettes, pipettors, syringes and microsyringes mostly measure the volume delivered from them: think about the requirements of the experiment.

Certain liquids may cause problems:

- High-viscosity liquids are difficult to dispense: allow time for the liquid to transfer.
- Organic solvents may evaporate rapidly, making measurements inaccurate: work quickly; seal containers quickly.
- Solutions prone to frothing (e.g. surfactant solutions) are difficult to measure and dispense: avoid forming bubbles; do not transfer quickly.

Pasteur pipettes

Hold correctly during use (Fig. 3.1) – keep the pipette vertical, with the middle finger gripping the barrel to support the pipette while the thumb and index finger provide controlled pressure on the bulb, and squeeze gently to provide individual drops.

To prevent liquid being sucked into the bulb and hence cross-contamination:

- Ensure that the capacity of the bulb does not exceed that of the barrel.
- Do not remove the tip of the pipette from the liquid while drawing up the liquid; the inrush of air may splash the liquid into the bulb. This is particularly true when you lose patience trying to draw up viscous liquids.
- Do not lie the pipette on its side during use.

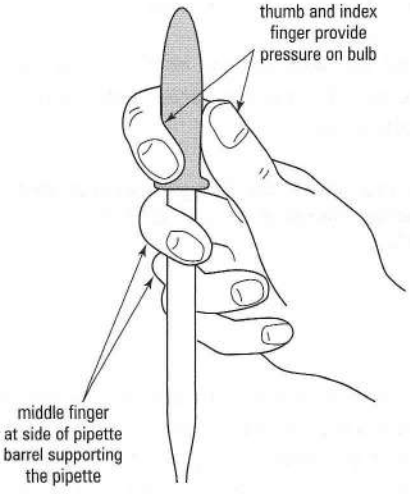


Fig. 3.1 How to hold a Pasteur pipette.

Conversely, if volatile liquids such as dichloromethane (DCM), ethanol, propanone (acetone) or diethylether (ether), for example, are to be

dispensed, the warmth of the glass pipette will cause the liquid to squirt from the pipette without any pressure on the bulb. To prevent this, suck up the liquid several times into the pipette so as to cool the glass and then dispense as normal.

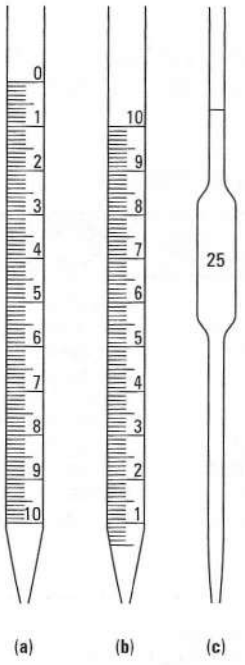


Fig. 3.2 Glass pipettes: (a) graduated pipette, reading from zero to shoulder; (b) graduated pipette, reading from maximum to tip, by gravity; (c) bulb (volumetric) pipette, showing volume on bulb.

Glass pipettes – always check that the pipette is a 'drain-down' type. There may be old 'blow-out' pipettes lurking in the back of drawers.

Conical flasks and beakers

These have approximate graduations and should only be used for measuring volumes of solutions/liquids where accuracy is unimportant.

Measuring cylinders and volumetric flasks

These must be used on a level surface (the laboratory bench) so that the scale is horizontal; you should first fill with solution until just below the desired mark, then fill slowly (e.g. using a Pasteur pipette) until the bottom of the meniscus is level with the mark. Remember to allow time for the solution to run down the walls of the vessel and to bend down so that your eyes are level with the graduation mark(s) and the meniscus.

Burettes

These must be mounted vertically in a clamp – don't over-tighten the clamp (see p. 26) – or in a burette holder, on a stand. First ensure that the tap is closed and, using a funnel, add a little of the solution to be dispensed, rinse the burette and discard the washings through the tap: this is vital in titrations where a little water in the burette will alter the concentration of the solution. Refill the burette with solution, open the tap and allow the liquid to fill the barrel below the tap, then take a meniscus reading, noting the value in your notebook. Dispense the solution via the tap and measure the new meniscus reading. The volume dispensed is the difference between the two readings.

Pipettes

There are various designs, including graduated and bulb (volumetric) pipettes (Fig. 3.2). Take care to look at the volume scale before use: some graduated pipettes empty from full volume to zero, others from zero to full volume; some scales refer to the shoulder of the tip, others to the tip by gravity. Never blow out volumetric (bulb) pipettes, just touch the tip against the inside wall of the vessel.

Rinse out pipettes with a little of the solution to be delivered before commencing the accurate measurement. To prevent cross-contamination, never draw the solution into the pipette filler.

KEY POINT **For safety reasons, it is no longer permissible to mouth pipette – various aids (pipette fillers) are available, such as the rubber-bulb and Pi-Pump® (Fig. 3.3).**

Pipettors (autopipettors)

There are two basic types:

1. Air displacement pipettors. For routine work with dilute aqueous solutions. One of the most widely used is the Gilson Pipetman® (Fig. 3.4).
2. Positive displacement pipettors. For non-standard applications, including dispensing viscous, dense or volatile liquids where an air displacement pipettor might create aerosols leading to errors.

Using a pipettor – check your technique (precision) by dispensing volumes of distilled water and weighing on a balance, assuming 1 mg = 1 μL = 1 mm^3. For small volumes, measure several aliquots together, e.g. 10 × 15 μL = 150 mg. Aim for accuracy of ±1%.

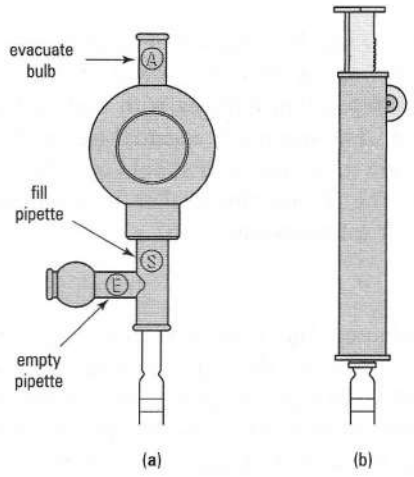


Fig. 3.3 Pipette fillers: (a) rubber-bulb type; (b) Pi-Pump®.

Using syringes – take great care when handling syringe needles. They are very sharp and may be contaminated by chemicals.

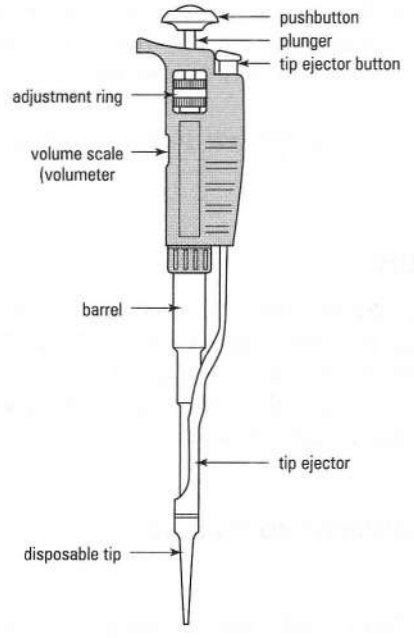


Fig. 3.4 A pipettor – the Gilson Pipetman®.

Air displacement and positive displacement pipettors may be:

- Fixed volume: capable of delivering a single factory-set volume.
- Adjustable: where the volume delivered is determined by the operator across a particular range of values.
- Pre-set: movable between a limited number of values.
- Multichannel: able to deliver several replicate volumes at the same time.

Whichever type of these routine but expensive devices you use, you must ensure that you understand the operating principles of the volume scale and the method for changing the volume delivered – some pipettors are easily misread.

A pipettor must be fitted with the correct disposable tip before use and each manufacturer produces different tips to fit particular models. Specialized tips are available for particular applications.

If you accidentally draw liquid into the barrel, seek assistance from your demonstrator/supervisor since the barrel will need to be cleaned before further use (to prevent cross-contamination) and unskilled dismantling of the device will cause irreparable damage.

Syringes

Syringes should be used by placing the tip of the needle into the solution and slowly drawing the plunger up to the required point on the scale. Check the barrel to make sure no air bubbles have been drawn up, and expel the solution slowly, touching the needle tip on the side of the vessel to remove any adhering solution. If there is air in the barrel, fill past the mark, invert the syringe and push the plunger to the mark so that the air and a little of the solution are expelled into a waste collection vessel. Then dispense the solution. The use of syringes for dispensing air-sensitive reagents is described in Chapter 18.

Microsyringes should always be cleaned before and after use by repeatedly drawing up and expelling pure solvent. The dead space in the needle can occupy up to 4% of the nominal syringe volume. Some microsyringes have a fine wire attached to the plunger, which fills the dead space. Never pull the plunger out of the barrel.

Balances

These can be used to weigh accurately (p. 22) how much liquid you have dispensed. Convert mass to volume using the equation:

$$\text{Mass/density} = \text{volume}$$

e.g. a liquid (9.0 g) of density ($1.2\,\text{g}\,\text{mL}^{-1}$) = 7.5 mL. Densities of common solvents and common chemicals can be found in Lide (2000). You will also need to know the liquid's temperature, since density is temperature dependent.

Holding and storing liquids

Test tubes

Both 'normal' and the much smaller 'semi-micro' test tubes are used for small-scale reactions and tests, e.g. qualitative analysis (p. 135) or solvent selection for recrystallization (p. 93).

Beakers

Beakers are used for general purposes, e.g. heating a non-volatile solvent while the solute dissolves, 'working up' a reaction where liquid/solid products need to be accessible for manipulation (stirring with a glass rod), or titrations using electrodes where a wide opening is essential (see p. 229). The volume graduations on the side are inaccurate and should only be used where approximations will suffice. The lip on the beaker is specifically designed to aid quantitative transfer of solutions (see p. 18).

Conical (Erlenmeyer) flasks

These can be used for general purposes, but they have more specialist applications. The narrow mouth and sloping shoulders reduce losses on stirring/swirling and evaporation and make them easier to seal. The absence of a lip does not favour quantitative transfer: useful in manual titrations (p. 145) and recrystallizations (p. 96). Volume markings are approximate.

Bottles and vials

Storing light-sensitive chemicals – use a brown-glass vessel or wrap aluminium foil around a clear vessel *and* its stopper.

These can be used when the solution needs to be sealed for safety, storage or to prevent evaporation or oxidation. They usually have a screwtop, plastic cap or stopper or a ground-glass stopper and come in various styles and sizes: from 2.5 L glass bottles used for storing large volumes of solutions to small plastic-capped vials (5 mL) for saving small amounts of reaction products.

Seal the vessels in an appropriate manner, using a stopper, cap or sealing film such as Parafilm® or Nescofilm®, bearing in mind the nature of the contents – sealing film should only be used for water solutions since it dissolves in some organic solvents and plasticizers may be extracted. Do not use corks, they are not air-tight. Do not use rubber bungs to seal containers containing organic solvents, they can swell even over a short period of time making removal very difficult.

You should clearly label all stored solutions (see p. 21) including all relevant hazard information.

Creating specialized apparatus

Glassware systems incorporating ground-glass connections, such as Quickfit®, are essential for setting up combinations of standard glass components for reactions, distillation, etc. In project work you may need to adapt standard forms of glassware for a special need. It may be necessary to contact a glassblowing service to make special items to order.

Table 3.2 Spectral cutoff values for glass and plastics (λ_{50} = wavelength at which transmission of electromagnetic radiation is reduced to 50%)

Material	λ_{50} (nm)
Routine glassware	340
Pyrex® glassware	292
Polycarbonate	396
Acrylic	342
Polyester	318
Quartz	220

Choosing between glass and plastic containers

Bear in mind the following points:

- Reactivity. Plastic vessels often distort at relatively low temperature, may be inflammable, may dissolve in certain organic solvents and may be affected by prolonged exposure to ultraviolet (UV) light.
- Opacity. Both glass and plastic absorb light in the UV range of the electromagnetic spectrum (Table 3.2). Quartz should be used where this is important, e.g. in cells for UV spectrophotometry (see p. 165) or photochemistry.

- Contamination. Some plasticizers may leach from vessels, especially with some organic solvents, such as DCM. Glass may adsorb ions and other molecules and then leach them into solutions, especially under acidic or alkaline conditions. Pyrex® glass is stronger than ordinary soda glass (rarely found except in specific items such as Pasteur pipettes and melting point tubes, but check if you are not sure) and can withstand temperatures up to 500 °C.
- Rigidity and resilience. Plastic vessels are not recommended where volume is critical as they may distort through time. Glass vessels are more easily broken than plastic.
- Disposability. Plastic items may be cheap enough to make them disposable, an advantage where there is a risk of chemical contamination.

Cleaning glass and plastic

Special cleaning of glass – for an acid wash use dilute acid, rinse with tap water (three times) to remove the washing solution and then rinse thoroughly at least three times with distilled or deionized water. To remove acidic deposits, wash with KOH/ethanol solution followed by rinsing with deionized water. Glassware, which must be exceptionally clean, should be washed in a chromic acid bath, but this must only be made up and used under supervision.

In quantitative analytical work, beware of contamination arising from prior use of chemicals or inadequate rinsing following washing. A thorough rinse with distilled or deionized water immediately before use will remove dust and other soluble deposits, but ensure that the rinsing solution is not left in the vessel. For analyses on the 'μg scale' and below, you should clean glass and plastic containers by immersion in nitric acid (10% v/v) overnight and then rinsing with a large volume of distilled or deionized water. The clean vessels should be stored either upside down or covered with Clingfilm®, to prevent dust contamination.

For general work, 'strong' basic detergents (e.g. Decon® or Pyroneg®) are good for solubilizing acidic deposits and an acid wash will remove remaining basic residues. A rinse with ethanol or propanone (acetone) will remove many organic deposits.

Safety with glass

Many minor accidents in the laboratory are due to lack of care with glassware. You should follow these general precautions:

- Wear eye protection at all times.
- Don't use chipped or cracked glassware and examine the equipment for 'star' cracks – it may break under very slight strains and should be disposed of in the broken glassware bin. All laboratories will have a waste bin dedicated to broken glass. Never put broken glass into other bins.
- If heating glassware, use a 'soft' Bunsen flame (half-open air vent) or 'wave' the flame around the heating point – this avoids creating a hot spot where cracks may start. Always use special heat-resistant gloves or rubber 'fingers' (see p. 36) when handling hot glassware.
- When clamping glassware (see p. 26) ensure that the clamp has a cork, rubber or plastic 'cushion' in the jaws to prevent breakages. There must be no metal–glass contact and you must not over-tighten the clamp.
- Take care when attaching rubber or plastic tubing to glass tubes, condensers, etc., and inserting thermometers and glass tubes into screwcap adapters (see p. 43). Always hold the tube and the 'hole' close together (Fig. 3.5) and wear thick gloves where appropriate.
- Don't force bungs too firmly into bottles (see p. 12) they can be difficult to remove. If you need a tight seal, use a screwtop bottle, with a rubber or plastic seal, Parafilm® or ground-glass jointware, such as Quickfit®.

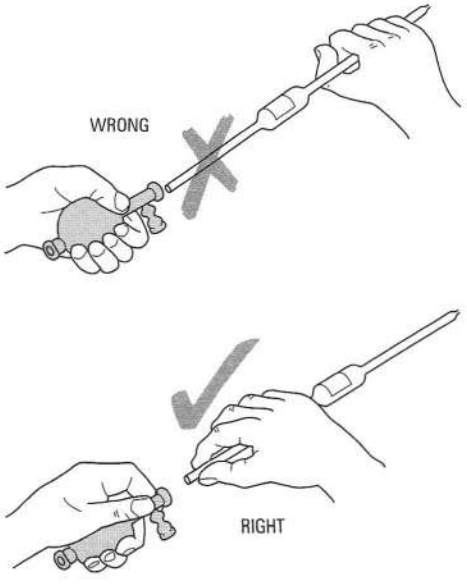


Fig. 3.5 Handling glass tubing.

- Never carry large bottles (> 1 L) by their necks – carry them in a bottle basket.
- Dispose of broken glass thoroughly and with great care – use disposable paper towels, tongs or dust-pan and brush and thick gloves. Always put pieces of broken glass in the correct bin.

4 Basic laboratory procedures I

Using chemicals

Safety aspects

The *Merck Index* (Budavari, 1999) and the *CRC Handbook of Chemistry and Physics* (Lide, 2000) are useful sources on the chemical and physical properties of elements and compounds, including melting and boiling points, solubility and toxicity, etc. Manufacturers' catalogues now include hazard data and disposal procedures.

In practical classes, the person in charge has the responsibility to inform you of any hazards associated with the use of chemicals. In project work, your first duty when using an unfamiliar chemical is to find out about its properties, especially those relating to safety. For routine practical procedures, a risk assessment may have been carried out by a member of staff and the relevant safety information may be included in the practical schedule: an example is shown in Table 4.1. If not, you will have to carry out a risk assessment before you begin. Before you use any of the chemicals, you *must* find out whether safety precautions need to be taken and complete the appropriate forms confirming that you appreciate the risks involved. Your department must provide the relevant information to allow you to do this. If your supervisor has filled out the form, read it carefully before signing.

Table 4.1 Representative risk assessment information for a practical exercise in organic chemistry: the synthesis of *N*-phenylethanamide (acetanilide)

Substance	Hazards/comments
Aminobenzene	Toxic, harmful by skin absorption. Wear gloves, dispense in fume cupboard
Ethanoic anhydride	Corrosive, flammable, toxic, reacts with water. Wear gloves, dispense in fume cupboard

Key safety points when handling chemicals are:

- Treat all chemicals as potentially dangerous.
- Wear a lab coat, with the buttons fastened, at all times.
- Wear eye protection at all times.
- Make sure you know where safety devices such as eye bath, fire extinguisher, first aid kit are kept before you start work in the lab.
- Wear gloves for toxic, irritant or corrosive chemicals and carry out procedures with them in the fume cupboard.
- Use appropriate aids such as spatulae, pipette fillers, Pasteur pipettes etc., to minimize risk of contact.
- Label all solutions, samples, etc., appropriately (see p. 22).
- Extinguish all naked flames when working with flammable substances.
- Never drink, eat or smoke where chemicals are being handled.
- Report all spillages and clean them up appropriately.
- Dispose of chemicals in the correct manner.

Using chemicals – be considerate to others: always return storeroom chemicals promptly to the correct place. Report when supplies are getting low to the person responsible for looking after the store. If you empty an aspirator or wash bottle, fill it up from the appropriate source.

Selection

Chemicals are supplied in varying degrees of purity and this is always stated on the manufacturer's containers. Suppliers differ in the names given to the grades and there is no conformity in purity standards. Very pure chemicals cost more, often very much more, and should only be used when the situation demands. If you need to order a chemical, your department will have a defined procedure for doing this.

Preparing solutions

Molar solutions – you will find that chemists talk about '0.1 Molar solutions' or you may see '0.1 M' as a concentration written on flasks or in books and journals. The term 'Molar' (abbreviated to M) means number of moles per litre. Hence an aqueous solution of hydrochloric acid (0.1 M) has a concentration of 0.1 mol L^{-1} equivalent to 3.65 g of hydrogen chloride per litre of solution.

Solutions are usually prepared with respect to their molar concentrations (e.g. mol L^{-1}, mol dm^{-3}, or mol m^{-3}) or mass concentrations (e.g. g L^{-1}, or kg m^{-3}): both can be regarded as an amount (usually mass) per unit volume, in accordance with the relationship:

$$\text{concentration} = \frac{\text{amount}}{\text{volume}} \qquad [4.1]$$

Percentage concentration – you may find that the concentration of a solution is expressed in percentage terms. Thus sodium carbonate (5% w/v) – the symbol *w* indicates *weight* of solute and *v* is the *volume* of solution. The resulting solution is a general-purpose dilute aqueous solution of sodium carbonate prepared from sodium carbonate (5 g) made up to 100 mL in water and used for neutralizing acid.

The levels of accuracy of solution preparation required are usually indicated in the protocol or by the nature of the experiment. Look for phrases such as 'accurately weighed' which means to four decimal places on an analytical balance, together with quantitative transfer. Volumes quoted as 250.00 mL, 100.00 mL, 25.00 mL imply the use of volumetric flasks and pipettes.

The most important aspect of eqn [4.1] is to recognize clearly the units involved, and to prepare the solution accordingly: for molar concentrations you will need the relative molecular mass of the chemical, so that you can calculate the mass of substance required. Further advice on concentrations and interconversion of units is given on p. 45.

In general there are two levels of accuracy required for the preparation of solutions:

1. General-purpose solutions – solutions of chemicals used in qualitative and preparative procedures (p. 17) when the concentration of the chemical need not be known to more than one or two decimal places. For example:
   (a) solutions used in extraction and washing processes, e.g. hydrochloric acid (0.1 mol L^{-1}), sodium hydroxide (2 M), sodium carbonate (5% w/v);
   (b) solutions of chemicals used in preparative experiments where the techniques of purification – distillation, recrystallization, filtration, etc. – introduce intrinsic losses of substances that make accuracy to any greater level meaningless.
2. Analytical solutions – solutions used in quantitative analytical procedures when the concentration needs to be known to an accuracy of four decimal places (e.g. 0.0001 mol L^{-1}). For example in:
   (a) volumetric procedures (titrations) and gravimetric analysis, where the concentrations of standard solutions of reagents and compounds to be analysed need to be accurately known;
   (b) spectroscopy, e.g. quantitative UV and visible spectroscopy, atomic absorption spectroscopy and flame photometry;
   (c) electrochemical measurements: pH titrations, conductance measurements and polarography;
   (d) chromatographic methods.

The procedures for weighing and the glassware used in the preparation of solutions differ according to the level of accuracy required.

Preparation of general-purpose solutions

Box 4.1 shows the steps involved in making up general-purpose aqueous solutions.

The concentration you require is likely to be defined by the protocol you are following and the grade of chemical and supplier may also be specified. To avoid waste, think carefully about the volume of solution you require, though it is always advisable to err on the high side because you may spill some, make a mistake when dispensing or need to repeat part of the experiment. Choose one of the standard volumes for vessels, as this will make measuring out easier.

Use distilled or deionized water to make up aqueous solutions and stir with a clean Pyrex® glass rod or magnetic stirrer bar ('flea') until all the chemical is dissolved. Magnetic stirrers are a convenient means of stirring solutions but precautions should be taken to prevent losses by splashing. Add the flea to the empty beaker or conical flask, add the chemical and then some water. Place the vessel centrally on the stirrer plate, switch on the stirrer and gradually increase the speed of stirring. When the crystals or powder have dissolved, switch off the stirrer and remove the flea with a magnet. Take care not to contaminate your solution when you do this and rinse the flea into the solution with distilled water. In general it is convenient to use glass rods with

Box 4.1 How to make up an aqueous solution of known concentration from a solid chemical

1. **Find out or decide the concentration of chemical required** and the degree of purity necessary.
2. **Decide on the volume of solution required.**
3. **Find out the relative molecular mass of the chemical (M_r).** This is the sum of the atomic (elemental) masses of the component element(s) and can usually be found on the container. If the chemical is hydrated, i.e. has water molecules associated with it, these must be included when calculating the mass required.
4. **Work out the mass of chemical that will give you the *concentration* desired in the *volume* required,** bearing in mind the quoted percentage purity of the chemical.

   Example 1: Suppose your procedure requires you to prepare 250 mL of 0.1 mol L^{-1} sodium chloride solution.

   (a) Begin by expressing all volumes in the same units, either millilitres or litres (e.g. 250 mL as 0.25 L).

   (b) Calculate the number of moles required from eqn [4.1]: 0.1 = amount (mol) ÷ 0.25. By rearrangement, the required number of moles is thus 0.1 × 0.25 = 0.025 mol.

   (c) Convert from mol to g by multiplying by the relative molecular mass (M_r for NaCl = 58.44 g mol^{-1}).

   (d) Therefore, you need to make up 0.025 × 58.44 = 1.46 g up to 250 mL of solution, using distilled water.

   Example 2: Suppose you are required to make up 100 mL of sodium carbonate (2 M).

   (a) Convert 2 M into mol L^{-1}; concentration required = 2 mol L^{-1}.

   (b) Express all volumes in the same units: therefore 100 mL = 0.1 L.

   (c) Calculate the number of moles required from eqn [4.1]: 2 = amount (mol) ÷ 0.1. By rearrangement, the required number of moles is thus 2 × 0.1 = 0.2 mol.

   (d) Convert from mol to g by multiplying by the M_r but note from the container that the compound is $Na_2CO_3.10H_2O$. Therefore the M_r required must include the water of crystallization and M_r = 286.14 g mol^{-1}.

   (e) Therefore, you need to make up 0.2 × 286.14 = 57.2 g up to 100 mL of solution using distilled water.
5. **Weigh out the required mass of chemical to an appropriate accuracy.** If the mass is too small to weigh with the desired degree of accuracy, consider the following options:

   (a) Make up a greater volume of solution.

   (b) Make up a more concentrated solution, which can be diluted at a later stage.

   (c) Weigh the mass first, and calculate what volume to make the solution up to afterwards using eqn 4.1.
6. **Add the chemical to a beaker or conical flask and then add a little less water than the final volume required.** If some of the chemical sticks to the weighing receptacle, use some of the water to wash it off. For accurate solutions, see p. 19 for accurate weighing and quantitative transfer.
7. **Stir and, if necessary, heat the solution to ensure all the chemical dissolves.** You can determine visually when this has happened by observing the disappearance of the crystals or powder. Allow the solution to cool, if heated.
8. **Make up the solution to the desired volume.** If the concentration needs to be accurate, use a volumetric flask (see p. 19 for accurate weighing and quantitative transfer); if a high degree of accuracy is not required, use a measuring cylinder.

   (a) Pour the solution from the beaker into the measuring vessel using a funnel to avoid spillage, using water to rinse out the vessel.

   (b) Make up the volume so that the meniscus comes up to the appropriate measurement line. If accuracy is not a major concern, the graduation marks on the beaker or conical flask may be used to establish the approximate volume.
9. **Transfer the solution to a reagent bottle or conical flask and label the vessel clearly, including hazard information, where appropriate.** Do not use water in this final transfer since you will alter the concentration of the solution by dilution.

beakers – ease of access for stirring – and magnetic fleas with conical flasks – lower losses through splashing – but often it is a matter of your preference and laboratory skills.

'Obstinate' solutions may require heating, but only do this if you know that the chemical will not be damaged at the temperature used. Use a stirrer–

heater to keep the solution mixed as you heat it. Allow the solution to cool to room temperature before you finalize its volume.

Preparation of analytical solutions

The key features in the preparation of solutions for analytical purposes are:

- Make sure that you have the most accurate available knowledge of masses of the chemicals used.
- Ensure that you have the most accurate available knowledge of the volumes of solutions used.

To achieve these features exact techniques of weighing and solution transfer must be used and the procedure is illustrated in the following example.

> 'Prepare a standard solution (250.00 mL) of ammonium ferrous sulphate (approximately 0.1 M), which is to be used to determine the concentration of a solution of potassium permanganate by titration'.

You must be aware of the following embedded information:

- This is a quantitative experiment, therefore requiring an analytical solution to be prepared.
- You must use a 250.00 mL volumetric flask, which you should note is calibrated at 20 °C.
- You must weigh accurately, to four decimal places, the mass of the chemical.
- It is almost impossible to weigh the exact mass of chemical for a specific concentration. For example, the mass of $(NH_4)_2FeSO_4.6H_2O$ required to prepare 250.00 mL of 0.1 M solution is 9.8035 g and you cannot weigh out this exact mass. However, you *can* weigh out a known mass to four decimal places accurately. From this you can then calculate the exact concentration of the chemical in solution, since you will know both mass and volume to a high degree of accuracy.

A **standard solution** is one in which the solute is weighed out to an accuracy of 4 decimal places and is made up in a volumetric flask.

A **stock solution** is one from which dilutions are made.

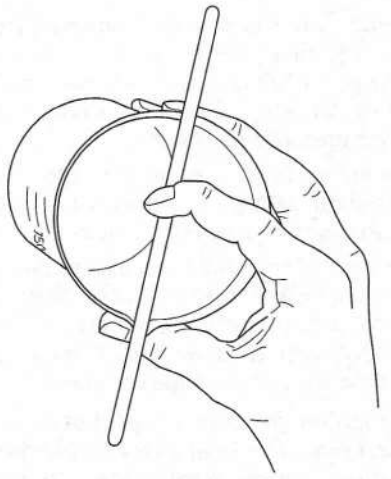


Fig. 4.1 Pouring a solution using a glass rod.

Box 4.2 shows the method for the preparation of the standard solution. The main practical point is that you must not lose, by splashing or failure to transfer by inadequate rinsing, *any* of the solution being prepared in the beaker and you must transfer *all* of the solution, by repeated rinsing, into the volumetric flask. Therefore it is good practice to use only a glass rod to stir the solution gently to dissolve the solid and to use the glass rod, as shown in Fig. 4.1, to pour the solution into the filter funnel. This technique, with practice, prevents losses of solution down the side of the beaker via the spout; rinsing with water can be achieved by use of a wash bottle squirted directly into the beaker.

You should not use a flea to stir a solution in the preparation of a standard solution, since this introduces more washing steps – washing the flea and the 'flea extractor' – and you still need to use the glass rod for quantitative transfer.

Procedure required with analytical solutions prepared from liquids

Many experiments in analytical chemistry, such as chromatography and spectroscopy, require the preparation of a standard solution of a liquid organic compound. Therefore you must know accurately the mass of the liquid. The compound can be dispensed by the methods described in Chapter 3, provided that the pipette, syringe, etc., is accurate, and thus the mass = volume × density, bearing in mind the temperature factor.

Box 4.2 How to make up an aqueous solution of known concentration from a solid chemical for use in quantitative analysis

Example: Suppose you are to prepare a standard solution (250.00 mL) of ammonium ferrous sulphate (approximately 0.1 M), which is to be used to determine the concentration of a solution of potassium permanganate.

1. **This is a quantitative experiment so the ammonium ferrous sulphate must be of the highest purity available to you.**
2. **Work out the mass of chemical that will give you the *concentration* desired in the *volume* required.**
   (a) Convert 1.0 M into mol L^{-1}; concentration required = 1.0 mol L^{-1}.
   (b) Express all volumes in the same units: therefore 250.00 mL = 0.25 L.
   (c) Calculate the number of moles required from eqn [4.1]: 1.0 = amount (mol) ÷ 0.25. By rearrangement, the required number of moles is thus 1.0 × 0.25 = 0.025 mol.
   (d) Convert from mol to g by multiplying by the M_r, but note from the container that the compound is $(NH_4)_2FeSO_4.6H_2O$. Therefore the M_r required must include the water of crystallization and M_r = 392.14 g mol^{-1}.
   (e) Therefore, you need to weigh out 0.025 × 392.14 = 9.8035 g of $(NH_4)_2FeSO_4.6H_2O$.
3. **Place a clean, dry weighing boat or appropriately sized sample tube onto a simple two-decimal-place balance** (see p. 22) and zero (tare) the balance and weigh about 9.80 g of the chemical.
4. **Carefully transfer the sample tube plus chemical to a four-decimal-place analytical balance** (see p. 23) and record the accurate mass: say 11.9726 g.
5. **Remove the sample and container from the balance and tip the contents into a clean, dry beaker (400 mL)**, ensuring that there is no spillage outside the beaker. *Do not attempt to wash out the sample tube with water.*
6. **Immediately reweigh the sample tube on the analytical balance:** say 2.1564 g. This is the mass of the container together with a few crystals of the chemical which have remained in the container. However, you now know exactly the mass of the chemical in the beaker: 11.9726 − 2.1564 = 9.8162 g of $(NH_4)_2FeSO_4.6H_2O$.
7. **Add deionized or distilled water** (about 100 mL) to the beaker and stir the mixture gently with a clean Pyrex® glass rod until all the solid has dissolved. *Do not splash or spill any of this solution* or you cannot calculate its concentration. Remove the glass rod from the solution, rinsing it with a little distilled water into the solution.
8. **Clamp a clean volumetric flask** for support (see p. 26) and place a clean, dry filter funnel in the top supported by a ring. Carefully pour the solution into the volumetric flask, ensuring no spillage of solution by using the technique illustrated in Fig. 4.1 and pouring slowly so that no air-lock is formed and no solution runs down the side of the beaker. When the addition is complete, *do not move the beaker from its position over the funnel.*
9. **Rinse the inside of the beaker several times with a distilled water wash bottle** to transfer all of the solution into the volumetric flask, paying particular attention to the 'spout' and glass rod. Then place the beaker aside and lift the funnel from the flask while rinsing it with distilled water. You have now achieved a *quantitative transfer.* Swirl the liquid in the flask to prevent density gradients.
10. **Make the solution up to the mark using distilled water**, stopper the flask and mix thoroughly by gentle inversion (10 times) of the flask while holding the stopper in place.

    You now have a solution (250.00 mL), which contains $(NH_4)_2FeSO_4.6H_2O$ (9.8162 g).

    The concentration of this solution is expressed as:

    250.00 mL of the solution contains 9.8162 g of $(NH_4)_2FeSO_4.6H_2O$

    Therefore:

    1000.00 mL of solution contains

    (4 × 9.8162) = 39.2648 g of $(NH_4)_2FeSO_4.6H_2O$

    The concentration of the solution is

    39.2648 g L^{-1} = 39.2648 ÷ 392.14 = 0.1001 mol L^{-1} = 0.1001 M

Alternatively you can use a weighing bottle as shown in Fig. 4.2. The liquid is placed in the bottle, weighed accurately and then the approximate amount required is added to the volumetric flask containing some solvent. The volumetric flask is stoppered immediately, the weighing bottle reweighed and the weight of liquid dispensed is calculated. The volumetric flask is then

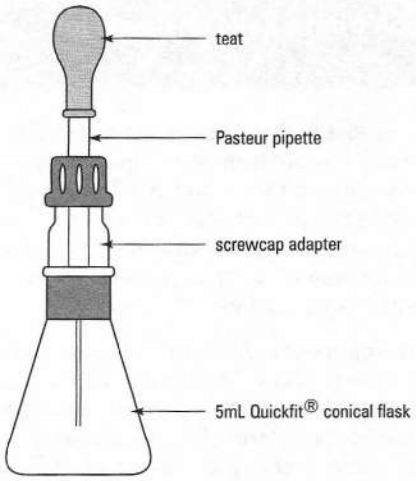


Fig. 4.2 A weighing bottle.

made up to the mark and stoppered. You now know the concentration of the standard solution to four decimal places.

Preparing dilutions

Making a single dilution

In analytical work, you may need to dilute a standard solution to give a particular mass concentration or molar concentration. Use the following procedure.

1. Transfer an appropriate volume of standard solution to a volumetric flask, using appropriate equipment (Table 3.1).
2. Make up to the calibration mark with solvent – add the last few drops from a Pasteur pipette until the bottom of the meniscus is level with the calibration mark.
3. Mix thoroughly, either by repeated inversion (holding the stopper firmly) or by prolonged stirring, using a magnetic stirrer. Make sure that you add the magnetic flea *after* the volume adjustment step.

For general-purpose work using dilute aqueous solutions where the higher degree of accuracy is not required, it may be acceptable to substitute conical flasks, beakers or test tubes for volumetric flasks and use measuring cylinders for volume measurements. In such cases you would calculate the volumes of 'stock' solutions (usually 'bench' reagents) and diluent required, with the assumption that the final volume is determined by the individual volumes of stock solution and diluent used. Thus a two-fold dilution would be prepared by using one volume of stock solution and one volume of diluent. The dilution factor is obtained from the initial concentration of the stock solution and the final concentration of the diluted solution. The dilution factor can be used to calculate the volumes and stock and diluent required in a particular instance. For example, suppose you wanted to prepare 100 mL of a solution of NaOH at 0.1 mol L^{-1}. Using the bench reagent, commonly containing 2.0 mol L^{-1} (2.0 M), the dilution factor is $0.1 \div 2.0 = 0.05 = 1/20$ (a twenty-fold dilution). Therefore the amount of stock solution required is 1/20th of 100 mL = 5 mL and the amount of diluent needed is 19/20th of 100 mL = 95 mL.

Making a dilution – use the relationship $[C_1]V_1 = [C_2]V_2$ to determine volume or concentration (see p. 46).

Using the correct volumes – it is important to distinguish between the volumes of the various liquids: a one-in-ten dilution is obtained using one volume of stock solution plus nine volumes of diluent (1 + 9 = 10).

Preparing a dilution series

Dilution series are used in a wide range of procedures including the preparation of standard curves for the calibration of analytical instruments (p. 171). A variety of different approaches can be used but the most common is a *linear dilution series*.

In a linear dilution series the concentrations are separated by an equal amount, e.g. a series containing cadmium at 0, 0.2, 0.4, 0.6, 0.8, 1.0 mmol L^{-1} might be used to prepare a calibration curve for atomic absorption spectroscopy (p. 170) when assaying polluted soil samples. Use $[C_1]V_1 = [C_2]V_2$ to calculate the volume of standard solution for each member of the series and pipette or syringe the calculated volume into an appropriately sized volumetric flask as described above. Remember to label clearly each diluted solution as you prepare it, since it is easy to get confused. The process is outlined in Box 4.3.

KEY POINT **Make all the dilutions from the working stock solution to the required solution. Do not make a solution of lower dilution from one already prepared: if you have made an error in the first dilution, it will be repeated for the second dilution.**

Box 4.3 How to make up a linear dilution series for use in quantitative analysis

The experimental protocol states: 'Prepare a standard solution (250.00 mL; 0.01 M) of cadmium ions using cadmium nitrate and use this solution to produce a linear dilution series of solutions (100.00 mL) of accurately known concentrations of approximately 0.0, 0.2, 0.4, 0.6, 0.8, 1.0 mmol L^{-1}'.

1. **Calculate the amount of cadmium nitrate required for the standard solution:** cadmium nitrate is supplied as the tetrahydrate $Cd(NO_3)_2.4H_2O$; $M_r = 308.47\ g\ mol^{-1}$. Therefore, using eqn [4.1], you should calculate 250.00 mL of an 0.01 M solution of cadmium ions requires $0.01 \times 0.25 = 0.0025$ mol $= 0.0025 \times 308.47 = 0.07712$ g of $Cd(NO_3)_2.4H_2O$. *Remember*: this solution will contain 0.0025 moles of 'cadmium nitrate' or 0.0025 moles of cadmium ions and 0.005 moles of nitrate ions.
2. **Make the standard solution** by the quantitative method described in Box 4.2 and calculate its concentration to four decimal places.
3. **Calculate the volume of solution required for the dilution series** using $[C_1]V_1 = [C_2]V_2$, where V_1 = volume (mL) of standard solution required; C_1 = concentration of standard solution; V_2 = volume of diluted solution (100.00 mL) and C_2 = concentration of diluted solution.
4. **Express the concentrations in the same units**, the most convenient in this case being mol L^{-1}. Therefore, $C_1 = 0.01\ M = 1 \times 10^{-2}$ mol L^{-1} and for the diluted solution of concentration 0.2 mmol L^{-1}, $C_2 = 0.2 \times 10^{3}$ mol L^{-1}. By rearrangement

$$V_1 = \{[C_2]V_2\} \div [C_1]$$

$$= \frac{0.2 \times 10^{3}\ \text{mol L}^{-1} \times 100.00\ \text{mL}}{1 \times 10^{2}\ \text{mol L}^{-1}} = 2.00\ \text{mL}$$

5. **Transfer the standard solution (2.00 mL) to the volumetric flask (100.00 mL)** and make up to the mark with distilled water.
6. **Repeat the calculation for each of the other diluted solutions as required**, but note that a short cut is possible *in this case*: since you require stock solution (2.00 mL) for the diluted solution of concentration 0.2 mmol L^{-1}, you will need 4.00 mL for the 0.4 mmol L^{-1} solution etc. Use pure distilled water for the solution of concentration 0.0 mol L^{-1}.
7. **Calculate the exact concentrations of the diluted solutions** using $[C_1]V_1 = [C_2]V_2$, since the concentration of the standard solution is most unlikely to be exactly 0.0100 mol L^{-1} (see Box 4.2). For example, if the concentration of the standard solution $[C_1]$ is actually 0.009 87 mol $L^{-1} = 9.87 \times 10^{3}$ mol L^{-1}, then for the dilute solution of approximate concentration 0.2 mmol L^{-1}, the actual concentration $[C_2]$ is:

$$[C_2] = \{V_1 \times [C_1]\} \div V_2$$

$$= \frac{0.2\ \text{mL} \times 9.87 \times 10^{-3}\ \text{mol L}^{-1}}{100.00\ \text{mL}}$$

$$= 2 \times 9.87 \times 10^{-5}\ \text{mol L}^{-1} = 0.1974\ \text{mmol L}^{-1}.$$

Note: It is much simpler to measure out whole-number volumes (2.00 mL, 4.00 mL, etc.) using a pipette and produce diluted solutions of accurately known concentrations (but not necessarily whole numbers) rather than to try to produce whole-number concentrations by measuring out non-whole-number volumes.

Sealing flasks – think carefully about the sealing system for a flask to be stored at low temperature. Cooling will reduce the volume of vapour (including air) in the flask and create a partial vacuum. Rubber bungs can be irremovable after cooling (another good reason for never using them) and even ground-glass joints can seize up. Plastic stoppers, screwtops or lightly greased glass stoppers, as appropriate, are recommended.

Storing chemicals and solutions

Chemicals which decompose easily (labile chemicals) may be stored in a fridge or freezer. Take special care when using chemicals which have been stored at low temperature: the container and its contents must be allowed to warm up to room temperature before use, otherwise water will condense onto the chemical. This may render accurate weighing impossible and you may ruin the chemical.

Chemicals and solutions to be stored at low temperatures must be in stoppered or sealed vessels. Do not store aqueous solutions below 0 °C since freezing can occur and, with the resulting expansion of the volume, the vessel may crack. Solutions containing flammable solvents should only be stored in specialized 'spark-proof' fridges: consult your laboratory instructor.

You must be aware of the particular problems of storing solutions in flasks with ground-glass joints. If you are using aqueous solutions you should

Greasing joints – if you are using solutions of NaOH or KOH you *must* grease the ground-glass joint and stopper since the surfaces are attacked by strong alkalis.

lightly grease the joint and stopper with petroleum jelly, since the water will not dissolve the grease as it is poured from the flask. The stopper can be removed easily and the solution will be uncontaminated.

Conversely, if you are using solutions made up from organic solvents, you should *not* grease the joints since the organic solvent will dissolve the grease as you pour it from the flask and contaminate the solution. Moreover, you should not allow the solution to come in contact with the ungreased joints, since the solvent will evaporate and leave the solute to 'weld' the stopper to the flask. Fill the flask with solution, using a filter funnel with the stem of the funnel positioned well below the joint. See p. 43 for use of ground-glass jointware.

KEY POINT **Always label all stored chemicals clearly with the following information: the name (if a solution, state solute(s) and concentration(s)), plus any relevant hazard warning information, the date made up, and your name.**

Balances and weighing

Electronic single-pan balances with digital readouts are now favoured over mechanical types and are common in most laboratories. There are essentially two types of balance:

1. General purpose balances which weigh to the nearest 0.01 g with a capacity of about 300 g. Chemicals may be dispensed for weighing, into a suitable weighing container, directly onto these balances.
2. Analytical four-figure balances for quantitative work, which weigh to the nearest 0.0001 g (0.1 mg) and have a maximum capacity of about 100 g. Chemicals must not be transferred onto the balance at any time and analytical balances must only be used for weighing by difference.

Weighing – *never* weigh anything directly onto a balance's pan: you may contaminate it for others. Use an appropriate weighing container such as a weighing boat, sample tube, weighing paper, conical flask, beaker.

Both types are illustrated in Fig. 4.3 and you should familiarize yourself with their operation before use.

General-purpose balances

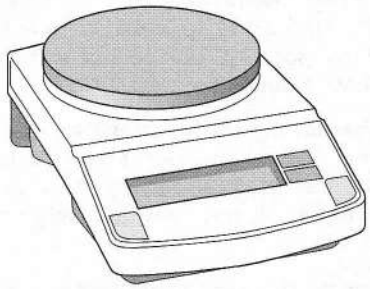
'Weighing paper' – It is common practice to put a piece of paper onto the pan of general-purpose balances. The mass of the paper is then 'tared off' before the weighing container is placed on the balance pan. The paper protects the balance pan from corrosion by spillages and also allows you to discard easily any material spilt without affecting the weighing.

The most useful feature of this type of balance is the electronic zero facility (self-taring), which means the mass of the weighing container can be subtracted automatically before weighing chemicals.

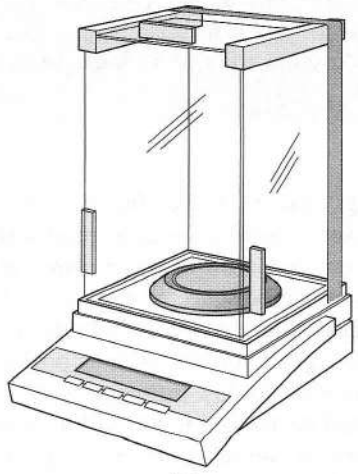
To operate a standard self-taring balance:

1. Check that it is level, using the adjustable feet to centre the bubble in the spirit level (usually at the back of the machine). For relatively accurate work or when using in a fume cupboard, make sure that the draught shield is in place.
2. Ensure that the balance is switched on: the display should be lit.
3. Place an empty weighing container (see p. 24) centrally on the balance pan and allow the reading to stabilize. *If the object is larger than the pan, take care that no part rests on the body of the balance or the draught shield as this will invalidate the reading.* Press the tare bar to bring the reading to zero.
4. Place the chemical or object carefully in the weighing vessel:
   (a) Solid chemicals should be dispensed with a suitably sized clean spatula.

It is poor experimental technique to use large containers to weigh out small masses: you are attempting to measure small differences between large numbers. For small masses, use a small weighing container (Fig. 4.4).



(a)



(b)

Fig. 4.3 Single-pan balance: (a) general purpose, two decimal places; (b) analytical, four decimal places.

(b) Non-volatile liquids should be dispensed using a Pasteur pipette but take the weighing container off the balance pan before dispensing; then reweigh the liquid plus container. Repeat until the desired weight is obtained.

5. Allow the reading to stabilize and make a note of the reading.
6. If you have added excess chemical, take great care when removing it. Remove the container from the balance, remove the solid (with a spatula) or liquid (with a Pasteur pipette) and reweigh.
7. If you need to clean any deposit accidentally left on or around the balance, switch off the balance.

Take care not to exceed the limits for the balance: while most have devices to protect against overloading, you may damage the mechanism.

Analytical balances

These are delicate precision instruments and as such are likely to be found away from the open laboratory in draught-free conditions on a vibration-dampened surface. Analytical balances are maintained to the highest specifications and should need no adjustments on your part, such as levelling and zero adjustment. The key points for using an analytical balance are summarized below:

- No chemicals must be transferred within the weighing compartment of the balance.
- If it has a 'locking' function, the balance pan must always be 'locked' when placing and removing objects onto and from the balance pan.
- The doors of the balance must always be closed when taking measurements.

The procedure for weighing a solid chemical for the preparation of an analytical standard solution is shown in Box 4.4.

Weighing containers

These come in various materials, shapes and sizes: from glass weighing boats to beakers and even special glazed paper. The weighing container to be used depends on several factors:

- The amount of chemical to be weighed.
- The properties of the chemical to be weighed: is it solid, liquid, volatile, corrosive, deliquescent, hygroscopic?
- How and into what type of vessel it is to be transferred.
- The accuracy to which it is to be weighed.

Some of the common types of weighing container are shown in Fig. 4.4.

For analytical procedures, only weighing boats, weighing bottles or glass or plastic sample tubes should be used. Weighing boats are used to transfer a solid directly into a volumetric flask via the neck of the weighing boat: this procedure is recommended when the chemical is known to be totally soluble in the solvent and allows you to omit the solution preparation stage in a beaker or conical flask (see Box 4.2). You must ensure that the neck of the weighing boat will fit well inside the ground-glass joint of the neck of the volumetric flask so that all the chemical can be washed down the sides of the volumetric flask and does not stick to the ground-glass joint during the quantitative transfer.

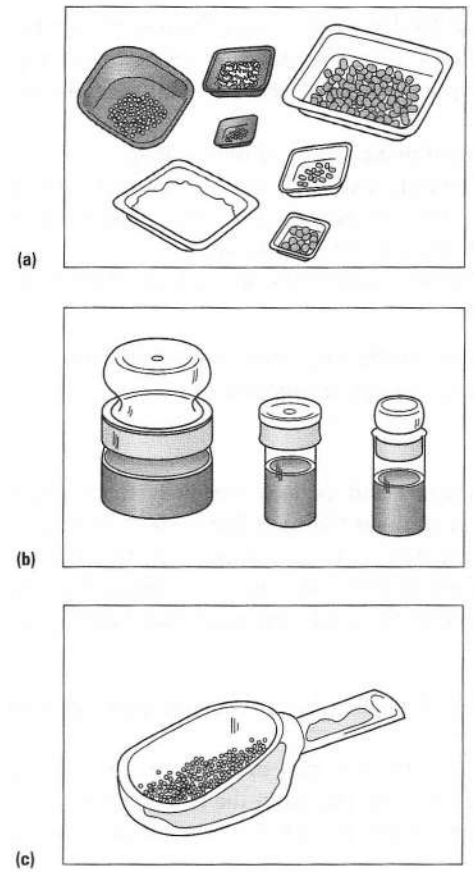


Fig. 4.4 Weighing containers: (a) plastic weighing dishes; (b) weighing bottles; (c) weighing boat.

Box 4.4 How to weigh out a sample of a solid for use in quantitative analysis

1. **Place the clean, dry weighing boat or sample tube on a general-purpose two-decimal-place balance** and zero the balance.
2. **Weigh out the calculated amount of chemical** within the accuracy of the balance.
3. **Check the zero reading on the analytical balance** by pressing the bar/button with the balance doors closed.
4. **Relock the balance pan** by pressing the bar/button.
5. **Carefully transfer the weighing boat or sample tube to the balance pan of the analytical balance** (for very accurate work use tweezers or fine tongs since the sweat from your fingers will contribute to the weight recorded) and close the balance door.
6. **Release the balance pan by pressing the bar/button**, allow the balance to stabilize and record the weight of the chemical and container. If the last decimal place 'cycles' between two or three numbers, determine the mid-point of the 'cycle' and record this value as the weight.
7. **Lock the balance pan by pressing the bar/button,** remove the sample container and transfer the solid to your volumetric flask, beaker or conical flask by pouring, but do not wet the weighing boat or sample tube with solvent.
8. **Replace the weighing container on the analytical balance pan,** close the balance door and weigh the container. Again decide on the mid-point weight if the last decimal place 'cycles' and record this value as the weight of the 'empty' weighing container.
9. **Lock the balance pan by pressing the bar/button** and remove the weighing container from the balance.
10. **Subtract the weight of the 'empty' weighing container from that of the weighing container plus sample** and you now know the mass of chemical, to an accuracy of four decimal places, which has been transferred into your volumetric flask, beaker or conical flask.

Fig. 4.5 Transferring a solid using glazed paper.

For general-purpose work, weighings can be made directly into pre-weighed or 'tared' conical flasks or beakers, again to avoid a transfer stage. Much more common is the use of disposable plastic weighing dishes of the appropriate size. The edges of these dishes can be squeezed together to form a 'funnel' to prevent losses when transferring the solid. Remember that plastic disposable dishes may dissolve in organic solvents such as propanone (acetone), toluene, etc., and should not be used for low-melting organic solids or liquids. Watch- and clock-glasses should be avoided if you wish to transfer the solid into narrow-necked vessels such as conical flasks or sample tubes since it very difficult to direct the solid into the narrow opening of the vessel from the large 'flat' surface of the watch- or clock-glass. In such cases, or when large amounts of solid are to be transferred, it is advisable to use a wide-necked filter funnel called a 'powder funnel'.

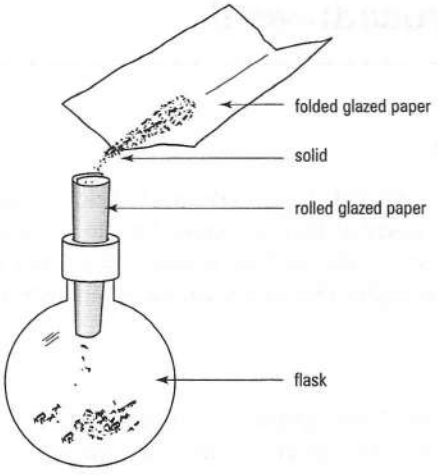


Fig. 4.6 Transferring a solid to a narrow-necked flask.

In many preparative experiments, which are carried out on a small scale (involving 1 g to 10 g of solids), the most useful weighing container is special glazed paper, provided that the chemicals do not react with the paper. A creased square of glazed paper is 'tared' on the balance pan and the solid weighed out directly onto it. The chemical can then be allowed to flow down the crease into the vessel (Fig. 4.5). Furthermore, when attempting to transfer small amounts of solid in vessels with narrow-bore ground-glass joints (see p. 43) it is important not to allow the solid to contact the joint, because the joint will not seal correctly. Use a filter funnel or roll a piece of glazed paper into a funnel, insert the stem of the paper funnel to below the joint and then run in the solid from the creased weighing paper (Fig. 4.6). Paper used in this manner is much cheaper than proprietary weighing dishes and is a useful method of recycling out-of-date manufacturers' catalogues!

5 Basic laboratory procedures II

Clamps and support stands

When carrying out experiments it is vital that all apparatus is held in place securely during the procedure. It is essential that you know how to assemble supporting and securing equipment to the highest possible standards of safety. The most common types of supporting and securing equipment are shown in Fig. 5.1.

Support stands

These are also known as 'retort stands' and comprise an aluminium or steel rod screwed into a heavy metal base. Always check that the base sits level and that the rod is tightened fully in place.

Clamp holders

These are also described as 'clamp bosses' or 'bosses'. Make sure that the locking screws move freely and are not distorted. When you attach the clamp holder to the support stand, tighten the screw firmly and ensure that the open 'slot' to be used for the clamp is pointing upwards (Fig. 5.2).

Clamps

General-purpose clamps are used for securing glassware – therefore make sure that the inner surfaces of the clamp 'jaws' and the 'fingers' are covered with cork or rubber to provide a cushion for the glass: there must be no metal to glass contact in case you overtighten the clamp and crush the glass. Tighten the clamp firmly and ensure that the clamped glassware does not move.

Conical flasks should be clamped at the neck and ground-glass jointware should be clamped at the joint – this usually has the greatest thickness of glass.

KEY POINT **Take particular care when using parallel-sided separatory funnels and chromatography columns (Fig. 5.3), where clamping in the middle of the funnel can be the same as squeezing the middle of a large-diameter glass tube. Clamp at the ground-glass joint using a 'well-cushioned' clamp.**

In most clamps, only *one* of the jaws moves when turning the screw. When you use the clamp in a horizontal position, make sure that the movable jaw is at the *top* (see p. 109).

Burette clamps are specially designed to hold the burette vertically. Springs hold the burette at two points about 5 cm apart – again check for the presence of a rubber or plastic 'cushion' at the points of contact – to prevent slipping and lateral movement. Since the burette clamp slides down the rod of the support stand, then provided the support stand is vertical, the burette will be vertical.

Support rings

These metal rings come in various diameters to support filter funnels and separatory funnels. Often these support rings are coated in plastic to provide the cushion between metal and glass.

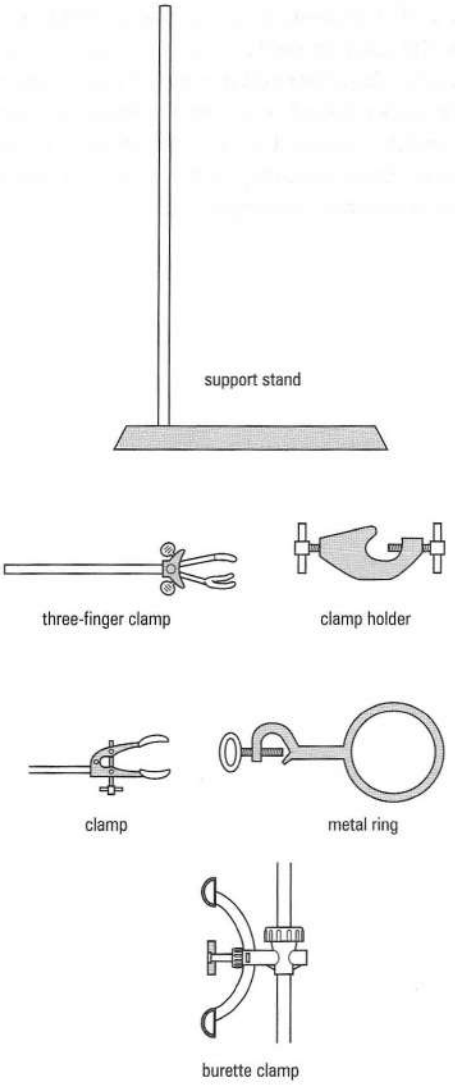


Fig. 5.1 Clamps and supporting equipment.

Using support rings – make sure that the tap on the separating funnel will go through the ring.

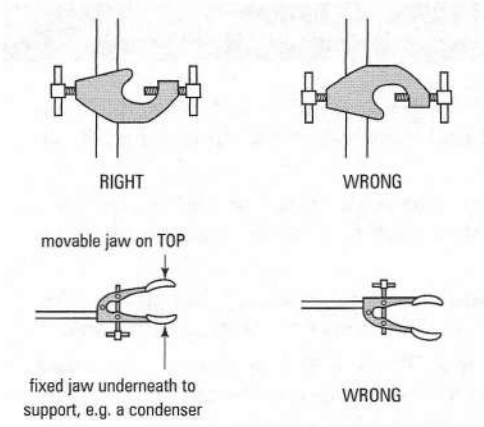


Fig. 5.2 Right and wrong ways of using support stands, clamp holders and clamps.

If your support ring is metal, you can make a 'cushion' by finding a piece of thin-walled rubber tubing of the same bore as the metal of the ring, cutting it to a length equivalent to the circumference of the ring and then cutting down the length of the rubber tubing. You can then slide the tubing around the ring to provide the 'cushion'.

KEY POINT **When using clamps, support rings and support stands make sure that the clamped/supported apparatus is always in position above the base of the support stand (Fig. 5.4) to prevent the stand toppling over.**

Cork rings

These are used to hold round-bottom flasks on flat surfaces while manipulations are being carried out. Since they are light in weight they can be used to hold round-bottom flasks on a general-purpose balance.

Filtration

Filtration is the physical separation of a solid from a liquid and is a process encountered in experimental procedures such as gravimetric analysis (p. 139), recrystallization (p. 92), and solvent drying (p. 41). In principle, the mixture of the solid and liquid is passed through a porous material, filter paper or sintered glass, and the solid is trapped on the porous material while the liquid passes through.

The type of filtration equipment you select for use depends upon which of the two components, the solid or the liquid, you are trying to isolate. In general:

- If you wish to isolate the liquid – *use gravity filtration.*
- If you wish to isolate the solid – *use suction (vacuum) filtration*

There are a few occasions when these rules do not apply, e.g. when the solid is to be decomposed by heat and the filter paper destroyed, a process known as 'ashing'. Some gravimetric analyses, such as the determination of sulphate as barium sulphate, require retention of the precipitate from gravity filtration since $BaSO_4$ is too fine to be collected on a vacuum filtration system. $BaSO_4$ is thermally stable up to 600 °C, so the filter paper can be burned away.

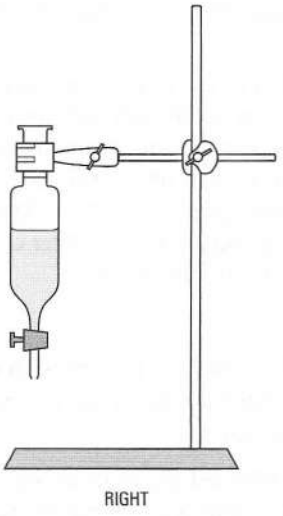


Fig. 5.3 Clamping a parallel-sided funnel.

Gravity filtration

In gravity filtration you need to pass the liquid through the porous material and retain all the unwanted solid in the filter. In general, the best material to use is a filter paper of the appropriate porosity to trap all the solid particles and with the greatest surface area to allow the liquid to pass through quickly. The apparatus required for gravity filtration is shown in Fig. 5.5. The filter funnels are usually made of glass, but if organic solvents are not involved in the filtration, plastic funnels can be used. Glass filter funnels with the pipe cut off are known as 'stemless' filter funnels and have a specific use in hot filtration (p. 98).

The key to successful gravity filtration is the fluted filter paper. A fluted filter paper decreases the area of contact between the filter paper and the funnel, thus allowing rapid filtration. If you use 'traditional' cone-folded filter paper, note that all sides of the paper are touching the sides of the funnel and on half the filter paper the liquid has to pass through three thicknesses of paper, all of which slow the rate of filtration. Slow filtration can lead to disaster in hot filtration during recrystallization (p. 100).

Since filter funnels and filter papers come in different sizes, choose a filter paper of diameter just less than twice the diameter of the funnel. When fluted, the filter paper will be just below the rim of the funnel. There are many ways to fold (flute) a filter paper, but one of the simplest is shown in Box 5.1.

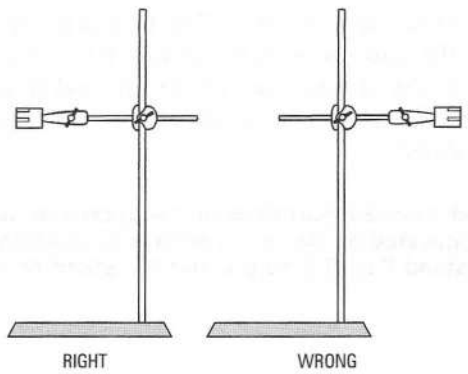


Fig. 5.4 Correct use of support stands.

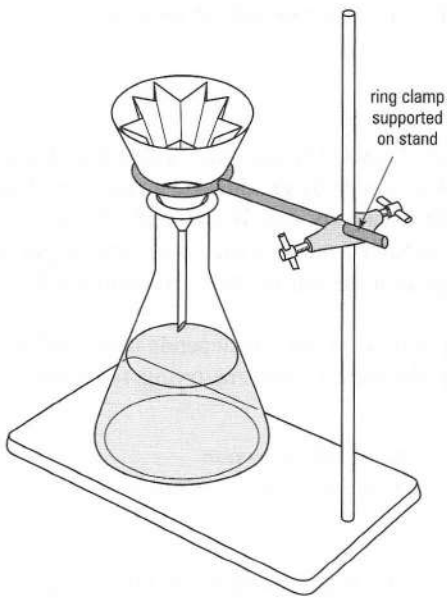
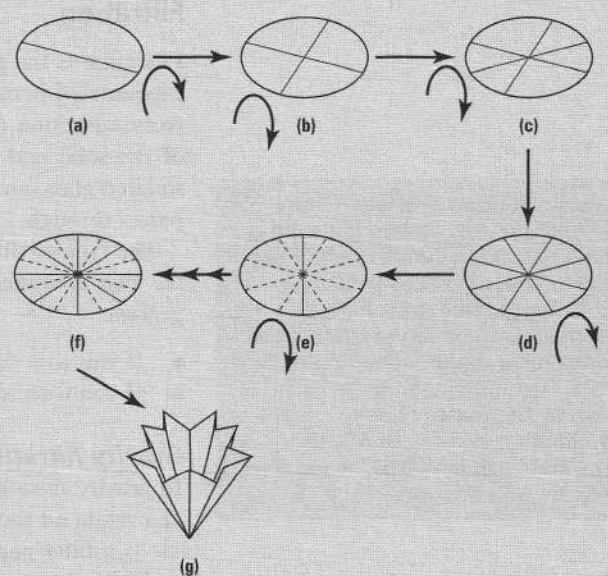


Fig. 5.5 Gravity filtration.

Filtering solids – if you allow the solid to settle you will have difficulty pouring the thick slurry of solid and liquid from the bottom of the beaker or conical flask into the filter cone and you will need to use more filtrate to complete the filtration effectively.

Box 5.1 How to flute a filter paper for gravity filtration

1. **Fold the filter paper in half (a)**, open it out then fold into quarters (b), open it out and then fold into eighths (c and d), all in the same dierction.
2. **Turn the paper over** and fold each sector in half (e); you are creating sixteenths but with each fold in the opposite direction to (a), (b), (c), and (d).
3. **Finally, fold the paper into a cone (g)** ensuring that all the folds are sharp and that the base of the cone comes to a sharp point.
4. The flutes ensure that the filter paper has minimum contact with the filter funnel and the sharp point ensures that the liquid flows rapidly out of the cone and out of the funnel.
5. If your cone point is blunt it will cover the stem of the funnel and so all the liquid must pass through this part of the filter paper, slowing the filtration.



To filter the mixture, swirl the suspension of the solid in the liquid so that there is a fairly even distribution of solid in the liquid, and then pour the mixture into the filter cone, making sure that you do not pour any of the mixture outside the filter paper otherwise you will need to repeat the filtration, and do not overfill the filter cone. Transfer all the mixture in this way and finally wash the last bit of solid and liquid into the filter cone with a *small amount* of filtered solution and then *a little pure solvent*.

Suction filtration

This technique is used for the isolation of a *solid* from a suspension of a solid in a liquid and relies on producing a partial vacuum in the receiving flask. The essential components of a suction filtration system are:

- A ceramic funnel containing a flat perforated plate: there are two types based on size and shape called Büchner funnels or Hirsch funnels. When you are filtering, the perforated plate is covered by a filter paper.

Filtering hot mixtures – *never* attempt to use suction filtration on a *hot* mixture of solid and liquid. As the liquid filters through into the vacuum it may boil under the reduced pressure and be drawn into the pressure tubing. If the liquid is saturated with the solid, the evaporating liquid will deposit the solid in the holes of the perforated plate and block them.

Filtering charcoal – *never* attempt to remove finely divided charcoal, used in decolourization during recrystallization or as a catalyst support, by suction filtration. It is a very fine powder and will *always* leak into the filtrate. Filter off charcoal by gravity filtration.

- A receiver flask with a side arm for attachment of the vacuum source. Büchner flasks are conical flasks made from thickened glass, and Hirsch tubes (also known as side-arm boiling tubes or test tubes depending upon size) are capable of withstanding weak vacuum, e.g. a water pump.
- A flexible seal between the ceramic funnel and the receiving vessel: a Büchner collar or filter seal.
- A source of vacuum, usually a water pump (water aspirator) which is connected to the receiving flask by thick walled rubber tubing (pressure tubing). Sometimes there will be a trap between the water pump and the receiving flask.

The various types of these components are shown in Fig. 5.6: typical apparatus is shown in Fig. 5.7 and the general procedure for suction filtration is described in Box 5.2.

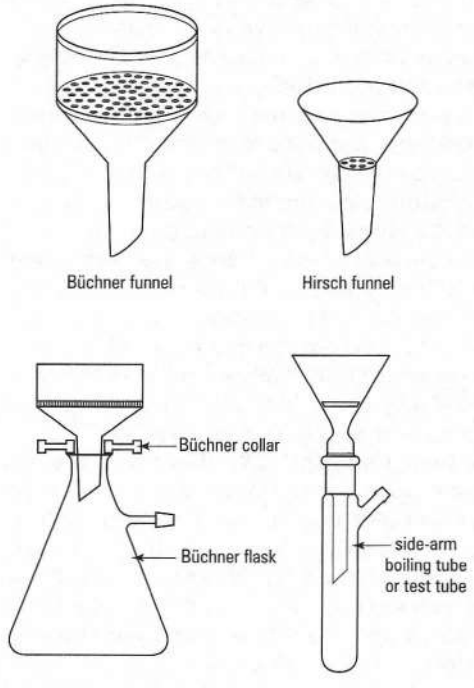


Fig. 5.6 Equipment for suction filtration.

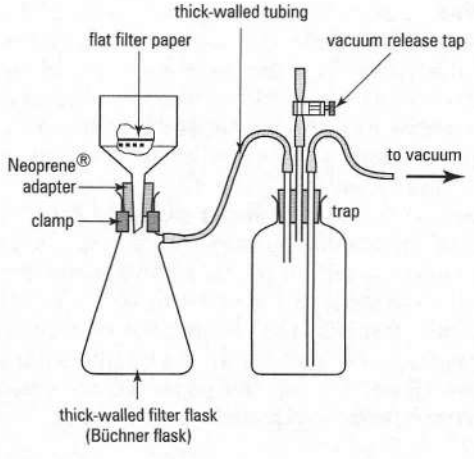


Fig. 5.7 Suction filtration using a Büchner funnel.

Box 5.2 Isolation of a solid by suction filtration

1. **Select the appropriate size of apparatus based on the amount of solid you expect to isolate and the volume of liquid to be collected in the receiver flask.** Consider the following points:
   - **A.** There is no point in using a large Büchner funnel for a small amount of solid since you will collect a layer of solid 'one molecule thick' and be unable to scrape it from the filter paper cleanly. If there is too much solid for the size of the funnel, you will have to repeat the filtration with a second set of apparatus or the solid may not suck dry quickly.
   - **B.** If you use a side-arm boiling tube to try to collect 100 mL of liquid, you will overfill the tube and liquid: (i) will flow into the pressure tubing, contaminating it for your fellow students: (ii) may fill the intermediate trap, if there is one, and you will need to dismantle and clean it; (iii) may be sucked into the water pump causing corrosion and loss of performance.
2. **Clean and dry all the apparatus to be used.**
3. **Clamp the receiving vessel to a support stand:** pressure tubing is heavy and even large Büchner flasks will fall over: do not think that a test tube rack will hold a side-arm boiling tube safely.
4. **Place the correct-sized Büchner collar in the neck of the receiving flask:** it should sit well into the neck and fit the funnel to form a good seal.
5. **Place the funnel into the collar/seal:** note that the funnel has a 'point' at the bottom of the stem. Make sure that this 'point' is as far away as possible from the vacuum attachment side arm of the receiver flask, since the filtering liquid runs off this 'point' and if the point is near the vacuum inlet, the liquid may be drawn into the side arm and then into the trap or water pump (see **1B** above).
6. **Select a filter paper, which fits exactly over the perforation in the base of the funnel.** The filter paper should not fold or crease up the sides of the funnel because the solid will be sucked round the edge of the paper into the receiver flask. If the paper does not fit exactly, trim to size with scissors.
7. **Place the paper into the funnel and wet it with a few drops of liquid** – the same liquid which is to be used in the filtration.
8. **Switch on the tap for the water pump to provide gentle suction.** If your system has a trap, don't forget to close the tap on the trap and connect the rubber tubing to the side arm of the receiver. Do not force the rubber tubing too far onto the side arm – you may need to pull it off quickly if something goes wrong (see **1B** and **5**). The filter paper will be pulled down onto the perforated plate by the vacuum.
9. **Turn on the tap to the water pump to the *maximum water flow*.** If you do not do this, the water pump is not working at its maximum efficiency and the vacuum created in your filtration system may cause water to be sucked into a trap, or receiving flask, from the water pump. This is called 'suck-back'.
10. **Swirl the mixture to be filtered and then slowly pour it into the Büchner or Hirsch funnel at such a rate so that the filtration is rapid.** Note that the rate of filtration may slow as the 'cake' of solid on the filter becomes thicker.
11. **To transfer the last of the solid/liquid from its beaker or conical flask into the funnel use a little of the filtrate in the receiving flask.** Release the vacuum by opening the tap on the trap or pull off the vacuum tubing, but *do not turn off the tap on the water pump* (there is a possibility of 'suck-back' (see **9** above)). Dismantle the apparatus, pour a little of the filtrate into the beaker or conical flask, reassemble the apparatus and continue the filtration. Repeat until all the material has been filtered. Use the filtrate to wash down any of the solid sticking to the sides of the funnel onto the filter 'cake' – it will not dry quickly on the sides of the funnel.
12. **Release the vacuum, by pulling the vacuum tubing from the flask or opening the tap on the trap and turn down the water pressure on the water pump.** Transfer the filtrate to a clean beaker or conical flask. Add a little pure, ice-cold solvent to the filter cake and reconnect the vacuum to provide gentle suction. This will wash the solid. Turn up the vacuum to maximum and suck air through the solid to dry it as much as possible. If 'cracks' appear in the filter 'cake', close them by pressing gently with a clean spatula and repeat until no more filtrate appears to be sucked out.
13. **When drying is complete, release the vacuum, turn off the water tap and remove the filter funnel from the apparatus.** The solid is best removed as a complete 'cake' by lifting the edge of the filter paper with spatula, inverting the funnel over a watch-glass of clock-glass and the cake should fall out. Peel the filter from the top of the 'cake', break up the 'cake' using a spatula and dry it appropriately.
14. **Evaporate the filtrate to half volume (see p. 121)** and cool to obtain a second crop of crystals.
15. **Wash out and clean all the apparatus and dispose of the liquid filtrate safely.**

Never use a sintered-glass funnel to remove finely divided charcoal. It is *always* absorbed into the pores of the sinter and almost impossible to remove easily.

Instead of a filter paper on a porous plate, sintered-glass can be used as the porous material for filtration. Sintered-glass funnels come in various types and sizes (Fig. 5.8) with different porosity (size of holes) of the sintered glass. Sintered glass is also used in crucibles (Fig. 5.8) in gravimetric analysis (p. 139).

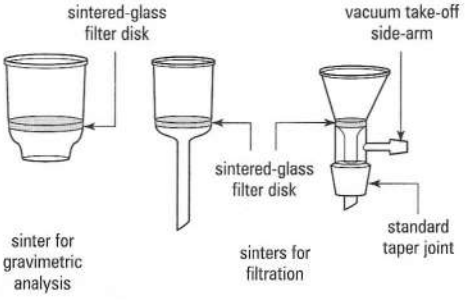


Fig. 5.8 Typical sintered-glass funnels.

Removing charcoal from a cold solution by suction filtration – cover the filter paper with a 'filter aid' such as Celite®. This absorbs the fine particles.

When sintered-glass funnels are used instead of Büchner or Hirsch funnels the solid is collected directly on the sintered glass: no filter paper is used. As a result cleaning sintered-glass funnels is a major problem if the solid has been drawn into the pores of the sintered-glass. Therefore if you are to use a sintered glass funnel, check with your instructor on the appropriate method for cleaning the funnel, before and after use, so that your product will not be contaminated. On the other hand, if particle sizes are large enough to prevent this problem, sintered-glass funnels are very effective in suction filtration.

Heating

Safety note *Never* use gas burners to heat flammable chemicals in open containers, in particular solvents, which are usually used in relatively large volumes.

In the laboratory you will be required to heat chemicals in dissolution of a solid, promotion of reaction (reflux), distillation of pure compounds and mixtures, extraction, coagulation of precipitates, drying solid compounds, etc. Your choice of heat source depends upon several factors:

- First and foremost, the *flammability* and *volatility* of the chemical and solvent.
- The operation to be carried out, e.g. simple preparation of a solution, reflux or distillation.
- The temperature required for the process.
- The amount of chemical or solvent to be heated.

Bumping

Before you attempt to heat any liquid or solution you must take precautions to prevent 'bumping'. This is when the liquid suddenly boils without any warning and results in hot liquid and vapour shooting uncontrollably out of the container. 'Bumping' can occur during simple heating in a test tube, conical flask or beaker or in more complex situations such as reflux and distillation. It is necessary to provide a point in the liquid or solution where vaporization of the liquid can occur in a controlled manner.

Before you start heating a liquid or solution you must *always* take *one* of the following 'anti-bumping' precautions:

- Add one or two 'boiling stones' or 'anti-bumping granules'; these can be filtered off later in the process.

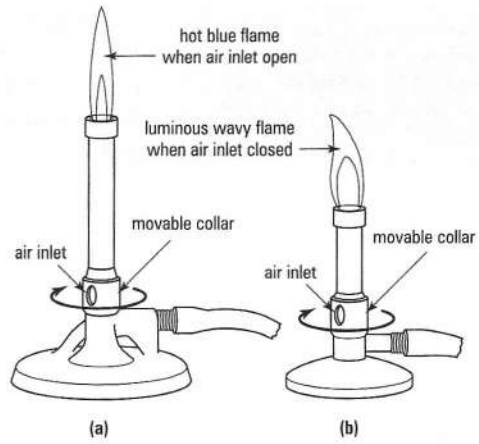


Fig. 5.9 (a) Bunsen burner; (b) microburner.

- Add a Pyrex® glass rod to the beaker or conical flask. The rod must be longer than the container so that it can be removed and rinsed before the solution is used further.
- Add a 'boiling stick': these are thin pieces of wood sold as 'wooden applicators', but you must be sure that nothing will be extracted from the wood into your solution.
- Stir the liquid or solution with a magnetic 'flea' (see p. 35) which should be removed before further processing of the chemicals.
- Stir the liquid or solution with a mechanical stirrer (see p. 118).
- Use an air or inert gas (nitrogen) capillary bleed during vacuum distillation (see p. 110).

Using a microburner – do not place the microburner under the flask and leave it there: you will have no control over the rate of heating.

Heating a test tube – you must 'wave' the test tube in the flame to prevent localized heating, which will cause the liquid to 'bump' out of the tube. Also use an appropriate anti-bumping device and make sure that the tube is not pointing at anyone.

KEY POINT **Make sure that you add the anti-bumping device *before* you start heating since uncontrolled boiling may occur immediately. You *must* allow the liquid to cool back to room temperature and then add the anti-bumping device.**

Burners

Gas burners come in two common forms: large burners called Bunsen burners and small burners known as microburners (Fig. 5.9). Bunsen burners are commonly used for heating aqueous solutions in flat-bottomed Pyrex® vessels supported on a tripod and wire gauze (Fig. 5.10) but for many other heating applications Bunsen burners do not provide adequate control. Microburners may be used for direct heating of round-bottom or pear-shaped glassware in small-scale operations where good control of the heating rate is required, such as distillation or determination of melting point (see p. 87). When using a microburner for heating make sure that you do not create a 'hot spot', which may result in uneven heating of the liquid and 'bumping', by 'waving' the flame around the flask starting just below the level of the liquid and working down to the bottom of the flask and back again.

Microburners are also useful for heating boiling tubes and test tubes, sealing the ends of melting point tubes (see p. 88), making micropipettes for chromatography (see p. 217) and bending the ends of Pasteur pipettes for special purposes (see p. 112).

To use a burner, first make sure that the gas piping, is attached securely to both the burner and the gas tap, is of the correct type of

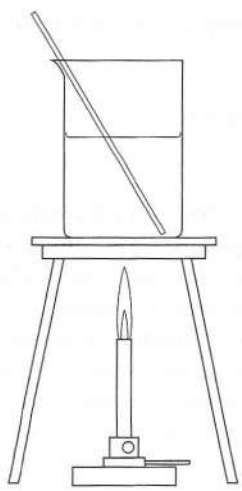


Fig. 5.10 Heating an aqueous solution using a Bunsen burner.

Tubing for gas burners – this must be of medium wall thickness so that it does not kink or compress easily. With thin-walled tubing, you could accidentally lean on the tubing, cutting off the gas supply, which extinguishes the burner. When you release the constriction, gas will then flow into the laboratory.

Safety note Before lighting a gas burner make sure that nobody is using flammable solvents in the laboratory.

Safety note Take particular care when you turn off the gas supply to a Bunsen or microburner. Usually the gas tap is situated at the back of the laboratory bench, so make sure you do not reach over the flame or knock over your apparatus.

rubber tubing, and is not damaged. Close the movable collar and light the gas with a gas igniter: do not use a splint or a piece of burning paper in case you cause a fire in the waste bin when disposing of the paper. Open the collar to produce a hot blue flame and adjust the gas flow to give the required size of flame. If your work is interrupted, close the collar a little to produce a luminous flame. Finally, when the operations are complete, turn off the gas and do not pick up the burner by the barrel, or put it into a cupboard, until it has cooled.

The advantages of the burner are that the heat source can be removed instantly from the apparatus and that the flame is visible: in contrast you often cannot distinguish between a cold and hot metal surface. The disadvantages are those of an open flame.

Steam baths and water baths

These are heat sources, which have a maximum temperature of 100 °C; they are safe to use even with most flammable chemicals and solvents and they differ only in the way the steam is produced.

Water baths are the more common of the two, and the steam is generated by heating water with an electric element – just like a kettle. The element may have a thermostatic control, which can control the temperature of the water to some extent. Most water baths have a constant level device on the side of the bath, which supplies water to the bath to a fixed level above the heating element and prevents the water bath from boiling dry. Water baths are 'single hole' or 'multiple hole' types, and the holes are covered by concentric metal or plastic rings, which allows you to vary the size of the hole.

Do not confuse a *water bath*, which is a general-purpose heating device, with a *constant temperature bath*, which is a precision device used to control the temperature of the liquid in it (usually water, but not always) to within 0.5 °C.

When you are to use a water bath, make sure that water is flowing into and out of the bath via the constant level device – check that water is flowing from the pipe into the drain. Turn on the controller to the level you require – the power can be turned down once the bath is boiling.

Safety note If you need to increase or decrease the size of the 'hole' by adding or removing rings, do it before you start heating or use tongs to prevent scalding by the steam.

A steam bath looks like the 'single hole' water bath except that there is no heating element and no constant level device. Steam baths require piped steam as their source of heat usually provided by a steam line, which is a permanent supply in the laboratory.

Using a 'multiple hole' water bath – make sure all the other 'holes' are covered otherwise the steam will escape through them and heating your flask will be very slow.

Beakers and conical flasks sit firmly on the rings, while round-bottom and pear-shaped flasks should have about half the surface of the flask immersed in the steam (Fig. 5.11).

The main advantage of a water bath is the minimal risk of fire, while the major disadvantages are the maximum temperature available (~100 °C) and the special precautions needed if anhydrous reaction conditions are required: remember that steam will condense down the *inside* of reflux condensers.

Safety note Steam lines are *very dangerous* and you should not attempt to use them without direct supervision from your laboratory instructor.

Hot plates and stirrer hot plates

These consist of a flat metal or ceramic plate, which is heated electrically, and varies in size for use by an individual or by several people at the same time. The small versions normally have a built-in magnetic stirrer, which can be used to stir the liquid with a magnetic 'flea'.

Diethyl ether (ether) and hydrocarbons (petroleum spirit) must *never* be heated on a hot plate. Diethyl ether (b.pt. 35 °C) should always be heated under reflux using warm water and petroleum hydrocarbons should be heated under reflux on a water bath or oil bath.

Hot plates of both types should only be used for heating flat-bottom vessels such as beakers or conical flasks and only when the liquid being heated is non-flammable. The vapour of a flammable liquid may 'run' down the outside of the vessel and ignite on the hot metal of the heating surface. Since the contact area between the heating surface and a round-bottom flask

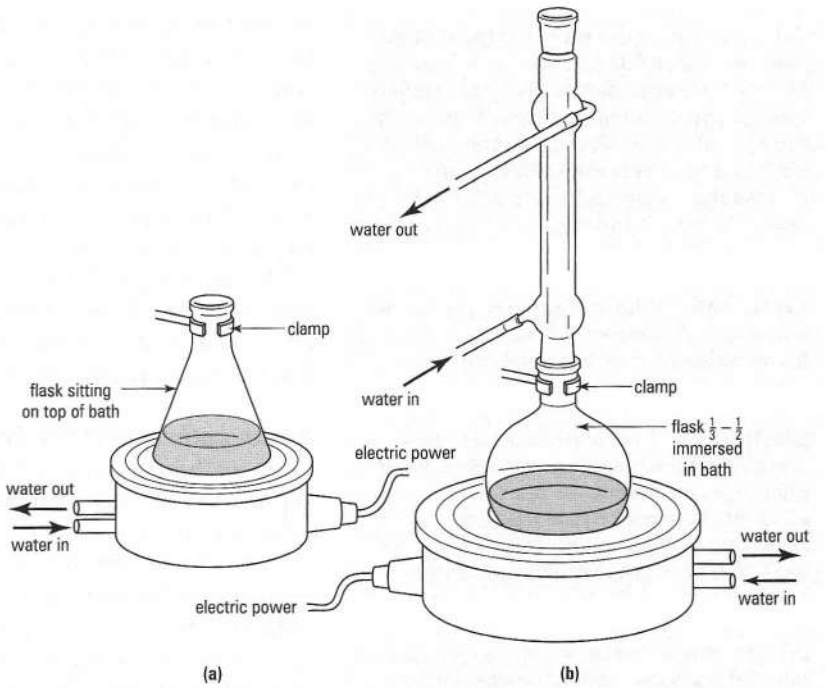


Fig. 5.11 (a) Heating a conical flask on a water bath; (b) heating a reflux set-up on a water bath.

is very small, your attempts to heat these flasks effectively will require excessive temperatures of the heating surface, which increases the dangers due to lack of control of the heating process. Therefore round-bottom flasks should only be heated using an oil bath (below) or a mantle (p. 35).

Using hot plates – *never* wrap the power cable round the heating plate. It may be hot and melt the flex, exposing the wires.

The flat exposed heating plate is extremely dangerous when hot: *always* check that the plate is cool by passing your hand over the plate without touching it or by placing a drop of water on the plate. If you have to pick up a hot plate, hold it by the sides and do not touch the plate; it may burn. Typical uses of hot plates are illustrated in Fig. 5.12a.

Oil baths

These are mostly used to heat round-bottom flasks at temperatures above 100 °C. The oil bath, containing the heating fluid, is usually a non-ferrous metal or Pyrex® dish and heated on a stirrer hot plate, and the temperature of the bath is measured with a thermometer. The oil bath should never be more than half-full, to allow a margin of safety for thermal expansion of the oil, and stirred with a magnetic 'flea' to ensure even heating. The equipment used in a typical oil bath is shown in Fig. 5.12b.

Stirring in an oil bath – the stirrer hotplate will stir both the 'flea' in the oil bath and the 'flea' in the reaction vessel.

The nature of the oil used in the bath depends upon the temperature range required and a selection of liquids is shown in Table 5.1.

You are most likely to encounter paraffin oil baths during your laboratory work and the following safety points should be considered:

Using oil baths – oil baths should be used in the fume cupboard because prolonged heating may cause unpleasant and possibly toxic fumes to be released from the oil.

- Paraffin oil discolours rapidly with prolonged heating. If the oil is dark, replace it with fresh oil. Dispose of the old oil in an appropriate manner (check with laboratory staff).
- Check that there is no water in the bottom of the oil bath: look for a separate layer or globules of liquid in the bottom of the bath. If you heat

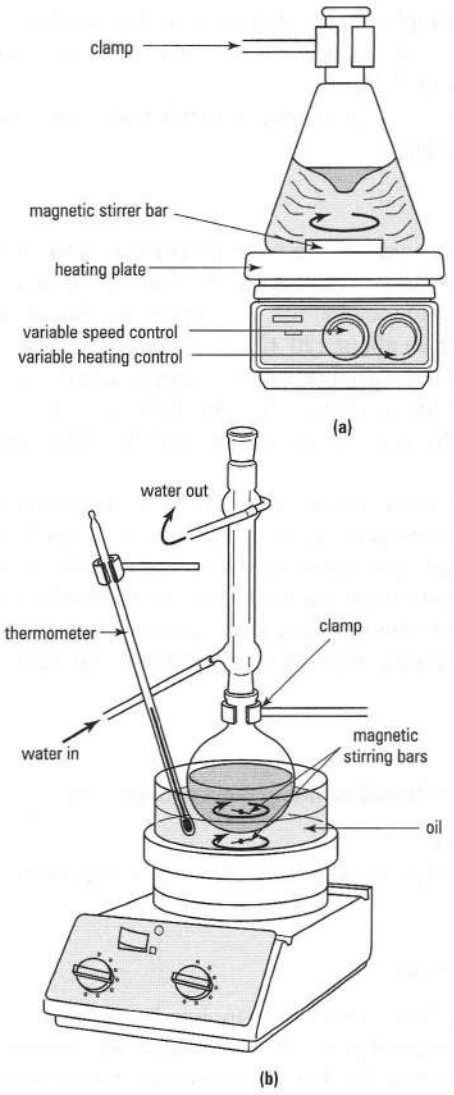


Fig. 5.12 (a) A stirrer hot plate. (b) Using a stirrer hot plate with an oil bath to heat a round-bottom flask.

Safety note *Never* connect an electric heating mantle, requiring an external controller, directly to the mains electricity supply.

Table 5.1 Oil bath liquids

Material	Usable range (°C)	Comments
Paraffin oil (mineral oil)	0–200	Flammable, cheap, produces acrid smoke above 220 °C
Ethylene glycol	0–150	Flammable, cheap, low flash point
Polyethylene glycol 400	0–250	Water soluble
Silicone oil	0–250	Expensive
Glycerol	0–250	Water soluble

the bath above 100 °C, the water will boil and may spatter hot oil over you and the apparatus. Dispose of the contaminated oil into the appropriate waste container, clean the bath with paper towels to absorb the water and refill with fresh oil once *completely* dry.

- If the oil bath is a Pyrex® dish, check the dish carefully for cracks and 'star' cracks because you do not do want the dish of hot oil to break.
- Support the stirrer hot plate on a 'labjack' so that you can quickly remove the heat source if the reaction goes wrong (see mantles, p. 36).
- When the heating process is finished, allow the bath to cool and raise the flask, let the oil drain from it into the bath and then wipe the flask with a cloth or paper towel. Otherwise your hands, gloves, apparatus, compounds, etc., may become contaminated with oil.

Electric heating mantles

These are used for heating round-bottom flasks *only* and come in various sizes. Always used a heating mantle appropriate to the size of the flask you are going to heat, since you need to *control* the heating process. The flask should fit snugly into the mantle with the top half of the flask above the case of the mantle. If the mantle is too small, heating will be inefficient, whereas if the mantle is too big, the flask will be overheated and decomposition of the contents may occur.

Electric heating mantles comprise an electric heating element wrapped in a glass fibre covering, protected by an earth screen and enclosed in an aluminium or, more commonly, a polypropylene case to allow handling at moderate temperatures. Heating control is provided either by a regulator built into the heating mantle (Fig. 5.13a) or by an external controller, which is connected to the mantle by a plug.

Some mantles, 'stirrer mantles', have a built-in magnetic stirrer just like a stirrer hot plate and can be used to mix the liquid using a magnetic flea or bar (Fig. 5.13b). Stirrer mantles have *two* controls on the side: make sure that you know the function of each, since one controls the extent of heating and the other the stirrer speed.

When using mantles make the following safety checks:

- Make sure that the heating element is not damaged or worn. If in doubt consult your instructor and get a replacement.
- Plug in and test the mantle controls ensuring that both heater and stirrer are working. If fumes are given off from the heating elements someone has spilt chemicals into the mantle: switch off, report the fault to your instructor and obtain a replacement.
- Mantles are relatively slow to react to changes in the heater control setting and it is easy to 'overshoot' the desired temperature. Therefore always make small incremental changes in the heating control and if a temperature below the boiling point of the liquid is required, make sure that a thermometer is incorporated in the apparatus.

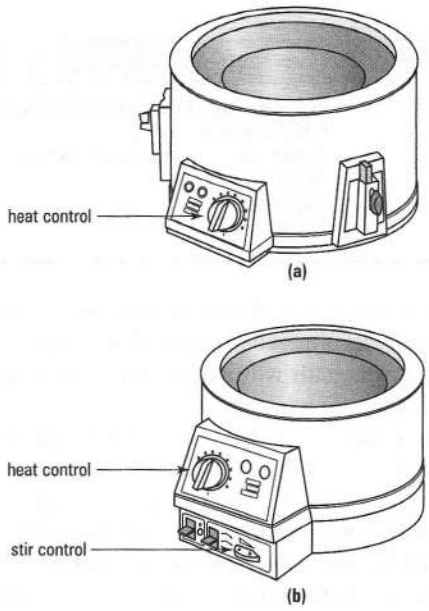


Fig. 5.13 Mantles: (a) heating mantle; (b) stirrer mantle.

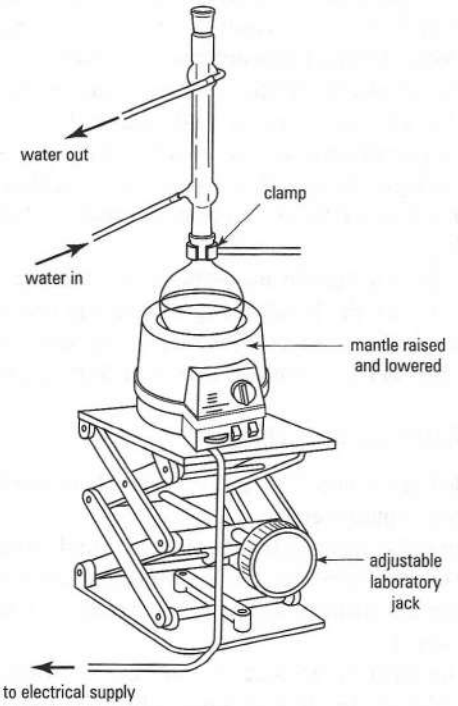


Fig. 5.14 Heating using a stirrer mantle.

- When using a mantle with complex apparatus such as for distillation (p. 107), support the mantle on a 'labjack' so that it can be removed quickly if overheating occurs (Fig. 5.14).
- If the mantle is not equipped with a stirrer, remember to add anti-bumping granules (p. 31) to the liquid.

Hot-air guns

These can be used instead of Bunsen burners or microburners provided that the temperature required is not too high. The main uses of hot-air guns are for drying glassware and as a heat source for distillation of liquids at relatively moderate temperatures up to about 120 °C.

Hot-air guns produce heated air, usually at two temperatures, and cold air. As with a burner, the heat transferred to the flask being heated can be controlled by 'waving' the hot air stream around the flask (see p. 32).

Remember that the hot-air gun has a hot-wire heating element; therefore do not use it in the presence of flammable vapours. The nozzle of the hot-air gun will become very hot and can cause burns or even ignite some solvents for some time after it has been switched off. You should switch the gun to the cold-air mode for a few minutes after you have finished heating and place it in a 'holster' made from a support ring before finally turning off the gun.

Handling hot glassware

The safety precautions necessary for handling hot glassware depend upon:

- The temperature of the glassware.
- The type of glassware: test tube, beaker, conical flask, round-bottom flask, etc.
- The size of the glassware.
- The manipulation being carried out.

Normally you do not need any hand protection to handle glassware at temperatures up to 50 °C. For general-purpose work, such as removing glassware from the drying oven, assembling hot glassware and manoeuvring hot beakers and conical flasks to and from a burner, steam bath or hot plate, heat-resistant gloves are suitable and should be available in the laboratory. Where more intricate processes are required, such as hot filtration, then 'rubber fingers' made from medium-wall rubber tubing (Fig. 5.15) give adequate protection up to about 120 °C and are less cumbersome than insulated gloves. 'Rubber fingers' are useful for small volumes, up to 150 mL, of liquids when the flask or beaker can be easily held by one hand. If larger volumes are being manipulated then two hands are required and heat-resistant gloves are essential.

The following specific techniques should be noted:

- *Test tubes*: should be held by a wooden test tube holder (Fig. 5.16), which provides adequate insulation and grip. You should *never* use folded pieces of paper or metal tongs.
- *Conical flasks*: are often clamped to a support stand during heating and you should *never* attempt to use the clamp as a device to hold the flask when removed from the support stand. If you place the flask on the laboratory bench with the clamp attached it will fall over because of the weight of the clamp. Furthermore, you will have little control over the

Safety note *Never* attempt to lift and manipulate a volume of liquid/solution that you cannot carry *easily*; if in doubt divide equally between two flasks.

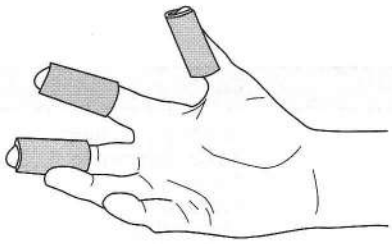


Fig. 5.15 Rubber 'fingers'.

Cooling reactions – the heat from exothermic reactions is absorbed by the cooling medium, otherwise the reaction rate will increase rapidly with the rising temperature and may result in violent boiling or even explosion.

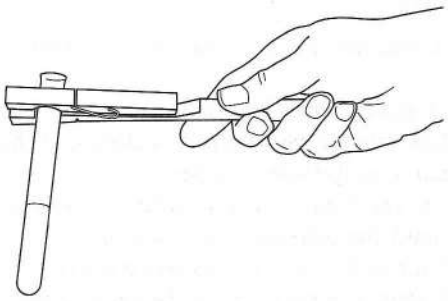


Fig. 5.16 Holding a test tube.

Controlling temperature – temperature control may be necessary to achieve the desired reaction. For example, chemicals may react to give different products at different temperatures.

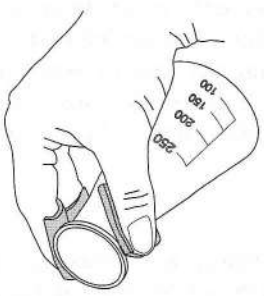


Fig. 5.17 Pouring a hot solution.

pouring process. Use 'rubber fingers' or an insulated glove and *never* use folded pieces of paper or metal tongs (Fig. 5.17).

- *Beakers*: these have specific problems since they have no narrow neck which can be gripped for lifting. Small beakers of volumes up to 400 mL capacity can easily be gripped in one hand protected by an insulated glove or 'rubber fingers'.
- *Round-bottom flasks*: small flasks of capacity up to 250 mL should be held at the neck, gripped in one hand protected by an insulated glove or 'rubber fingers' and when moving and pouring from larger flasks they should be held by the neck and supported underneath. Do not use a clamp round the neck of the flask as a support.

Cooling

During laboratory work you will be required to carry out experiments at temperatures below room temperature. The most common situations where cooling is required are:

- Cooling solutions during recrystallization.
- Completion of precipitation in quantitatative (gravimetric) analysis and preparations.
- Cooling exothermic reactions.
- Carrying out reactions at low temperatures.

There are three cooling media commonly used in the laboratory: crushed ice, solid carbon dioxide (Dry Ice®, Drikold®, Cardice®) and liquid nitrogen. You are unlikely to use liquid nitrogen in the undergraduate laboratory – if it is required you must consult your demonstrator about the special safety protocols required for its use.

The most suitable containers for cooling baths are plastic bowls (ice baths), Pyrex® dishes (solid CO_2 baths) and Dewar flasks (solid CO_2 and liquid nitrogen baths). If Pyrex® dishes are to be used below −20 °C, an insulated container can be prepared by placing a smaller Pyrex® dish inside a larger one and filling the space between with an insulating material such as cotton wool, cork chips or polyurethane foam chips. Remember that foam chips will dissolve if they come into contact with many organic solvents.

If the temperature of the liquid or solution being cooled is critical, *do not assume* that the temperature of the liquid or solution is the same as that of the cooling bath: place a thermometer in the flask and remember that for temperatures lower than −5 °C you should use an alcohol thermometer (red thread) or a thermocouple-type thermometer (after checking that the probe will not react with the contents of the flask).

Ice baths

A slurry of crushed ice and water can be used to give a cooling bath in the range 0 °C to 5 °C. Pure crushed ice does not give good contact with the glassware and inefficient cooling results.

If temperatures below 0 °C are required, mixtures of crushed ice and various inorganic salts can be used as shown in Table 5.2. Note that these mixtures contain no liquid and therefore cooling is inefficient and the temperatures indicated in the table are the lowest attainable under ideal conditions.

Table 5.2 Ice–salt mixtures

Salt	Ratio (salt : ice)	Temperature
$CaCl_2.6H_2O$	1 : 2.5	−10 °C
NH_4Cl	1 : 4	−15 °C
NaCl	1 : 3	−20 °C
$CaCl_2.6H_2O$	1 : 0.8	−40 °C

Safety note If the cooling bath is more than half-full of solvent, it will 'boil' out of the bath as you add the first few lumps of solid CO_2.

Using cooling baths – remember that a cooling bath will condense water from the atmosphere and therefore lose its effectiveness over a period of time.

Solid CO_2 baths

Solid CO_2, when mixed with organic solvents, provides cooling media of temperatures ranging from −15 °C to −100 °C. Some common mixtures together with the minimum achievable temperatures are shown in Table 5.3. The CO_2 and the organic solvent form a 'slush', which gives excellent contact with flasks and, therefore, efficient cooling.

Table 5.3 Solid CO_2– solvent mixtures

Solvent	Temperature	Comments
Ethylene glycol	−15 °C	Ice/NH_4Cl cheaper
Acetonitrile	−40 °C	Toxic, flammable
Chloroform	−60 °C	Toxic
Ethanol	−72 °C	Flammable
Acetone	−78 °C	Flammable
Diethyl ether	−100 °C	Highly flammable

Solid CO_2 is supplied as large hard blocks or small quantities can be prepared from cylinders of liquid CO_2 as a 'snow'. Skin contact with solid CO_2 will cause frostbite and it *must* be handled with *insulated gloves*. The cooling bath must be an insulated container, which will need to be topped up with solid CO_2 at regular intervals to maintain the temperature, or, if prolonged cooling is required, a Dewar flask in which the coolant will maintain its temperature for 12 hours or so.

To prepare a solid CO_2 cooling bath:

- Choose the appropriate solvent and remember to take into account the hazards associated with its use.
- Break the solid CO_2 into small pieces. The CO_2 'snow' can be broken with a spatula, but the hard blocks should be wrapped in cloth and then broken into pieces with a wooden or polyethylene mallet.
- Half fill the bath with the solvent and then, using an insulated glove, add small pieces of the solid CO_2 until the mixture stops 'boiling' and then add a little more solid CO_2 and stir with a glass rod to give a slurry.
- Use an alcohol or thermocouple thermometer to check the temperature of the bath.
- Top up the cooling bath with solid CO_2 if the temperature begins to rise.

KEY POINT **When using an internal thermometer to measure the temperature of a liquid or solution which is being stirred with a magnetic flea or stirring bar, ensure that the thermometer bulb does not come into contact with it.**

To prevent the condensation of water into your flask, you should have an inert gas flowing through it (see p. 126) and prepare the cold bath around the flask and its contents so that slow cooling occurs. Sudden immersion of a relatively 'hot' flask into the cold bath will cause the bath to 'boil' and air (containing water vapour) will be sucked into the apparatus despite the inert atmosphere. Alternatively a 'loosely packed' $CaCl_2$ guard tube (see p. 117) will suffice if cooling is not too rapid.

Cooling probes

These are rigid or flexible metal probes, which are connected to a refrigeration compressor. The probe is placed in the cooling bath and covered with a suitable solvent, which is then cooled to the temperature desired.

Cooling probes are commonly found in constant temperature baths, when a temperature below ambient temperature is required, and probes can be used instead of solid CO_2 to produce temperatures down to $-100\,^{\circ}C$. Cooling probes are expensive pieces of equipment; therefore you will find them dedicated to a specific experiment and they are not usually available for basic laboratory operations.

Drying

During your laboratory course it will be necessary to dry glassware, analytical standard compounds (see p. 144), chemicals you have synthesized, crucibles used in gravimetric analysis (see p. 139) and solvents. Drying solvents is described on p. 127.

Drying glassware

For most general laboratory applications glassware can be dried in an electric oven between 80 °C and 90 °C or by rinsing the glassware with a small amount of water-miscible solvent, such as acetone or ethanol, and then evaporating the solvent using a compressed-air jet. Remember to remove all plastic components, taps and stoppers from the glassware *before* you put it in the oven.

If glassware is required for anhydrous reactions, it must be heated in the oven above 100 °C, assembled while hot and allowed to cool while a stream of inert gas is passed through it (see p. 126).

Never place chemicals in the oven on a piece of filter paper. If your chemical melts it will run through the paper and may contaminate the samples of fellow students. If it is on a watch-glass it may be recovered if it has not decomposed.

Drying solids

Here the term 'drying' means removal of a solvent, not specifically water, from a solid by evaporation. The rate of evaporation and thus the rate of drying can be increased by one (or all) of the following:

- Heating the chemical.
- Using a drying agent in a closed container to absorb the solvent.
- Reducing the atmospheric pressure.

Only chemicals which are *thermally stable* should be dried by heating. Most inorganic compounds, which are salts with relatively high melting points, can be dried in an electric oven to remove water, whereas organic compounds, many of which have relatively low melting points, need to be treated with more care and the oven temperature should be set between 30 °C and 50 °C *below* the melting point of the chemical. Chemicals must be placed in the oven on a clock-glass or watch-glass and be spread as thinly as possible, to increase the rate of solvent evaporation.

If you cannot dry your compound in the oven, then use a *desiccator*. Desiccators are made from glass or plastic and some, vacuum desiccators, are equipped with a tap to allow evacuation as shown in Fig. 5.18. The bottom of the desiccator is filled with a drying agent (desiccant) and the chemical, on a watch-glass or clock-glass, is placed on the mesh shelf above and the desiccator closed by sliding the lid onto the desiccator to provide an air-tight seal. The desiccant absorbs the solvent from the 'atmosphere' in the desiccator as it evaporates from the solid. The nature of the desiccant depends upon the solvent to be removed (Table 5.4).

The most common drying agents for removal of water are anhydrous $CaCl_2$ and self-indicating silica gel. The $CaCl_2$ should have the appearance of 'chalky' lumps. Self-indicating silica gel is blue in the 'active' state and pink

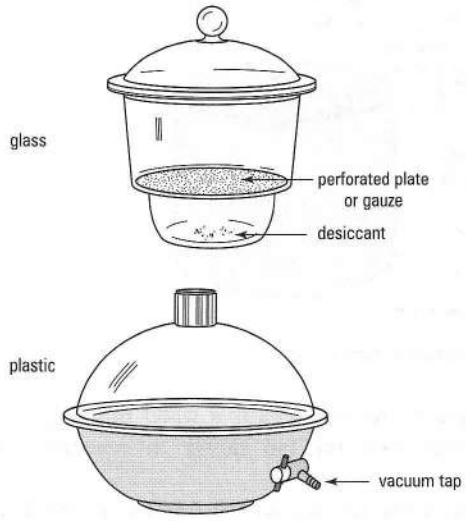


Fig. 5.18 Desiccators.

when its capacity for water absorption is 'exhausted'. The water can be removed from the pink silica gel by heating in an oven above 100 °C and restoration of the blue colour indicates reactivation.

Table 5.4 Drying agents for desiccators

Solvent to be removed	Drying agent	Comments
H_2O	Silica gel	
	$CaCl_2$	
	solid KOH	Corrosive
	P_2O_5	Corrosive
	Conc. H_2SO_4	Corrosive liquid
EtOH, MeOH	$CaCl_2$	
Hydrocarbons	Paraffin wax	

Using corrosive acids – P_2O_5 and concentrated H_2SO_4 should be avoided unless there is no suitable alternative. Consult your instructor for their disposal.

The rate of drying can be increased by evacuating the desiccator and vacuum desiccators are specially designed for this purpose. The principal steps for the use of a vacuum desiccator are as follows and it is essential that you follow the order of the operations:

- Check that the appropriate desiccant is present and active.
- Check that the desiccator seals perfectly: ensure that the ground-glass edges to the lid are greased lightly or, if the desiccator has a rubber gasket, carry out a trial evacuation to ensure that the vacuum seals the desiccator by gently pressing the lid onto the gasket – listen for air being sucked around the seal.
- Place the sample onto a watch- or clock-glass or a beaker covered with tissue paper (secure with an elastic band) and place it on the shelf.
- Place the lid on the desiccator and open the tap and cover the desiccator with an appropriately sized safety cage (Fig. 5.19) to prevent injury from flying glass in the case of an implosion.

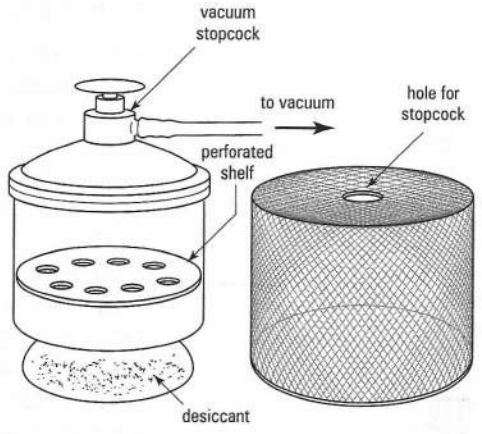


Fig. 5.19 Vacuum desiccator (with mesh safety cage).

Using vacuum desiccators – *never* switch off the vacuum supply before disconnecting the pipe from the desiccator since you will suck water or oil into the supply pipe (see p. 30).

Using vacuum desiccators – *never* disconnect the vacuum supply with the tap on the desiccator open. Air will rush into the desiccator and blow your dry compound around the inside.

- Connect the tap to an operating source of vacuum: a water pump (p. 30) or vacuum pump (p. 110) and open the tap slowly to evacuate the desiccator.
- When drying appears complete, close the tap on the dessicator and then disconnect the vacuum supply.

- Place a small piece of filter paper on the end of the tap and open the tap slowly. The filter paper will stick to the tap as air is drawn slowly through it. When the air has completely filled the desiccator, the filter paper will fall off and it is safe to open the desiccator.

Using water pumps for prolonged drying – this uses too much water: evacuate the desiccator, close the tap and disconnect and turn off the water pump. Check periodically that the vacuum is preserved using the filter paper technique. Re-evacuate if necessary.

If you need to heat the compound under vacuum, then you will need to use a vacuum oven (for large quantities of solids). The principles of operation of these pieces of equipment are similar to those of a vacuum desiccator, except that an electric heater is incorporated. Always allow the apparatus to cool to room temperature before releasing the vacuum.

Drying liquids

Drying solvents – the drying agent used for solutions or pure liquids is not usually suitable for drying solvents for inert atmosphere reactions.

This usually means removing water from a liquid chemical or a solution of a chemical in a water-immiscible solvent. You will always need to dry solutions after a liquid–liquid extraction (p. 102) and you may need to dry liquids after evaporation (p. 121) or distillation (p. 107). In both cases the liquid is placed in direct contact with the solid drying agent, i.e. the drying agent is added to the liquid or solution. Ideally the drying agent should be totally insoluble in the liquid, should not react with it, absorb the water quickly and efficiently, and be easily filtered off. A list of the common drying agents is given in Table 5.5.

Table 5.5 Drying agents for liquids and solutions

Drying agent	Capacity	Speed	Efficiency	Comments
$MgSO_4$	High	Fast	Good	Best general use
Na_2SO_4	High	Slow	Poor	Useful
$CaCl_2$	High	Slow	Poor	Reacts with O and N compounds
$CaSO_4$	Low	Fast	Good	Useful
K_2CO_3	High	Fast	Good	Reacts with acidic compounds
Molecular sieve	Moderate	Fast	Good	Must be activated at 300 °C

Capacity: amount of water taken up.
Speed: rate of water absorption.
Efficiency: extent of drying after treatment.

Molecular sieves are synthetic calcium and sodium aluminosilicates, which have 'holes' of specific sizes to allow the absorption of molecules of similar dimensions. Types 3A and 4A, with 0.3 nm and 0.4 nm pores respectively, are used for drying.

Drying solutions and liquids – note that phrases such as 'drying *over* magnesium sulphate' mean that the $MgSO_4$ is *added* to the solution.

You must remember that the drying agent will absorb some of the liquid or solvent being dried as well as the water. If you wish to dry a small volume of liquid, it is better to dissolve it in a low-boiling water-immiscible solvent and dry the solution by the procedure described in Box 5.3.

Box 5.3 How to dry a solution over magnesium sulphate

1. **Place the solution to be dried in a clean, dry conical flask.** The flask should not be more than half-full.
2. **Add small quantities of $MgSO_4$ (between 0.1 g and 1.0 g depending upon the volume of solvent) and swirl the conical flask between each addition.** At first the $MgSO_4$ will 'stick' to the sides and bottom of the flask where it contacts with the water, but on further additions it will eventually form a free-flowing powder in the liquid and the solution will appear very clear and bright.
3. **Allow the mixture to stand for 10 minutes.**
4. **Gravity filter the dried solvent layer through a fluted filter paper into a clean, dry flask.** The fluted filter paper should contain a small amount of fresh $MgSO_4$, as a safety precaution, in case a few drops of water are floating in the surface of a denser solvent than water.
5. **Rinse the $MgSO_4$ in the conical flask with a few millilitres of pure dry solvent and filter it,** to ensure that you recover all the solute, and then rinse the $MgSO_4$ on the filter paper.
6. **Remove the solvent** by rotary evaporation (p. 121) or distillation (p. 108).

Jointed glassware

This type of glassware, commonly known as Quickfit®, comprises a complete range of components fitted with standard-taper ground-glass joints. The joints are fully interchangeable with those of the same size and apparatus for a whole range of experiments can be assembled from the simple components without the need to use rubber bungs, corks, etc. Where there is a mismatch between the sizes of the joints of the pieces of glassware, reduction and expansion adapters can be used. A typical range of jointed glassware is illustrated in Fig. 5.20.

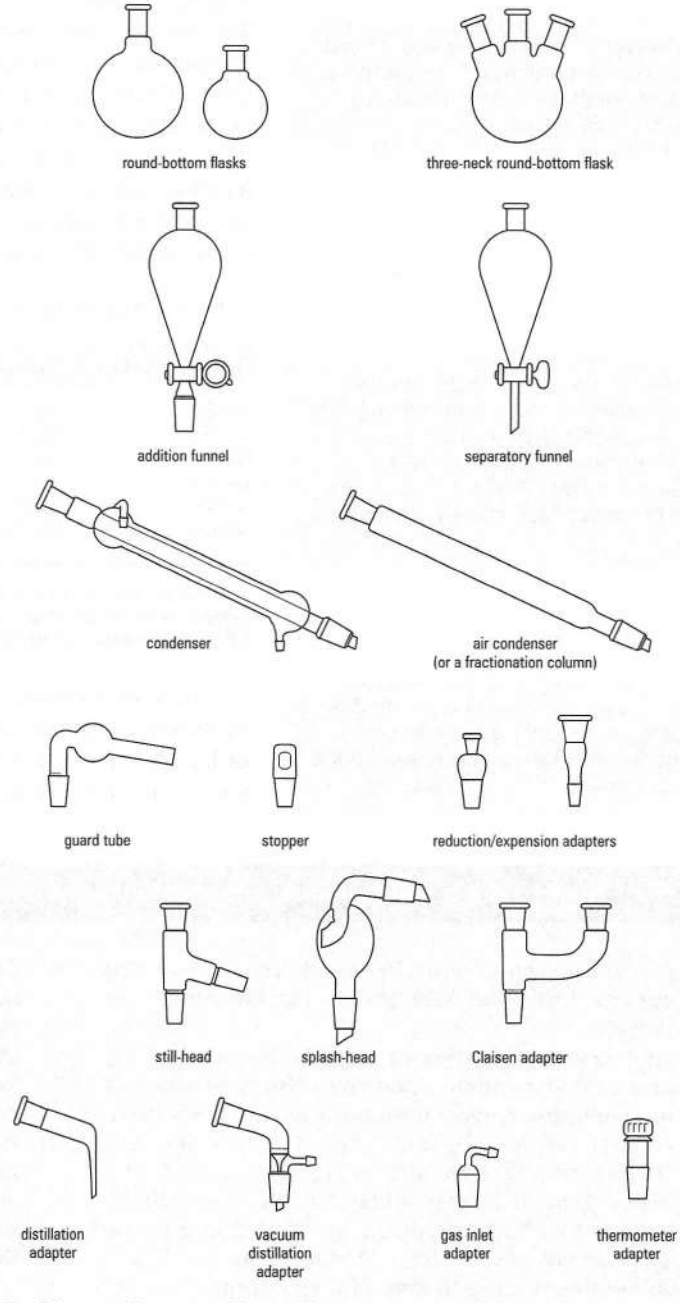


Fig. 5.20 Glass equipment with standard-taper ground-glass joints.

Do not attempt to use non-standard ground-glass joints in the standard jointware, it will not fit correctly. For example, ground-glass stoppers from volumetric flasks do not seal Quickfit® separatory funnels.

The ground-glass joint on the glassware is classified according to the diameter of the joint at its widest point (internal diameter) and the length of the ground-glass portion of the joint. Thus a 14/23 joint has a maximum internal diameter of 14 mm and a length of 23 mm. Other common joint sizes you will frequently encounter are 19/26, 24/29 and 35/39. The joint size is always etched into glass on the side of or near to the joint. For obvious reasons, joints are categorized as 'female' and 'male'.

Care of jointed glassware

Jointed glassware is much more expensive than ordinary glassware because of the precision required in fabricating the joints. If the joints 'seize' and cannot be separated the glassware cannot be used again and you may have the problem of a stoppered flask containing a volatile organic solvent, which somebody has to open! If this happens, consult your instructor for help and further advice.

There are two main causes of 'seized' joints:

1. Using solutions of potassium hydroxide or sodium hydroxide in water or other solvents, which attack the glass.
2. Trapping chemicals, including solids and solutions of solids, in the ground-glass joints.

Greasing joints – you *must* grease all the joints when carrying out a *vacuum distillation.*

If you are using jointed glassware with strong alkalis (NaOH, KOH), you *must* grease the joints. In most cases a simple hydrocarbon-based grease, such as petroleum jelly, will suffice, since it is easily removed from the joints by wiping with a cloth wet with a hydrocarbon solvent (petroleum spirit, b.pt. 60–80 °C). Avoid silicone-based grease, since this is difficult to remove, soluble in some organic solvents and may contaminate your reaction products.

Separating joints – *always* separate ground-glass joints as soon as you have finished with the apparatus and wipe the joints clean.

To grease a joint, put a small smear of grease on the *upper* part of the 'male' joint, push it into the 'female' joint with a twisting movement and the joint should become 'clear' from the top to about half-way down. If more than half the joint has become 'clear', you have used too much grease: separate the joints, clean with a solvent-soaked cloth and repeat the process.

If you break a piece of jointed glassware, *do not* throw the item into the broken-glass bin. The glass blower may be able to re-use it.

To avoid trapping chemicals in the ground-glass joints, fill flasks etc. using a long-stemmed filter funnel or paper cone, which extends past the joint into the flask (see p. 25).

Screwcap adapters

Screwcap or thermometer adapters allow you to place thermometers, glass tubes or air bleeds into jointed glassware flasks. The screwcap adapter works by using the screwcap to compress a rubber ring round the thermometer or glass tube and thus hold it in place. The flexibility of the system allows the height of the thermometer/glass tube to be varied. The adapters come in different joint sizes and varying hole sizes to accommodate different-diameter thermometers, tubes, Pasteur pipettes, etc. The component parts of a screwcap adapter are shown in Fig. 5.21.

To use the screwcap adapter with a thermometer:

Using screwcap adapters – if the rubber ring is not present in the screwcap adapter, the thermometer will slide out of the adapter and may smash both the flask and the thermometer.

- *Always* disassemble the adapter to ensure that the rubber ring and the Teflon® seal are present. If they are missing, get replacements before use. The Teflon® seal is to protect the rubber ring from corrosive and solvent vapours.
- Ease the rubber ring onto the thermometer (see p. 13) and slide the Teflon® seal on *below* the ring.

Fig. 5.21 The screwcap adapter.

- Slide the screwcap over the top of the thermometer and then screw the whole assembly onto the base of the adapter and tighten the screw slightly, just enough to hold the thermometer.
- Trial fit the adapter and thermometer into the apparatus and adjust the height of the thermometer by disassembling the adapter and moving the rubber ring up or down. Reassemble the adapter.
- Check for final fit and tighten the screwcap firmly, but do not over-tighten.

Joint clips

Plastic joint clips or Keck® clips (Fig. 5.22a) are used for holding ground-glass joints firmly together and may be used to replace clamps and support stands at certain points when building apparatus (see p. 109) and are essential when using rotary evaporators (p. 122). The main weakness of these otherwise useful devices is that they soften at about 130 °C and this may allow the joints to separate. Therefore they should never be used at the 'hot end' of a distillation, for example. The clip should be used to hold a distillation adapter on the end of a water condenser, or the flasks onto a fraction collector, but *never* on the distilling flask or to hold the condenser onto the still head (Fig. 5.22b).

When using joint clips always:

- Check that you are using the appropriate size of clip for the joints being held. The clips are often colour coded.
- Check that the clip is not cracked or split.
- Check that the wide 'lower jaw' of the clip fits under the rim of the 'female' joint and the 'upper jaw' fits round the male joint.
- Support the joints with your hand as you push the clip into place. If in doubt use a protective insulated glove.

Fig. 5.22 Joint clips and their use.

6 Principles of solution chemistry

Preparing solutions – practical advice is given on p. 15.

A solution is a homogeneous liquid, formed by the addition of solutes to a solvent. The behaviour of solutions is determined by the type of solutes involved and by their proportions, relative to the solvent. Many laboratory exercises involve calculation of concentrations, e.g. when preparing an experimental solution at a particular concentration, or when expressing data in terms of solute concentration. Make sure that you understand the basic principles set out in this chapter before you tackle such exercises.

Solutes can affect the properties of solutions in several ways, as follows.

Definition

Electrolyte – a substance that dissociates, either fully or partially, in water to give two or more ions.

Electrolytic dissociation

This occurs where a substance dissociates to give charged particles (ions). For a strong electrolyte, e.g. Na^+Cl^-, dissociation is essentially complete. In contrast, a weak electrolyte, e.g. ethanoic acid, will be only partly dissociated, depending upon the pH and temperature of the solution (p. 57).

Osmotic effects

Do not confuse the solubility of a chemical with its strength as an electrolyte. Ethanoic acid is completely soluble with water in all proportions, yet it is a weak electrolyte because it is only partially dissociated. Barium hydroxide is very insoluble in water, but the small quantity which does dissolve (see K_s below) is dissociated completely into Ba^{2+} and OH^- ions; thus it is a strong electrolyte.

These are the result of solute particles lowering the effective concentration of the solvent (water). These effects are particularly relevant to biological systems since membranes are far more permeable to water than to most solutes. Water moves across biological membranes from the solution with the higher effective water concentration to that with the lower effective water concentration (osmosis).

Ideal/non-ideal behaviour

This occurs because solutions of real substances do not necessarily conform to the theoretical relationships predicted for dilute solutions of so-called ideal solutes. It is often necessary to take account of the non-ideal behaviour of real solutions, especially at high solute concentrations (see Lide (2000) for appropriate data).

Concentration

Expressing solute concentrations – you should use SI units wherever possible. However, you are likely to meet non-SI concentrations and you must be able to deal with these units too.

In SI units (p. 71) the concentration of a solute is expressed in $mol\,m^{-3}$, which is *essential* for calculating specific parameters for substances (e.g. p. 73), but which is *inconvenient* when dealing with solutions in the laboratory. A cubic metre (m^3) of water weighs approximately 1 ton! A common unit of volume used in chemistry is the litre (L): this is a non-SI unit and is converted to the SI unit of volume (m^3) using $1.0\,L = 10^{-3}\,m^3$. The concentration of a solute is usually symbolized by square brackets, e.g. [NaCl]. Details of how to prepare solutions are given on pp. 17, 19.

A number of alternative ways of expressing the relative amounts of solute and solvent are in general use, and you may come across these terms in your practical work or in the literature.

Molarity

Example A 1.0 molar solution of NaCl would contain 58.44 g NaCl (the molecular mass) per litre of solution.

This is the term used to denote molar concentration, $[C]$, expressed as moles of solute per litre volume of solution ($mol\,L^{-1}$). This non-SI term continues to find widespread usage, in part because of the familiarity of working scientists with the term, but also because laboratory glassware is calibrated in

Box 6.1 Useful procedures for calculations involving molar concentrations

1. **Preparing a solution of defined molarity.** For a solute of known relative molecular mass (M_r), the following relationship can be applied:

$$[C] = \frac{\text{mass of solute}/M_r}{\text{volume of solution}} \quad [6.1]$$

So, if you wanted to make up 200 mL (0.2 L) of an aqueous solution of NaCl ($M_r = 58.44$ g) at a concentration of 500 mmol L^{-1} (0.5 mol L^{-1}), you could calculate the amount of NaCl required by inserting these values into eqn [6.1]:

$$0.5 = \frac{\text{mass of solute}/58.44}{0.2}$$

which can be rearranged to

$$\text{mass of solute} = 0.5 \times 0.2 \times 58.44 = 5.844\,\text{g}$$

The same relationship can be used to calculate the concentration of a solution containing a known amount of a solute, e.g. if 21.1 g of NaCl were made up to a volume of 100 mL (0.1 L), this would give:

$$[\text{NaCl}] = \frac{21.1/58.44}{0.1} = 3.61\,\text{mol}\,\text{L}^{-1}$$

2. **Dilutions and concentrations.** The following relationship is very useful if you are diluting (or concentrating) a solution:

$$[C_1]V_1 = [C_2]V_2 \quad [6.2]$$

where $[C_1]$ and $[C_2]$ are the initial and final concentrations, while V_1 and V_2 are their respective volumes: each pair must be expressed in the same units. Thus, if you wanted to dilute 200 mL of 0.5 mol L^{-1} NaCl to give a final molarity of 0.1 mol L^{-1}, then, by substitution into eqn [6.2]:

$$0.5 \times 200 = 0.1 \times V_2$$

Thus $V_2 = 1000$ mL (in other words, you would have to add water to 200 mL of 0.5 mol L^{-1} NaCl to give a final volume of 1000 mL to obtain a 0.1 mol L^{-1} solution).

3. **Interconversion.** A simple way of interconverting amounts and volumes of any particular solution is to divide the amount and volume by a factor of 10^3: thus a molar solution of a substance contains 1 mol L^{-1}, which is equivalent to 1 mmol mL^{-1}, or 1 μmol μL^{-1}, or 1 nmol nL^{-1}, etc. You may find this technique useful when calculating the amount of substance present in a small volume of solution of known concentration, e.g. to calculate the amount of NaCl present in 50 μL of a solution with a concentration (molarity) of 0.5 mol L^{-1} NaCl:

   (a) this is equivalent to 0.5 μmol μL^{-1};

   (b) therefore 50 μL will contain $50 \times 0.5\,\mu\text{mol} = 25\,\mu\text{mol}$.

The 'unitary method' (p. 263) is an alternative approach to these calculations.

millilitres and litres, making the preparation of molar and millimolar solutions relatively straightforward. However, the symbols in common use for molar (M) and millimolar (mM) solutions are at odds with the SI system and many people now prefer to use mol L^{-1} and mmol L^{-1} respectively, to avoid confusion. Box 6.1 gives details of some useful approaches to calculations involving molarities.

Molality

This is used to express the concentration of solute relative to the *mass* of solvent, i.e. mol kg^{-1}. Molality is a temperature-independent means of expressing solute concentration, rarely used except when the osmotic properties of a solution are of interest (p. 49).

Example A 0.5 molal solution of NaCl would contain $58.44 \times 0.5 = 29.22$ g NaCl per kg of water.

Per cent composition (% w/w)

This is the solute mass (in g) per 100 g solution. The advantage of this expression is the ease with which a solution can be prepared, since it simply requires each component to be pre-weighed (for water, a volumetric measurement may be used, e.g. using a measuring cylinder) and then mixed together. Similar terms are parts per thousand (‰), i.e. mg g^{-1}, and parts per million (ppm), i.e. μg g^{-1}.

Example A 5% w/w NaOH solution contains 5 g NaOH and 95 g water (= 95 mL water, assuming a density of 1 g mL^{-1}) to give 100 g of solution.

Per cent concentration (% w/v and % v/v)

For solutes added in solid form, this is the number of grams of solute per 100 mL solution. This is more commonly used than per cent composition, since solutions can be accurately prepared by weighing out the required amount of solute and then making this up to a known volume using a volumetric flask. The equivalent expression for liquid solutes is % v/v.

Example A 5% w/v KOH solution contains 5 g KOH in 100 mL of solution. A 5% v/v glycerol solution would contain 5 mL glycerol in 100 mL of solution.

Note that when water is the solvent this is often not specified in the expression, e.g. a 20 mL v/v ethanol solution contains 20% ethanol made up to 100 mL of solution using water.

The principal use of mass/mass or mass/volume terms (including $g\,L^{-1}$) is for solutes whose molecular mass is unknown (e.g. polymers), or for mixtures of certain classes of substance (e.g. total salt in sea water). You should *never* use the per cent term without specifying how the solution was prepared, i.e. by using the qualifier w/w, w/v or v/v. For mass concentrations, it is often simpler to use mass per unit volume, e.g. $mg\,L^{-1}$, $\mu g\ \mu L^{-1}$, etc.

Parts per million concentration (ppm)

This is a non-SI weight per volume (w/v) concentration term commonly used in quantitative analysis such as flame photometry, atomic absorption spectroscopy and gas chromatography, where low concentrations of solutes are to be analysed. The term ppm is equivalent to the expression of concentration as $\mu g\,mL^{-1}$ ($10^{-6}\,g\,mL^{-1}$) and a 1.0 ppm solution of a substance will have a concentration of $1.0\,\mu g\,mL^{-1}$ ($1.0 \times 10^{-6}\,g\,mL^{-1}$). A typical procedure for calculations in terms of ppm is shown in Box 6.2.

Note that ppm may be a weight/weight (w/w) expression. The origin of the term ppm derives from a solution whose concentration is 1 ppm if it contains 1 g of solute for each million (10^6) g of solvent.

Parts per billion (ppb) is an extension of this concentration term as $ng\,mL^{-1}$ ($10^{-9}\,g\,mL^{-1}$) and is commonly used to express concentrations of very dilute solutions. For example, the allowable concentration of

Box 6.2 How to convert ppm into mass of chemical required

Example: Suppose you are asked to prepare an aqueous solution of sodium ions (250.00 mL) of approximate, but accurately known, concentration of 10 ppm from either sodium chloride or anhydrous sodium carbonate.

1. **Convert the ppm concentration into $g\,L^{-1}$.** Thus $1.0\,ppm = 1.0 \times 10^{-6}\,g\,mL^{-1}$ and hence a solution of $10\ ppm = 10 \times 10^{-6}\,g\,mL^{-1} = 10^3 \times 10 \times 10^{-6}\,g\,L^{-1} = 10 \times 10^{-3}\,g\,L^{-1}$.
2. **Convert the concentration from $g\,L^{-1}$ to $mol\,L^{-1}$.** The A_r for sodium ion is 23 g and for a litre of 10 ppm solution you need $10 \times 10^{-3} \div 23 = 0.435 \times 10^{-3}$ mol of sodium ions.
3. **Convert the number of moles per litre into moles in the volume required (250.00 mL).** Since 1.0 litres of 10 ppm solution of sodium ions requires 0.435×10^{-3} mol of sodium ions, then 250.00 mL (0.25 L) of solution will need $0.25 \times 0.435 \times 10^{-3}$ mol $= 0.1087 \times 10^{-3}$ mol of sodium ions.
4. **Calculate the mass of either sodium chloride or sodium carbonate required to make up the solution:**
   (a) Sodium chloride (NaCl), $M_r = 58.5$: therefore you need to weigh out $58.5 \times 0.1087 \times 10^{-3} = 6.359 \times 10^{-3}$ g of sodium chloride to be made up to 250.00 mL for a 10 ppm solution of sodium ions.
   (b) Sodium carbonate (Na_2CO_3), $M_r = 106$: you *must* note that each 'molecule' of sodium carbonate contains *two* sodium ions; thus the number of moles of sodium carbonate required for a 10 ppm solution of sodium ions is $0.1087 \times 10^{-3} \div 2 = 0.05435 \times 10^{-3}$ mol. You must therefore weigh out $106 \times 0.05435 \times 10^{-3} = 5.7611 \times 10^{-3}$ g of sodium carbonate to be made up to 250.00 mL for a 10 ppm solution of sodium ions.
5. **Decide how you are to prepare these solutions** using the procedures outlined in Boxes 4.1, 4.2 and 4.3, since the calculation shows that you will need to weigh out small masses of chemicals, which are at the limit of accuracy of an analytical balance.

arsenic in water may be 0.05 ppm, but it is more conveniently expressed as 50 ppb.

Activity (a)

This is a term used to describe the *effective* concentration of a solute. In dilute solutions, solutes can be considered to behave according to ideal (thermodynamic) principles, i.e. they will have an effective concentration equivalent to the actual concentration. However, in concentrated solutions ($\geqslant 0.5\,\text{mol}\,\text{L}^{-1}$), the behaviour of solutes is often non-ideal, and their effective concentration (activity) will be less than the actual concentration [*C*]. The ratio between the effective concentration and the actual concentration is called the activity coefficient (γ) where

$$\gamma = \frac{a}{[C]} \qquad [6.3]$$

Equation [6.3] can be used for SI units ($\text{mol}\,\text{m}^{-3}$), molarity ($\text{mol}\,\text{L}^{-1}$) or molality ($\text{mol}\,\text{kg}^{-1}$). In all cases, γ is a dimensionless term, since a and [*C*] are expressed in the same units. The activity coefficient of a solute is effectively unity in dilute solution, decreasing as the solute concentration increases (Table 6.1). At high concentrations of certain ionic solutes, γ may increase to become greater than unity.

Table 6.1 Activity coefficient of NaCl solutions as a function of molality. Data from Robinson and Stokes (1970)

Molality	Activity coefficient at 25 °C
0.1	0.778
0.5	0.681
1.0	0.657
2.0	0.668
4.0	0.783
6.0	0.986

Example A solution of NaCl with a molality of 0.5 mol kg^{-1} has an activity coefficient of 0.681 at 25 °C and a molal activity of $0.5 \times 0.681 = 0.340\,\text{mol}\,\text{kg}^{-1}$.

KEY POINT **Activity is often the correct expression for theoretical relationships involving solute concentration (e.g. where a property of the solution is dependent on concentration). However, for most practical purposes, it is possible to use the *actual* concentration of a solute rather than the activity, since the difference between the two terms can be ignored for dilute solutions.**

Equivalent mass (equivalent weight)

Equivalence and normality are outdated terms, although you may come across them in older texts. The magnitude of an equivalent mass (equivalent weight) can be simply identified from the balanced equation for the reaction being considered. Remember that the equivalent mass can *change*, depending on the reaction, as the following reactions illustrate.

Examples For carbonate ions (CO_3^{2-}), with a molecular mass of 60.00 and a valency of 2, the equivalent mass is $60.00/2 = 30.00\,\text{g eq}^{-1}$.

For sulphuric acid (H_2SO_4, molecular mass 98.08), where two hydrogen ions are available, the equivalent mass is $98.08/2 = 49.04\,\text{g eq}^{-1}$.

For:

$$HCl + NaOH \rightarrow NaCl + H_2O$$

1 mol of HCl reacts with 1 mol of NaOH, the equivalent mass of HCl is $M_r = 36.5$ and the equivalent mass of NaOH is also its $M_r = 40$.

For:

$$H_2SO_4 + 2NaOH \rightarrow Na_2SO_4 + 2H_2O$$

since 1 mol of H_2SO_4 reacts with 2 mol of NaOH, the equivalent mass of H_2SO_4 is $M_r \div 2 = 98 \div 2 = 49$, while the equivalent mass of NaOH is still $M_r = 40$.

For:

$$5FeSO_4 + KMnO_4 \rightarrow Fe_2(SO_4)_3 + 2MnSO_4$$

since 1 mol of $KMnO_4$ reacts with 5 mol of $FeSO_4$, then the equivalent mass of $KMnO_4$ is $M_r \div 5 = 158 \div 5 = 31.6$, and that of $FeSO_4$ is still $M_r = 152$.

But, for:

$$H_2SO_4 + Na_2CO_3 \rightarrow Na_2SO_4 + CO_2 + H_2O$$

since the reaction is 1:1, the equivalent masses of H_2SO_4 and Na_2CO_3 are their M_r values, 98 and 106 respectively.

As a result of this possible confusion, the concept of equivalent mass (weight) is rarely used.

Example A 0.5 N solution of sulphuric acid would contain $0.5 \times 49.04 = 24.52\,g\,L^{-1}$.

Normality

A 1 normal solution (1 N) is one that contains one equivalent mass of a substance per litre of solution. The general formula is:

$$\text{normality} = \frac{\text{mass of substance per litre}}{\text{equivalent mass}} \qquad [6.4]$$

Example Under ideal conditions, 1 mol of NaCl dissolved in water would give 1 mol of Na^+ ions and 1 mol of Cl^- ions, equivalent to a theoretical osmolarity of $2\,osmol\,L^{-1}$.

Osmolarity

This non-SI expression is used to describe the number of moles of osmotically active solute particles per litre of solution ($osmol\,L^{-1}$). The need for such a term arises because some molecules dissociate to give more than one osmotically active particle in aqueous solution.

Example A $1.0\,mol\,kg^{-1}$ solution of NaCl has an osmotic coefficient of 0.936 at 25 °C and an osmolality of $1.0 \times 2 \times 0.936 = 1.872\,osmol\,kg^{-1}$.

Osmolality

This term describes the number of moles of osmotically active solute particles per unit mass of solvent ($osmol\,kg^{-1}$). For an ideal solute, the osmolality can be determined by multiplying the molality by n, the number of solute particles produced in solution (e.g. for NaCl, $n = 2$). However, for real solutes, a correction factor (the osmotic coefficient, ϕ) is used:

$$\text{osmolality} = \text{molality} \times n \times \phi \qquad [6.5]$$

Table 6.2 Osmotic coefficients of NaCl solutions as a function of molality. Data from Robinson and Stokes (1970)

Molality	Osmotic coefficient at 25 °C
0.1	0.932
0.5	0.921
1.0	0.936
2.0	0.983
4.0	1.116
6.0	1.271

If necessary, the osmotic coefficients of a particular solute can be obtained from tables (e.g. Table 6.2): non-ideal behaviour means that ϕ may have values > 1 at high concentrations. Alternatively, the osmolality of a solution can be measured using an osmometer.

Example A $1.0\,mol\,kg^{-1}$ solution of NaCl at 25 °C has an osmolalilty of $1.872\,osmol\,kg^{-1}$ and an osmotic pressure of $1.872 \times 2.479 = 4.641$ MPa.

Osmotic pressure

This is based on the concept of a membrane permeable to water, but not to solute molecules. For example, if a sucrose solution is placed on one side and pure water on the other, then a passive driving force will be created and water will diffuse across the membrane into the sucrose solution, since the effective water concentration in the sucrose solution will be lower. The tendency for water to diffuse into the sucrose solution could be counteracted by applying a hydrostatic pressure equivalent to the passive driving force. Thus, the osmotic pressure of a solution is the excess hydrostatic pressure required to prevent the net flow of water into a vessel containing the solution. The SI unit of osmotic pressure is the pascal, Pa ($= kg\,m^{-1}\,s^{-2}$). Older sources may use atmospheres, or bars, and conversion factors are given in Box 9.1 (p. 72). Osmotic pressure and osmolality can be interconverted using the expression $1\,osmol\,kg^{-1} = 2.479$ MPa at 25 °C.

The use of osmotic pressure has been criticized as misleading, since a solution does not exhibit an 'osmotic pressure' unless it is placed on the other side of a selectively permeable membrane from pure water!

Colligative properties and their use in osmometry

Several properties vary in direct proportion to the effective number of osmotically active solute particles per unit mass of solvent and can be used to determine the osmolality of a solution. These colligative properties include freezing point, boiling point and vapour pressure.

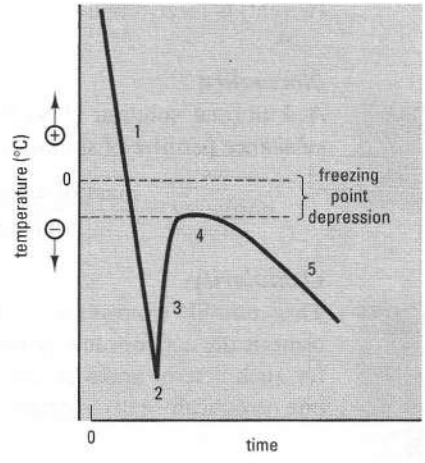


Fig. 6.1 Temperature responses of a cryoscopic osmometer. The response can be subdivided into:
1. initial supercooling
2. initiation of crystallization
3. crystallization/freezing
4. plateau, at the freezing point
5. slow temperature decrease

Using an osmometer – it is vital that the sample holder and probe are clean, otherwise small droplets of the previous sample may be carried over, leading to inaccurate measurement.

An osmometer is an instrument which measures the osmolality of a solution, usually by determining the freezing point depression of the solution in relation to pure water, a technique known as cryoscopic osmometry. A small amount of sample is cooled rapidly and then brought to the freezing point (Fig. 6.1), which is measured by a temperature-sensitive thermistor probe calibrated in mosmol kg^{-1}. An alternative method is used in vapour pressure osmometry, which measures the relative decrease in the vapour pressure produced in the gas phase when a small sample of the solution is equilibrated within a chamber.

Solubility

Insolubility – no solute can be shown to be completely insoluble in a given solvent, but for practical purposes, a compound which has less than 0.01% (w/w) solubility in a solvent can be considered to be insoluble in that solvent.

Variation of solubility – the solubility of a chemical may vary in different solvents. For example, NaCl is soluble in water but insoluble in DCM whereas for naphthalene the opposite is true.

Saturated solutions – theoretically, a saturated solution is one in which the solution is in dynamic equilibrium with the undissolved solute.

Solubility – remember to use mass = volume × density when converting solubilities from grams of solute per 100 g of solvent to $g\,L^{-1}$, when using solvents other than water.

The extent to which a solute will dissolve in a solvent is called its solubility. The solubility of a chemical is conventionally expressed as the maximum number of grams of a chemical that will dissolve in 100 g of solvent but conversion to $mol\,L^{-1}$ or $g\,L^{-1}$ is simple and may be appropriate for some applications (see below). Since solubility is temperature dependent, is always quoted at a specific temperature. With a very few exceptions, increasing the temperature of a solvent increases the solubility of the solute.

Saturated solutions

For practical purposes, a saturated solution is one in which no more solute will dissolve. For example, the solubility of sodium chloride in water is 35.6 g per 100 g at 25 °C and 39.1 g per 100 g at 100 °C and both solutions are saturated solutions at their respective temperatures. If the 100 °C solution is cooled to 25 °C, then 3.5 g of NaCl crystals will precipitate from the solution, because the solution at 25 °C requires only 35.6 g of NaCl for saturation. This process is the basis of purification of compounds by recrystallization (see p. 92).

Solubility product

In dilute aqueous solutions, it has been demonstrated experimentally for poorly soluble ionic salts (solubilities less than $0.01\,mol\,L^{-1}$) that the mathematical product of the total molar concentrations of the component ions is a constant at constant temperature. This product, K_s is called the *solubility product*. Thus for a saturated solution of a simple ionic compound AB in water, we have the dynamic equilibrium:

$$AB_{solid} \rightleftharpoons A^+_{(aq)} + B^-_{(aq)}$$

where AB represents the solid which has not dissolved, in equilibrium with its ions in the aqueous saturated solution. Then:

$$K_s = [A^+] \times [B^-]$$

For example, silver chloride is a solid of solubility 0.000 15 g per 100 mL of water in equilibrium with silver cations and chloride ions. Then:

$$K_s = [Ag^+] \times [Cl^-]$$

The solubility of AgCl is 0.0015 g mL^{-1} (10 × solubility per 100 g, assuming that the density of water is 1.0 g mL^{-1}) and therefore the solubility of AgCl is $0.0015 \div 143.5 = 1.05 \times 10^{-5}$ mol L^{-1}. Thus the saturated solution contains 1.05×10^{-5} mol L^{-1} of Ag^+ ions and 1.05×10^{-5} mol L^{-1} of Cl^- ions and the solubility product K_s is

$$K_s = (1.05 \times 10^{-5}) \times (1.05 \times 10^{-5}) = 1.1 \times 10^{-10} \text{ mol}^2 \text{ L}^{-2}$$

If the solid does not have a simple 1:1 ratio of its ionic components, e.g. $PbCl_2$, then the solubility product is given by:

$$K_s = [Pb^{2+}] \times [Cl^-]^2$$

In general terms, the solubility product for a compound M_yN_x, is given by

$$K_s = [M^+]^y \times [N^-]^x$$

Example A 0.5 molal solution of NaCl would contain 58.44 × 0.5 = 29.22 g NaCl per kg of water.

The practical effects of solubility products are demonstrated in the detection of anions and cations by precipitation (p. 135) and in quantitative gravimetric analysis (p. 139). For example, if dilute aqueous solutions of silver nitrate (solubility 55.6 g per 100 g of water) and sodium chloride (solubility 35.6 g per 100 g of water) are mixed, an immediate white precipitate of AgCl is produced because the solubility product of AgCl has been exceeded by the numbers of Ag^+ and Cl^- ions in the solution, even though the ions come from different 'molecules'. A saturated solution of AgCl is formed and the excess AgCl precipitates out. The solubility product of the other combinations of ions is not exceeded and thus sodium and nitrate ions remain in solution. Even if the concentration of Ag^+ is extremely low, the solubility product for AgCl can be exceeded by the addition of an excess of Cl^- ions, since it is the multiplication of these two concentrations which defines the solubility product. Thus soluble chlorides can be used to detect the presence of Ag^+ ions and, conversely, soluble silver salts can be used to detect Cl^- ions, both quantitatively and qualitatively.

Reactions of ions in solution

There are essentially only four basic reactions of ions in solution:

1. Acid–base reactions.
2. Precipitation reactions.
3. Complexation reactions
4. Reduction–oxidation (redox) reactions.

Acid–base, precipitation and complexation reactions are all examples of exchange (metathesis) reactions in which ions in solution 'exchange partners', for example:

$$A^+Y^- + B^+Z^- \rightarrow A^+Z^- + B^+Y^-$$

Metathesis reactions are really equilibria between the ionic species, which are displaced to the right (to the reaction product) by a feature which defines the classification of the reaction type.

Acid–base reactions

The most common acid–base reactions are exemplified by the neutralization reaction between hydrogen ions and hydroxide ions; for example, the reaction between dilute hydrochloric acid and dilute sodium hydroxide:

$$H^+Cl^-_{(aq)} + \boldsymbol{Na^+OH^-_{(aq)}} \rightarrow \text{H-}\mathbf{OH}_{(liq)} + \boldsymbol{Na^+}Cl^-_{(aq)}$$

Since water is essentially a covalent compound (see p. 56) its formation effectively removes H^+ and OH^- from the equilibrium and drives the reaction to completion. Other examples of this general type of reaction include the removal of a molecule as a gas, such as reactions of acids with carbonates and bicarbonates, where unstable H_2CO_3 decomposes to H_2O and CO_2.

Precipitation reactions

In these reactions between ions, one substance is removed from the ionic equilibrium by precipitation (see solubility product, p. 50) and drives the equilibrium to the right (see p. 51).

Complexation reactions

A complex ion is formed by the reaction of a metal cation, in particular transition metals, with an electron donor molecule (ligand), which can be neutral or have a negative charge. The cation can accept an electron pair and the ligand donates an electron pair to form a covalent donor (co-ordinate) bond between the ligand and the metal ion. The ligands are said to co-ordinate with the metal ion to give a complex. Many ligands are more powerful electron donors than water and thus the addition of a ligand to an aqueous solution of a metal cation displaces the equilibrium towards the more stable complex ion. (See p. 151 for stability constants and complexes.) The effects of complex formation are illustrated in Box 6.3.

The overall effect of complex formation is to 'remove' a hydrated metal ion from the mixture of ions in solution by displacing the equilibrium in favour of the complex, cf. the similar process in the formation of water in acid–base titrations and precipitation reactions.

Reduction–oxidation (redox) reactions

The concepts of oxidation and reduction are defined in terms of complete electron transfer from one atom, ion or molecule of a chemical to another:

- Chemical oxidized – chemical loses electron(s).
- Chemical reduced – chemical gains electron(s).

This approach is generally applicable to most reactions and avoids complications of the older definitions involving hydrogen and oxygen. You should realize that if a chemical is oxidized during a reaction, then another *must* be reduced: oxidation and reduction always occur together. Furthermore:

- Oxidizing agent – gains electron(s) and is therefore reduced.
- Reducing agent – loses electron(s) and is therefore oxidized.

The following reaction between magnesium metal and dilute acid illustrates these concepts:

$$Mg_{(s)} + 2H^+_{(aq)} \rightarrow Mg^{2+}_{(aq)} + H_{2(g)}$$

Box 6.3 An example of complex formation

If an aqueous solution of ammonia is added to an aqueous solution of copper (II) sulphate the following changes are observed.

A. On addition of the ammonia solution to the pale-blue copper solution a white precipitate forms.

B. As addition is continued, the white precipitate dissolves and a royal-blue solution is formed, which does not change on further addition of ammonia solution.

These changes can be explained as follows:

1. Ammonia solution is an equilibrium mixture, which lies well to the left. Consequently a dilute solution of ammonia contains a little OH^- and lots of free NH_3:

$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^-$$

2. Copper (II) sulphate comprises the $Cu(H_2O)_4^{2+}$ ion and SO_4^{2-} ions in solution.
3. As the ammonia solution is added, the solubility product of $Cu(OH)_2$ is exceeded, even by the low concentration of OH^- ions and the white solid, $Cu(OH)_2$, precipitates from the saturated solution (see p. 51).
4. As addition of the ammonia solution is continued, the free NH_3 molecules displace the water molecules from the pale-blue $Cu(H_2O)_4^{2+}$ complex ion to form the royal-blue $Cu(NH_3)_4^{2+}$ complex, which is more stable than the water complex (larger stability constant).
5. Since the insoluble $Cu(OH)_2$ is in equilibrium with the $Cu(H_2O)_4^{2+}$, which is being removed as the $Cu(NH_3)_4^{2+}$ complex, the $Cu(OH)_2$ reverts to $Cu(H_2O)_4^{2+}$ which then forms the $Cu(NH_3)_4^{2+}$ complex. Thus the white precipitate dissolves leaving the royal-blue solution of the $Cu(NH_3)_4^{2+}$ complex.

Magnesium metal has lost two electrons in forming Mg^{2+} ions and has therefore been *oxidized*. The two protons have each gained an electron to form hydrogen atoms (and then one hydrogen molecule) and have been *reduced*. Since magnesium metal has been oxidized, it is a *reducing agent* and because H^+ has gained an electron, it is an *oxidizing agent*.

The stoichiometry of a redox reaction is defined by the number of electrons transferred between the oxidizing agent and the reducing agent since the number of electrons lost by the reducing agent *must* equal the number of electrons gained by the reducing agent, e.g.

$$2Mg_{(s)} + O_{2(g)} \rightarrow 2MgO_{(s)}$$

So that you can work out titrations involving redox reactions, you will find it necessary to balance redox equations, and while it is easy for simple reactions such as those above, more complex redox reactions, such as the one below, require more thought and work.

$$2KMnO_4 + 5H_2O_2 + 3H_2SO_4 \rightarrow 2MnSO_4 + K_2SO_4 + 5O_2 + 8H_2O$$

Such problems can be broken down into several simple steps, each with its own set of rules:

- Identify the atoms, ions or molecules which have been oxidized and reduced.
- Identify the ionic half-reactions for the species being oxidized and reduced and combine them.
- Balance the ionic half-reactions and combine them to give a balanced equation for the reaction.

The species which are oxidized and reduced can be identified using the concept of *oxidation numbers*. The rules for determining oxidation numbers and examples are given in Box 6.4 and the application of ionic half-reactions

to balance redox equations is shown in Box 6.5. Note that the result of the use of partial ionic equations gives a balanced ionic equation for the redox reaction.

KEY POINT **In simple acid–base, precipitation and complexation reactions, no change of oxidation number occurs at any of the atoms involved.**

Box 6.4 The use of oxidation numbers to identify redox systems

The oxidation number is a hypothetical charge assigned to atoms in molecules and ions using a set of specific rules. Since redox reactions involve transfers of electrons, identification of the atoms which change oxidation number will show the atoms, ions or molecules which are specifically involved in the redox process.

Rules for oxidation numbers

1. **For an atom in its elemental form, the oxidation number is *always* 0.** Thus Cl in Cl_2 has an oxidation number 0, as does Na metal, and carbon in charcoal, graphite or diamond.
2. **For any monatomic ion, the oxidation number is the same as the charge on the ion.** Thus Na^+ has an oxidation number of +1, Cl^- is −1, Al^{3+} is +3, S^{2-} is −2, etc.
3. **Non-metals usually have negative values**, but there are some exceptions:
   (a) The oxidation number of fluorine is *always* −1 in *all* compounds.
   (b) The oxidation number of oxygen is always −2, except when bonded to fluorine (OF_2), in peroxides (O_2^{2-}), where each oxygen atom is −1 and superoxides (O_2^-), where each oxygen atom is $-\frac{1}{2}$.
   (c) The oxidation number of the other halogens is always −1, except when bonded to atoms of greater electronegativity, e.g. Cl in ClF_3 is +3, Br in BrCl is +1, etc.
   (d) The oxidation number of hydrogen is always +1, except when bonded to electropositive metals, when it is −1, e.g. in HCl it is +1 in NH_3 it is +1 and in NaH, MgH_2 and AlH_3 it is −1.
4. **The sum of the oxidation numbers of all atoms in a neutral compound is zero**, e.g. in $KClO_4$, K is +1 (rule **2**), four oxygen atoms, $4 \times -2 = -8$ (rule **3b**); therefore Cl must be +7 (rule **3c**).
5. **The sum of the oxidation numbers of all the atoms in a polyatomic ion is equal to the charge on the ion**, e.g. in CO_3^{2-} ion, each oxygen is −2 (rule **3b**); thus $3 \times -2 = -6$. Since the charge on the CO_3^{2-} ion is 2−, then carbon must be +4.
6. **If the oxidation number of an atom becomes more positive during the reaction**, it has lost electrons and has been *oxidized*.

   If the oxidation number of an atom becomes more negative during the reaction, it has gained electrons and has been *reduced*.

Example: If you consider the unbalanced equation for the reaction shown on p. 53:

$$KMnO_4 + H_2O_2 + H_2SO_4 \rightarrow MnSO_4 + K_2SO_4 + O_2 + H_2O$$

you can now calculate that the only atoms which have changed oxidation number are *manganese*, which has changed from +7 in MnO_4^- to +2 as Mn^{2+} in $MnSO_4$, and *oxygen*, which has changed from −1 in H_2O_2 (rule **3b**) to 0 in O_2. Thus Mn has gained electrons and been *reduced* and oxygen has lost electrons and been *oxidized*. Furthermore, the Mn atom has gained five electrons and the O_2^{2-} ion has lost two electrons so five molecules of H_2O_2 should react with two molecules of $KMnO_4$.

The two partial ionic reactions can now be identified:

For oxidation $MnO_4^- \rightarrow Mn^{2+}$
For oxidation $H_2O_2 \rightarrow O_2$

and the equation balanced as shown in Box 6.5.

Box 6.5 How to balance redox equations from partial ionic equations using the ion–electron method

Example: You are to produce a balanced equation from the partial ionic equations deduced in Box 6.4.

1. **Balance the atom which changes oxidation number** in each partial ionic equation:

   $MnO_4^- \rightarrow Mn^{2+}$ no change necessary in either equation since Mn and O are balanced

   $H_2O_2 \rightarrow O_2$ on each side of the equation

2. **Balance the oxygen atoms** on each side of each equation.
   (a) **If the reaction occurs in acid or neutral solution,** for each O atom deficient, add one molecule H_2O to the side deficient.
   (b) **If the reaction occurs in alkaline solution,** for each O deficient add *two* OH^- to the side deficient and one molecule of H_2O to the other.

   Your reaction occurs in acid solution, so:

   $$MnO_4^- \rightarrow Mn^{2+} + 4H_2O$$
   $$H_2O_2 \rightarrow O_2$$

3. **Balance the hydrogen** by addition of H^+ to the side deficient:

   $$8H^+ + MnO_4^- \rightarrow Mn^{2+} + 4H_2O$$
   $$H_2O_2 \rightarrow O_2 + 2H^+$$

4. **Balance the charge** on each side of the equation by the addition of electrons, each electron having a charge of −1:

   $$5e^- + 8H^+ + MnO_4^- \rightarrow Mn^{2+} + 4H_2O$$
   $$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^-$$

5. **Balance the electrons in each equation,** since number of electrons gained by oxidizing agent *must* equal number of electrons lost by reducing agent. Therefore multiply top equation by 2 and bottom equation by 5:

   $$10e^- + 16H^+ + 2MnO_4^- \rightarrow 2Mn^{2+} + 8H_2O$$
   $$5H_2O_2 \rightarrow 5O_2 + 10H^+ + 10e^-$$

6. **Add the equations together:**

   $$10e^- + 16H^+ + 5H_2O_2 + 2MnO_4^- \rightarrow 2Mn^{2+} + 5O_2 + 10H^+ + 8H_2O + 10e^-$$

7. **Cancel terms on opposite sides of the new equation:**

   $$6H^+ + 5H_2O_2 + 2MnO_4^- \rightarrow 2Mn^{2+} + 5O_2 + 8H_2O$$

This *ionic equation* is sufficient to work out the mole ratio of the reacting species, i.e. 5 moles of H_2O_2 will react with 2 moles of MnO_4^-. All the other ions, K^+ and SO_4^{2-}, remain in solution and are unchanged by the reaction. If the fully balanced equation is required, the ions can be added to the ionic equation at the end of the process and the numbers adjusted:

$$3H_2SO_4 + 5H_2O_2 + 2KMnO_4 \rightarrow 2MnSO_4 + 5O_2 + K_2SO_4 + 8H_2O$$

7 pH and buffer solutions

Definitions

Acid – a compound that acts as a proton donor in aqueous solution.

Base – a compound that acts as a proton acceptor in aqueous solution.

Conjugate pair – an acid together with its corresponding base.

Alkali – a compound that liberates hydroxyl ions when it dissociates. Since hydroxyl ions are strongly basic, this will reduce the proton concentration.

Ampholyte – a compound that can act as both an acid and a base. Water is an ampholyte since it may dissociate to give a proton and a hydroxyl ion (amphoteric behaviour).

pH is a measure of the amount of hydrogen ions (H^+) in a solution. It is usual to think of aqueous solutions as containing H^+ ions (protons), though protons actually exist in their hydrated form, as hydronium ions (H_3O^+). The proton concentration of an aqueous solution [H^+] is affected by several factors:

- Ionization (dissociation) of water, which liberates protons and hydroxyl ions in equal quantities, according to the reversible relationship:

$$H_2O \rightleftharpoons H^+ + OH^- \qquad [7.1]$$

- Dissociation of acids, according to the equation:

$$H\text{–}A \rightleftharpoons H^+ + A^- \qquad [7.2]$$

  where H–A represents the acid and A^- is the corresponding conjugate base. The dissociation of an acid in water will increase the amount of protons, reducing the amount of hydroxyl ions as water molecules are formed (eqn [7.1]). The addition of a base (usually, as its salt) to water will decrease the amount of H^+, owing to the formation of the conjugate acid (eqn [7.2]).
- Dissociation of alkalis, according to the relationship:

$$X^+OH^- \rightleftharpoons X^+ + OH^- \qquad [7.3]$$

  where X^+OH^- represents the undissociated alkali. Since the dissociation of water is reversible (eqn [7.1]), in an aqueous solution the production of hydroxyl ions will effectively act to 'mop up' protons, lowering the proton concentration.

Many compounds act as acids, bases or alkalis: those which are almost completely ionized in solution are usually called strong acids or bases, while weak acids or bases are only slightly ionized in solution (p. 45).

In an aqueous solution, most of the water molecules are not ionized. In fact, the extent of ionization of pure water is constant at any given temperature and is usually expressed in terms of the ion product (or ionization constant) of water, K_w:

$$K_w = [H^+]\,[OH^-] \qquad [7.4]$$

where [H^+] and [OH^-] represent the molar concentration (strictly, the activity) of protons and hydroxyl ions in solution, expressed as mol L^{-1}. At 25 °C, the ion product of pure water is 10^{-14} mol^2 L^{-2} (i.e. 10^{-8} mol^2 m^{-6}). This means that the concentration of protons in solution will be 10^{-7} mol L^{-1} (10^{-4} mol m^{-3}), with an equivalent concentration of hydroxyl ions (eqn [7.1]). Since these values are very low and involve negative powers of 10, it is customary to use the pH scale, where:

$$pH = -\log_{10}[H^+] \qquad [7.5]$$

and [H^+] is the proton activity (see p. 48).

Example The pH of 0.02 mol L^{-1} HCl can be calculated as follows: HCl is a strong acid giving [H^+] = [Cl^-] = 0.02 mol L^{-1}. Therefore $pH = -\log_{10}(0.02) = 1.7$.

Example The pH of a solution is 6.4. Therefore the [H^+] = 10^{-pH}, i.e. 3.98×10^{-7} mol L^{-1}.

Table 7.1 Effects of temperature on the ion product of water (K_w), H^+ ion concentration and pH at neutrality. Values calculated from Lide (2000).

Temp. (°C)	K_w (mol^2 L^{-2})	[H^+] at neutrality (nmol L^{-1})	pH at neutrality
0	0.11×10^{-4}	33.9	7.47
4	0.17×10^{-4}	40.7	7.39
10	0.29×10^{-4}	53.7	7.27
20	0.68×10^{-4}	83.2	7.08
25	1.01×10^{-4}	100.4	7.00
30	1.47×10^{-4}	120.2	6.92
37	2.39×10^{-4}	154.9	6.81
45	4.02×10^{-4}	199.5	6.70

Table 7.2 Properties of some pH indicator dyes

Dye	Acid–base colour change	Useful pH range
Thymol blue (acid)	Red–yellow	1.2–6.8
Bromophenol blue	Yellow–blue	1.2–6.8
Methyl orange	Red–yellow	2.8–4.0
Congo red	Blue–red	3.0–5.2
Bromocresol green	Yellow–blue	3.8–5.4
Methyl red	Red–yellow	4.3–6.1
Litmus	Red–blue	4.5–8.3
Chlorophenol red	Yellow–red	4.8–6.3
Bromocresol purple	Yellow–purple	5.2–6.8
Bromothymol blue	Yellow–blue	6.0–7.6
Neutral red	Red–yellow	6.8–8.0
Phenol red	Yellow–red	6.8–8.2
1-Naphthol-phthalein	Yellow–blue	7.2–8.6
Phenol-phthalein	None–red	8.3–10.0

KEY POINT **While pH is strictly the negative logarithm (to the base 10) of H^+ activity, in practice H^+ concentration in mol L^{-1} (equivalent to kmol m^{-3} in SI terminology) is most often used in place of activity, since the two are virtually the same, given the limited dissociation of H_2O. The pH scale is not SI: nevertheless, it continues to be used widely in chemistry.**

The value where an equal amount of H^+ and OH^- ions are present is termed neutrality: at 25 °C the pH of pure water at neutrality is 7.0. At this temperature, pH values below 7.0 are acidic while values above 7.0 are alkaline. However, the pH of a neutral solution changes with temperature (Table 7.1), owing to the enhanced dissociation of water with increasing temperature. This must be taken into account when measuring the pH of any solution and when interpreting your results.

Always remember that the pH scale is a logarithmic one, not a linear one: a solution with a pH of 3.0 is not twice as acidic as a solution of pH 6.0, but a thousand times as acidic (i.e. contains 1000 times the amount of H^+ ions). Therefore, you may need to convert pH values into proton concentrations before you carry out mathematical manipulations (see Box 40.2). For similar reasons, it is important that pH change is expressed in terms of the original and final pH values, rather than simply quoting the difference between the values: a pH change of 0.1 has little meaning unless the initial or final pH is known.

Measuring pH

pH electrodes

Accurate pH measurements can be made using a pH electrode, coupled to a pH meter. The pH electrode is usually a combination electrode, comprising two separate systems: an H^+-sensitive glass electrode and a reference electrode which is unaffected by H^+ ion concentration (see Fig. 7.2). When this is immersed in a solution, a pH-dependent voltage between the two electrodes can be measured using a potentiometer. In most cases, the pH electrode assembly (containing the glass and reference electrodes) is connected to a separate pH meter by a cable, although some hand-held instruments (pH probes) have combination electrodes and meter within the same assembly, often using an H^+-sensitive field effect transistor in place of a glass electrode, to improve durability and portability.

Box 7.1 gives details of the steps involved in making a pH measurement with a glass pH electrode and meter.

pH indicator dyes

These compounds (usually weak acids) change colour in a pH-dependent manner. They may be added in small amounts to a solution, or they can be used in paper strip form. Each indicator dye usually changes colour over a restricted pH range (Table 7.2): universal indicator dyes/papers make use of a combination of individual dyes to measure a wider pH range. Dyes are not suitable for accurate pH measurement as they are affected by other components of the solution including oxidizing and reducing agents and salts. However, they are useful for:

- estimating the approximate pH of a solution;
- determining a change in pH, e.g. at the end-point of a titration.

Definition

Buffer solution – one which resists a change in H^+ concentration (pH) on addition of acid or alkali.

Buffers

Rather than simply measuring the pH of a solution, you may wish to *control* the pH, during EDTA complexation titrations (see p. 152) or preparative experiments involving carbonyl compounds, and one of the most effective ways to control pH is to use a buffer solution.

A buffer solution is usually a mixture of a weak acid and its conjugate base. Added protons will be neutralized by the anionic base while a reduction in protons, e.g. due to the addition of hydroxyl ions, will be counterbalanced by dissociation of the acid (eqn [7.2]); thus the conjugate pair acts as a 'buffer' to pH change.

The British standard for the pH scale is an aqueous solution of potassium hydrogen phthalate (0.05 M), which has a pH of 4.001 at 20 °C and is often used as a calibration solution for pH meters.

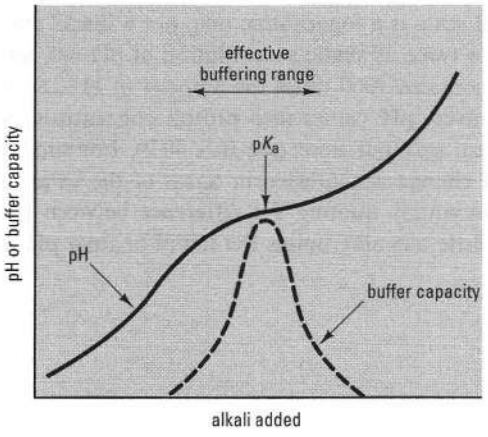


Fig. 7.1 Theoretical pH titration curve for a buffer solution. pH change is lowest and buffer capacity is greatest at the pK_a of the buffer solution.

Buffer capacity and the effects of pH

The extent of resistance to pH change is called the buffer capacity of a solution. The buffer capacity is measured experimentally at a particular pH by titration against a strong acid or alkali: the resultant curve will be strongly sigmoidal, with a plateau where the buffer capacity is greatest (Fig. 7.1). The mid-point of the plateau represents the pH where equal quantities of acid and conjugate base are present, and is given the symbol pK_a, which refers to the negative logarithm (to the base 10) of the acid dissociation constant, K_a, where

$$K_a = \frac{[H^+]\,[A^-]}{[HA]} \qquad [7.6]$$

By rearranging eqn [7.6] and taking negative logarithms, we obtain:

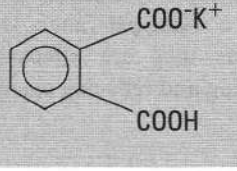
$$pH = pK_a + \log_{10}\frac{[A^-]}{[HA]} \qquad [7.7]$$

This relationship is known as the Henderson–Hasselbalch equation and it shows that the pH will be equal to the pK_a when the ratio of conjugate base to acid is unity, since the final term will be zero. Consequently, the pK_a of a buffer solution is an important factor in determining the buffer capacity at a particular pH. In practical terms, this means that a buffer solution will work most effectively at pH values about one unit either side of the pK_a.

Selecting an appropriate buffer

When selecting a buffer, you should be aware of certain limitations to its use. Citric acid and phosphate buffers readily form insoluble complexes with divalent cations, while phosphate can also act as a substrate, activator or inhibitor of certain enzymes. Both of these buffers contain biologically significant quantities of cations, e.g. Na^+ or K^+. TRIS (Table 7.3) is often toxic to biological systems: owing to its high lipid solubility it can penetrate membranes, uncoupling electron transport reactions in whole cells and isolated organelles. In addition, it is markedly affected by temperature, with a 10-fold increase in H^+ concentration from 4 °C to 37 °C. A number of zwitterionic molecules (possessing both positive and negative groups) have been introduced to overcome some of the disadvantages of the more traditional buffers. These newer compounds are often referred to as 'Good buffers', to

Potassium hydrogen phthalate (KHP) is the mono potassium salt of a weak organic dibasic acid.



Box 7.1 Using a glass pH electrode and meter to measure the pH of a solution

The following procedure should be used whenever you make a pH measurement: consult the manufacturer's handbook for specific information, where necessary. Do not be tempted to miss out any of the steps detailed below, particularly those relating to the effects of temperature, or your measurements are likely to be inaccurate.

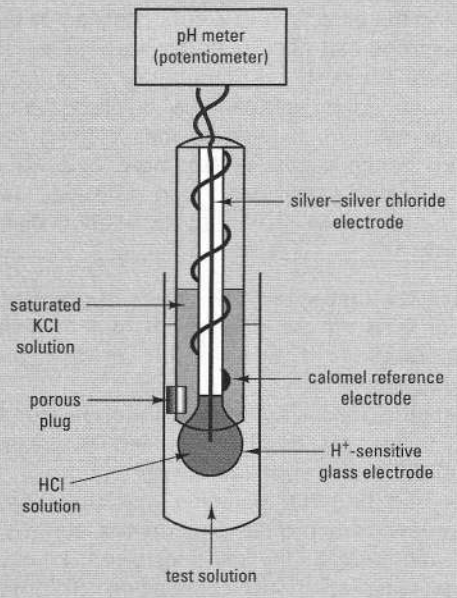


Fig. 7.2 Measurement of pH using a combination pH electrode and meter. The electrical potential difference recorded by the potentiometer is directly proportional to the pH of the test solution.

1. **Stir the test solution thoroughly before you make any measurement**: it is often best to use a magnetic stirrer. Leave the solution for sufficient time to allow equilibration at lab temperature.
2. **Record the temperature of every solution you use**, including all calibration standards and samples, since this will affect K_w, neutrality and pH.
3. **Set the temperature compensator on the meter to the appropriate value**. This control makes an allowance for the effect of temperature on the electrical potential difference recorded by the meter: it does *not* allow for the other temperature-dependent effects mentioned elsewhere. Basic instruments have no temperature compensator, and should only be used at a specified temperature, either 20 °C or 25 °C, otherwise they will not give an accurate measurement. More sophisticated systems have automatic temperature compensation.
4. **Rinse the electrode assembly with distilled water** and gently dab off the excess water onto a clean tissue: check for visible damage or contamination of the glass electrode (consult a member of staff if the glass is broken or dirty). Also check that the solution within the glass assembly is covering the metal electrode.
5. **Calibrate the instrument:** set the meter to 'pH' mode, if appropriate, and then place the electrode assembly in a standard solution of known pH, usually pH 7.00. This solution may be supplied as a liquid, or may be prepared by dissolving a measured amount of a calibration standard in water: calibration standards are often provided in tablet form, to be dissolved in water to give a particular volume of solution. Adjust the calibration control to give the correct reading. Remember that your calibration standards will only give the specified pH at a particular temperature, usually either 20 °C or 25 °C. If you are working at a different temperature, you must establish the actual pH of your calibration standards, either from the supplier, or from literature information.
6. **Remove the electrode assembly from the calibration solution and rinse again with distilled water**: dab off the excess water. Basic instruments have no further calibration steps (single-point calibration), while the more refined pH meters have additional calibration procedures.

   If you are using a basic instrument, you should check that your apparatus is accurate over the appropriate pH range by measuring the pH of another standard whose pH is close to that expected for the test solution. If the standard does not give the expected reading, the instrument is not functioning correctly: consult a member of staff.

   If you are using an instrument with a slope control function, this will allow you to correct for any deviation in electrical potential from that predicted by the theoretical relationship (at 25 °C, a change in pH of 1.00 unit should result in a change in electrical potential of 59.16 mV) by performing a two-point calibration. Having calibrated the instrument at pH 7.00, immerse in a second standard at the same temperature as that of the first standard, usually buffered to either pH 4.00 or pH 9.00, depending upon the expected pH of your samples. Adjust the slope control until the exact value of the second standard is achieved (Fig. 7.3). A pH electrode and meter calibrated using the two-point method will give accurate readings over the pH range from 3 to 11: laboratory pH electrodes are not accurate outside this range, since the theoretical

Box 7.1 (continued)

relationship between electrical potential and pH is no longer valid.

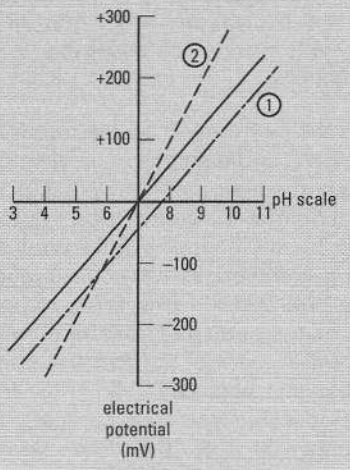


Fig. 7.3 The relationship between electrical potential and pH. The solid line shows the response of a calibrated electrode while the other plots are for instruments requiring calibration: 1 has the correct slope but incorrect isopotential point (calibration control adjustment is needed); 2 has the correct isopotential point but incorrect slope (slope control adjustment is needed).

7. **Once the instrument is calibrated, measure the pH of your solution(s)**, making sure that the electrode assembly is rinsed thoroughly between measurements. You should be particularly aware of this requirement if your solutions contain organic biological material, e.g. soil, tissue fluids, protein solutions, etc., since these may adhere to the glass electrode and affect the calibration of your instrument. If your electrode becomes contaminated during use, check with a member of staff before cleaning: avoid touching the surface of the glass electrode with abrasive material. Allow sufficient time for the pH reading to stabilize in each solution before taking a measurement: for unbuffered solutions, this may take several minutes, so do not take inaccurate pH readings due to impatience!
8. **After use, the electrode assembly must not be allowed to dry out.** Most pH electrodes should be stored in a neutral solution of KCl, either by suspending the assembly in a small beaker, or by using an electrode cap filled with the appropriate solution (typically 1.0 mol L^{-1} KCl buffered at pH 7.0). However, many labs simply use distilled water as a storage solution, leading to loss of ions from the interior of the electrode assembly. In practice, this means that pH electrodes stored in distilled water will take far longer to give a stable reading than those stored in KCl.
9. **Switch the meter to zero (where appropriate), but do not turn off the power:** pH meters give more stable readings if they are left on during normal working hours.

   Problems (and solutions) include: inaccurate and/or unstable pH readings caused by crosscontamination (rinse electrode assembly with distilled water and blot dry between measure-ments); development of a protein film on the surface of the electrode (soak in 1% w/v pepsin in 0.1 mol L^{-1} HCl for at least an hour); deposition of organic or inorganic contaminants on the glass bulb (use an organic solvent, such as acetone, or a solution of 0.1 mol L^{-1} disodium ethylenediamine-tetraacetic acid, respectively); drying out of the internal reference solutions (drain, flush and refill with fresh solution, then allow to equilibrate in 0.1 mol L^{-1} HCl for at least an hour); cracks or chips to the surface of the glass bulb (use a replacement electrode).

acknowledge the early work of Dr N.E. Good and co-workers: HEPES is one of the most useful zwitterionic buffers, with a pK_a of 7.5 at 25 °C.

These zwitterionic substances are usually added to water as the free acid: the solution must then be adjusted to the correct pH with a strong alkali, usually NaOH or KOH. Alternatively, they may be used as their sodium or potassium salts, adjusted to the correct pH with a strong acid, e.g. HCl. Consequently, you may need to consider what effects such changes in ion concentration may have in a solution where zwitterions are used as buffers.

Fig. 7.4 shows a number of traditional and zwitterionic buffers and their effective pH ranges. When selecting one of these buffers, aim for a pK_a which is in the direction of the expected pH change (Tables 7.2, 7.3). For example, HEPES buffer would be a better choice of buffer than PIPES for use at

Table 7.3 pK_a values at 25 °C of some acids and bases (upper section) and some large organic zwitterions (lower section) commonly used in buffer solutions. For polyprotic acids, where more than one proton my dissociate, the pK_a values are given for each ionization step. Only the trivial acronyms of the larger molecules are provided: their full names can be obtained from the catalogues of most chemical suppliers

Acid or base	pK_a value(s)
Acetic acid	4.8
Carbonic acid	6.1, 10.2
Citric acid	3.1, 4.8, 5.4
Glycylglycine	3.1, 8.2
Phthalic acid	2.9, 5.5
Phosphoric acid	2.1, 7.1, 12.3
Succinic acid	4.2, 5.6
TRIS*	8.3
Boric acid	9.2
MES	6.1
PIPES	6.8
MOPS	7.2
HEPES	7.5
TRICINE	8.1
TAPS	8.4
CHES	9.3
CAPS	10.4

*Note that this compound is hygroscopic and should be stored in a desiccator.

Table 7.4 Preparation of sodium phosphate buffer solutions for use at 25 °C. Prepare separate stock solutions of (a) disodium hydrogen phosphate and (b) sodium dihydrogen phosphate, both at 0.2 mol L^{-1}. Buffer solutions (at 0.1 mol L^{-1}) are then prepared at the required pH by mixing together the volume of each stock solution shown in the table, and then diluting to a final volume of 100 mL using distilled or deionized water

Required pH (at 25 °C)	Volume of stock (a) Na_2HPO_4 (mL)	Volume of stock (b) NaH_2PO_4 (mL)
6.0	6.2	43.8
6.2	9.3	40.7
6.4	13.3	36.7
6.6	18.8	31.2
6.8	24.5	25.5
7.0	30.5	19.5
7.2	36.0	14.0
7.4	40.5	9.5
7.6	43.5	6.5
7.8	45.8	4.2
8.0	47.4	2.6

pH 7.2 for experimental systems where a pH increase is anticipated, while PIPES would be a better choice for where acidification is expected.

Preparation of buffer solutions

Having selected an appropriate buffer, you will need to make up your solution to give the desired pH. You will need to consider two factors:

1. The ratio of acid and conjugate base required to give the correct pH.
2. The amount of buffering required; buffer capacity depends upon the absolute quantities of acid and base, as well as their relative proportions.

In most instances, buffer solutions are prepared to contain between 10 mmol L^{-1} and 200 mmol L^{-1} of the conjugate pair. While it is possible to calculate the quantities required from first principles using the Henderson–Hasselbalch equation, there are several sources which tabulate the amount of substance required to give a particular volume of solution with a specific pH value for a wide range of traditional buffers (e.g. Perrin and Dempsey, 1974). For traditional buffers, it is customary to mix stock solutions of acidic and basic components in the correct proportions to give the required pH (Table 7.4). For zwitterionic acids, the usual procedure is to add the compound to water, and then bring the solution to the required pH by adding a specific amount of strong alkali or acid (obtained from tables). Alternatively, the required pH can be obtained by dropwise addition of alkali or acid, using a meter to check the pH, until the correct value is reached. When preparing solutions of zwitterionic buffers, the acid may be relatively insoluble. Do not wait for it to dissolve fully before adding alkali to change the pH – the addition of alkali will help bring the acid into solution (but make sure it has all dissolved before the desired pH is reached).

Finally, when preparing a buffer solution based on tabulated information, always confirm the pH with a pH meter before use.

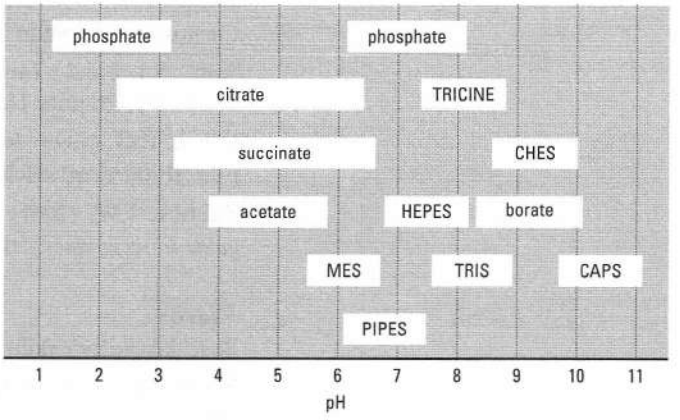


Fig. 7.4 Useful pH ranges of some commonly used buffers.

Resources for fundamental laboratory techniques

Books

Anon. (1989) *Safe Practices in Chemical Laboratories*, Royal Society of Chemistry, London.

Anon. (1989) *COSHH in Laboratories*, Royal Society of Chemistry, London.

Bennett, S.W. and O'Neale, K. (1999) *Progressive Development of Practical Skills in Chemistry. A guide to early-undergraduate experimental work*, Royal Society of Chemistry, Cambridge.

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Sharp, J.T., Gosney, I. and Rowley, A.G. (1989) *Practical Organic Chemistry*, Chapman and Hall, London.

Suib. S.L. and Tanaka, J. (1999) *Experimental Methods in Inorganic Chemistry*, Prentice Hall, Harlow, Essex.

Urben, P.G. (1999) *Bretherick's Handbook of Reactive Chemical Hazards, 6th* Edn, Butterworth-Heinemann, London.

Zubrick, J.W. (2001) *The Organic Chem Lab Survival Manual. A student's guide to techniques*, 5th Edn, John Wiley and Sons Inc., Chichester.

Videos

Basic laboratory skills, LGC, Royal Society of Chemistry, Cambridge (1998).

Further Laboratory Skills, LGC, Royal Society of Chemistry, Cambridge (1998).

Software

SoftCOSHH 2000, Royal Society of Chemistry, Cambridge.

The investigative approach

8 Making and recording measurements

Definition

Variable – any characteristic or property which can take one of a range of values.

Parameter – a numerical constant or mathematical function used to describe a particular population (e.g. the mean height of 18-year-old females).

Statistic – an estimate of a parameter obtained from a sample (e.g. the height of 18-year-old females based on those in your class).

The term data (singular = datum, or data value or variate) refers to measurements of a particular characteristic, or variable, classified as:

- Quantitative: where the individual values are described on a numerical scale which may be either (i) continuous, taking any value on the measurement scale, or (ii) discontinuous (or discrete), where only integer values are possible. Many of the variables measured in chemistry are continuous and quantitative, e.g. weight, temperature, time, amount of product formed in an enzyme reaction.
- Ranked: where the data values can be listed in order of magnitude. Where such data are given numbered ranks, they are sometimes called 'semi-quantitative data'. Note that such ranks cannot be treated as 'real' numbers and they should not be added, averaged, etc.
- Qualitative: where individual values are assigned to a descriptive category, e.g. the detection of the presence or absence of a chemical by a colour test or precipitate.

Variables may be independent or dependent. Usually, the variable under the control of the experimenter (e.g. time, reagent concentration, pH, etc.) is the independent variable, while the variable being measured is the dependent variable (p. 76). Sometimes, it is inappropriate to describe variables in this way, and they are often referred to as interdependent. Another group of values, often termed derived (or computed) data, are calculated from two or more individual measurements, and these include ratios, percentages and rates.

Measurement scales

Examples A nominal scale for temperature is not feasible, since the relevant descriptive terms can be ranked in order of magnitude.

An ordinal scale for temperature measurement might use descriptive terms, ranked in ascending order, e.g. cold = 1, cool = 2, warm = 3, hot = 4.

The Celsius scale is an interval scale for temperature measurement, since the arbitrary zero corresponds to the freezing point of water (0 °C).

The Kelvin scale is a ratio scale for temperature measurement since 0 K represents a temperature of absolute zero (for information, the freezing point of water is 273.15 K on this scale).

Variables may be measured on different types of scale:

- Nominal – where classification is based on a descriptive characteristic (e.g. colour). This is the only scale for qualitative data.
- Ordinal – this classifies by numerical rank, from smallest to greatest, but with no assumption of equal spacing between ranks.
- Interval – for certain quantitative variables, where numbers on an equal unit scale are related to an arbitrary zero, e.g. temperature in °C.
- Ratio – similar to the interval scale, except that the zero point represents an absence of that character, i.e. it is an absolute zero.

You should aim to do quantitative measurements using a ratio scale whenever possible, to allow you to use the broadest range of mathematical operations and statistical procedures. For example, if you are measuring the surface area of a sample of graphite you could give the area as 200 m^2 but, if you know the mass of the sample (10 g), you should quote the surface area as 20 $m^2 g^{-1}$.

Accuracy and precision

Accuracy is the closeness of a measured or derived data value to its true value, while precision is the closeness of repeated measurements to each other (Fig. 8.1). A balance with a fault in it (i.e. a bias, see below) could give precise (i.e. very repeatable) but inaccurate (i.e. untrue) results. Unless there is bias in a measuring system, precision will lead to accuracy and it is

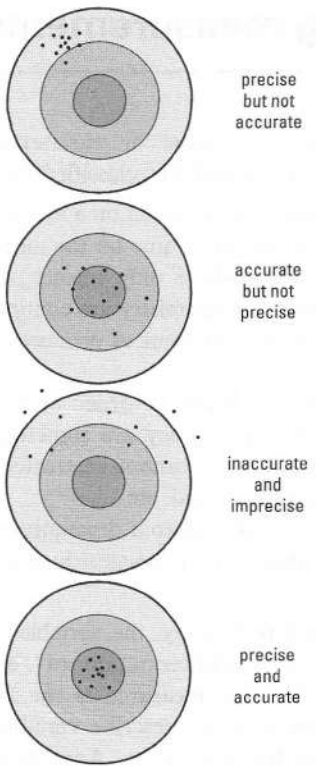


Fig. 8.1 'Target' diagrams illustrating precision and accuracy.

Minimizing errors – determine early in your study what the dominant errors are likely to be and concentrate your time and effort on reducing these.

Working with derived data – special effort should be made to reduce measurement errors because their effects can be magnified when differences, ratios, indices or rates are calculated.

Recording primary data – never be tempted to jot down data on scraps of paper: you are likely to lose them, or to forget what individual values mean.

precision that is generally the most important practical consideration, if there is no reason to suspect bias. You can investigate the precision of any measuring system by repeated measurements of the same sample: the nearer the replicate values are to each other, the more precise the measurement.

Absolute accuracy and precision are impossible to achieve, owing to the limitations of measuring systems. It is particularly important to avoid spurious accuracy in the presentation of results; include only those digits which the accuracy of the measuring system implies. This type of error is common when changing units (e.g. inches to metres) and in derived data, especially when calculators give results to a large number of decimal places.

Bias (systematic error)

Bias is a systematic or non-random inaccuracy and is one of the most troublesome difficulties in using numerical data. Biases may be associated with incorrectly calibrated instruments, e.g. a faulty electrode or syringe, or with experimental manipulations, e.g. decomposition of chemical compound on storage. Bias in measurement can also be subjective, or personal, e.g. an experimenter's preconceived ideas about an 'expected' result.

Bias can be minimized by using a carefully standardized procedure, with fully calibrated instruments. Investigate bias in 'trial runs' by measuring a variable in several different ways, to see if the same result is obtained.

To avoid personal bias, 'blind' measurements should be made where the identity of each sample is unknown to the operator, e.g. use a coding system.

Measurement (random) error

All measurements are subject to error, but the dangers of misinterpretation are reduced by recognizing and understanding the likely sources of error and by adopting appropriate protocols and calculation procedures.

A common source of random error is carelessness, e.g. reading a scale in the wrong direction or parallax errors. This can be reduced greatly by careful recording and may be detected by repeating the measurement. Other errors arise from faulty or inaccurate equipment, but even a perfectly functioning machine has distinct limits to the accuracy and precision of its measurements. These limits are often quoted in manufacturers' specifications and are applicable when an instrument is new; however, you should allow for some deterioration with age.

One major influence virtually impossible to eliminate is the effect of the investigation itself: even putting a thermometer in a liquid may change the temperature of the liquid. The very act of measurement may give rise to a confounding variable (p. 76) as discussed in Chapter 10. You should include descriptions of the possible sources of error(s) and estimates of their importance in any report and these should not be used as an excuse for poor technique or inadequacies in your experimental design.

Collecting and recording primary data

When carrying out lab work or research projects, you will need to master the important skills of recording and managing data. Individual observations (e.g. laboratory temperature) can be recorded in the text of your notes, but tables are the most convenient way to collect large amounts of information.

KEY POINT **A good set of lab notes should:**

- **outline the purpose of your experiment or observation;**
- **set down all the information required to describe your experimental section;**
- **record all relevant information about your results or observations and provide a visual representation of the data;**
- **note your immediate conclusions and suggestions for further experiments.**

When preparing a table for data collection, you should:

1. Use a concise title or a numbered code for cross-referencing.
2. Decide on the number of variables to be measured and their relationship with each other and lay out the table appropriately:
   (a) The first column of your table should show values of the independent (controlled) variable, with subsequent columns for the individual (measured) values for each replicate or sample.
   (b) If several variables are measured for the same organism or sample, each should be given a row.
   (c) In time-course studies, put the replicates as columns grouped according to treatment, with the rows relating to different times.

Designing a table for data collection – make sure there is sufficient space in each column for the values; if in doubt, err on the generous side.

3. Make sure the arrangement reflects the order in which the values will be collected. Your table should be designed to make the recording process as straightforward as possible, to minimize the possibility of mistakes. For final presentation, a different arrangement may be best (Chapter 37).
4. Consider whether additional columns are required for subsequent calculations. Create a separate column for each mathematical manipulation, so the step-by-step calculations are clearly visible. Use a computer spreadsheet (p. 307) if you are manipulating lots of data.
5. Use a pen to record data.
6. Take sufficient time to record quantitative data unambiguously – use large, clear numbers, making sure that individual numerals cannot be confused.

Recording numerical data – write down only those numbers that can be justified by your measurement technique (significant figures).

7. Record numerical data to an appropriate number of significant figures, reflecting the accuracy and precision of your measurement (p. 65). Do not round off data values, as this might affect the subsequent analysis.
8. Record the actual observations, not your interpretation, e.g. the colour of a particular chemical test, rather than whether the test was positive or negative. Take care not to lose any of the information content of the data: for instance, if you only write down means and not individual values, this will affect your ability to carry out subsequent statistical analyses.
9. Prepare duplicated recording tables/checklists for repeated experiments.
10. Explain any unusual results in a footnote. Don't rely on memory.

Recording details of project work

The recommended system is one where you make a dual record.

Primary record

Choosing a lab notebook – a spiral-bound notebook is good for making a primary record: it lies conveniently open on the bench and provides a simple method of dealing with major mistakes!

The primary record is made at the bench and you must concentrate on the detail of materials, methods and results. Include information that would not be used elsewhere, but which might prove useful in error tracing: for example, if you note how a solution was made up (exact

volumes and weights used rather than concentration alone), this could reveal whether a miscalculation had been the cause of a rogue result. Note the origin, type and state of the chemicals used. In the experimental section, the basic rule is to record enough information to allow a reasonably competent scientist to repeat your work exactly. You must tread a line between the extremes of pedantic, irrelevant detail and the omission of information essential for proper interpretation – better perhaps to err on the side of extra detail to begin with. An experienced worker can tell you which subtle shifts in technique are important (e.g. batch numbers for an important chemical, or when a new stock solution is prepared). Many important scientific advances have been made because of careful observation and record taking and because coincident data were recorded that did not seem of immediate value. Make rough diagrams to show the arrangement of replicates, equipment, etc. If forced to use loose paper to record data, make sure each sheet is dated and taped to your lab book, collected in a ring binder, or attached with a treasury tag. The same applies to traces, printouts and graphs.

The basic order of the primary record should mirror that of a research report (see p. 332), including: the title and date, brief introduction, a comprehensive experimental section, the data and short conclusions.

Secondary record

You should make a secondary record concurrently or later in a bound book and it ought to be neater, in both organization and presentation. This book will be used when discussing results with your supervisor, and when writing up a report or thesis, and may be part of your course assessment. Writing a second, neater version forces you to consider again details that might have been overlooked in the primary record and provides a duplicate in case of loss or damage. While these notes should retain the essential features of the primary record, they should be more concise and the emphasis should move towards analysis of the experiment. Don't repeat the experimental section for a series of similar experiments; use devices such as 'method as for Expt B4'. A photocopy may be sufficient if the method is derived from a text or article (check with your supervisor). Outline the aims more carefully at the start and link the experiment to others in a series (e.g. 'Following the results of Expt D24, I decided to test whether...'). You should present data in an easily digested form, e.g. as tables of means or as summary graphs. Use appropriate statistical tests (p. 271) to support your analysis of the results. Always analyse and think about data immediately after collecting them as this may influence your subsequent activities. Write down any conclusions: sometimes those which seem obvious at the time of doing the work are forgotten when the time comes to write up a report or thesis. Likewise, ideas for further studies may prove valuable later. Even if your experiment appears to be a failure, suggestions as to the likely causes might prove useful.

Using communal records

If working with a research team, you may need to use their communal databases. These avoid duplication of effort and ensure uniformity in techniques. They may also form part of the legal safety requirements for lab work. They might include:

- a shared notebook of common techniques (e.g. solutions or calibration technique);
- a set of simplified step-by-step instructions for use of equipment;
- an alphabetical list of suppliers of equipment and consumables;
- a list of chemicals required by the group and where they are stored;
- the risk assessment sheets for dangerous procedures (p. 7);
- a record of the use and disposal of chemicals and solvents.

9 SI units and their use

Dimensionless measurements – some quantities can be expressed as dimensionless ratios or logarithms (e.g. absorbance and pH), and in these cases you do not need to use a qualifying unit.

When describing a measurement, you normally state both a number and a unit (e.g. 'the length is 1.85 metres'). The number expresses the ratio of the measured quantity to a fixed standard, while the unit identifies that standard measure or dimension. Clearly, a single unified system of units is essential for efficient communication of such data within the scientific community. The Système International d'Unités (SI) is the internationally ratified form of the metre–kilogram–second system of measurement and represents the accepted scientific convention for measurements of physical quantities.

Another important reason for adopting consistent units is to simplify complex calculations where you may be dealing with several measured quantities (see p. 260). Although the rules of the SI are complex and the scale of the base units is sometimes inconvenient, to gain the full benefits of the system you should observe its conventions strictly.

The description of measurements in SI involves:

- seven base units and two supplementary units, each having a specified abbreviation or symbol (Table 9.1);
- derived units, obtained from combinations of base and supplementary units, which may also be given special symbols (Table 9.2);
- a set of prefixes to denote multiplication factors of 10^3, used for convenience to express multiples or fractions of units (Table 9.3).

Table 9.1 The base and supplementary SI units

Measured quantity	Name of SI unit	Symbol
Base units		
Length	metre	m
Mass	kilogram	kg
Amount of substance	mole	mol
Time	second	s
Electric current	ampere	A
Temperature	kelvin	K
Luminous intensity	candela	cd
Supplementary units		
Plane angle	radian	rad
Solid angle	steradian	sr

Table 9.2 Some important derived SI units

Measured quantity	Name of unit	Symbol	Definition in base units	Alternative in derived units
Energy	joule	J	$m^2\,kg\,s^{-2}$	N m
Force	newton	N	$m\,kg\,s^{-2}$	$J\,m^{-1}$
Pressure	pascal	Pa	$kg\,m^{-1}\,s^{-2}$	$N\,m^{-2}$
Power	watt	W	$m^2\,kg\,s^{-3}$	$J\,s^{-1}$
Electric charge	coulomb	C	A s	$J\,V^{-1}$
Electric potential difference	volt	V	$m^2\,kg\,A^{-1}\,s^{-3}$	$J\,C^{-1}$
Electric resistance	ohm	Ω	$m^2\,kg\,A^{-2}\,s^{-3}$	$V\,A^{-1}$
Electric conductance	siemens	S	$s^3\,A^2\,kg^{-1}\,m^{-2}$	$A\,V^{-1}$ or Ω^{-1}
Electric capacitance	farad	F	$s^4\,A^2\,kg^{-1}\,m^{-2}$	$C\,V^{-1}$
Luminous flux	lumen	lm	cd sr	
Illumination	lux	lx	$cd\,sr\,m^{-2}$	$lm\,m^{-2}$
Frequency	hertz	Hz	s^{-1}	
Radioactivity	becquerel	Bq	s^{-1}	
Enzyme activity	katal	kat	mol substrate s^{-1}	

Table 9.3 Prefixes used in the SI

Multiple	Prefix	Symbol	Multiple	Prefix	Symbol
10^{-3}	milli	m	10^3	kilo	k
10^{-6}	micro	μ	10^6	mega	M
10^{-9}	nano	n	10^9	giga	G
10^{-12}	pico	p	10^{12}	tera	T
10^{-15}	femto	f	10^{15}	peta	P
10^{-18}	atto	a	10^{18}	exa	E

Example 10 μg is correct, while 10μg, 10 μg. and 10μ g are incorrect; 2.6 mol is right, but 2.6 mols is wrong.

Recommendations for describing measurements in SI units

Basic format

- Express each measurement as a number separated from its units by a space. If a prefix is required, no space is left between the prefix and the unit it refers to. Symbols for units are only written in their singular form and do not require full stops to show that they are abbreviated or that they are being multiplied together.

Example n stands for nano and N for newtons.

- Give symbols and prefixes appropriate upper or lower case initial letters as this may define their meaning. Upper case symbols are named after persons but when written out in full they are not given initial capital letters.
- Show the decimal sign as a full point on the line. Some metric countries continue to use the comma for this purpose and you may come across this in the literature: commas should not therefore be used to separate groups of thousands. In numbers that contain many significant figures, you should separate multiples of 10^3 by spaces rather than commas.

Compound expressions for derived units

- Take care to separate symbols in compound expressions by a space to avoid the potential for confusion with prefixes. Note, for example, that 200 m s (metre seconds) is different from 200 ms (milliseconds).
- Express compound units using negative powers rather than a solidus (/): for example, write $\mathrm{mol\,m^{-3}}$ rather than $\mathrm{mol/m^3}$. The solidus is reserved for separating a descriptive label from its units (see p. 251).
- Where there is a choice, select relevant (natural) combinations of derived and base units, e.g. you might choose units of $\mathrm{Pa\,m^{-1}}$ to describe a hydrostatic pressure gradient rather than $\mathrm{kg\,m^{-2}\,s^{-1}}$, even though these units are equivalent and the measurements are numerically the same.

Use of prefixes

Examples 10 μm is preferred to 0.000 01 m or 0.010 mm.

$1\,\mathrm{mm^2} = 1 \times 10^{-3}\,\mathrm{m^2} = 1 \times 10^{-6}\,\mathrm{m^3}$ (not one-thousandth of a square metre).

$1\,\mathrm{dm^3}$ (1 litre) is more properly expressed as $1 \times (10^{-1}\,\mathrm{m})^3 = 1 \times 10^{-3}\,\mathrm{m^3}$.

Avogadro's constant is $6.022\,174 \times 10^{23}\,\mathrm{mol^{-1}}$.

State as $\mathrm{MW\,m^{-2}}$ rather than $\mathrm{W\,mm^{-2}}$.

- Use prefixes to denote multiples of 10^3 (Table 9.3) so that numbers are kept between 0.1 and 1000.
- Treat a combination of a prefix and a symbol as a single symbol. Thus, when a modified unit is raised to a power, this refers to the whole unit including the prefix.
- Avoid the prefixes deci (d) for 10^{-1} and centi (c) for 10^{-2} as they are not strictly SI.
- Express very large or small numbers as a number between 1 and 10 multiplied by a power of 10 if they are outside the range of prefixes shown in Table 9.3.
- Do not use prefixes in the middle of derived units: they should be attached only to a unit in the numerator (the exception is in the unit for mass, kg).

In this book, we use L and mL where you would normally find equipment calibrated in that way, but use SI units where this simplifies calculations. In formal scientific writing, constructions such as $1 \times 10^{-6}\,\mathrm{m^3}$ (= 1 mL) and $1\,\mathrm{mm^3}$ (= 1 μL) may be used.

KEY POINT **For the foreseeable future, you will need to make conversions from other units to SI units, as much of the literature quotes data using imperial, c.g.s. or other systems. You will need to recognize these units and find the conversion factors required. Examples relevant to chemistry are given in Box 9.1. Table 9.4 provides values of some important physical constants in SI units.**

Some implications of SI in chemistry

Volume

The other common non-SI unit of volume is the cubic centimetre, $\mathrm{cm^3}$, $(10^{-2}\,\mathrm{m})^3$. Even though they are not *exactly* the same, mL, and $\mathrm{cm^3}$ are used interchangeably, as are cubic decimetre $\mathrm{dm^3}(10^{-1}\,\mathrm{m})^3 = 10^{-3}\,\mathrm{m^3}$ and litre (L).

The SI unit of volume is the cubic metre, $\mathrm{m^3}$, which is rather large for practical purposes. The litre (L) and the millilitre (mL) are technically obsolete, but are widely used and glassware is still calibrated using them.

Table 9.4 Some physical constants in SI terms

Physical constant	Symbol	Value and units
Avogadro's constant	N_A	$6.022\,174 \times 10^{23}\ mol^{-1}$
Boltzmann's constant	k	$1.380\,626\ J\ K^{-1}$
Charge of electron	e	$1.602\,192 \times 10^{-19}\ C$
Gas constant	R	$8.314\,43\ J\ K^{-1}\ mol^{-1}$
Faraday's constant	F	$9.648\,675 \times 10^{4}\ C\ mol^{-1}$
Molar volume of ideal gas at STP	V_0	$0.022\,414\ m^3\ mol^{-1}$
Speed of light *in vacuo*	c	$2.997\,924 \times 10^{8}\ m\ s^{-1}$
Planck constant	h	$6.626\,205 \times 10^{-34}\ J\ s$
Acceleration due to gravity	g	$9.807\ m\ s^{-1}$
Atomic mass unit	m_u	$1660\,540\,2 \times 10^{-27}\ kg$
Rydberg constant	R_∞	$1.097 \times 10^{7}\ m^{-1}$
Permitivity of vacuum	ε_0	$8.854 \times 10^{-12}\ F\ m^{-1}$

Box 9.1 Conversion factors between some redundant units and the SI

Quantity	SI unit/symbol	Old unit/symbol	Multiply number in old unit by this factor for equivalent in SI unit*	Multiply number in SI unit by this factor for equivalent in old unit*
Area	square metre/m^2	acre	$4.046\,86 \times 10^{3}$	$0.247\,105 \times 10^{-3}$
		hectare/ha	10×10^{3}	0.1×10^{-3}
		square foot/ft^2	0.092 903	10.763 9
		square inch/in^2	645.16×10^{-9}	$1.550\,00 \times 10^{6}$
		square yard/yd^2	0.836 127	1.195 99
Angle	radian/rad	degree/°	$17.453\,2 \times 10^{-3}$	57.295 8
Energy	joule/J	erg	0.1×10^{-6}	10×10^{6}
		kilowatt hour/kWh	3.6×10^{6}	$0.277\,778 \times 10^{-6}$
Length	metre/m	Ångstrom/Å	0.1×10^{-9}	10×10^{9}
		foot/ft	0.304 8	3.280 84
		inch/in	25.4×10^{-3}	39.370 1
		mile	$1.609\,34 \times 10^{3}$	$0.621\,373 \times 10^{-3}$
		yard/yd	0.914 4	1.093 61
Mass	kilogram/kg	ounce/oz	$28.349\,5 \times 10^{-3}$	35.274 0
		pound/lb	0.453 592	2.204 62
		stone	6.350 29	0.157 473
		hundredweight/cwt	50.802 4	$19.684\,1 \times 10^{-3}$
		ton (UK)	$1.016\,05 \times 10^{3}$	$0.984\,203 \times 10^{-3}$
Pressure	pascal/Pa	atmosphere/atm	101 325	$9.869\,23 \times 10^{-6}$
		bar/b	100 000	10×10^{-6}
		millimetre of mercury/mmHg	133.322	$7.500\,64 \times 10^{-3}$
		torr/Torr	133.322	$7.500\,64 \times 10^{-3}$
Radioactivity	becquerel/Bq	curie/Ci	37×10^{9}	$27.027\,0 \times 10^{-12}$
Temperature	kelvin/K	centigrade (Celsius) degree/°C	°C + 273.15	K − 273.15
		Fahrenheit degree/°F	(°F + 459.67) × 5/9	(K × 9/5) − 459.67
Volume	cubic metre/m^3	cubic foot/ft^3	0.028 316 8	35.314 7
		cubic inch/in^3	$16.387\,1 \times 10^{-6}$	$61.023\,6 \times 10^{3}$
		cubic yard/yd^3	0.764 555	1.307 95
		UK pint/pt	$0.568\,261 \times 10^{-3}$	1 759.75
		US pint/liq pt	$0.473\,176 \times 10^{-3}$	2 113.38
		UK gallon/gal	$4.546\,09 \times 10^{-3}$	219.969
		US gallon/gal	$3.785\,41 \times 10^{-3}$	264.172

*In the case of temperature measurements, use formulae shown.

Mass

The SI unit for mass is the kilogram (kg) rather than the gram (g): this is unusual because the base unit has a prefix applied.

Amount of substance

You should use the mole (mol, i.e. Avogadro's constant, see Table 9.4) to express very large numbers. The mole gives the number of atoms in the atomic mass, a convenient constant.

Concentration

The SI unit of concentration, $mol\,m^{-3}$, is not convenient for general laboratory work. It is equivalent to the non-SI term 'millimolar' (mM) while 'molar' (M) becomes $kmol\,m^{-3}$. If the solvent is not specified, then it is assumed to be water (see Chapter 6).

Time

In general, use the second (s) when reporting physical quantities having a time element. Hours (h), days (d) and years should be used if seconds are clearly absurd (e.g. samples were taken over a 5-year period). Note, however, that you may have to convert these units to seconds when doing calculations.

Definition

STP – Standard Temperature and Pressure = 293.15 K and 101 325 Pa (or 101.325 kPa or 0.101325 M Pa).

Temperature

The SI unit is the kelvin, K. The degree Celsius scale has units of the same magnitude, °C, but starts at 273.15 K, the melting point of ice at STP. Temperature is similar to time in that the Celsius scale is in widespread use, but note that conversions to K may be required for calculations. Note also that you must not use the degree sign (°) with K and that this symbol must be in upper case to avoid confusion with k for kilo; however, you *should* retain the degree sign with °C to avoid confusion with the coulomb, C.

Box 9.2 How to interconvert SI units

Example: You are required to calculate the molecular weight of a polymer by measurements of its osmotic pressure in solution. At infinite dilution, measured graphically from your experiments, the equation below applies:

$$\frac{\Pi}{c} = \frac{RT}{M_r}$$

where Π = osmotic pressure at infinite dilution (Pa), R = gas constant ($J\,K^{-1}\,mol^{-1}$), T = temperature (K), c = concentration of solution ($kg\,m^{-3}$) and M_r = molecular weight.

1. **Rearrange the equation for M_r:**

$$M_r = \frac{RTc}{\Pi}$$

2. **Look at the units and decide which are common:** since the gas constant is expressed in joules, you should convert the osmotic pressure term into joules. The derived unit for pressure is $N\,m^{-2}$ and, since the derived units for N are $J\,m^{-1}$, the full derived unit of pressure is $(J\,m^{-1}) \times m^{-2} = J\,m^{-3}$.
3. **Substitute the units into the equation for M_r:**

$$M_r = \frac{RTc}{\Pi} = \frac{J\,K^{-1}mol^{-1} \times K \times kg\,m^{-3}}{J\,m^{-3}} = kg\,mol^{-1}$$

4. **Substitute the appropriate numerical values into the equation for M_r:** you know that the units of the calculation will be correct since the molecular weight is the weight of 1 mole of polymer, expressed in kg.

Interconversion of SI units

You will find that the use of SI units simplifies mathematical manipulations and ensures that you obtain the correct units for the parameter being calculated. Remember that you must convert all units into the appropriate SI units, e.g. masses must be expressed as kg, volumes as m^3 and concentrations as $kg\,m^{-3}$ or $mol\,m^{-3}$, etc., and that you may need to use alternatives in derived units (Table 9.2). The application of these principles is shown in Box 9.2.

10 Scientific method and design of experiments

Definition

Hypothesis – One possible explanation for an observed event. A mechanistic hypothesis is one based on some intuition about the mechanism underlying a phenomenon.

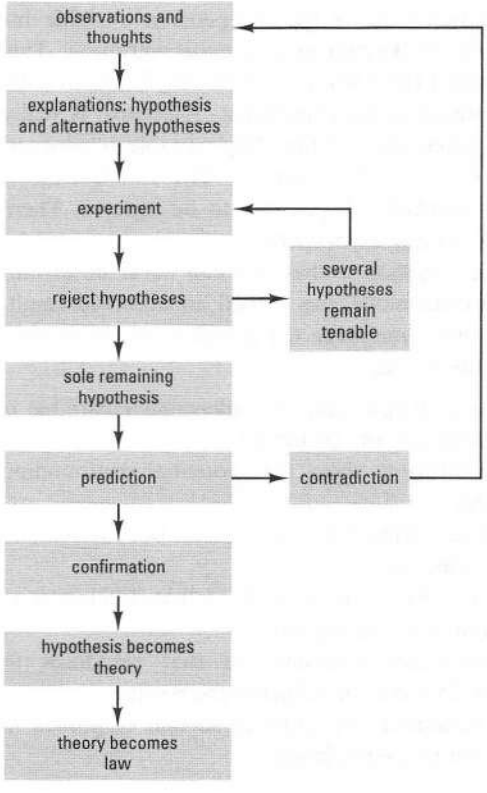


Fig. 10.1 How scientific investigations proceed.

Definition

Mathematical model – an algebraic summary of the relationship between the variables in a system.

Science is a systematized body of knowledge derived from observation and experiment. Scientists carry out experiments, make observations and attempt to explain the results; these tentative explanations are called hypotheses and their validity is tested by systematically forming and rejecting alternative explanations.

Some branches of chemistry are designed to provide fundamental information, rather than to test a particular hypothesis: for example, the purification and characterization of a newly discovered naturally occurring molecule. In contrast, an *experiment* is a contrived situation designed to test one or more hypothesis under conditions controlled by the investigator. Any hypothesis that cannot be rejected from the results of an experiment is provisionally accepted. This 'sieve' effect leaves us with a set of current explanations for our observations. These explanations are not permanent and may be rejected on the basis of a future investigation. A hypothesis that has withstood many such tests and has been shown to allow predictions to be made is known as a theory, and a theory may generate such confidence through its predictive abilities to be known as a law (Fig. 10.1).

Observations are a prelude to experimentation, but they are preconditioned by a framework of peripheral knowledge. While there is an element of luck in being at the right place and time to make important observations, as Pasteur stated, 'chance favours only the prepared mind'. A fault in scientific method is that the design of the experiment and choice of method may influence the outcome – the decisions involved may not be as objective as some scientists assume. Another flaw is that radical alternative hypotheses may be overlooked in favour of a modification to the original hypothesis, and yet just such leaps in thinking have frequently been required before great scientific advances.

No hypothesis can ever be rejected with certainty. Statistics allow us to quantify as vanishingly small the probability of an erroneous conclusion, but we are nevertheless left in the position of never being 100% certain that we have rejected all relevant alternative hypotheses, nor 100% certain that our decision to reject some alternative hypotheses was correct! However, despite these problems, experimental science has yielded and continues to yield many important findings.

KEY POINT **The fallibility of scientific 'facts' is essential to grasp. No explanation can ever be 100% certain as it is always possible for a new alternative hypothesis to be generated. Our understanding of chemistry changes all the time as new observations and methods force old hypotheses to be retested.**

Quantitative hypotheses involve a mathematical description of the system. They can be formulated concisely by mathematical models. Models are often useful because they force deeper thought about mechanisms and encourage simplification of the system. A mathematical model:

- is inherently testable through experiment;
- identifies areas where information is lacking or uncertain;
- encapsulates many observations;
- allows you to predict the behaviour of the system.

Remember, however, that assumptions and simplifications required to create a model may result in it being unrealistic. Further, the results obtained from any model are only as good as the information put into it.

Experimentation and variables

Definitions

Treatment – a particular set of conditions applied to one or more experimental subjects.

Block – a group of replicates: a sub-division of the entire set of experimental subjects (the field). This terminology originates from agricultural experiments.

In many experiments, the aim is to provide evidence for causality. If x causes y, we expect, repeatably, to find that a change in x results in a change in y. Hence, the ideal experiment of this kind involves measurement of y, the dependent (measured) variable, at one or more values of x, the independent variable, and subsequent demonstration of some relationship between them. Experiments therefore involve comparisons of the results of treatments – changes in the independent variable as applied to an experimental subject. The change is engineered by the experimenter under controlled conditions. Experimental subjects given the same treatment are known as replicates.

Interpretation of experiments is seldom clear-cut because uncontrolled variables always change when treatments are given.

Confounding variables

Example In an experiment using a reagent prepared in an organic solvent, the concentration of solvent will vary alongside the concentration of reagent. A control, using solvent alone (usually called a 'blank'), will allow its effects to be determined.

These increase or decrease systematically as the independent variable increases or decreases. Their effects are known as systematic variation. This form of variation can be disentangled from that caused directly by treatments by incorporating appropriate controls in the experiment. A control is really just another treatment where a potentially confounding variable is adjusted so that its effects, if any, can be taken into account. The results from a control may therefore allow an alternative hypothesis to be rejected. There are often many potential controls for any experiment.

The consequence of systematic variation is that you can never be certain that the treatment, and the treatment alone, has caused an observed result. By careful design, you can, however, 'minimize the uncertainty' involved in your conclusion. Methods available include:

- Ensuring, through experimental design, that the independent variable is the only major factor that changes in any treatment.
- Incorporating appropriate controls to show that potential confounding variables have little or no effect.
- Selecting experimental subjects randomly to cancel out systematic variation arising from biased selection.
- Matching or pairing individuals among treatments so that differences in response due to their initial status are eliminated.
- Arranging subjects and treatments randomly so that responses to systematic differences in conditions do not influence the results.
- Ensuring that experimental conditions are uniform so that responses to systematic differences in conditions are minimized.

Avoiding personal bias – it may be necessary to encode the subjects, so that the investigator is 'blind' as to which subject is in which treatment regime.

Nuisance variables

These are uncontrolled variables which cause differences in the value of y independently of the value of x, resulting in random variation. Nuisance variables are not common in chemistry except where molecules from natural sources are used. To reduce and assess the consequences of nuisance variables:

- incorporate replicates to allow random variation to be quantified;
- choose experimental subjects that are as similar as possible;
- control random fluctuations in environmental conditions.

Constraints on experimental design

Evaluating design constraints – a good way to do this is by processing an individual subject through the experimental procedures – a 'preliminary run' can help to identify potential difficulties.

Box 10.1 outlines the important stages in designing an experiment. At an early stage, you should find out how your resources may constrain the design. For example, limits may be set by availability of subjects, cost of treatment, availability of a chemical or bench space. Logistics may be a factor (e.g. time taken to record or analyse data), or your equipment and facilities may affect design because you cannot regulate conditions as well as you might desire. You should also consider what statistical tests you intend to make (p. 271), as this is an important part of experimental design.

Using replicates

Using independent replicates – remember that the degree of independence of replicates is important: sub-samples cannot act as replicate samples; they tell you about variability in the measurement method but not in the quantity being measured.

Replicate results show how variable the response is within treatments. They allow you to compare the differences among treatments in the context of the variability within treatments – you can do this via statistical tests such as analysis of variance (Chapter 41). Larger sample sizes tend to increase the precision of estimates of statistical parameters and increase the chances of showing a significant difference between treatments if one exists. For statistical reasons (weighting, ease of calculation, fitting data to certain tests), it is best to keep the number of replicates even.

Box 10.1 Checklist for designing and executing an experiment

1. **Preliminaries**
   - (a) **Read background material** and decide on a subject area to investigate.
   - (b) **Formulate a simple hypothesis to test.** It is preferable to have a clear answer to one question than to be uncertain about several questions.
   - (c) **Decide which dependent variable you are going to measure and how**: is it relevant to the problem? Can you measure it accurately, precisely and without bias?
   - (d) **Think about and plan the statistical analysis of your results.** Will this affect your design?
2. **Designing**
   - (a) **Find out the limitations on your resources.**
   - (b) **Choose treatments which alter the minimum of confounding variables.**
   - (c) **Incorporate as many effective controls as possible.**
   - (d) **Keep the number of replicates as high as is feasible.**
   - (e) **Ensure that the same number of replicates is present in each treatment with random allocation to individual treatments.**
3. **Planning**
   - (a) **List all the materials you will need.** Order any chemicals and make up solutions; grow, collect or prepare the experimental material you require; check equipment is available.
   - (b) **Organize space and/or time** in which to do the experiment.
   - (c) **Account for the time taken to apply treatments and record results.** Make out a timesheet if things will be hectic.
4. **Carrying out the experiment**
   - (a) **Record the results and make careful notes of everything you do.** Make additional observations to those planned if interesting things happen.
   - (b) **Repeat experiment** if time and resources allow.
5. **Analysing**
   - (a) **Graph data as soon as possible** (during the experiment if you can). This will allow you to visualize what has happened and make adjustments to the design (e.g. timing of measurements).
   - (b) **Carry out any planned statistical analysis.**
   - (c) **Jot down conclusions and new hypotheses** arising from the experiment.

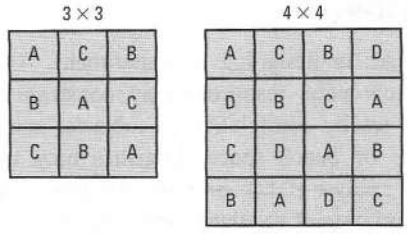


Fig. 10.2 Examples of Latin square arrangements for three and four treatments. Letters indicate treatments; the number of possible arrangements increases greatly as the number of treatments increases.

Example If you knew that soil type varied in a graded fashion across a field, you might arrange blocks to be long thin rectangles at right angles to the gradient to ensure conditions within the block were as even as possible.

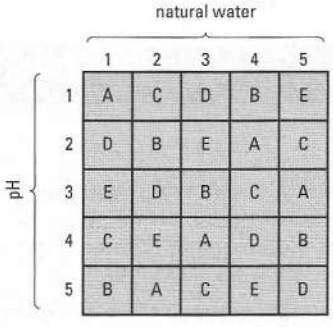


Fig. 10.3 The experimenter wishes to investigate the effect of pH A–E on the extraction of phenols from five natural waters. Each water sample is extracted at a different pH and the influence of pH on the recovery of phenols noted.

Definition

Interaction – where the effects of treatments given together are greater or less than the sum of their individual effects.

If the total number of replicates available for an experiment is limited by resources, you may need to compromise between the number of treatments and the number of replicates per treatment. Statistics can help here, as it is possible to work out the minimum number of replicates you would need to show a certain difference between pairs of means (say 10%) at a specified level of significance (say $P = 0.05$). For this, you need to obtain a prior estimate of variability within treatments (see Miller and Miller, 2000).

Randomization of treatments

For relatively simple experiments, you can adopt a completely randomized design; here, the position and treatment assigned to any subject is defined randomly. You can draw lots, use a random number generator on a calculator, or use the random number tables which can be found in most books of statistical tables.

A completely randomized layout has the advantage of simplicity but cannot show how confounding variables alter in space or time. This information can be obtained if you use a blocked design in which the degree of randomization is restricted. Here, the experimental space or time is divided into blocks, each of which accommodates the complete set of treatments. When analysed appropriately, the results for the blocks can be compared to test for differences in the confounding variables and these effects can be separated out from the effects of the treatments. The size and shape (or timing) of the block you choose is important: besides being able to accommodate the number of replicates desired, the suspected confounding variable should be relatively uniform within the block.

A Latin square is a method of placing treatments so that they appear in a balanced fashion within the experimental area. Treatments appear once in each column and row (see Fig. 10.2), so the effects of confounding variables can be 'cancelled out' in two directions at right angles to each other. This is effective if there is a smooth gradient in some confounding variable over the experimental area. It is less useful if the variable has a patchy distribution, where a randomized design might be better.

Latin square designs are useful in serial experiments where different treatments are given to the same subjects in a sequence (e.g. Fig. 10.3). A disadvantage of Latin squares is the fact that the number of columns and rows is equal to the number of replicates, so increases in the number of replicates can only be made by the use of further Latin squares.

Multifactorial experiments

The simplest experiments are those in which one treatment (factor) is applied at a time to the samples. This approach is likely to give clear-cut answers, but it could be criticized for lacking realism. In particular, it cannot take account of interactions among two or more conditions that are likely to occur in real life. A multifactorial experiment (Fig. 10.4) is an attempt to do this; the interactions among treatments can be analysed by specialized forms of analysis of variance.

Multifactorial experiments are economical on resources because of 'hidden replication'. This arises when two or more treatments are given to a subject because the result acts statistically as a replicate for each treatment. Choice of relevant treatments to combine is important in multifactorial experiments; for instance, an interaction may be present at certain concentrations of a chemical but not at others (perhaps because the response is saturated). It is

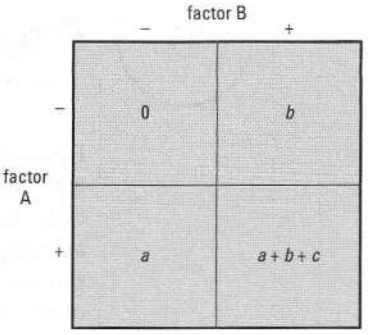


Fig. 10.4 Design of a simple multifactorial experiment. Factors A and B have effects *a* and *b* when applied alone. When both are applied together, the effect is denoted by $a + b + c$.

- If $c = 0$, there is no interaction (e.g. $2 + 2 + c = 4$).
- If c is positive, there is a positive interaction (synergism) between A and B (e.g. $2 + 2 + c = 5$).
- If c is negative, there is a negative interaction (antagonism) between A and B (e.g. $2 + 2 + c = 3$).

also important that the measurement scale for the response is consistent, otherwise spurious interactions may occur. Beware when planning a multifactorial experiment that the numbers of replicates do not get out of hand: you may have to restrict the treatments to 'plus' or 'minus' the factor of interest (as in Fig. 10.4).

Repetition of experiments

Even if you have taken great care to ensure that your experiment is well designed and statistically analysed, you are limited in the conclusions that can be made. Firstly, what you can say is valid for a particular place and time, with a particular investigator, experimental subject and method of applying treatments. Secondly, if your results were significant at the 5% level of probability, there is still an approximately 1 in 20 chance that the results did arise by chance. To guard against these possibilities, it is important that experiments are repeated. Ideally, this would be done by an independent scientist with independent materials. However, it makes sense to repeat work yourself so that you can have full confidence in your conclusions. Many scientists recommend that all experiments are carried out three times in total. This may not be possible in undergraduate practical classes or project work!

Reporting results – it is good practice to report how many times your experiments were repeated (in the experimental section); in the results section, you need either a statement saying that the illustrated experiment is representative or one explaining the differences between results obtained.

11 Project work

Research projects are an important component of the final-year syllabus for most degree programmes in chemistry, while shorter projects may also be carried out during courses in earlier years. Project work presents difficulties at many stages but can be extremely rewarding. The assessment of your project is likely to contribute significantly to your degree grade, so all aspects of this work should be approached in a thorough manner.

Deciding on a topic to study

The Internet as an information source – since many university departments have home pages on the World Wide Web, searches using relevant key words may indicate where research in your area is currently being carried out. Academics usually respond positively to e-mailed questions about their area of expertise.

Asking around – one of the best sources of information about supervisors, laboratories and projects is past students. Some of the postgraduates in your department may be products of your own system and they could provide an alternative source of advice.

Assuming you have a choice, this important decision should be researched carefully. Make appointments to visit possible supervisors and ask them for advice on topics that you find interesting. Use library texts and research papers to obtain further background information. Perhaps the most important criterion is whether the topic will sustain your interest over the whole period of the project. Other things to look for include:

- Opportunities to learn new skills. Ideally, you should attempt to gain experience and skills that you might be able to 'sell' to a potential employer.
- Ease of obtaining valid results. An ideal project provides a means to obtain 'guaranteed' data for your report, but also the chance to extend knowledge by doing genuinely novel research.
- Assistance. What help will be available to you during the project? A busy lab with many research students might provide a supportive environment should your potential supervisor be too busy to meet you often; on the other hand, a smaller lab may provide the opportunity for more personal interaction with your supervisor.
- Impact. It is not outside the bounds of possibility for undergraduate work to contribute to research papers. Your prospective supervisor can alert you to such opportunities.
- Success. You are doing a research project and it may not always provide a positive result. Negative results are just as useful.

Planning your work

Liaising with your supervisor(s) – this is essential if your work is to proceed efficiently. Specific meetings may be timetabled, e.g. to discuss a term's progress, review your work plan or consider a draft introduction. Most supervisors also have an 'open-door' policy, allowing you to air current problems. Prepare well for all meetings: have a list of questions ready before the meeting; provide results in an easily digestible form (but take your lab notebook along); be clear about your future plans for work.

As with any lengthy exercise, planning is required to make the best use of the time allocated. This is true on a daily basis as well as over the entire period of the project. It is especially important not to underestimate the time it will take to write and produce your thesis (see below). If you wish to benefit from feedback given by your supervisor, you should aim to have drafts in his/her hands in good time. Since a large proportion of marks will be allocated to the report, you should not rush its production.

If your department requires you to write an interim report, look on this as an opportunity to clarify your thoughts and get some of the time-consuming preparative work out of the way. If not, you should set your own deadlines for producing drafts of the introduction, materials and methods section, etc.

Project work can be very time consuming at times. Try not to neglect other aspects of your course – make sure your lecture notes are up to date and collect relevant supporting information as you go along.

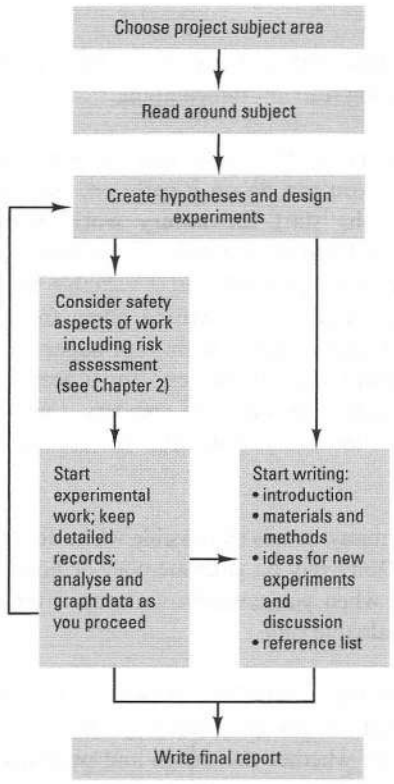


Fig. 11.1 Flowchart showing a recommended sequence of events in carrying out an undergraduate research project.

Getting started

Fig. 11.1 is a flowchart illustrating how a project might proceed; at the start, don't spend too long reading the literature and working out a lengthy programme of research. Get stuck in and do an experiment. There's no substitute for 'getting your hands dirty' for stimulating new ideas:

- even a 'failed' experiment will provide some useful information which may allow you to create a new or modified hypothesis;
- pilot experiments may point out deficiencies in experimental technique that will need to be rectified;
- the experience will help you create a realistic plan of work.

Designing experiments or sampling procedures

Design of experiments is covered in Chapter 10. Avoid being too ambitious at the start of your work! It is generally best to work with a simple hypothesis and design your experiments or sampling around this. A small pilot experiment or test sample will highlight potential stumbling blocks including resource limitations, whether in materials or time or both.

Working in a laboratory environment

During your time as a project student, you are effectively a guest in your supervisor's laboratory.

- Be considerate – keep your 'area' tidy and offer to do your share of lab duties such as calibrating the pH meter, replenishing stock solutions, distilled water, cleaning used glassware, etc.
- Use instruments carefully – they could be worth more than you'd think. Careless use may invalidate calibration settings and ruin other people's work as well as your own.
- Do your homework on techniques you intend to use – there's less chance of making costly mistakes if you have a good background understanding of the methods you will be using.
- Always seek advice if you are unsure of what you are doing.

KEY POINT **It is essential that you follow all the safety rules applying to the laboratory or field site. Make sure you are acquainted with all relevant procedures – normally there will be prominent warnings about these. If in doubt, ask!**

Keeping notes and analysing your results

Tidy record keeping is often associated with good research, and you should follow the advice and hints given in Chapter 8. Try to keep copies of all files relating to your project. As you obtain results, you should always calculate, analyse and graph data as soon as you can (see Fig. 11.1). This can reveal aspects that may not be obvious in numerical or readout form. Don't be worried by negative results – these can sometimes be as useful as positive results if they allow you to eliminate hypotheses – and don't be dispirited if things do not work first time. Thomas Edison's maxim 'Genius is one per cent inspiration and ninety-nine per cent perspiration' certainly applies to research work!

Writing the report

The structure of scientific reports is dealt with in Chapter 52. The following advice concerns methods of accumulating relevant information.

Brushing up on IT skills – word processors and spreadsheets are extremely useful when producing a thesis. Chapters 48 and 49 detail key features of these programs. You might benefit from attending courses on the relevant programs or studying manuals or texts so that you can use them more efficiently.

Introduction This is a big piece of writing that can be very time-consuming. Therefore, the more work you can do on it early on, the better. You should allocate some time at the start for library work (without neglecting benchwork), so that you can build up a database of references (p. 319). While photocopying can be expensive, you will find it valuable to have copies of key reviews and references handy when writing away from the library. Discuss proposals for content and structure with your supervisor to make sure your effort is relevant. Leave space at the end for a section on aims and objectives. This is important to orientate readers (including assessors), but you may prefer to finalize the content after the results have been analysed!

Experimental You should note as many details as possible when doing the experiment or making observations. Don't rely on your memory or hope that the information will still be available when you come to write up. Even if it is, chasing these details might waste valuable time.

Using drawings or photographs – these can provide valuable records of sampling sites or experimental set-ups and could be useful in your report. Plan ahead and do the relevant work at the time of carrying out your research rather than afterwards.

Results Show your supervisor graphed and tabulated versions of your data promptly. These can easily be produced using a spreadsheet (p. 307), but you should seek your supervisor's advice on whether the design and print quality is appropriate to be included in your thesis. You may wish to access a specialist graphics program to produce publishable-quality graphs and charts: allow some time for learning its idiosyncrasies! If you are producing a poster for assessment (Chapter 54), be sure to mock up the design well in advance. Similarly, think ahead about your needs for any seminar or poster you will present.

Discussion Because this comes at the end of your thesis, and some parts can only be written after you have all the results in place, the temptation is to leave the discussion to last. This means that it can be rushed – not a good idea because of the weight attached by assessors to your analysis of data and thoughts about future experiments. It will help greatly if you keep notes of aims, conclusions and ideas for future work *as you go along* (Fig. 11.1). Another useful tip is to make notes of comparable data and conclusions from the literature as you read papers and reviews.

Acknowledgements Make a special place in your notebook for noting all those who have helped you carry out the work, for use when writing this section of the report.

References Because of the complex formats involved (p. 319), these can be tricky to type. To save time, process them in batches as you go along.

KEY POINT **Make sure you are absolutely certain about the deadline for submitting your report and try to submit a few days before it. If you leave things until the last moment, you may find access to printers, photocopiers and binding machines is difficult.**

Resources for the investigative approach

Books

Adams, M.J. (1995) *Chemometrics in Analytical Chemistry*, Royal Society of Chemistry, Cambridge.

Crawford, K. and Heaton, A. (1999) *Problem Solving in Analytical Chemistry*, Royal Society of Chemistry, Cambridge.

Massart, D.L., Vandeginste, B.G.M., Buydens, L.M.C., de Jong, S., Lewi, P.J. and Smeyers-Verbeke, J. (1997) *Handbook of Chemometrics and Qualimetrics: Part A*, Elsevier Science B.V., Amsterdam.

Meier, P.C. (2000) *Statistical Methods in Analytical Chemistry*, 2nd Edn, John Wiley and Sons Ltd, Chichester.

Miller, J.N. and Miller, J.C. (2000) *Statistics and Chemometrics for Analytical Chemistry*, 4th Edn, Prentice Hall, Harlow, Essex.

Laboratory techniques

12 Melting points

Purity of solids – melting points do not vary significantly with changes in atmospheric pressure, whereas the boiling points of pure liquids are not constant: they vary with changes in atmospheric pressure and cannot be used as a measure of purity.

Melting points are measured (determined) for four reasons:

1. The melting range and upper limit are an indication of the purity of the sample.
2. Comparison of the melting point with the literature may indicate the identity of the compound or confirm that it is not the compound required.
3. If the compound is new, other scientists will need the information.
4. The compound can be identified with reasonable certainty by taking a mixed melting point (p. 90).

Criterion of purity

Melting point depression – only impurities, which will dissolve in the compound, will lower its melting point, e.g. reactants, by-products, solvents. Insoluble impurities, e.g. salts, charcoal, filter paper, grit, etc., will not dissolve in the melted compound.

When you synthesize compounds in the laboratory, you will always be asked to determine their melting points so that your practical expertise can be assessed.

Pure solid covalent organic compounds and many inorganic complexes incorporating organic ligands have definite melting points. The pure solid will melt reproducibly over a narrow temperature range, usually less than 1 °C, and this melting range is known as the melting point. If the compound is not pure, the melting range will increase significantly and the upper end of the melting range will be lowered. Thus the melting point (m.pt.) of a compound is a measure of its purity (p. 90). Other methods are used routinely to estimate purity of solid compounds, such as NMR (p. 190), and the presence of a single 'spot' or a single peak on a chromatogram from thin-layer (p. 216), gas–liquid (p. 211) or high-performance liquid (p. 218) chromatography, but melting point remains the standard measure of purity.

KEY POINT **The term melting point really means the melting range of a chemical and in your laboratory report you should always quote the measured melting range under the heading 'melting point'.**

Melting point apparatus

The equipment for measuring the melting point of a solid varies in complexity from a simple oil bath heated with a microburner to a microscope with a heated stage as shown in Fig. 12.1. The essential components of a melting point apparatus are:

Thermometers – make sure that you use a partial-immersion thermometer not a total-immersion thermometer. The type is written on the back of the thermometer.

- A sample holder: usually a glass capillary tube sealed at one end in the case of the oil bath and heated block systems, or a pair of microscope slides on an electrically heated plate in the Kofler block.
- A temperature recording device, placed as near to the sample as possible. Usually this is a thermometer but it can be a thermocouple probe with digital readout.
- A heat source with fine control to allow a gradual increase in temperature. These sources vary with the sophistication of the equipment.

In the undergraduate laboratory you are likely to use only the simpler systems such as the oil bath or heated block apparatus.

Capillary tubes

Capillary tubes for melting point measurement are available commercially and are supplied open at both ends or closed at one end or closed at both

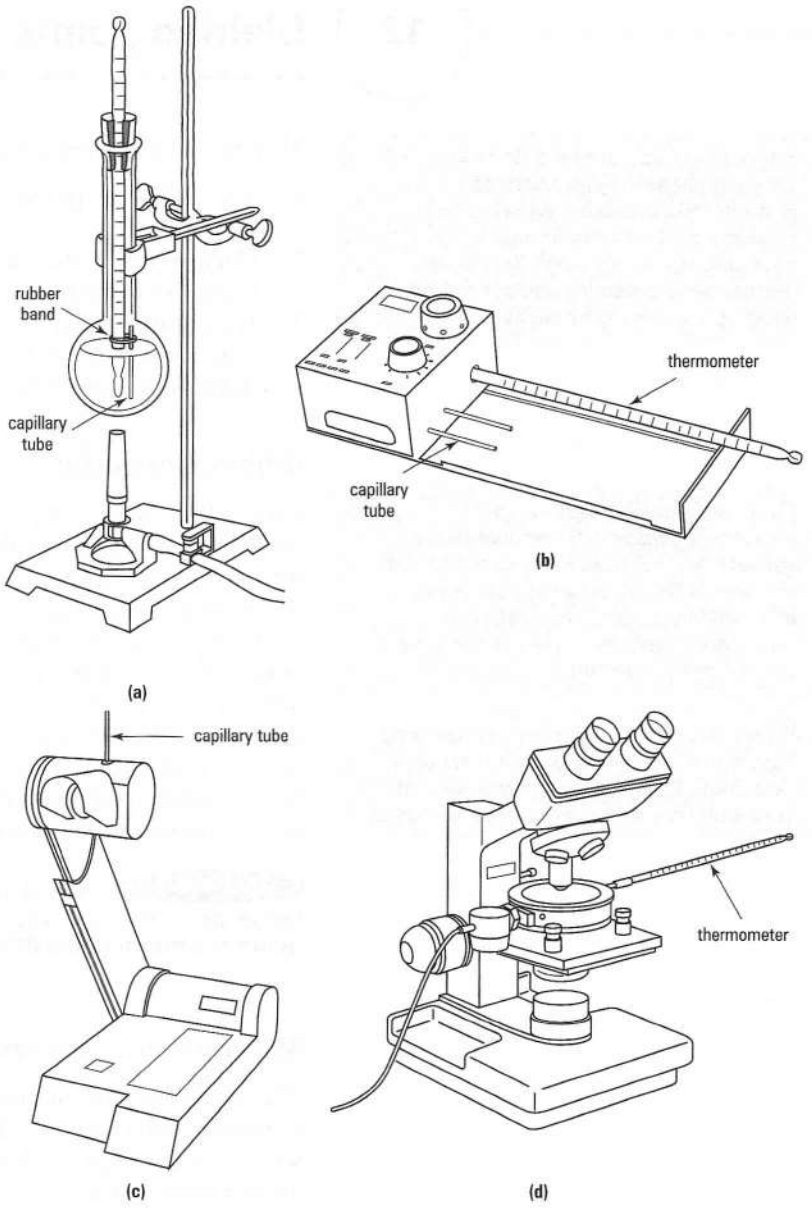


Fig. 12.1 Melting point apparatus: (a) oil bath; (b) heated block – thermometer readout; (c) heated block – digital readout; (d) Kofler hot-stage microscope.

Breaking capillary tubes – if the capillary tube is sealed at both ends, you can break it into two half-sized single-end-sealed melting point tubes by scoring the mid-point of the tube with a glass file and then snapping the tube at the score mark. If you don't score the glass, the edges of the break will be uneven and the tube will be difficult to fill.

ends. To seal one end of an open capillary tube, just touch the end of the capillary tube onto the outer 'layer' of the hot flame of a microburner (see Fig. 12.2). The end of the tube will collapse in and seal the tube. Make sure that the tube is sealed, i.e. there is not a fine line in the sealed end, and that there is no large globule of glass on the end of the tube, otherwise it may not fit into the hole in the heating block of the melting point apparatus. Similarly, if you push the tube too far into the flame, the tube will bend and therefore not fit into the heating block.

To put the compound into the capillary tube, place a little of the dry compound in a small heap on a watch-glass and press the open end of the tube into the heap, trapping a plug of chemical in the opening. The chemical can be

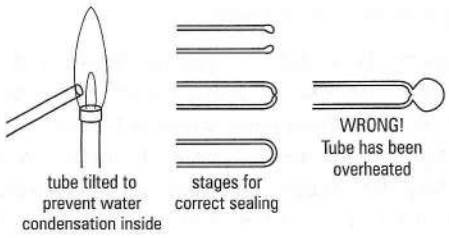


Fig. 12.2 Sealing a melting point capillary tube.

Safety note The 'notch' in the cork is essential: it allows the thermometer to be read over all its length, it allows the thermometer to be gripped by the cork and most importantly, it allows the heated air to escape from the apparatus. **Never** use a cork without the 'notch'.

If the rubber band comes into contact with the oil it will expand – the capillary tube will drop off into the oil bath and it will discolour the oil so that you will not be able to see when your sample melts.

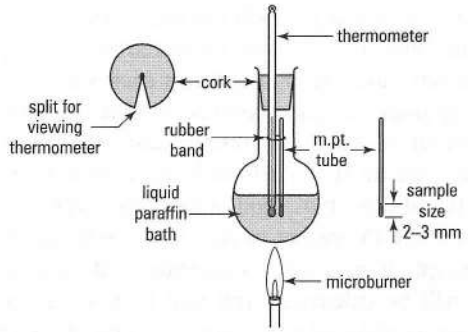


Fig. 12.3 Components of an oil bath melting point apparatus.

Safety note Mercury vapour is a severe cumulative and chronic hazard and the normal vapour pressure of mercury, at room temperature, is many times above the control limit (CL) of 0.05 mg m^{-3} If you *break a thermometer* or *find* or *suspect* the presence of mercury, inform your instructor *immediately*.

moved to the sealed end by turning the tube over and tapping it on the bench, or by vibrating it by rubbing it against the thread of the screw on a clamp, or dropping it down a long glass tube onto the laboratory bench. Remember: you only need 2–3 mm of sample in the bottom of the capillary tube.

Oil bath apparatus

The component parts of a typical oil bath melting point apparatus are shown in Fig. 12.3.

- Check that the mineral oil is clean and contains no water (p. 34) and that the bulb is only two-thirds full, to allow for expansion. Clamp the oil bath to a support stand (p. 26).
- Check that your thermometer is of the appropriate range, that the mercury thread is intact and the glass, in particular the bulb, is not cracked.
- Attach the capillary to the thermometer with a rubber ring, making sure that the compound is next to the thermometer bulb and remember to hold the thermometer near the bulb while attaching the capillary tube (p. 13).
- Press the thermometer into the 'notched' cork, making sure that you can see the thermometer scale, and trial fit the thermometer, sample and cork into the oil bath making sure that the thermometer bulb and sample are in the centre of the oil bath and that the rubber band is not in the oil, and will not be covered by oil, when the oil expands on heating.
- If adjustment is needed, carefully slide the cork up or down the thermometer (p. 13).
- You can attach a melting point capillary to both sides of the thermometer bulb to carry out two separate melting point determinations simultaneously.

Electric heated block apparatus

The heating block usually has three holes, to permit three simultaneous measurements of melting points. Always ensure that:

- The heating block is at room temperature at the outset.
- The light in the heating block works.
- The thermometer is undamaged (as above) and fits snugly into its hole in the heating block.
- All heating controls are set at zero.
- The capillary tubes slide easily into their holes in the heating block.

The usual problems encountered with this equipment are broken capillary tubes or broken thermometer bulbs in the heating block. Consult your instructor in these cases.

Melting point determination

The general procedure is to heat the oil bath with the microburner using the technique described on p. 32, or to use the heating control to give a temperature rise of about 10 °C per minute. When the temperature is about 20 °C below the melting point, the rate of heating must be reduced to about 2 °C per minute and continued at this rate until the compound has melted. The melting point is the *temperature range* from where the first drop of liquid appears to where the last crystal dissolves into the liquid.

Heating too fast – if you record a melting point which is higher than the literature value, you are implying that your compound has a purity >100%, which is impossible. Therefore it must be a different compound to the one expected.

It is normal practice to make at least two measurements of the melting point, one approximate and at least one accurate reading.

The following points of technique should be considered:

- The most common error is heating the sample *too quickly*. There is often a lag between slowing the heating rate and the reduction in temperature increase, resulting in a reading of the melting point which is *too high*.
- If you do not know what the compound is, you will not know its expected melting point. Therefore, you should determine an approximate value and then repeat the procedure to give an accurate measurement, by reducing the heating rate, particularly for the final stage near the melting point. The boost heater on the electrical apparatus can be used for finding the approximate melting point but *not* for accurate determination.
- If you are carrying out several melting point measurements, it is common sense to measure the melting point of the lowest melting compound first – less time for the apparatus to cool to room temperature.
- If you miss the melting point, do not allow the sample to solidify and then retake the melting point with the same sample: some decomposition may have occurred on melting.

Thermometer calibration

Calibrated thermometers – if your compound is only slightly impure and has a melting point 2 °C lower than the literature value, a thermometer reading 10 °C low will give you an error of 12 °C, indicating a low level of purity and hence a low grade or false information to fellow scientists.

Your thermometer may be a major source of error in melting point measurement. Occasionally thermometers for routine laboratory use may not be accurate and may read up to 10 °C high or low. To avoid this problem you should always calibrate your thermometer to determine any error and be able to correct for it.

To calibrate your thermometer you must measure the melting points of a series of very pure compounds, available commercially, having a range of melting points similar to the range over which you will use the thermometer; a general-purpose series is shown in Table 12.1. Having measured the melting points of each of the pure compounds, take the mid-point of each value for the thermometer reading of melting point for each compound and the mid-point of the literature melting point of each compound and plot them graphically using literature temperature as the y-axis and the thermometer reading as the x-axis. The straight-line plot will obey the equation $y = mx + c$, where y = real temperature (literature m.pt.), x = thermometer reading, m = slope and c = intercept. If you use a computer and suitable program, the values of m and c will be calculated and you then solve the equation for using the thermometer reading (x-axis values) to find the real temperature of melting (y-axis values) to find the true melting range.

Of course, if you break or lose your thermometer, you must calibrate another.

Table 12.1 A typical series of standards for thermometer calibration

Compound	Literature m.pt.
Naphthalene	79–80 °C
Benzoic acid	121 °C
4-Nitroaniline	147 °C
4-Toluic acid	180–182 °C
Anthracene	216–218 °C

Mixed melting point determination

You can confirm the identity of a compound by determining a mixed melting point. If you prepare a mixture of your unknown chemical and the one you suspect it may be and measure the melting point of the mixture then there are two possible results:

1. The melting point of the mixture is the same as the pure compound, which means that the unknown compound and the known compound are the *same*.

2. The melting point of the mixture is lower than either of the two pure components and the melting range is large. This is because the two compounds are different with the result that one is an impurity in the other.

For example, both benzoic acid and mandelic acid are white crystalline solids which melt at 121 °C. However a 1 : 1 mixture of the two compounds begins to melt at about 80 °C.

The usefulness of mixed melting points is limited in that you must have some idea of the chemical nature of your unknown compound and a sample of the suspected compound must be available.

13 Recrystallization

Recrystallization involves allowing a hot solution of the required compound to cool. **Crystallization** implies allowing the solvent to evaporate from a solution of the compound. Crystallization will *not* remove solvent-soluble impurities since they will be deposited as the solvent evaporates.

The products from many synthetic preparations are seldom pure and the technique of recrystallization, which involves dissolving the impure material in a hot solvent and then cooling the solution to produce crystals, is routinely used to purify covalent organic and inorganic solids.

In general there are three types of impurities, which are removed by the recrystallization process:

1. Insoluble material: anti-bumping granules, pieces of filter paper, traces of drying agents, grit, hair and other materials which may have been present in the starting chemicals.
2. Small quantities of unreacted starting chemicals and/or by-products from side reactions or other isomers.
3. Very small amounts of coloured by-products resulting from oxidation or polymerization of the chemicals used.

Limits of purification – crude solids containing only up to 10–15% impurities can be purified by recrystallization. Otherwise chemical purification or chromatography (p. 217) will be required to produce a compound, which can then be further purified by recrystallization.

Recrystallization is designed to remove all these types of impurity and provide a pure product suitable for melting point measurement. Purification by recrystallization is based on the theory of saturated solutions (p. 50) and a suitable recrystallization solvent is one in which the chemical to be purified is *insoluble* in the *cold solvent* and *soluble* in the *hot solvent*.

When the crude reaction product is dissolved in the hot solvent the insoluble impurities (type 1 above) can be removed by hot filtration (p. 98). When the hot solution is allowed to cool, the solution becomes saturated with the desired compound and it precipitates from the cold solution. The cold solution does not become saturated with the lower concentration of the contaminants of type 2, which therefore remain in solution. Coloured impurities (type 3) can be removed by absorption using charcoal as described on p. 96.

Practice in the technique of recrystallization is important, since the aim of the procedure is to produce the maximum quantity of the highest quality product. Poor technique often results in *low recovery* of *high-quality* product or *high recovery* of *low-quality* product.

The recrystallization process can be divided into three separate steps:

1. Selection of a suitable solvent.
2. Recrystallization of the crude compound.
3. Drying the purified solid.

Solvents for recrystallization

You can decide on a suitable solvent for recrystallization of your crude chemical product in several ways:

- The experimental protocol may tell you which solvent to use.
- If you know the identity of the compound you have made, reference texts may indicate a suitable solvent (e.g. Lide, 2000; Buckingham and Macdonald, 1995).
- If you are not sure of the identity of the compound you have prepared or if it is a new compound, you must carry out a 'solvent selection' to find out which solvent is the most appropriate (p. 93).

When selecting a suitable solvent for recrystallization, chemists work to the general rule 'like dissolves like' when considering the polarity of the chemical to be recrystallized and the polarity of the solvent. In general solvents for recrystallization are classified in terms of polarity and miscibility. Solvent polarity depends upon the overall distortion of the electron clouds in the covalent bonds within the solvent molecules, resulting in a dipole. The greater

the dipole, the greater the polarity of the solvent, e.g. trichloromethane has three polarized C–Cl bonds, giving an overall dipole to the molecule and producing a good solvent for many molecules, but in tetrachloromethane all of the C–Cl dipoles cancel out, giving a non-polar solvent with different solvent properties in comparison. An additional feature that adds solvating power is *hydrogen bonding*. Thus water, a polarized molecule, can form hydrogen bonds with oxygen and nitrogen atoms in solutes, dissolving them efficiently.

When you are choosing a solvent for recrystallization, look for the following general characteristics:

- A high dissolving power for the solute at high temperature and a low dissolving power at room temperature or below, so that a high recovery of purified compound can be achieved.
- A high or negligible dissolving power for the impurities, so that they will either be filtered off or remain in solution.
- A relatively low boiling point, to facilitate drying the purified compound.

Yields from recrystallization – some of the compound that you require will remain dissolved in the solvent, because it has to form a saturated solution. Therefore you can *never* recover 100% of your compound by recrystallization.

Definition

Solvents which mix in all proportions, such as water and ethanol, are said to be *miscible*, whereas solvents which do not mix, e.g. water and petrol, are *immiscible*.

The miscibility of solvents with each other must be taken into account when attempting *mixed-solvent recrystallizations* (see p. 95); the properties of some common recrystallization solvents, which you are likely to encounter in your laboratory work, are shown in Table 13.1. Remember that this is only a general list of solvents; further information on solvent properties can be found in standard textbooks such as Harwood *et al.* (2000, p. 133), Loewenthal (1990, p. 146) and Furniss *et al.* (1989, p. 137).

Table 13.1 Selected solvent properties

Solvent	b.pt. (°C)	Polarity	Miscibility with water	Comments: class of organic compounds recrystallized
Water	100	V. high	Yes	Always use when suitable: salts, aromatic acids
Ethanol	78	High	Yes	Flammable: alcohols, acids, amides
Propanone	56	High	Yes	Flammable: carbonyl compounds
Ethyl ethanoate	78	Medium	No	Flammable: esters
Dichloromethane	41	Medium	No	Toxic: halogen compounds, ethers
Toluene	110	Low	No	Flammable: hydrocarbons
Light petroleum	40–60	V. low	No	Flammable: hydrocarbons

Solvent selection

To find a suitable solvent for recrystallization you must carry out a series of tests measuring the solubility of your crude compound in a series of solvents (cold and hot) of varying solvent polarity. This series of tests is called 'solvent selection' and is carried out on a test tube scale but, as your technique improves, you can carry out the tests using semi-micro scale since you will use less of your compound during the process. The procedure for solvent selection at the test tube scale is described in Box 13.1 and modification of the procedure to semi-micro scale requires only a corresponding reduction of the quantities of solvent and solute used.

KEY POINT **When carrying out the solvent selection experiments you must always cool the solution after heating. Do not assume that if the compound is insoluble in the cold solvent and dissolves when heated, it will always precipitate on cooling.**

Box 13.1 How to carry out a solvent selection for recrystallization of an unknown compound

A suitable range of solvents for general use, in order of decreasing polarity, is: water, ethanol, propanone, ethyl ethanoate, dichloromethane, toluene and light petroleum (b.pt. 40–60 °C).

1. **Clean and dry six Pyrex® test tubes** to ensure that you will have no problems with contaminated solvents when carrying out the solubility tests.
2. **Add a small sample of the compound under test to each tube,** using just enough compound to cover the bottom of the test tube.
3. **Add about 2.0 mL of a pure solvent to the first tube and observe the effect.** You should look for the following features, which may help in the next recrystallization stage:
   (a) **The solid does not dissolve, but it is 'wetted' by the solvent.** This implies that the solvent *may* be suitable for use in recrystallization.
   (b) **The compound dissolves easily.** Therefore the solvent is *unsuitable* for recrystallization and there is no need to continue tests with this solvent.
   (c) **Most of the compound dissolves but leaves a small amount of insoluble residue.** This means that there are solvent-insoluble impurities present in your compound, but the solvent itself is *unsuitable* for use in recrystallization.
   (d) **Some colour is released into the solvent and the compound becomes lighter in colour.** This means that there could be a coloured impurity present in your compound and it will be necessary to decolorize the product in the recrystallization experiment.
4. **Heat the mixture in the test tube** by an appropriate means, bearing in mind the flammability and toxicity of the solvent, to see if the solid dissolves in the hot solvent. For non-flammable solvents, remember to hold the test tube with a holder (see p. 37) and to 'wave' the test tube over the heat source to prevent 'bumping' (p. 31). Flammable solvents should be heated using a steam bath in a fume cupboard: *do not* lower the test tube into the steam bath and leave it there. Instead you must 'wave' the test tube in the steam escaping from the bath to achieve controlled heating. This is particularly important with low-boiling-point flammable or toxic solvents such as propanone, dichloromethane and light petroleum (b.pt. 40–60 °C). You should look for the following results:
   (a) **The solid does not dissolve in the hot solvent,** which is therefore *unsuitable* for recrystallization and there is no need to continue tests with this solvent.
   (b) **The solid dissolves** and thus the solvent *may be* useful for recrystallization.
   (c) **Most of the compound dissolves but leaves a small amount of insoluble residue.** This means that there are solvent-insoluble impurities present in your compound and the solvent *may be* suitable for use in recrystallization.
   (d) **The solid melts and floats on the meniscus at the top of the solvent** giving the *appearance* of having dissolved. This is a common feature of low-melting-point hydrocarbons such as naphthalene and other non-polar aromatic compounds: shake the test tube to see if oily globules are released from the meniscus. If this occurs, the solvent is *unsuitable* for recrystallization.
5. **Cool the test tube** in an ice-water bath or under a cold stream of water ensuring that no water gets into the test tube. There are several possible results:
   (a) **The compound recrystallizes in high yield on cooling:** often there will appear to be more solid than you started with because of the fine crystals produced. This solvent is *suitable.*
   (b) **The compound recrystallizes in low yield on cooling:** the solvent is *unsuitable* unless no better alternative can be found.
   (c) **The compound remains in solution after cooling:** the solvent is *unsuitable* or a supersaturated solution has been formed. To check if the formation of a supersaturated solution has occurred, gently scratch the inside of the test tube at the surface of the solution with a Pyrex® rod. The scratches provide points of nucleation for crystal growth and crystals should form rapidly if the solution is supersaturated. Take care when scratching the test tube with the glass rod (see p. 100). If a supersaturated solution is formed, then the solvent is *suitable* for recrystallization.
6. **Repeat the test using the other solvents** and record your results in tabular form to include your experimental observations and conclusions.
7. **If you have found several suitable solvents,** then select one after considering factors such as flammability, toxicity and boiling point, since you will need to use much larger volumes of solvent in the recrystallization process, with consequent complications in the equipment to be used (see Table 13.2).
8. **If you have not found a suitable solvent,** then you will need to carry out a *mixed-solvent* recrystallization and you must find out which combination of solvents will be suitable (see Box 13.2).

Mixed solvents

When no single solvent is found to be suitable for recrystallization, then a mixed solvent system must be used. There are three essential properties required for a pair of solvents to be used in a mixed-solvent system:

1. The two solvents must be miscible in all proportions over the temperature range to be used.
2. The solute must be insoluble in one of the solvents.
3. The solute must be soluble in the other solvent.

Using mixed solvents for recrystallization – never assume that a mixed solvent system is a mixture of *equal volumes* of the two solvents. The ratio of the two solvents is established practically during the recrystallization experiment.

It is an advantage if the two solvents have similar boiling points within 20–30 °C.

Suitable common solvent pairs from the solvents given in Table 13.1 are water/ethanol and dichloromethane/light petroleum (b.pt. 40–60 °C), but many other combinations are possible. One of the most frequently encountered mixed solvent systems is 'aqueous ethanol' (water/ethanol) in which the compound to be recrystallized is insoluble in water and very soluble in ethanol.

You can identify the solubility characteristics of your compound using the solvent selection procedure shown in Box 13.1 and modifications for mixed solvent selection are given in Box 13.2.

The recrystallization process

Having chosen a suitable solvent system, the process to be used to purify the bulk of your impure compound can be separated into several distinct steps:

Box 13.2 How to carry out a mixed-solvent selection for recrystallization of an unknown compound

From your solvent selection tests (Box 3.1) you will have discovered the individual solvents in which your compound is soluble (good solvents) and in which it is insoluble (poor solvents), when the solvents are cold. Proceed as follows:

1. **Choose a miscible solvent pair**, in which the solubility of your compound is appropriate.
2. **Place a small amount (about 0.2 g) of the compound in a clean dry Pyrex® test tube** and add a good solvent (about 2.0 mL) in which it is soluble.
3. **Heat the test tube by an appropriate method**, dependent on the solvent used, until the solid has dissolved. Remember that your original solvent selection may have shown that solvent-insoluble impurities are present.
4. **Add a few drops of the poor solvent from a Pasteur pipette.** A slight 'cloudiness' or precipitate should form, since the solubilizing power of the good solvent has been decreased by the poor solvent and the solution will have been cooled slightly.
5. **Reheat the solution until the cloudiness or precipitate disappears** and then add a few more drops of the poor solvent to produce a precipitate.
6. **Repeat the heating and solvent addition process** until the point is reached when the precipitate *just* dissolves when the solution is heated. You now have a hot solution of your compound with the correct ratio of the two solvents.
7. **If you have added too much poor solvent and the precipitate will not redissolve on heating**, add enough good solvent to dissolve the precipitate and then continue to add poor solvent and heat as before.
8. **Cool the test tube in an ice–water bath**, to ensure that crystals are formed.
9. **If an oil is formed on cooling**, which may happen if the melting point of the solid is below the boiling points of the solvents used, you should use slightly more of the good solvent (or less of the poor solvent) or try a different combination of solvents.
10. **If crystals do not form on cooling**, you may have formed a supersaturated solution and you should 'scratch' with a Pyrex® glass rod, as described for single solvent selection (Box 13.1).

- Dissolution of the solid using a single-solvent or a mixed-solvent system.
- Decolorization using charcoal: even if your compound is white, decolorization will improve its appearance significantly.
- Hot filtration (p. 98) to remove solvent-insoluble impurities and charcoal.
- Cooling, to produce the crystals.
- Collection of the crystals by suction filtration (p. 28).
- Drying (p. 39).

It is important to remember that for a successful recrystallization, you need to use equipment of a size appropriate to the amount of solid and the volume of solvent you are likely to use. You can estimate the volume of solvent to be used by extrapolation of the data from your solvent selection tests. In general terms, conical flasks, beakers and round-bottom flasks should never be more than half-full of solution but, on the other hand, using small volumes of solutions in large flasks will result in losses of the compound on the sides of the vessels.

Dissolution

To carry out a single-solvent recrystallization (Box 13.3) you must get the compound into solution and this is achieved by suspending it in the appropriate cold solvent, found in the solvent selection process, and then heating the mixture to dissolve the solid. The equipment used will depend on the boiling point of the solvent, its flammability and toxicity. Some general systems are shown in Table 13.2.

Table 13.2 Solvents for recrystallization

Solvent	b.pt. (°C)	Glassware	Heat source	Containment
Water	100	Conical flasks, beakers	Burner, hot plate	None
Ethanol	78	Conical flasks	Water bath	Fume cupboard
Propanone	56	Conical flasks	Water bath	Fume cupboard
Ethyl ethanoate	78	Conical flasks	Water bath	Fume cupboard
Dichloromethane	41	Reflux	Water bath	Fume cupboard
Toluene	110	Reflux	Hot plate, mantle	Fume cupboard
Light petroleum	40–60	Reflux	Water bath	Fume cupboard

When heating solvents in conical flasks and beakers you should cover the top of the flask with a clock-glass or watch-glass to prevent excessive evaporation of the solvent, resulting in the formation of crystals on the sides of the flask above the surface of the solution. *Do not forget* to *take the appropriate 'anti-bumping' precautions* (p. 31), because you may need to boil the mixture for several minutes to achieve complete dissolution of the chemical(s). You should use a glass rod, a wooden 'boiling stick' or anti-bumping chips in conical flasks of volume up to 500 mL; magnetic 'fleas' or anti-bumping granules in round-bottom flasks in reflux apparatus (p. 116); magnetic 'fleas' or stirring bars in large-volume glassware (> 500 mL).

Decolourization

Small amounts of coloured impurities can be removed from your product by absorption on finely divided charcoal, which is then removed in the hot filtration process (p. 98). In general, you should use charcoal in *every*

Box 13.3 How to carry out a single-solvent recrystallization

Examples: Suppose that you have prepared a crude sample (about 4.0 g) of *N*-phenylethanamide (acetanilide) and you are to purify if by recrystallization from water. The melting point of pure *N*-phenylethanamide is 114 °C (Lide, 2000).

1. **Weigh the crude sample** and retain a few crystals in case seeding is required.
2. **Transfer the solid**, using glazed paper or a solids funnel (p. 25), into a clean, dry conical flask (250 mL).
3. **Add cold water** (about 100 mL) and a glass rod as an anti-bumping device to the flask and then heat the mixture on a hot plate until the compound has dissolved completely. Then add more water (about 10 mL), to ensure that precipitation of the solute does not occur during the following stages.
4. **Remove the flask from the heat** and allow it to cool for 2 minutes.
5. **Add a small amount of decolorizing charcoal (about 0.01 g) to the solution**, place a watch-glass on top of the conical flask and heat the mixture gently for 5 minutes.
6. **Prepare another clean, dry conical flask (250 mL)** and add water (about 20 mL) and a glass rod and then heat to boiling on the hot plate with a stemless funnel and fluted filter paper just above the neck (Fig. 13.1), so that the steam heats the funnel and filter paper.
7. **Filter the recrystallization solution through the filter paper** using hand protection (rubber fingers or an insulated glove) and keep the filter topped up with solution to prevent cooling. At the same time, keep the recrystallization solution hot during the filtration by putting it back onto the hot plate.
8. **When filtration is complete**, remove the collection flask from the heat, take out the glass rod and clamp the flask in an ice–water bath, covering the top of the flask with a watch-glass.
9. **When the solution is cold** (about 5 °C), collect the solid by suction filtration (Box 5.2), using the filtrate, not fresh water, to transfer the solid completely from the collection flask. If crystals do not form, either add a few crystals of the crude solid to 'seed' the supersaturated solution or 'scratch' with a Pyrex® glass rod to induce recrystallization.
10. **Rinse the compound on the filter** using a little (about 5 mL) ice-cold water and continue suction, to make the crystals as dry as possible.
11. **Transfer the crystals to a clock-glass** using a spatula and spread them in a thin layer.
12. **Dry the pure compound in the oven** at 70 °C for about 30 minutes and test them with a spatula to check their dryness: they should not stick together.
13. **Allow the crystals to cool**, covering them with another clock-glass to prevent contamination.
14. **Transfer the purified crystals to a 'tared' watch-glass** on a balance to determine their weight and then transfer them to a labelled sample tube using folded glazed paper (p. 24).
15. **Calculate the efficiency of the process:**

$$\% \text{ recovery} = \frac{\text{weight of pure compound}}{\text{weight of crude compound}} \times 100$$

Coloured compounds – you should know something about the *colour* of the compound you are trying to purify. There is no point in trying to *remove* the colour from a coloured compound. However, charcoal will improve significantly the appearance of a coloured compound.

recrystallization since the colour of white and coloured compounds is improved by 'decolorization'.

The amount of charcoal used should be about 2% by weight of the sample to be recrystallized. Note the following important points:

- Add the charcoal only when the solution has been formed. If you add charcoal to the cold solution, you will not be able to see when all of the compound has dissolved.
- Do not add charcoal to a boiling or a very hot solution otherwise the solution will boil extremely vigorously, usually boiling out of the flask or reflux apparatus.
- To add charcoal to a hot solution, *remove* the flask from the heat source and then let it cool a little (not enough to cause precipitation). Add the charcoal either from a spatula (into a conical flask or beaker), a paper funnel (p. 25) or powder funnel (into a round-bottom flask with a ground-glass joint in reflux apparatus), and then reheat the solution.

Solvent-insoluble impurities – remember that if you have found solvent-insoluble impurities, not *all* your 'compound' will dissolve in the hot solvent. You can confirm the presence of these by adding an excess of hot 'selected' solvent to a small sample of the crude compound. If it does not dissolve completely, solvent-insoluble impurities are present.

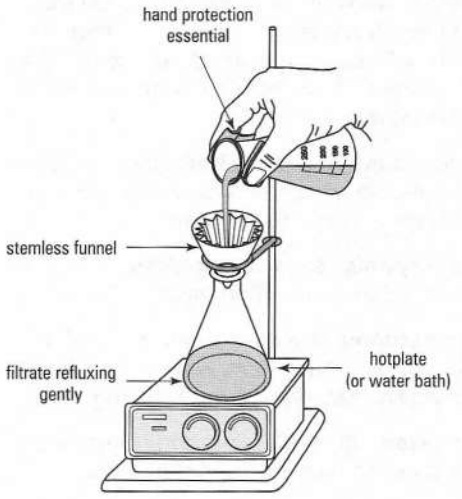


Fig. 13.1 Filtration of a hot solution.

Hot filtration

This is a modification of gravity filtration (p. 28), designed to remove solvent-insoluble impurities, charcoal, anti-bumping granules or magnetic 'fleas' from the hot solution before cooling the solution to form the crystals of purified product.

KEY POINT **Hot filtration is used to prevent cooling of the solution during the filtration process, which would result in the formation of crystals in the filter paper.**

For a successful hot filtration the solution must pass through the filter paper and filter funnel into the collection flask as quickly as possible so that cooling and crystallization do not occur. The following points should be noted:

- *Always* use a fluted filter paper (p. 28).
- *Always* use a 'stemless' glass filter funnel because cooling and thus crystallization may occur in the funnel stem causing a blockage.
- *Always* heat the filter funnel and filter paper, either by pre-heating them in an oven or by using boiling solvent in the collecting flask (Fig. 13.1).
- If filtration is rapid, keep the filter cone 'topped up' with the hot solution being filtered, since this keeps the filter cone and filter funnel hot. If you allow the filter cone to empty of liquid, cooling and crystallization may occur.
- When attempting a hot filtration with a mixed-solvent system, always ensure that the 'ideal' solvent ratio has not been reached, i.e. there is not enough of the poor solvent to cause immediate precipitation as a result of slight cooling during the hot filtration. The solvent ratio can be adjusted to the 'ideal' ratio for maximum recovery, after filtration and before cooling (Box 13.4).

Cooling

Rapid cooling in an ice–water bath ('crash-crystallization') usually produces small crystals occluded with mother-liquor, whereas slow cooling by allowing the collection flask to stand on the laboratory bench often produces large well-defined crystals. Remember to:

- cover the top of the collection flask with a watch- or clock-glass to prevent solvent evaporation and entry of dust into the flask: *do not use* rubber bungs or corks since they may be pulled into the flask as it cools (p. 21);
- clamp the flask in place, if you use an ice–water bath, otherwise it may fall over as the ice melts and the volume of water increases;
- make sure that the solution is cold, even after slow cooling, so that maximum precipitation of the solid occurs.

If no crystals appear on cooling, you will have formed a supersaturated solution and, to induce precipitation of the solute, you must provide sites for nucleation and crystal growth. This can be achieved by either *seeding* the solution by adding a few crystals ('dust') of the crude compound or *scratching* the inside of the flask at the surface of the liquid, using a Pyrex® glass rod (Fig. 13.2).

Collection of the crystals

You should collect the purified crystals by suction filtration by the procedure described in Box 5.2 (p. 30). Remember to transfer the crystals from the

Box 13.4 How to carry out a mixed-solvent recrystallization

Examples: Suppose that you have prepared a crude sample (e.g. ~ 4.0 g) of *N*-phenylbenzamide (benzanilide). Your solvent selection tests have shown that it is insoluble in water and fairly soluble in cold ethanol. The melting point of pure *N*-phenylbenzamide is 158 °C (Lide, 2000). Proceed as follows:

1. **Weigh the crude sample** and retain a few crystals in case seeding is required.
2. **Transfer the solid**, using glazed paper or a solids funnel (p. 25), into a clean, dry conical flask (250 mL).
3. **Add cold ethanol** (about 25 mL) and a glass rod as an anti-bumping device to the flask and then heat the mixture on a water bath until the compound has dissolved completely.
4. **Add cold water** (about 5 mL) to the hot ethanol solution and a slight precipitate should form, which then redissolves – this is often seen as a 'flash' of white in the solution. Reheat the solution and add more water (5 mL). The precipitate will remain for a little longer and take more time to disappear on reheating.
5. **Repeat the water addition and reheating process,** but note that you will need to reduce the volume of water added at each consecutive addition, since the precipitate will take longer to redissolve as the solvating power of the ethanol is reduced by the water. Eventually, you will reach the situation where the precipitate just redissolves at the boiling point of the solvent mixture. You now have the ideal solvent mixture for recrystallization of *N*-phenylbenzamide and any slight cooling of the solution will result in its precipitation.
6. **Remove the solution from the heat**, add ethanol (5 mL), to ensure that the *N*-phenylbenzamide remains in solution if it cools a little, add charcoal (about 0.01 g), place a watch-glass on top of the flask and reheat the solution on the water bath for about 5 minutes.
7. **Prepare another clean, dry conical flask (250 mL)** and add ethanol (about 10 mL) and a glass rod and then heat to boiling on the water bath with a stemless funnel and fluted filter paper just above the neck (Fig. 13.1) so that the ethanol vapour heats the funnel and filter paper.
8. **Filter the recrystalization solution through the filter paper** using hand protection (rubber fingers or an insulated glove) and keep the filter topped up with solution to prevent cooling. At the same time, keep the recrystallization solution hot during the filtration by putting it back onto the hot plate.
9. **When filtration is complete**, remove the filter funnel and restart the water addition and reheating process until the ideal solvent ratio is reached once again.
10. **Remove the collecting flask from the heat**, take out the glass rod and clamp the flask in an ice–water bath, covering the top of the flask with a watch-glass.
11. **When the solution is cold** (about 5 °C), collect the solid by suction filtration (Box 5.2), using the filtrate, to transfer the solid completely from the collection flask. If crystals do not form, either add a few crystals of the crude solid to 'seed' the supersaturated solution or 'scratch' with a Pyrex® glass rod to induce recrystallization.
12. **Rinse the compound on the filter** using a little (about 5 mL) ice-cold water and continue suction, to make crystals as dry as possible.
13. **Transfer the crystals to a clock-glass** using a spatula and spread them out in a thin layer.
14. **Dry the pure compound in the oven** at 70 °C for about 30 minutes and test them with a spatula to check their dryness: they should not stick together.
15. **Allow the crystals to cool**, covering them with another clock-glass to prevent contamination.
16. **Transfer the purified crystals to a 'tared' watch-glass** on a balance to determine their weight and then transfer them to a labelled sample tube using folded glazed paper (p. 24).
17. **Calculate the efficiency of the process:**

$$\% \text{ recovery} = \frac{\text{weight of pure compound}}{\text{weight of crude compound}} \times 100$$

collecting flask to the filter using a little of the filtrate: *do not use fresh solvent*. The filtrate is a saturated solution of the compound being recrystallized and cannot dissolve any more solute, but fresh solvent will dissolve some of your product resulting in an inefficient recrystallization process.

Safety note Take great care when 'scratching' a supersaturated solution with a glass rod. Hold the flask or test tube at the *neck*, not at the bottom, and make short scratching movements on the side of the flask just above and below the surface of the liquid (Fig. 13.2). If you accidentally break the flask or test tube you will cut your hand if you hold the vessel at the bottom.

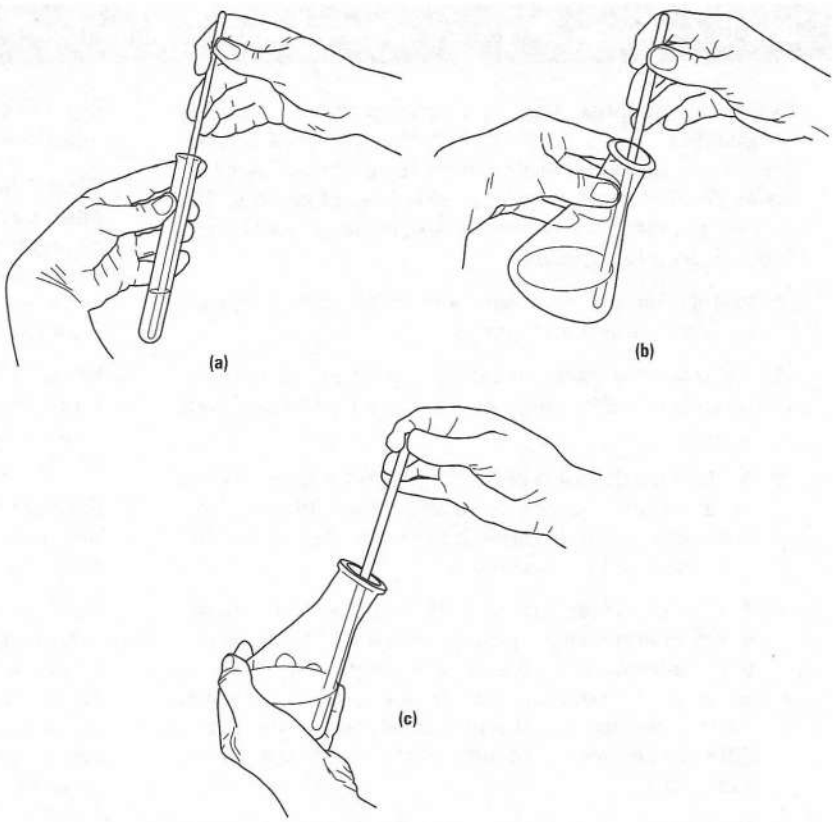


Fig. 13.2 'Scratching': (a) right; (b) right; (c) wrong.

Drying

The purified compound should be dried by the appropriate method as described on p. 39. If you do not know the melting point of your compound, you should always carry out a test by placing a small amount on a watch-glass in the oven, before committing the bulk of your chemical.

Detailed procedures for single-solvent and mixed-solvent recrystallizations are shown in Box 13.3 and Box 13.4 respectively and the modifications necessary for the use of other solvent systems can be worked out from the information in Table 13.2.

Problems in recrystallization

There are three common problems encountered during recrystallization:

1. The compound crystallizes in the filter funnel during hot filtration. This is because the solubility of the solute decreases rapidly with temperature and the slight cooling during hot filtration causes precipitation of the solid, even though you are heating the funnel. The answer is to use more than the minimum amount of solvent and then evaporate off the excess before cooling.
2. The compound does not recrystallize. There are two reasons: you have used too much solvent and you must evaporate off some solvent before cooling, or you have formed a supersaturated solution and you must 'seed' or 'scratch' the solution (see p. 98).

Crystallization in the filter paper:

prevention – keep everything hot and use a good fluted filter paper;

cure – wash through with hot solvent, evaporate most of the excess and repeat the hot filtration.

Evaporation of excess solvent – remove by rotary evaporation (p. 121) or N_2 blow-down (p. 123). This is the one occasion when water is the less than ideal solvent, since its evaporation is time consuming.

3. The compound precipitates as an oil. This is because compounds with low melting points often come out of a concentrated solution above their melting point. In such cases more solvent should be added and the compound redissolved and cooled so that precipitation is retarded to the temperature at which the crystalline solid comes out of solution. Often 'scratching' the hot solution as it cools can prevent 'oiling out'.

14 Solvent extraction

Extraction – making a cup of coffee involves extraction of the flavour chemicals and caffeine from the insoluble vegetable matter using hot water and is an example of liquid–solid extraction.

This technique separates the components of chemical mixtures by using the dissimilar solubility properties of the components of the mixture in different solvents. Extraction is used mainly to purify a reaction product partially before final purification by recrystallization (p. 92) or distillation (p. 107). The two common types of extraction process used in the laboratory are:

1. Liquid–liquid extraction: this uses two immiscible solvents; the desired compound in solution or suspension in one solvent is extracted into the other solvent. For example, covalent organic compounds are extracted from aqueous solution into dichloromethane, leaving the ionic by-products or reagents in the aqueous phase.
2. Solid–liquid extraction: this involves the use of a solvent to remove solvent-soluble components of a solid mixture.

Liquid–liquid extraction

Several experimental processes in practical chemistry are based on liquid–liquid extraction:

- 'Extraction': where a solid or liquid suspended or dissolved in one solvent is extracted into another. This technique can be used to separate covalent molecules from ionic compounds in an aqueous solution or suspension.
- 'Washing': where ionic species are removed from a non-polar solvent by extraction into water.
- 'Acid–base extraction': where covalent molecules are converted into their salts and thus removed from a non-polar solvent into water, while neutral covalent species will remain in the non-polar solvent, as shown in Table 14.1.

Table 14.1 Examples of acid–base extraction chemistry

$ArCOOH$	+	RH	$\xrightarrow[CH_2Cl_2]{NaOH}$	$ArCOO^-\ Na^+$	+	RH
Acid insoluble in H_2O soluble in CH_2Cl_2		Neutral insoluble in H_2O soluble in CH_2Cl_2		Salt soluble in H_2O		Neutral insoluble in H_2O soluble CH_2Cl_2
$ArNH_2$	+	RH	$\xrightarrow[CH_2Cl_2]{HCl}$	$ArNH_3^+\ Cl^-$	+	RH
Amine insoluble in H_2O soluble in CH_2Cl_2		Neutral insoluble in H_2O soluble in CH_2Cl_2		Salt soluble in H_2O		Neutral insoluble in H_2O soluble CH_2Cl_2
$ArCOOH$	+	$ArOH$	$\xrightarrow[CH_2Cl_2]{Na_2CO_3}$	$ArCOO^-\ Na^+$	+	$ArOH$
Acid insoluble in H_2O soluble in CH_2Cl_2		Weak acid insoluble in H_2O soluble in CH_2Cl_2		Salt soluble in H_2O		Weak acid insoluble in H_2O soluble CH_2Cl_2

All of these processes involve mixing the two immiscible solvents, one of which contains the mixture, in a separatory funnel (p. 103) and shaking the funnel to promote the extraction process. The immiscible layers are allowed to reform and are then separated.

Safety note Because of its *flammability* and tendency to form *explosive peroxides*, the use of diethyl ether for extractions in many undergraduate laboratory has effectively been discontinued. Use dichloromethane or ethyl ethanoate instead.

Safety note Dichloromethane is effectively *non-flammable* and is often the solvent of choice, but it is a *liver toxin* and an *irritant*, and must be handled in the fume cupboard if volumes greater than 50 mL are to be used.

For liquid–liquid extraction, water is usually the polar solvent. Since most extractions involve getting the required compound into the organic solvent (or removing unwanted ionic chemicals from it), it should have good solvent power for the desired compound and a low boiling point for ease of removal and recovery of the compound. The common organic solvents used in liquid–liquid extraction are diethyl ether (ethoxyethane) b.pt. 34 °C, dichloromethane (DCM) b.pt. 41 °C and ethyl acetate (ethyl ethanoate) b.pt. 77 °C. Dichloromethane is denser than water and forms the lower layer, whereas diethyl ether and ethyl acetate float on water and are the upper layer.

Partition coefficients

The theory of liquid–liquid extraction is based on the equilibrium between the concentrations of dissolved component in the two immiscible liquids, when they are in contact. The equilibrium constant for this process is called the partition coefficient or distribution coefficient and is given by:

$$K = \frac{\text{concentration of solute in liquid 1}}{\text{concentration of solute in liquid 2}} \qquad [14.1]$$

You only need to calculate such quantities if:

- you are carrying out specific experiments to determine partition coefficients, when you will be given specific instructions or references to the appropriate literature;
- the solute has appreciable solubility in both solvents.

The reason calculation is not necessary is that, in the overwhelming majority of extractions you will carry out, the conditions used are designed to ensure that the components will be almost totally soluble in one of the liquids and almost insoluble in the other, since complete separation is required. The number of extractions needed to extract a water-soluble solute into an immiscible organic phase can be calculated from the following relationship:

Extraction calculations – it is necessary to calculate volumes of solvents and number of extractions when attempting to maximize the economics of an industrial-scale extraction process.

$$w_n = w_0\left(\frac{Kv}{Kv + s}\right)^n \qquad [14.2]$$

where K = partition coefficient of the solute, v = volume (mL) of aqueous solution of the solute, s = volume (mL) of immiscible organic solvent, w_0 = weight of solute initially in the aqueous layer, w_n = weight of solute remaining in the aqueous layer after n extractions, and n = number of extractions. Evaluation of this expression shows that, for a fixed volume of solvent, it is more efficient to carry out many small extractions than one big one.

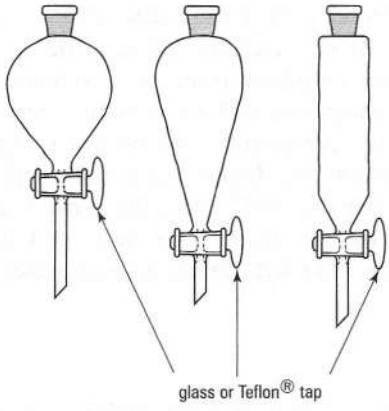
Separatory funnels

Separatory funnels – the coned-shaped funnels are specifically designed for extractions. Only use parallel-sided funnels when no alternative is available.

Safety note Extractions involving large volumes of solvents (> 100 mL) should *always* be carried out in cone-shaped separatory funnels supported by a ring, because there is then no danger of the funnel slipping from a clamp at its neck.

These come in a range of sizes from 5 mL to 5000 mL and there are two general types: parallel sided and cone shaped (Fig. 14.1). Cone-shaped separatory funnels are made of thin glass and should be supported in a ring (p. 104). Small-volume cone-shaped funnels (< 100 mL capacity) and parallel-sided separatory funnels should be clamped at the ground-glass joint at the neck (p. 27).

Separatory funnels will have glass or Teflon® taps with a rubber ring and clip or screw cap on the end to prevent the tap slipping from the barrel, or a



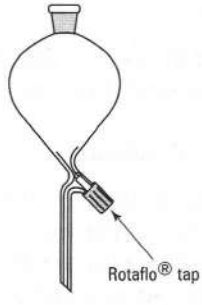


Fig. 14.1 Separatory funnels.

Drying separatory funnels in an oven – *always* disassemble the tap and *do not* place the tap and its plastic components in the oven. Dry them with tissue.

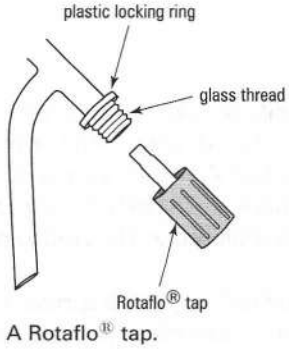


Fig. 14.2 A Rotaflo® tap.

Rotaflo® tap. You must ensure that the tap assembly is in good condition by making the following checks before starting work:

- For glass taps: disassemble the tap by first removing the clip and ring or cap from the tap (note the order of the component parts for reassembling). Dry the tap and barrel with tissue, add a light smear of grease to the tap (making sure you do not clog the hole in the tap) and reassemble the tap and fittings, turning the tap to ensure free movement. Support the separatory funnel in position and add some of the organic solvent to be used (2–3 mL) to the funnel, with the tap closed, to check that the tap does not leak. If the tap leaks, disassemble and regrease.
- For Teflon® taps: disassemble the tap, wipe the tap and barrel with clean tissue, reassemble without grease, check for free movement of the tap and for leakage as described above. When you have finished using the funnel, loosen the clip/cap on the tap since Teflon® will flow under pressure and the tap may 'seize' in the barrel.
- For a Rotaflo® tap: unscrew the tap from the funnel and ensure that the plastic locking thread is in place (Fig. 14.2). If it is not present, consult your instructor and obtain a replacement. Dry the barrel of the tap and the tap with a tissue and reassemble. Do not grease the Rotaflo® tap.

The general procedure for using a separatory funnel for extraction is described in Box 14.1 and there are five additional practical tips to aid your success:

1. Label *all* flasks to avoid confusion.
2. *Never throw away any of the separated liquids until you are absolutely sure of their identity.*
3. *Always* transfer solvents into the separatory funnel using a stemmed filter funnel so that solids and liquids will not stick to the inside of the joint and prevent a good seal when you insert the stopper and then invert the funnel.
4. *Always* place a *'safety' beaker* under the separatory funnel to collect liquid just in case the tap leaks (Fig. 14.3a).

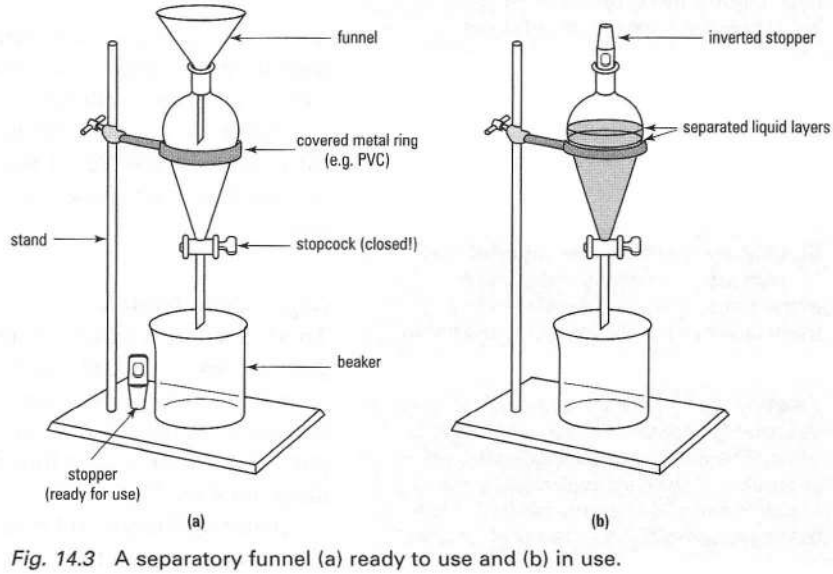


Fig. 14.3 A separatory funnel (a) ready to use and (b) in use.

Box 14.1 How to separate a carboxylic acid and a hydrocarbon using solvent extraction

This is an example of an acid–base extraction. The solid mixture (e.g. 4.0 g for the solvent volumes used below) of benzoic acid and naphthalene is soluble in dichloromethane but benzoic acid will dissolve in dilute aqueous sodium hydroxide (2 M) by forming the sodium salt (sodium benzoate). Naphthalene is insoluble in water.

1. **Dissolve the mixture in a clean, dry beaker in dichloromethane (50 mL).**
2. **Clean and dry the tap** of a separatory funnel (250 mL) and set up as shown in Fig. 14.3a.
3. **Make sure that the tap is *closed*** and then add the solution containing the mixture, using a stemmed funnel to prevent contamination of the joint, and rinse out the beaker with dichloromethane (~ 5 mL).
4. **Add sodium hydroxide solution (10 mL) to the separatory funnel**, place the stopper in the separatory funnel and gently invert it and hold it as shown in Fig. 14.4. *Do not shake the separatory funnel* since you do not know how much heat will be produced in the reaction, which will pressurize the separatory funnel.
5. **Open the tap**, to release any pressure caused by the heat of reaction.
6. **Close the tap, shake the mixture once** and open the tap to release any pressure.
7. **Close the tap, shake the mixture twice** and open the tap to release any pressure.
8. **Repeat** until no more vapour is expelled via the tap.
9. **Close the tap**, and replace the separatory funnel in the ring or clamp.
10. **Take out the stopper**, place it upside down in the top of the separatory funnel and allow the solvent layers to separate. The upper layer is the aqueous layer (10 mL compared with 50 mL of dichloromethane). Sometimes a few globules of dichloromethane will 'cling' to the surface of the water layer: these can be released by gently swirling the contents of the separatory funnel.
11. **When the liquids have stopped swirling, open the tap gently** and *slowly* run the dichloromethane lower layer into a clean conical flask and label it 'dichloromethane layer'. Avoid fast emptying of the funnel because a vortex may be formed which will cause the upper layer to run out with the lower layer.
12. **Run the remaining aqueous layer** into a clean, dry conical flask and label it 'sodium hydroxide layer'.
13. **Return the dichloromethane layer** to the separatory funnel and extract it with another portion (10 mL) of sodium hydroxide. Repeat the extraction process for a total of 40 mL (i.e. 4 × 10 mL) of the alkali, collecting all the sodium hydroxide extracts in the same flask.
14. **Finally, extract the dichloromethane with water (20 mL)**, to remove any traces of sodium hydroxide and add these 'washings' to the sodium hydroxide layer' flask.
15. **You now have** a solution of naphthalene in dichloromethane and a solution of sodium benzoate in sodium hydroxide ready for further processing.
16. **If an emulsion forms** – the layers do not have a well defined boundary: add a few drops of methanol to the upper layer down the inside wall of the funnel. This often 'breaks' the emulsion.

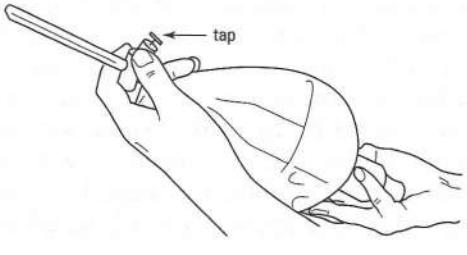


Fig. 14.4 Holding a separatory funnel.

Batch liquid–liquid extraction – the process described is inefficient if the material being extracted has appreciable solubility in both of the solvents used. In these situations a *continuous extraction system* is necessary and you should consult the specialist textbooks, e.g. Furniss *et al.* (1989, p. 160).

5. *Always* take the stopper from the separatory funnel before you attempt to allow liquid to run from the funnel. If you do not remove the stopper from the top of the funnel, a vacuum is formed in the funnel after a little of the liquid has run out. Air will be sucked into the funnel through the outlet stem causing bubbles, which will remix your separated layers. If your funnel is equipped with a Quickfit® stopper, it is good practice to take the stopper out of the top and put it back upside down (Fig. 14.3b). This ensures that no vacuum is formed and that organic vapours do not escape easily from the flask.

Solid–liquid extraction

In this process the components of a solid mixture are extracted into a solvent. The 'batch process', analogous to liquid–liquid extraction, involves grinding the solid to a fine powder, mixing it with the appropriate solvent, and filtering off the solid by gravity (p. 27) or under vacuum (p. 30) and then evaporating the solvent (p. 121) from the extract solution. However, a more

Box 14.2 How to set up a Soxhlet extraction system

1. **Select apparatus of the appropriate size for the amount of solid to be extracted.** Specifically, the Soxhlet thimble should fit below the siphon outlet and the volume of the solvent reservoir should be such that it is never more than half-full when all the solvent has siphoned from the extractor.
2. **Assemble the apparatus as shown in Fig. 14.5,** clamping at the joints at the flask and the *top* of the Soxhlet extractor. The best heat source to use for continuous operation over a long period is a mantle.
3. **Disassemble the apparatus,** leaving the clamps in position.
4. **Fill the flask to about one-third of its volume with solvent,** add some anti-bumping granules (or a magnetic 'flea', if a stirrer-mantle is being used) and clamp it into position in the mantle.
5. ***Lightly* grease the 'male' joint of the Soxhlet extractor** and attach it to the reservoir flask. Clamp the top of the extractor.
6. **Add solvent to the extractor until it siphons.** This ensures that the reservoir will never be dry. Check that the reservoir is now no more than half-full; if it is, replace with a larger flask.
7. **Half-fill the extraction thimble with the solid to be extracted** and plug the top of the thimble with white cotton wool to prevent any solid being carried over into the solvent.
8. **Place the thimble in the extractor.**
9. **Attach a water supply to the reflux condenser,** lightly grease the male joint and attach the condenser to the top of the extractor. Turn on the water, ensuring a steady flow.
10. **Switch on the heater and turn up the power so that the solvent refluxes** and drips into the extractor.
11. **Confirm that everything is running smoothly by watching at least two siphoning cycles** of the extraction and check the apparatus frequently.
12. **When the extraction is complete, allow the apparatus to cool and dismantle it.** Place the extraction thimble in the fume cupboard to allow the solvent to evaporate and the dispose of it appropriately. Gravity filter or decant the solvent in the reservoir flask to remove the anti-bumping granules of magnetic 'flea' and remove the solvent by distillation (p. 107) or rotary evaporation (p. 122).

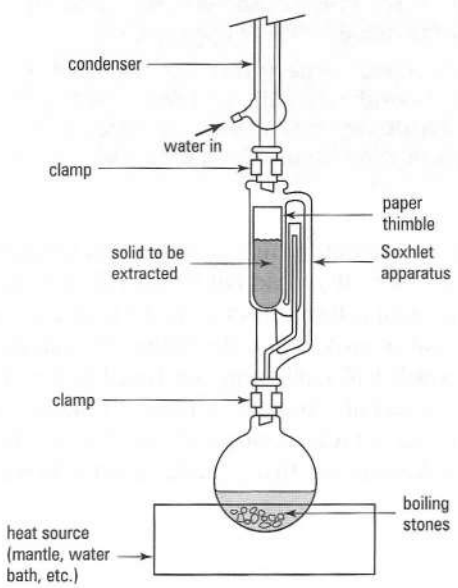


Fig. 14.5 A Soxhlet extraction system.

elegant 'continuous extraction process', called Soxhlet extraction, is available when the most appropriate solvent is known.

The apparatus for Soxhlet extraction is shown in Fig. 14.5 and comprises a flask containing the solvent, a Soxhlet extractor and a reflux condenser (p. 116). The solid to be extracted is placed in a porous thimble, made from hardened filter paper, and the solvent is heated so that its vapour flows past the thimble, condenses and fills the extractor with hot solvent to extract the solid. When the extractor is full, the solvent (together with the extracted material) siphons back into the solvent flask and the process is repeated automatically. The advantage of this procedure is that fresh solvent continually extracts the solid, which is concentrated in the flask. The disadvantage is that the compound extracted is kept at the boiling point of the solvent for a prolonged period. Soxhlet extractors come in sizes of 10 mL to 5000 mL, based on the volume of solvent contained in the extractor. The procedure for using a Soxhlet extraction system is described in Box 14.2.

15 Distillation

Working with azeotropes – not all liquid mixtures can be separated by distillation. In some cases an *azeotrope*, a mixture of the liquids of definite composition, which boils at a constant temperature, is formed. For example, an azeotrope containing 95.5% ethanol and 4.5% water boils at 78.15 °C, which is below the boiling point of pure ethanol (78.3 °C). Therefore 100% ethanol cannot be obtained by distillation of ethanol–water mixtures, even though their boiling points are about 22 °C apart.

Distillation is used to separate the components of a liquid mixture by vaporizing the liquids, condensing the vapours and collecting the liquid condensate. Separation is the result of the differing boiling points of the individual constituents of the mixture and the efficiency of the distillation column. You may be required to *purify* a liquid by *distillation*, which involves the removal of small quantities of impurities, or to *separate* a mixture of liquids by *fractional distillation*, each of which can then be purified by distillation.

You will meet several types of distillation process each applicable to different situations depending on the chemicals to be purified or separated.

- *Simple distillation*: used for separating liquids, boiling *below* 200 °C at atmospheric pressure, from other compounds. For effective separation there should be a difference in the boiling points of the components of at least 80 °C.
- *Fractional distillation*: used for separating components of liquid mixtures, which have boiling points differing by more than 25 °C, at temperatures *below* 200 °C.
- *Vacuum or reduced-pressure distillation*: used for separating liquids boiling *above* 200 °C, when decomposition may occur at the high temperature. The effect of distilling at reduced pressure is to lower the boiling point of a liquid. This technique can be applied to both simple distillation and fractional distillation.
- *Steam distillation*: used for separating mixtures of chemicals such as oils, resins, hydrocarbons, etc., which are essentially insoluble in water and may decompose at their boiling points. Heating the compounds with steam makes them distil *below* 100 °C.

Using steam distillation – in practice, the decision to use this approach to separate the components of a mixture is based on previous experience. Consult your instructor for advice.

Distillation equipment

Apparatus used for the various types of distillation has several general features:

- Distillation flask: usually round bottom or pear shaped with one or two necks (to allow a vacuum bleed to be fitted).
- Still-head: to hold the thermometer and to channel the vapour into the condenser. For fractional distillation, the fractionating column is fitted between the distillation flask and the still-head.
- Condenser: usually with circulating cold water.
- Take-off adapter: to allow the distillate to run into the collecting vessel. For vacuum distillation, the adapter will have a vacuum inlet tube and could have three 'arms' to allow three fractions to be collected without breaking the vacuum.
- Receiving (collection) vessel: this can be a test tube, a measuring cylinder, a conical flask, or a round-bottom flask with a ground-glass joint.

Setting up distillation apparatus – do not allow the support stands to move during the distillation, since this will allow the ground-glass joints connecting the still-head and condenser to separate. The hot vapours will then escape into the laboratory instead of going down the condenser.

Securing distillation components – do not use plastic joint clips on the 'hot end' of a distillation apparatus since they may melt and the joints may separate.

No matter what type of distillation you are attempting, it is essential that you assemble the apparatus correctly, since you are dealing with hot, often flammable, liquids and vapours. A typical simple distillation apparatus is shown in Fig. 15.1 and the method of assembly is described in Box 15.1.

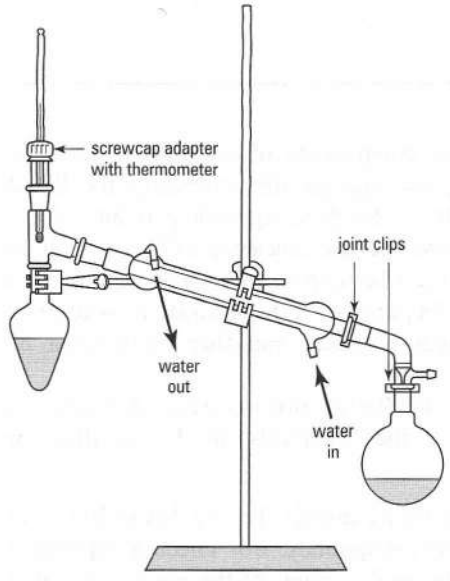


Fig. 15.1 Apparatus for simple distillation.

Heating a distillation flask – unlike when using a microburner, it is difficult to stop the heating process instantly when using an oil bath or mantle on a 'labjack'. Therefore have a clean receiver flask ready to collect the 'tailings' as soon as the temperature begins to rise.

Collecting fractions – as the temperature begins to rise 'between fractions' you will have 2 or 3 seconds to change receiving vessels before the liquid runs from the top to the bottom of the condenser. *Have three or four pre-weighed receivers ready to hand.*

Distillation in fume cupboards – it may be necessary to insulate the fractionating column from the 'wind' by wrapping it in aluminium foil, since the draught prevents equilibration in the column. Don't forget to leave a 'window' in the foil so that you can see the vapour condensing near the top of the column.

KEY POINT **You must ensure that all the ground-glass joints are seated properly and the apparatus is clamped firmly so that no movement of the joints will allow vapours to escape.**

Simple distillation

The procedure for doing a simple distillation is described in Box 15.2 and you should note the following points:

- Do not distil to dryness, i.e. no liquid remaining in the distillation flask, since this causes overheating and charring (decomposition) of the residue. *Always* leave 1 or 2 mL of liquid in the distillation flask.
- Initially, some liquid may distil while the temperature rises rapidly. This is termed 'forerun', for example often a small amount of solvent from an extraction process (p. 102): collect and then, when the temperature stabilizes, collect the desired compound in a clean receiving vessel.
- Towards the end of the distillation, the temperature may begin to rise again: this will be the higher boiling impurity. Stop heating, quickly remove the receiving flask containing your pure compound and collect these last few drops, termed 'tailings', in a fresh receiving vessel.

Fractional distillation

Simple distillation is not very effective in separating liquids unless their boiling points are at least 80 °C apart and a better separation can be achieved if a fractionating column is used. There are many types available (Fig. 15.2) and the device brings the ascending vapours into contact with the condensing (in the fractionating column) liquid and amounts to a succession of many simple distillations in which the descending liquid strips the high-boiling component from the ascending vapour. Overall, the lower boiling component distils first and cleanly. The column packing, usually glass beads, helices or 'fingers', gives a large surface area for contact of the ascending vapours and descending liquid. After the first fraction has distilled, the temperature will rise and the rate of distillation will slow. This is an intermediate fraction containing a little of both components of the mixture. Finally, the temperature will become constant and the pure higher boiling compound will distil. The procedure for fractional distillation is given in Box 15.3.

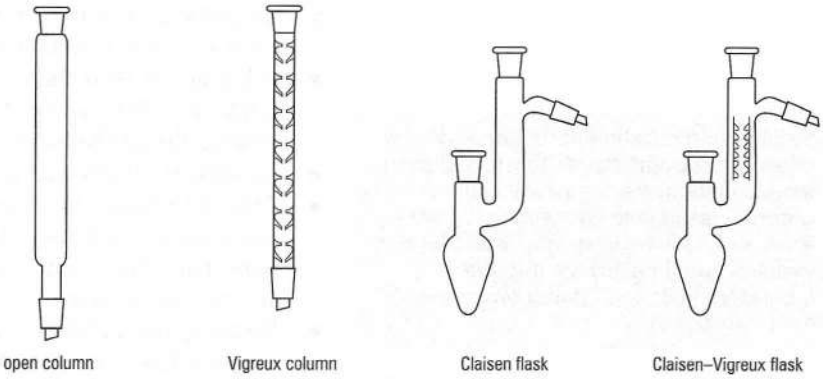


Fig. 15.2 Common fractionating columns.

KEY POINT **A successful fractional distillation relies on thermal equilibration of the components in the fractionating column. You must allow the column to be heated by the vapours of the mixture.**

Box 15.1 How to assemble the apparatus for a simple distillation

In general laboratory work you will usually be distilling small volumes of liquids (~ 25 mL). Standard glass joint-ware apparatus size 14/23 (p. 43) is appropriate. If you are to carry out larger scale distillations, you must consider the weight of the components, specifically the receiver flask, which should be supported on a 'labjack', since a joint clip may not be strong enough to hold a large flask (250 mL or larger).

1. **Clamp the distilling flask firmly to a support stand** ensuring that it is at the correct height to allow the heat source (microburner, oil bath or mantle on a 'labjack') to be removed if necessary.
2. **Insert the still-head** in the top of the flask.
3. **Carefully line up the condenser on a clamp** (attached to the same support stand as the distilling flask (Fig. 15.1), if possible) and adjust the height and angle of the clamp so that the condenser slides onto the still-head joint. Remember to have the non-moving jaw of the clamp at the bottom (p. 27) otherwise you will lift the condenser when you tighten the clamp and break the joint on the still-head or condenser or both. Carefully tighten the clamp and ensure that the joints are 'seated'.
4. **Attach the take-off adapter to the bottom of the condenser with a joint clip.** If you are using a Quickfit® flask as a receiving flask, *you must use a vacuum-type take-off adapter* (p. 42) otherwise you will be distilling in a closed system, which may explode when you begin to heat the distilling flask.
5. **Attach the Quickfit® receiver flask to the take-off adapter with a joint clip** or place the receiver flask underneath the take-off adapter, supported on a 'labjack' or clamped position. The outlet of the take-off adapter must be just inside the receiving vessel so that no spillage of distillate will occur. If you clamp the receiving vessel in position, use a separate support stand.
6. **Disassemble the apparatus in the reverse order to assembly** by opening the clamps – *do not move the clamps and stands.*
7. **Add the liquid to be distilled to the distillation flask** using a stemmed funnel, together with anti-bumping granules or boiling stick or a magnetic 'flea'. The flask should not be more than 50% full. Reclamp the flask in position.
8. **Lightly grease the 'male' joint on the still-head** and replace it in the flask.
9. **Insert a thermometer into a screwcap adapter** (p. 43) and adjust so that the bulb is just below the outflow from the still-head. Lightly grease the joint on the screwcap adapter and put it into the still-head.
10. **Fit the rubber tubing onto the condenser** (p. 117), lightly grease the joint on the still-head and refit the condenser, clamping it firmly into place.
11. **Connect the lower tube** to the water tap so that the water will flow up the condenser, and upper tube should be routed into the sink. Make sure that there are no kinks in the rubber tubes and, for safety, feed them over the protruding arms at the back of the clamps. Turn on the water tap gently and check that the water flows freely.
12. **Lightly grease the bottom joint of the condenser** and attach the take-off adapter using a joint clip.
13. **Attach the Quickfit® receiving flask to the take-off adapter with a joint clip** (after lightly greasing) or place the receiving vessel underneath the take-off adapter, supported on a 'labjack' or clamped into position. Turn up the water flow to give a steady stream from the condenser.

Box 15.2 How to carry out a simple distillation

1. **Set up the apparatus as described in Box 15.1** and make sure that all the joints in the distillation system are secured properly.
2. ***Slowly* apply the heat source to the distillation** until the liquid is boiling gently.
3. **Increase the heat slowly until the liquid starts to drip into the receiving vessel** at a rate of about one drop every 2 seconds. If the temperature is 'constant' (i.e. it does not vary more than 2–3 °C), collect the liquid and record the temperature range of the distillate.
4. **Remove the heat source**, allowing the apparatus to cool down and the remaining drops of distillate to run out of the condenser into the receiving flask.
5. **Seal and label the receiving flask(s).**
6. **When cool, dismantle the apparatus**, wash it out with an appropriate solvent and dispose of the washings in the correct solvent residues bottle.

Box 15.3 How to carry out a fractional distillation

1. **Set up the apparatus as described Box 15.1 but insert the fractionating column between the distilling flask and the still-head.** For extra stability, clamp the fractionating column at its top joint to support the stand carrying the distilling flask.
2. **Apply the heat source slowly until the liquid begins to boil gently.** Then slowly increase the heat until the column is warmed up and liquid is condensing from the thermometer bulb, *but not distilling over.*
3. **If the temperature is constant, increase the heat slightly until the first component distils over at a rate of about one drop every 2 seconds.** Collect this distillate as the first fraction, as long as the temperature is 'constant' (i.e. it does not vary more than 2–3 °C). Record the temperature range of the distillation.
4. **Change the receiving flask when the temperature begins to rise and the rate of distillation slows.**
5. **Increase the heat slowly** and collect the intermediate fraction until the temperature stabilizes again. Record the temperature range of the distillation.
6. **Change the receiving flask** and collect the new fraction (one drop every 2 seconds) for as long as the temperature remains 'constant' (i.e. it does not vary by more than 2–3 °C). Record the temperature of the distillation.
7. **Remove the heat source**, allowing the apparatus to cool down and the remaining drops of distillate to run out of the condenser into the receiving flask.
8. **Seal and label the receiving flask(s).**
9. **When cool, dismantle the apparatus**, wash it out with an appropriate solvent and dispose of the washings in the correct solvent residues bottle.

Vacuum or reduced-pressure distillation

Safety note You must check all flasks and glassware for 'star' cracks and chips. *If in doubt, replace.*

Working with reduced pressures – distillation flasks and receiving flasks must always be round bottom or pear shaped. Do not use flat-bottom or conical flasks under vacuum.

Measuring reduced pressures – note that despite SI nomenclature for pressure, practical work usually involves pressure measurement in mm of Hg. Atmospheric pressure is about 760 mm Hg. Pressures quoted in 'torr' (seen on some old vacuum equipment) are equivalent to mm Hg.

Moving a mercury manometer – always carry mercury manometers in a vertical position or the mercury may spill out.

Safety note Do not allow air to rush into an Anschutz manometer, otherwise the mercury in the left-hand limb of the manometer will shoot up the tube and may break the glass at the top.

Distillation at reduced pressure is used to distil liquids with few impurities or to fractionate the components of liquid mixtures with high boiling points, which would decompose if distilled at atmospheric pressure. The modifications to the distillation apparatus (Fig. 15.1) required for reduced-pressure distillation are:

- A vacuum source: this can be a water pump (see p. 29) with an 'anti-suck-back' trap producing a vacuum of 15–25 mm Hg, at best; or a rotary vacuum pump (consult your instructor about its use since it is an expensive piece of equipment and easily contaminated or damaged), which will evacuate down to 0.1 mm Hg. Two-stage dry vacuum pumps produce a vacuum of 1–5 mm Hg, are resistant to organic and acid vapours and are easy to use.
- A manometer to measure the pressure (vacuum) in the apparatus, since the boiling point of a liquid varies with pressure. Two types are in common use (Fig. 15.3): the Anschutz manometer, which gives a constant reading of the vacuum throughout the distillation, and the Vacustat®, which is used to take a 'sample' of the vacuum at a given instant. Vacustats® are very accurate and are more often used in combination with a rotary oil pump.
- A take-off adapter, which permits the collection of several fractions without stopping the distillation to change the receiving flasks. A receiving adapter, termed a 'pig' (Fig. 15.4), can be rotated on the end of the condenser to collect three fractions.
- Appropriate 'anti-bumping' measures: reduced-pressure distillations are notoriously prone to 'bumping' (p. 31) and anti-bumping granules are ineffective. You can use a magnetic 'flea' in conjunction with a hot plate-stirrer and oil bath; an air bleed, made by pulling out a Pasteur pipette into a fine capillary using a microburner (consult your instructor), inserting it into a screwcap adapter (p. 43) and placing it in the second neck of the distilling flask. The vacuum pulls a fine stream of air into the

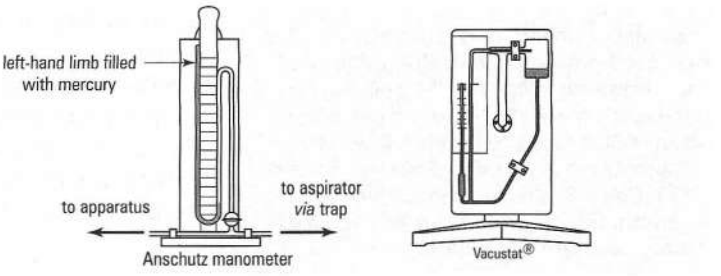


Fig. 15.3 Manometers for vacuum distillation.

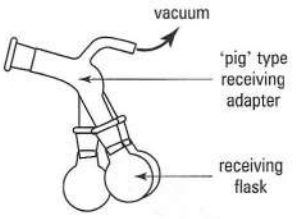


Fig. 15.4 'Pig'-type receiving flask adapter.

Safety note If, during the course of your vacuum distillation using a microburner and a boiling stick, the liquid stops boiling and appears 'quiescent', it is about to 'bump' vigorously. Stop heating, allow it to cool, 'break' the vacuum (Box 15.4), and add a new boiling stick.

flask and forms small bubbles which agitate the liquid during distillation; or a wooden boiling stick for small-scale distillations of short duration, since the vacuum will pull air from the stick and agitate the liquid.

- A three-way tap inserted between the vacuum source and the manometer, so that you can control the vacuum applied to the distillation system.

A typical system for reduced-pressure distillation is shown in Fig. 15.5 and the procedure, using a water pump, is described in Box 15.4.

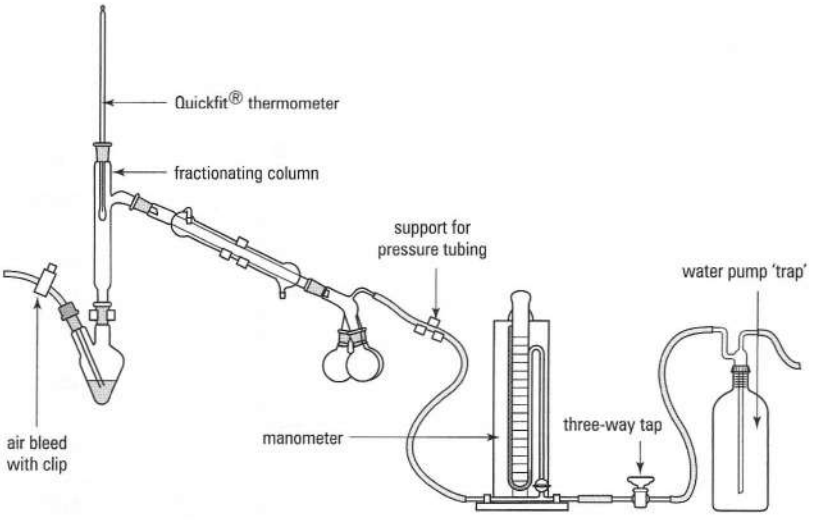


Fig. 15.5 Apparatus for vacuum distillation.

Ending a distillation – complete distillation of a liquid is not possible because of the need to leave a few millilitres in the distilling flask, to prevent overheating and due to the 'hold-up' volume of the flask and fractionating column.

Kugelrohr distillation

This is also known as short-path distillation or bulb-to-bulb distillation. It is a procedure for reduced-pressure distillation, which almost eliminates losses owing to the size of the apparatus, and is particularly useful for small volumes of liquids. The liquid sample is placed in a small round-bottom flask, connected to a series of bulbs (Fig. 15.6) and then heated and rotated (to prevent bumping)

Estimating the boiling point of a liquid at reduced pressure – a useful guide is: *if the pressure is halved, the boiling point is reduced by ~10 °C*. For example, if the b.pt is 300 °C at 760 mm Hg, it will be approximately 290 °C at 380 mm Hg and 190 °C at ~1 mm Hg. Alternatively a nomograph (Fig. 15.8) can be used for a more accurate estimation.

under vacuum in an electric oven. The liquid distils from the flask inside the oven to a cold bulb outside the oven. Since the distillation path is very short the length of a ground-glass joint losses are minimized. The use of several bulbs as receiver flasks allows a small volume of mixture to be fractionated by varying the temperature in the oven. The distillates can be removed from the bulbs by either washing into another flask with a low-boiling-point solvent or using a 'bent' Pasteur pipette (Fig. 15.7) since the bulbs can only be held horizontally.

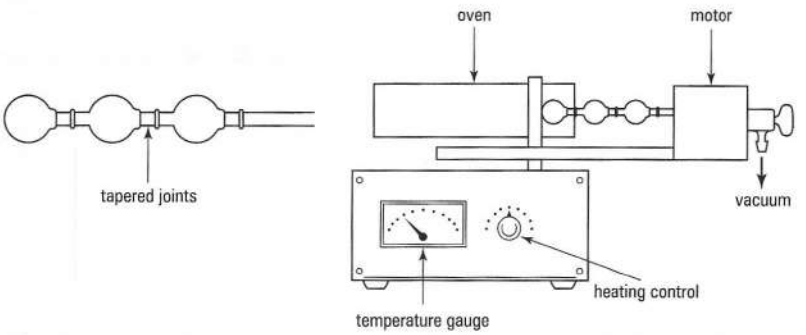


Fig. 15.6 Kugelrohr distillation apparatus.

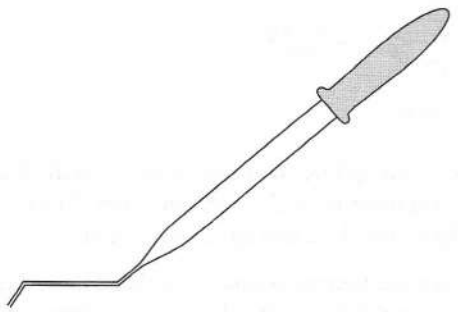


Fig. 15.7 A 'bent' Pasteur pipette.

Steam distillation

This process can be used to separate water-insoluble covalent compounds from crude reaction mixtures or to isolate volatile natural products, e.g. terpenes from plant tissue. The crude mixture is heated with water and steam and the steam-volatile material co-distils with the steam and is then condensed with the water and collected. You will then need to carry out an extraction (p. 102) to separate the water and the insoluble component.

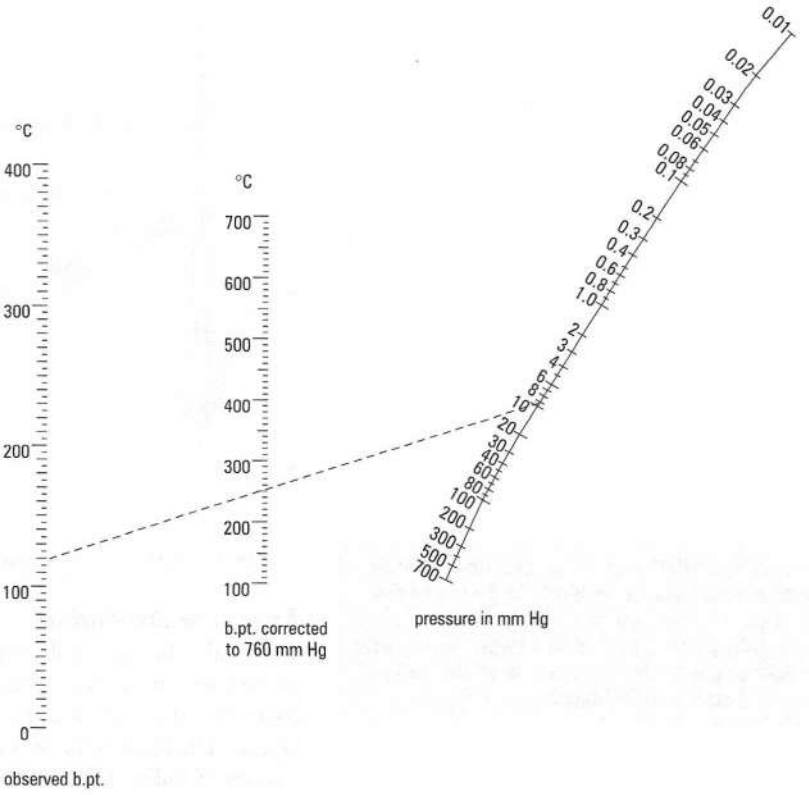


Fig. 15.8 Nomograph for estimating boiling point at reduced pressure. To use it, draw a line between the recorded pressure and the boiling point at 760 mm Hg, and then extend the line to the observed boiling point scale. This point is the boiling point at the reduced pressure. In this example, a liquid b.pt. of 250 °C at 760 mm Hg will boil at 118 °C at 10 mm Hg.

Box 15.4 How to carry out a reduced-pressure distillation using a water pump

1. **Set up the distillation apparatus as described in Box 15.1**, but use a two-necked distilling flask and include a short fractionating column between the top of the distilling flask and the still-head *or* use a Vigreux flask (Fig. 15.2) *or* use a Claisen still-head (Fig. 15.2).
2. **Connect three Quickfit® round-bottom receiving flasks to the receiving adapter or 'pig' using plastic joint clips** and attach the 'pig' to the bottom of the condenser. Support the receiving flasks on a 'labjack' if they are 100 mL size or larger. Rotation of the 'pig' allows three fractions to be collected without stopping the distillation – this is a major task when working under reduced pressure.
3. **Insert the air bleed into the 'spare' neck of the distilling flask** or into the lower joint on the Vigreux flask or Claisen head and make sure that the tip of the air bleed reaches the bottom of the distilling flask. If you are using boiling sticks as an 'anti-bumping' precaution, add the sticks, making sure that they reach to the bottom of the flask and do not float, and put stoppers in the unused joints.
4. **Insert a Quickfit® thermometer into the remaining** 'male' joint in the apparatus. *Do not use* an ordinary thermometer in a screwcap adapter: it may be sucked into the apparatus and break or crack the flask.
5. **Using thick-walled rubber pressure tubing, connect the water pump** (via the anti-suck-back trap) to the three-way tap and then to the Anschutz manometer. Connect another piece of pressure tubing to the manometer but do not connect it to the 'pig' for the moment.
6. **To check the available vacuum**, turn on the water pump fully, kink or seal the open pressure tubing on the manometer and turn the three-way tap so that a vacuum is created in the manometer. Slowly open the tap on the manometer and the mercury level should rise in one of the manometer 'arms'. When the mercury has stabilized, move the scale so that the zero is level with the lowest mercury level, and read off the upper mercury level. The pressure (vacuum) is the difference in levels in mm. The reading should be between 10–20 mm. Now close the tap on the manometer, unkink or unseal the pressure tubing, and turn the three-way tap so that air is admitted into the system.
7. **Connect the pressure tubing from the manometer to the 'pig'**. Pressure tubing is heavy and it should be supported with a clamp and stand, close to the 'pig', to prevent strain on the glassware.
8. ***Slowly* turn the three-way tap to evacuate the apparatus**. As the pressure decreases, bubbles will issue from the air bleed or the boiling stick and low-boiling solvents such as dichloromethane or diethyl ether will begin to boil, causing frothing in the flask. If this happens, stop applying the vacuum via the three-way tap and wait until the frothing has died down and then continue to lower the pressure until the three-way tap is fully open.
9. **Check whether the air bleed is too coarse**, producing large bubbles and vigorous splashing in the distillation flask. If so, place a piece of pressure tubing (about 2 cm long) on the end of the air bleed and constrict the tubing with a screw clip to reduce the air flow through the air bleed to an acceptable rate.
10. ***Slowly* open the tap on the manometer**, allow the mercury levels to stabilize and read the vacuum by moving the zero on the scale to the lower mercury level: the reading should be 15–25 mm or better. If this pressure is not obtained, there must be leaks in the system, which usually occur at the ground-glass joints or rubber-to-glass joints. Close the tap on the manometer, check the sealing of the joints by rotating each one in turn and also check the rubber-to-glass joints by carefully pushing the rubber pressure tubing a little further onto the glass. Open the tap on the manometer and recheck the pressure.
11. ***Gently* heat the distilling flask** and carry out a fractional distillation as described in Box 15.3, collecting the fractions by rotating the 'pig'. Remember to record the temperature *and pressure* at which the fractions distil. If the liquid in the distilling flask bumps over into the condenser, you will need to clean out the apparatus with a solvent, evaporate off the solvent to recover the chemicals and restart the whole process.
12. **When the distillation is complete, close the tap on the manometer** and allow the apparatus to cool. Support the 'pig' and receiving flasks (with your hand or a 'labjack') and slowly open the three-way tap to allow air into the system. Disconnect the pressure tubing from the 'pig' and place the 'pig' and receiving flasks in a safe place. Disconnect all other tubing and apparatus for washing or storage, turn off the water pump and finally, *very gently*, open the tap on the manometer to allow the mercury levels to equalize and then close the tap.

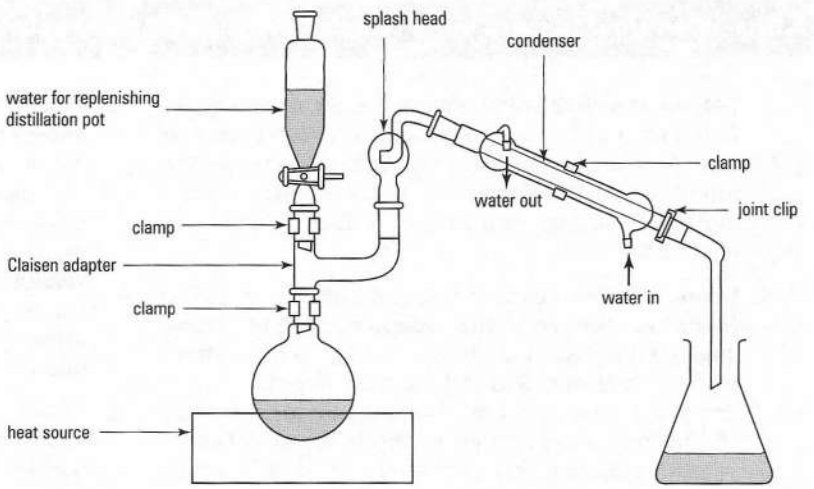


Fig. 15.9 Apparatus for steam distillation.

The steam required for a steam distillation can be provided by an external source, such as piped steam in the laboratory or steam generated by heating water in a flask, which is then piped into the distilling flask. Steam is very dangerous and the safest way to generate steam is to heat the compound with a vast excess of water in the distilling flask: steam is generated *in situ*. The equipment for steam distillation is illustrated in Fig. 15.9 and the procedure is described in Box 15.5.

KEY POINT **You must never attempt a distillation in a completely closed system. Always check that the expanding vapours can escape.**

Box 15.5 How to carry out a steam distillation

1. **Set up the apparatus for distillation** as described in Box 15.1 with the following modifications:
   (a) Use a Bunsen burner with tripod and gauze as the heat source.
   (b) Use a large three-necked flask as the distilling flask, usually 250 mL or 500 mL capacity for most laboratory procedures or a single necked flask with a Claisen head (Fig. 15.9).
   (c) Use a splash-head instead of a still-head since there is no point in recording the distillation temperature and it is more important not to contaminate the distillate by splashing from the distillation flask.
   (d) Insert an addition funnel (a separating funnel with a ground-glass joint on the stem) into the distilling flask and fit a stopper in the remaining neck of the flask.
   (e) Use a single-surface condenser: it is easier to unblock than a double-surface condenser.
   (f) Use a simple take-off adapter leading into a large conical flask since you will be collecting a large volume of water.
2. **Add the compound to be distilled to the flask via the spare neck**, half-fill the distillation flask with water, add a large portion of 'anti-bumping' granules and fill the addition funnel with water.
3. **Heat the flask until steady boiling commences and distillation of an oily emulsion begins.** Then continue the distillation adding water from the addition funnel to maintain the level of water in the distillation flask.
4. **If the distillate is a solid, it may clog and block the condenser.** If this occurs, turn off the condenser water, take the condenser tube off the tap to let the water drain out of the condenser and allow the steam to heat up the condenser and melt the solid, which will then flow into the receiving flask. Then carefully restore the water flow.
5. **The distillation is complete when no more oily emulsion is condensing** (check by collecting a few drops of distillate in a clean, dry test tube). Turn off the heat and allow the apparatus to cool before dismantling.
6. **Separate the product from the water** by extraction into a suitable organic solvent (p. 102).

16 Reflux

When carrying out reactions using volatile or dangerous chemicals or solvents – use a reflux system to keep the vapours in the apparatus even though the mixture is not heated to its boiling point.

Refluxing overnight – you *must* have the approval of your instructor for these operations and complete the necessary documentation for the night-staff. A special laboratory may be available for 'overnight' reactions.

Since the water pressure may change during the night, you must fix the coolant tubes in place, on the condenser and the tap, using copper wire twisted round the rubber tubing or plastic cable ties.

Stirring in pear-shaped flasks – special triangular-shaped 'fleas' are now available for the conical-base tubes used for evaporation and microscale reactions, but they are not yet common in the undergraduate laboratory.

Reflux is one of the most common techniques you will encounter in your chemistry laboratory classes. Since many reactions between covalent compounds are slow processes rather than instantaneous reactions, prolonged heating forces the equilibrium to give an acceptable amount of product. In the reflux process, the reactants are dissolved or suspended in a suitable solvent, the solvent is boiled and then condensed so that it returns to the reaction flask. Once set up, a reaction carried out under reflux can be run for minutes, hours or even days to promote the required reaction. The basic components for a reflux apparatus are:

- a reaction flask;
- a reflux condenser;
- a heat source (see p. 32);
- a coolant source, usually water, for the condenser.

The procedure for setting up a simple reflux apparatus (Fig. 16.1) is given in Box 16.1.

Reaction flasks

Round-bottom or pear-shaped reaction flasks are preferred, but note that stirring with the usual type of magnetic 'flea' is not possible in pear-shaped flasks. The flasks can have multiple necks so that the apparatus can be configured for temperature measurement, addition of solids or liquids, mechanical stirring and inert atmosphere work (p. 125). No matter which arrangement of components is used, always clamp the reaction flask at the neck and keep the heaviest components (such as an addition funnel containing another chemical) vertically above the flask. A condenser will still function at 30° from vertical and it is not very heavy.

Condensers

For general-purpose work, these are usually single-surface or double-surface types (Fig. 16.2): the double-surface condenser is used for low-boiling point solvents such as dichloromethane, diethyl ether or light petroleum (b.pt. 40–60 °C).

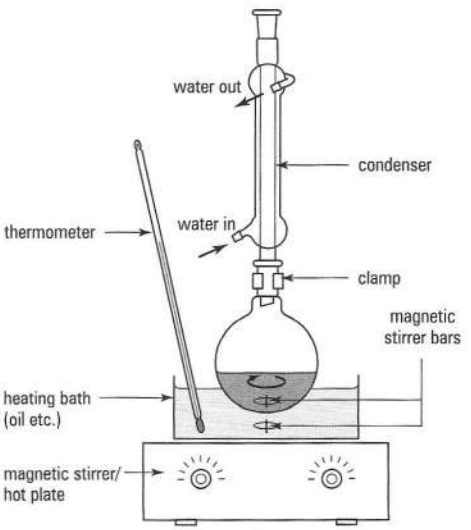


Fig. 16.1 Apparatus for simple reflux.

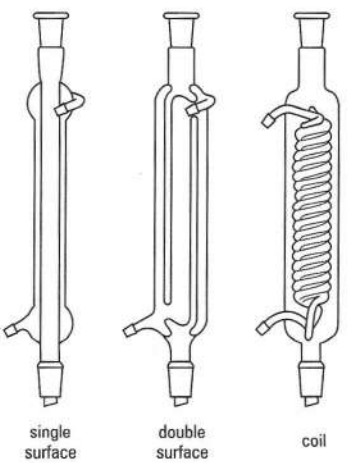


Fig. 16.2 Condensers.

Box 16.1 How to set up a simple reflux apparatus

1. **Choose a round-bottom or pear-shaped Quickfit® flask** of appropriate size so that it will be no more than half-full and clamp it to a support stand.
2. **Select the appropriate heat source and anti-bumping protection**, and adjust the height of the flask so that the heat source can be removed easily if something goes wrong.
3. **Choose a condenser of appropriate size** and type so that it will condense the volume of vapour formed in the reflux process. For example, do not use a small condenser with a 14/23 joint on a 250 mL flask, since it will not cope with the volume of vapour to be condensed, or a large condenser on a small flask, where all the solvent may be converted into vapour before condensation occurs.
4. **Add the liquids/solids and the solvent to the flask**, using the solids funnel, paper funnel or stemmed filter funnel (p. 24) so that you do not get chemicals on the inside of the ground-glass joint. If you do contaminate the joint, it may not allow the condenser to 'seat' properly and hot solvent vapour will escape into the atmosphere. Wipe the joint clean with tissue.
5. **Fit the rubber tubing to the condenser;** the lower tube is connected to the water tap so that the water flows up the condenser for the most efficient cooling.
6. **Lightly grease the joint on the condenser** and place it in the flask, rotating it to ensure a good seal. Do not clamp the condenser – gravity will keep it in place – but make sure that it is vertical. You may need to move the set-up quickly away from the heat source and it is easier to manipulate if only one clamp, on the neck of the flask, is present.
7. **Tidy the coolant pipes behind the clamp** ensuring that there are no kinks, the pipes are not touching the heat source and the outlet pipe is positioned in the drain or sink. Turn on the water gently to check for leaks and, if all is correct, turn up the water to give a steady flow.
8. **Apply enough heat to bring the liquid to the boil**, check that the solvent is refluxing at a steady rate and that the vapour is condensing no higher than one-third of the length of the condenser. The apparatus can then be left, with regular monitoring, for the reaction to proceed.

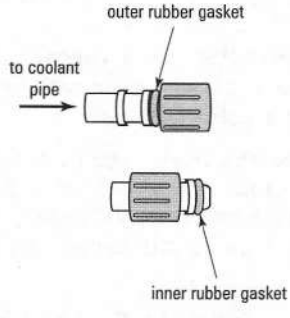


Fig. 16.3 Plastic connectors for condensers.

Refluxing anhydrous reactions – if it is necessary to exclude atmospheric water or oxygen or carbon dioxide, guard tubes are ineffective and the reaction must be carried out under an inert atmosphere (p. 125).

Safety note Always use a fresh guard tube because the action of water on the drying agent may form a solid 'cake' and seal the guard tube. This will act as a 'stopper' and pressurize the reflux apparatus when you begin heating and may cause an explosion. Do not pack the tube *too* tightly with drying agent.

Rubber tubing for coolant water is attached to the condenser in two ways:

1. If the condenser has glass inlet and outlet pipes with a 'knuckle', wet the inside of the rubber tube with a little water and slide it onto the pipe and past the 'knuckle'. The rubber tubing must be a tight fit otherwise it may slide off over a period of time.
2. Modern condensers have plastic adapters, which can be attached to the tubing and then screwed on the threaded inlet and outlet pipes. Slide the rubber tubing onto the moistened 'pipe' on the adapter, and then screw the adapter onto the condenser. You *must* ensure that the adapter has a rubber gasket on the inside of the threaded portion (Fig. 16.3), otherwise it will leak water at the condenser inlet or outlet.

KEY POINT **You should always attach rubber tubing to a condenser before fitting it to the apparatus.**

Drying tubes

Water can get into your reaction by condensation from the atmosphere or by condensation of the steam produced in a water bath. To exclude water, you should fit a guard tube containing a solid drying agent such as anhydrous calcium chloride or calcium sulphate to the top of the condenser. A typical guard tube is shown in Fig. 16.4; use a coarse-sized drying agent rather than a fine powder, which would 'cake' very quickly as it absorbs moisture.

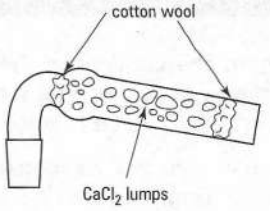


Fig. 16.4 A $CaCl_2$ guard tube.

Safety note Never attempt to use a pressure-equalizing dropping funnel as a separatory funnel because, when you invert the funnel, the solvent will flow down the side arm, past the tap and onto you or the laboratory bench!

Reflux with addition of chemicals

Instead of stopping the reaction and opening the apparatus, you can put in the new chemicals using an addition or 'dropping' funnel. Addition funnels are separatory funnels (see p. 119) with a ground-glass joint on the stem, and there are two types (Fig. 16.5):

1. Addition funnels: when you add a liquid or solution from the funnel to the reaction flask, you must take out the stopper, otherwise a vacuum is formed and the liquid does not flow out (see p. 105). This is a disadvantage when using compounds with irritating or toxic vapours or which are air sensitive. The simplest solution to this problem is to fit a guard tube, instead of a stopper, to the addition funnel and then the liquid or solution will flow easily into the reaction flask.
2. Pressure-equalizing dropping funnels: these have a side arm linking the reservoir of the funnel to the inlet stem below the tap. The pressures in the reservoir and the reaction flask are equal, and liquid will flow into the reaction flask even when the stopper is in place in the funnel. Pressure-equalizing dropping funnels are very expensive and are normally only used for inert atmosphere reactions (p. 119).

Box 16.2 How to set up the apparatus for reflux with mechanical stirring

1. **Clamp a multineck flask to a heavy support stand** at a height where you can put the heating source (mantle or oil bath) underneath, on a 'labjack'.
2. **Slide the stirring rod through the stirrer gland**, add the stirrer paddle and fit into the clamped joint of the flask. Lift the stirrer rod so that the paddle is not touching the bottom of the flask.
3. **Slide a piece of rubber pressure tubing** (about 40 mm long) half-way onto the drive shaft of the stirrer motor and fix the stirrer motor onto the support stand about 3 mm above the top end of the stirrer rod.
4. **Very carefully line up the centre of the pressure tubing on the motor** with the centre of the stirring rod and ensure that the drive shaft, stirring rod, stirrer gland and flask are aligned and vertical: look at the set-up from several different angles and adjust the clamps as appropriate until you are sure.
5. **Lift the stirring rod and slide it into the rubber tubing**, about 10 mm, and then raise the flask so that the stirrer paddle is about 2 mm above the bottom of the flask. Finally tighten all the clamps firmly.
6. **Make sure that the stirrer motor speed control is set to the minimum speed or zero.** Switch on the power and turn the speed control to the lowest setting. If the stirrer turns smoothly and slowly, all is well. You can increase the speed to check for vibration and 'whip' and then turn the speed down to zero. If the stirrer is reluctant to stir or seems 'stiff', switch off the power and readjust the apparatus.
7. **If the glass stirring rod 'slips' in the rubber tubing**, tighten the tubing round the glass rod with twisted copper wire or a plastic cable tie.
8. **Add the chemicals to the flask** (stemmed funnel) through one of the other necks, grease and fit a reflux condenser and additon funnel (if appropriate). Start the motor and increase the speed to give a steady stirring rate.
9. **Raise the heat source under the flask** and adjust the power to give steady boiling.
10. **When the reaction is complete, remove the heat source and allow the apparatus to cool to room temperature.** Switch off the stirrer and disconnect from the mains and remove the condenser and addition funnel. Carefully lower the reaction flask about 40 mm (the stirrer paddle will be lifted from the bottom of the flask by 40 mm), cut the copper wire or plastic tie holding the top of the stirrer rod in the rubber tube and *carefully* ease the stirrer rod out of the rubber connection. Unclamp the stirrer motor and put it aside. You can now remove the stirrer and stirrer gland from the reaction flask, rinsing the paddle into the flask with a little fresh solvent.

Adding a chemical to a refluxing reaction – many reactions are exothermic, so you should add the new chemical to the reaction at such a rate that the height of the refluxing vapour in the condenser does not change much.

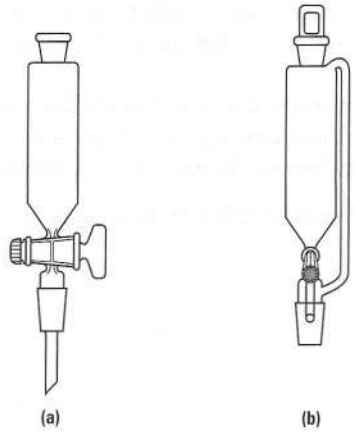


Fig. 16.5 Adding chemicals to a reflux apparatus: (a) addition funnel; (b) pressure-equalizing funnel.

The addition of dry solids to refluxing reactions requires special equipment and you should refer to the appropriate texts (Errington, 1997, p. 125; Harwood, *et al.*, 2000, p. 88; Furniss, *et al.*, 1989, p. 82). The simplest approach is to dissolve the solid in a small amount of the solvent being used and add it as a solution, using an addition funnel.

Reflux with mechanical stirring

When a magnetic stirrer is unsuitable, e.g. in reactions involving viscous liquids or mixtures of solids and liquids, a mechanical stirrer must be used. A mechanical or overhead stirrer system comprises:

1. An electric variable speed motor connected to the mains supply.
2. A flexible connector, usually a short length of rubber tubing.

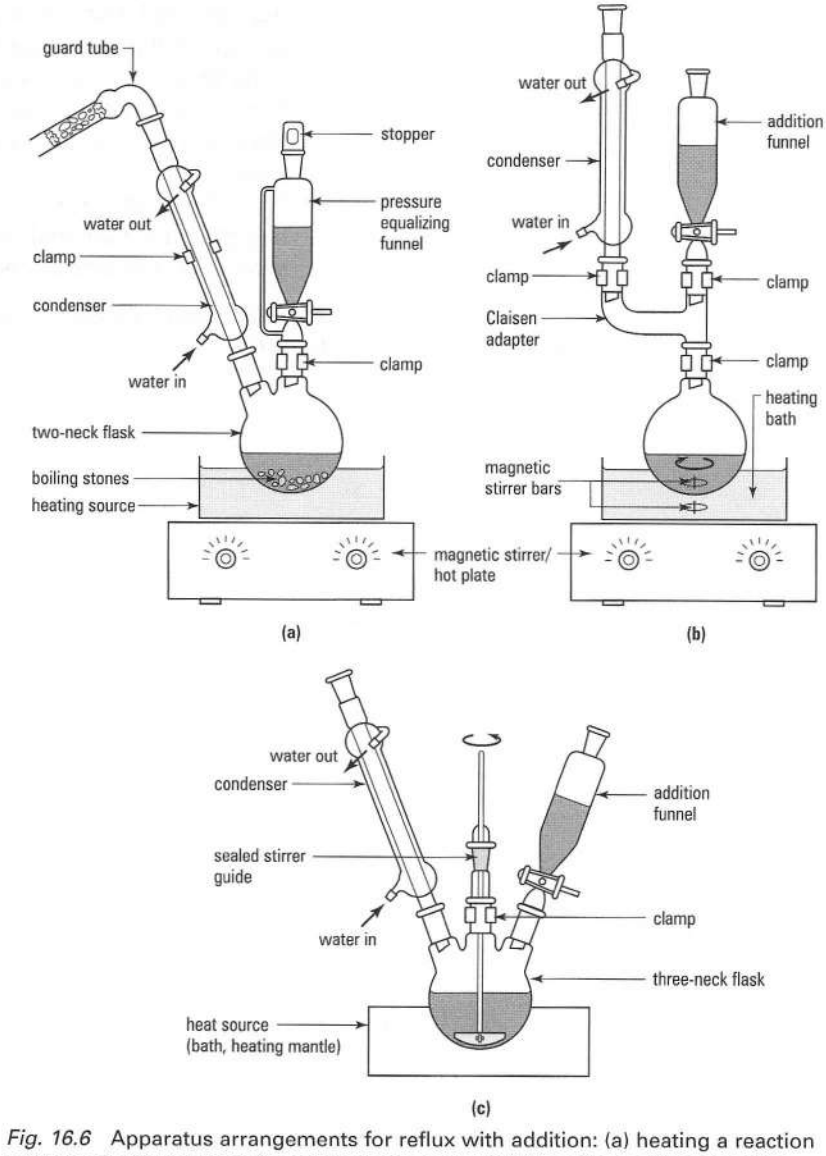


Fig. 16.6 Apparatus arrangements for reflux with addition: (a) heating a reaction mixture to reflux during the addition of a reagent; (b) heating a stirred reaction mixture during the addition of a reagent; (c) heating and overhead stirring of a reaction mixture during the addition of a reagent.

3. A stirrer gland, which fits into the top ground-glass joint of the flask acting as a seal for the refluxing vapour and a guide for the stirrer shaft. There are several types of gland available, but the best is now made from Teflon® and needs no lubrication. If this is not available, a screwcap adapter in which the rubber sealing ring has been lubricated with a touch of silicone oil can be used. Note that the stirrer shaft should rotate in the adapter, not the adapter in the joint of the flask, so do not tighten the screwcap too much.
4. A Teflon® paddle which swivels on the end of the stirrer shaft so that it can pass through the ground-glass joint on the top of the flask.

Setting up the apparatus for reflux with mechanical stirring is a precision task and is described in Box 16.2. The major problems encountered are:

- The weight of the stirrer motor high up on the support stand: use a large support stand with a heavy base or use a support framework, which is screwed to the laboratory bench. Besides the motor's weight, the torque of the motor can cause 'whipping' in the support stand.
- The motor, stirrer gland, stirrer shaft and reaction flask must be absolutely vertical and concentric, otherwise the glass stirrer shaft will snap.
- Since there will always be a little sideways movement when the stirrer is operating, the flask and the motor should be clamped in position *on the same stand.* Condensers and addition funnels should not be clamped.

A selection of configurations, suitable for most reflux procedures is shown in Fig. 16.6.

17 Evaporation

Evaporation is the process in which the solvent of a solution is converted into a vapour to leave a solid or liquid solute. There are many applications of evaporation, ranging from the slow evaporation of water from a solution of an ionic compound to leave hydrated crystals, e.g. crystallization of $CuSO_4.5H_2O$, to the evaporation of large volumes of low-boiling-point solvent under reduced pressure in the extraction of an organic compound. Since crystallization by evaporation is a specific technique for a relatively small range of compounds, the term evaporation is generally interpreted as the removal of the solvent from a solution.

Evaporation – the purpose of evaporation is to remove the solvent from a solution. Purification or separation of the components of the solute is by other means, such as recrystallization or distillation.

Evaporation of solvents

There are three commonly used techniques for solvent evaporation:

1. Evaporation from open flasks on a steam bath.
2. Rotary film evaporation.
3. Gas 'blow-down'.

All these techniques have advantages and disadvantages. Where your experimental protocol may simply state 'the solvent is evaporated off', you should select the most appropriate procedure based on:

- the volume of solvent to be removed;
- the amount of solute in solution;
- the relative boiling points of the solvent and solute;
- the next step in the experimental procedure.

Evaporation from open flasks

Safety note Clamp the conical flask in position on the steam bath. There is particular risk of the flask falling over when using small conical flasks (< 100 mL) and a glass rod.

This is useful for evaporating small volumes (~ 25 mL) of low-boiling-point solvents ($< 70\,^{\circ}C$) from solutions containing a solute which has a boiling point above 110 °C. Place the solution in a beaker or conical flask, containing a glass rod or boiling stick, onto a steam bath (maximum temperature achievable is 100 °C) in a fume cupboard and evaporate the solvent until boiling ceases. The obvious advantage is simplicity; the disadvantages include environmental concerns of release of solvents in the atmosphere, contamination of the solvent by condensed steam and incomplete solvent removal due to the 'hold-up' volume of the flask.

Rotary film evaporation

Using 'rovaps' – these are communal so make sure that the 'rovap' is clean before you use it and clean it up after use. Empty the solvent collection flask into the appropriate waste solvent bottles.

This method, which is also known as rotary evaporation or 'rovap', is the technique of choice for the removal of large volumes of volatile solvents from solutions, e.g. from extractions and column chromatography (p. 217). Rotary evaporators are now standard communal pieces of equipment in the laboratory and the operating principle is that of a reduced-pressure distillation except that the evaporation flask can be rotated. This rotation reduces the risk of 'bumping', inherent in all reduced-pressure distillations, and spreads the solution in a thin film on the walls of the flask. This effectively increases the surface area of the solution and increases the rate of evaporation, which is further enhanced by the use of a vacuum.

Box 17.1 How to use a rotary film evaporator

1. **Check that the apparatus is ready for use by ensuring that:**
   (a) The receiving flask is clipped in place and is empty.
   (b) You have available the correct-size ground-glass joint adapters to connect your flask to the rotating 'barrel' protruding from the motor of the evaporator. Many rotary film evaporators have an 'odd' joint size, usually 29/32, which is not common to the routine ground-glass flasks used in the laboratory. Alternatively, special bulb-shaped flasks with 29/32 joints may be available.
   (c) The rotating barrel is 'clipped' in place in the motor, by pulling it gently. Someone may have had to clean out and reassemble the 'rovap' and, if the barrel is not 'clipped' in place, it will slide out when you attach your flask. If the barrel slides out of the motor when you pull it, consult your instructor.
   (d) The rotating barrel is clean and dry.
   (e) Water is flowing steadily through the condenser. If it is not, adjust the water tap.
   (f) The temperature of the water in the water bath is about 20 °C below the boiling point of the solvent to be removed.
2. **Open the vacuum inlet adapter** at the top of the condenser, and turn the water pump to maximum (p. 30).
3. **Fill the rotating flask half-full or less**, using a stemmed filter funnel. You must not contaminate the joint of the rotating flask with solute, which will be deposited there during evaporation, since you may not be able to remove the flask after evaporation. If the flask is too full it may 'bump', sending solution up into the condenser and receiver. You will then have to dismantle and clean out the equipment with an appropriate solvent to recover your compound.
4. **Raise the apparatus using the lifting mechanism** so that, when the flask is attached, it is not touching the water in the bath. On modern equipment the lifting system is an electric motor controlled by an 'up-down' pressure switch, but on older apparatus the lifting device is either (i) a manual handle with a trigger, which is pulled to lift and released to lock it in place, or (ii) a lever with a twist-grip on the end. To operate the latter mechanism, twist the grip anti-clockwise to release the 'lock', pull the lever down to raise the apparatus and twist the grip clockwise to lock it in place.
5. **Attach the flask to the barrel** and put plastic joint clips on all the joints, while supporting the flask with your hand. If the weight of the flask and contents 'springs' any of the joints, it is too heavy: replace it with a smaller flask or remove some of the solution. *You must not rely on the power of the vacuum to hold your flask in place.*
6. **Turn on the motor**, slowly increasing the speed to the maximum.
7. **Close the vacuum inlet adapter slowly** until it is fully shut and observe the flask for a few seconds. If boiling occurs (liquid is condensed to the receiver flask) continue until boiling stops and then lower the flask so that it just touches the surface of the water, lock it in place and boiling should recommence. As the volume of solution decreases, you may need to lower the flask further into the water bath until all the solvent has been evaporated. If a white coating of frost forms on the flask evaporation may stop, because the flask is too cold: lower the flask into the water bath to warm it and evaporation should begin again.
8. **When evaporation is complete**, raise the flask from the bath, switch the motor off, open the vacuum inlet tap to allow air into the system and allow the flask to cool. Support the flask with your hand, take off the plastic joint clips, put the flask on one side and *only then* turn off the water pump. Turn off the water supply to the condenser.
9. **Unclip the receiving flask**, dispose of the solvent into a waste solvent bottle and reattach the receiving flask to the apparatus.

KEY POINT **When using a 'rovap' you must check that the reduced-pressure boiling point of the solute you are trying to isolate is below the temperature of the water bath.**

There are many variations in the details of the form of rotary film evaporators and a typical assembly is illustrated in Fig. 17.1. A general guide to the use of a rotary evaporator is given in Box 17.1.

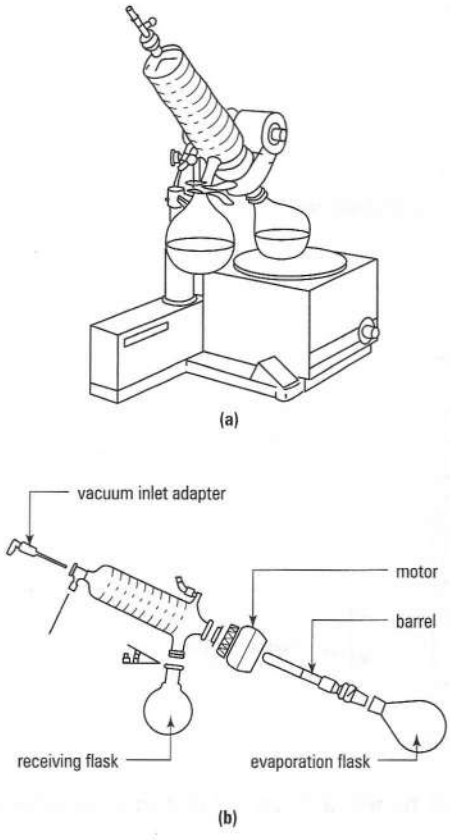


Fig. 17.1 (a) Typical examples of a rotary evaporator; (b) exploded view of glassware.

Transferring viscous liquids – it is often difficult to transfer small amounts of viscous liquids from a 'rovap' flask to a small sample tube. Dissolve the liquid in a small amount of dry solvent, transfer a little of this solution (1 or 2 mL) to a suitable small tube and 'blow off' the solvent with nitrogen.

Safety note Make sure that you do not put the tip of the Pasteur pipette too close to the liquid surface or you will blow the liquid out of the tube! Test the flow *first.*

When using rotary film evaporators you should take note of the following safety advice:

- Never use flat-bottom flasks or conical flasks under reduced pressure (p. 110).
- Always check your flask for 'star'-cracks.
- Always make sure that your solution has cooled to room temperature before you begin, otherwise it may boil vigorously and 'bump' when you apply the vacuum, before it is lowered into the water bath.
- Do not rush to lower the flask into the water bath: wait to see what happens to the extent of evaporation at room temperature.
- Always have the water bath just warm, not hot, at the start of the procedure. If the water bath is too hot, allow it to cool or add cold water or ice.
- Check that all joints are 'sealed' and that the water pump is producing a vacuum: it will change 'note' as the vacuum is produced, when it is working properly. If there is no vacuum, the solution may not boil and you will overheat it in trying to promote evaporation. The joints may suddenly seal and the solution will then boil vigorously under the reduced pressure and will 'bump' into the condenser and receiving flask.

If it is necessary to evaporate volumes of solvent greater than the capacity of the rotating flask, you can carry out the process batch-wise involving several separate evaporations or the rotary evaporator can be modified for continuous evaporation (Fig. 17.2). A thin Teflon® tube is attached to the vacuum inlet adapter so that it feeds down the condenser into the 'barrel' and another glass tube, dipping into the solution to be evaporated, is connected to the air inlet on the vacuum adapter. Once the rotary evaporator is operating, the tap on the vacuum adapter is opened a little. Solution is drawn up by the vacuum, runs into the rotating flask and is evaporated. Careful control of the tap allows a constant volume of solution to be sucked into the rotating flask and evaporated without overfilling it.

Gas 'blow-down'

This procedure is useful for removing very small volumes of solvents (about 2 mL) from solutes by blowing a stream of nitrogen over the surface of the solution, while warming the solution gently. The main application of the gas blow-down is in the isolation of small amounts of solute from rotary evaporation or small-scale liquid–liquid extraction, for further analysis by instrumental techniques, where the sample size may be 20 mg or less: for example, infrared spectroscopy (p. 180), NMR spectroscopy (p. 190), gas chromatography (p. 211) or liquid chromatography (p. 218).

The simplest system for evaporation by gas blow-down is shown in Fig. 17.3. A Pasteur pipette is connected by a flexible tube to a cylinder of nitrogen, which has a gas blow-off safety system (p. 125). The sample is placed in a special tube with a conical base, such as a ReactiVial®. Hold the Pasteur pipette and direct a gentle stream of nitrogen towards the side of the tube so that it flows over the surface of the liquid. As the solvent evaporates, the liquid and tube will cool and may condense atmospheric water into the tube. To prevent condensation, clamp the tube in a warm sand bath or above a closed steam bath or in the hole of a purpose-designed aluminium heating

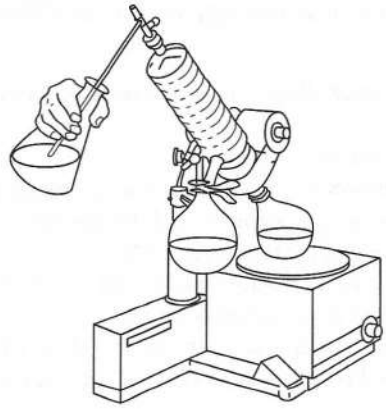


Fig. 17.2 The procedure for continuous solvent removal using a rotary evaporator.

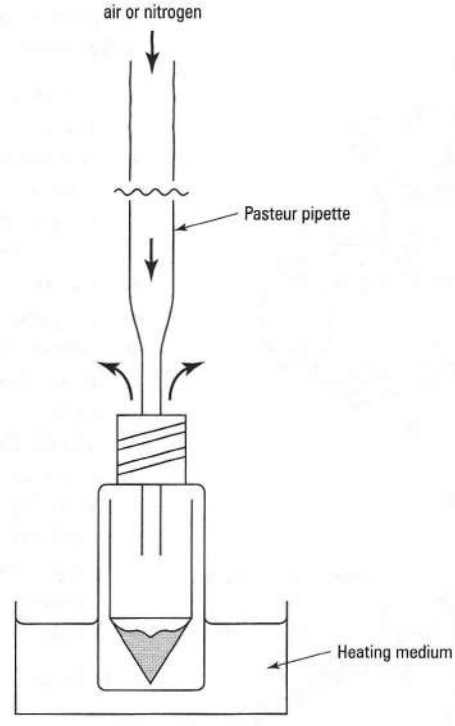


Fig. 17.3 Gas 'blow-down' evaporation.

block, The following points should be noted when using a gas blow-down system:

- Always carry out the operation in a fume cupboard.
- The solute should have negligible vapour pressure at room temperature, otherwise it may co-evaporate with the solvent.
- Do not heat the solution to boiling. Only apply enough heat to prevent condensation of atmospheric water vapour.

18 Inert atmosphere methods

Inert atmosphere reactions – these should be done in the fume cupboard, since most of the solvents and reagents used are flammable and/or toxic.

Nitrogen atmospheres – note that lithium metal reacts slowly with nitrogen.

Use of argon – reactions carried out under argon can be opened to the atmosphere briefly (~5 s), for the addition of other chemicals, without degradation of the inert atmosphere.

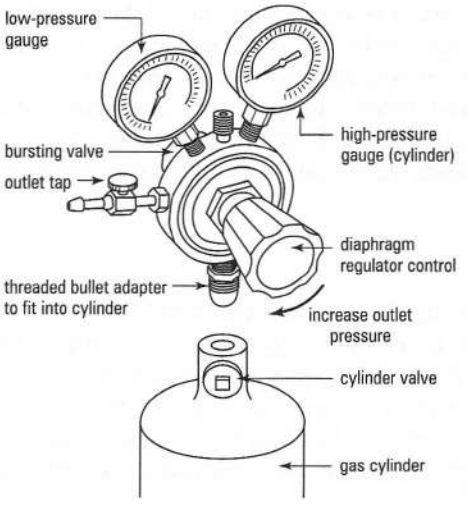


Fig. 18.1 The diaphragm pressure regulator.

Safety note Gas cylinders must be supported safely either by clamping to the bench using a special cylinder clamp or in a cylinder trolley.

Inert gas flow rate – only low flow rates are required to provide an inert atmosphere, once the apparatus has been 'swept' with the gas.

During your laboratory work you may need to carry out reactions using chemicals which are described as *air sensitive* or *moisture sensitive*. These compounds may react with water, oxygen, carbon dioxide and even nitrogen (e.g. lithium metal). The most sensitive chemicals may require special apparatus such as glove boxes or vacuum line equipment and you should consult the appropriate specialist literature (Errington, 1997, p. 56). On the other hand, many reactions involving some air-sensitive reagents (e.g. organolithium compounds or hydride reducing agent) can be done on a small scale using standard glassware with appropriate modifications. For simple apparatus for inert atmosphere reactions, the basic requirements are:

- Inert atmosphere, usually nitrogen or argon, piped into the apparatus. Nitrogen is the most commonly used inert gas, whereas argon is more expensive but does have the advantage that it is denser than nitrogen and is not lost from the apparatus as quickly. The inert atmosphere is maintained in the apparatus by the use of a 'bubbler' (see p. 126) on one of the outlets from the glassware – all other outlets must be stoppered or capped with septa.
- Appropriate glassware for the experiment (see Chapter 16) which must be dry.
- Dry solvents and chemicals (p. 127).
- Syringe techniques for dispensing and transferring chemicals to the apparatus (p. 127).

Inert atmosphere

The source of the inert atmosphere is usually a cylinder of nitrogen or argon gas under pressure, which should be placed as close to the apparatus as possible to avoid long 'runs' of connecting rubber tubing.

Gas cylinders

The gas flow rate from the cylinder is controlled by the cylinder head regulating valve (Fig. 18.1). Before you start make sure that the regulator outlet tap is off (turn anti-clockwise until it feels 'free') and then open the valve to the cylinder with the cylinder spanner (turn anti-clockwise) and the cylinder pressure should be indicated on the right-hand pressure dial. Switch on the gas at the regulator (turn slowly clockwise) until there is a reading on the left-hand dial. Use the minimum pressure required to provide a steady flow of gas. The gas flow rate from the regulator can be controlled further by a needle valve on the regulator outlet, if one is fitted. To switch off, reverse the instructions above.

Connection to the apparatus

Use clean, dry, thin-walled rubber tubing and special adapters with ground-glass joints to connect the tubing to your reaction flask or to the inlet pipe of a 'bubbler'. Where a single cylinder supplies several outlets, e.g. in a fume cupboard, the gas flow rate may change markedly when someone turns off one of the outlets, resulting in an increase in gas pressure to your equipment. You should, therefore, fit a gas 'blow-off' valve between your gas supply and

the apparatus. To do this, fit a glass tube 'T-piece' in the gas line to your apparatus and connect it to a Dreschel bottle containing mineral oil to a depth a little more than that of the mineral oil in the 'bubbler'. If there is a sudden upward change in gas pressure, the gas will be vented through the Dreschel bottle, as well as through your apparatus.

Gas 'bubblers'

Using a gas 'bubbler' – this ensures that your apparatus is not a sealed system, which will pressurize as you introduce the inert gas.

The exit of the inert gas from the apparatus must be protected by a gas 'bubbler'. The 'bubbler' allows you to monitor the flow of inert gas through the apparatus and prevents the entry of air into the apparatus. Several designs of gas 'bubbler' are available (Fig. 18.2) and it is usual to connect the 'bubbler' to the apparatus at the top of the condenser. You should make sure that the 'bubbler' contains enough mineral oil to create a seal from the atmosphere and that it has a bulb above the mineral oil to collect any mineral oil, which could be sucked back into your apparatus if there is a sudden contraction in the volume of inert gas in the apparatus. Such changes in volume will occur if you suddenly cool the apparatus without increasing the gas flow to compensate for the resulting reduction in inert atmosphere volume.

Apparatus

Guard tubes – these absorb relatively little atmospheric water and do not absorb oxygen and carbon dioxide. Always use a gas 'bubbler' to protect the exit from your apparatus.

Depending upon the type of reaction to be carried out, one of the assemblies shown on p. 119 may be used, with additional modifications for inert gas inlet and outlet. You should consider very carefully what is required: heating or cooling, magnetic or mechanical stirring, temperature measurement, etc. The gas inlet can be either directly into the reaction flask or into the inlet arm of the gas 'bubbler' – it depends on the number of 'necks' available on the reaction flask.

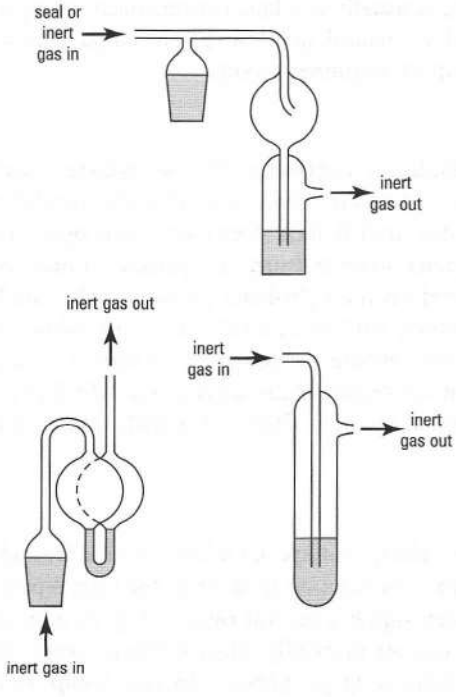


Fig. 18.2 Typical 'bubblers'.

Drying glassware

All equipment to be used should be dried (e.g. in an oven overnight at 125 °C – do not forget to remove all plastic or Teflon® components before placing the glassware in the oven). After drying, the apparatus should be greased, assembled hot, using heat-resistant gloves as protection, and allowed to cool with the inert gas flowing rapidly through it. Once cool, the water connections for the condenser should be fitted – screw-on water connectors (p. 118) are most useful in this context.

Addition of chemicals

Chemicals should be added to the reaction flask using a pressure-equalizing dropping funnel (p. 119). Liquids and solid compounds are best added as solutions in the solvent used in the reaction. If the solid is insoluble, a little solvent should be added to the reaction flask, the 'bubbler' outlet sealed, a stopper to the flask opened and the gas flow rate increased. The solid can then be added from a wide-stemmed filter funnel, protected by the inert gas and solvent vapour, so that air does not enter the apparatus. Then, simultaneously unseal the 'bubbler' and restopper the flask, and then turn down the gas flow. Alternatively a solids addition tube (Errington, 1997, p. 124; Harwood, *et al.*, 2000, p. 89; Furniss, *et al.*, 1989, p. 82) can be used.

Solvents and chemicals

Dry solvents – the term also implicitly means carbon dioxide-free and oxygen-free.

All chemicals and solvents used in inert atmosphere reactions must be dry. Most of these materials provided by suppliers are not dry enough, even solvents which you consider to be immiscible with water, and may contain enough moisture to hinder the reaction or reduce the yield of your product. Therefore you must ensure that all chemicals to be used in the process have been dried to the appropriate levels, as described below.

Solid chemicals

These should be dried by the methods outlined on p. 39. The most common approach is to dry the chemical in an oven and then allow it to cool in a vacuum desiccator (p. 40). Techniques for extremely air-sensitive solids can be found in the specialist literature.

Liquid chemicals

All liquids should be dried by a method appropriate to the amount of water they may contain (p. 41). Generally, the liquid should be dried with a solid drying agent (p. 41) which does not react with the chemical (consult the appropriate literature or your instructor), filtered, distilled (p. 107), then stored over molecular sieves (p. 41) in a bottle capped by a septum and redistilled before use. Alternatively, the liquid can be dissolved in a solvent, the solution dried (p. 41), the solvent removed by evaporation (p. 121) and the liquid distilled and stored as described above.

Solvents

Grignard reactions are relatively tolerant and dry solvent can be added to the apparatus using an oven-dried measuring cylinder.

The solvent will have the greatest volume in your reaction and it *must be dry*. Most laboratory-grade solvents, as supplied by manufacturers, contain varying amounts of water and therefore must be dried by the appropriate method before use. If you are required to dry the solvent, you should consult the literature (Errington, 1997; Harwood, *et al.*, 2000; Furniss, *et al.*, 1989).

Some manufacturers supply dry solvents in 2.5 L quantities for inert atmosphere reactions and HPLC (p. 218). These solvents are relatively expensive but may be economic in terms of time and expense if one-off reactions are required. However, such solvents should be treated with suspicion if the containers are less than half-full, since air and moisture may have been allowed into the container by previous users. If you have any doubts, dry the solvent.

Syringe techniques

Many air-sensitive chemicals are supplied as solutions in nitrogen-filled bottles, which are sealed by a septum, and small volumes (up to 25 mL) of these solutions are best transferred to the apparatus using glass syringes. Similarly, air-sensitive liquids can be added to the reaction using a syringe.

KEY POINT **When removing air-sensitive reagents from nitrogen-filled bottles, you must replace the volume of liquid removed with inert gas (nitrogen) from a gas cylinder or balloon, via a needle, otherwise air (water, oxygen and carbon dioxide) will be pulled into the bottle as a result of the vacuum you have created.**

Using syringes – always ensure that the syringe piston is the correct one for your syringe, since the components of the syringe are always separated for drying.

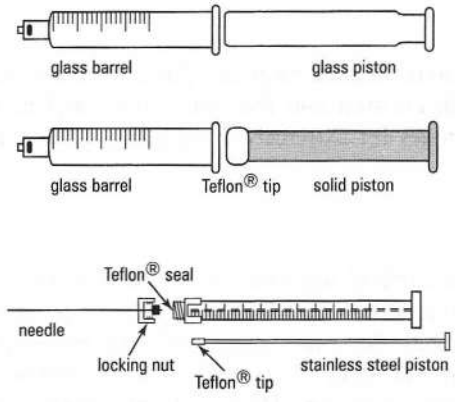


Fig. 18.3 Syringes for inert atmosphere work.

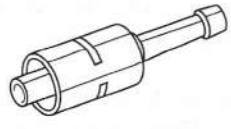


Fig. 18.4 Needle-to-tubing connector.

Syringes

Glass, gas-tight syringes with a Luer lock fitting are the most versatile type of syringe and they come in a range of sizes. The Luer lock enables the stainless steel needle to be locked in place on the end of the syringe so that there is no danger of the needle dropping off the syringe during the transfer process (Fig. 18.3). Variations in syringe types include those with Teflon®-tipped pistons (plungers), which are somewhat more expensive.

Before using a syringe, always check that it is working by sucking up a little of the solvent to be used, ensuring that air is not sucked into the syringe either via the Luer lock or down a gap between the syringe and piston. If all is correct, disassemble the syringe and needle, dry in an oven at 120 °C (not if Teflon® tipped) and allow to cool in a desiccator. Once you have transferred the air-sensitive reagent, you must clean out the syringe and needle immediately, by the appropriate method, as air will get into the needle and syringe and decompose the reagent causing the syringe to jam or the needle to block.

Needle-to-tubing connectors

These adapters allow a Luer lock syringe needle to be connected to rubber tubing (Fig. 18.4). An inert gas supply can then be piped, via the needle, into a bottle to allow the transfer of large volumes of solvent or air-sensitive reagents to the apparatus. Alternatively a balloon can be taped to a short piece of thick-walled rubber pressure tubing to provide a supply of nitrogen when withdrawing air-sensitive reagents using a syringe (Fig. 18.5).

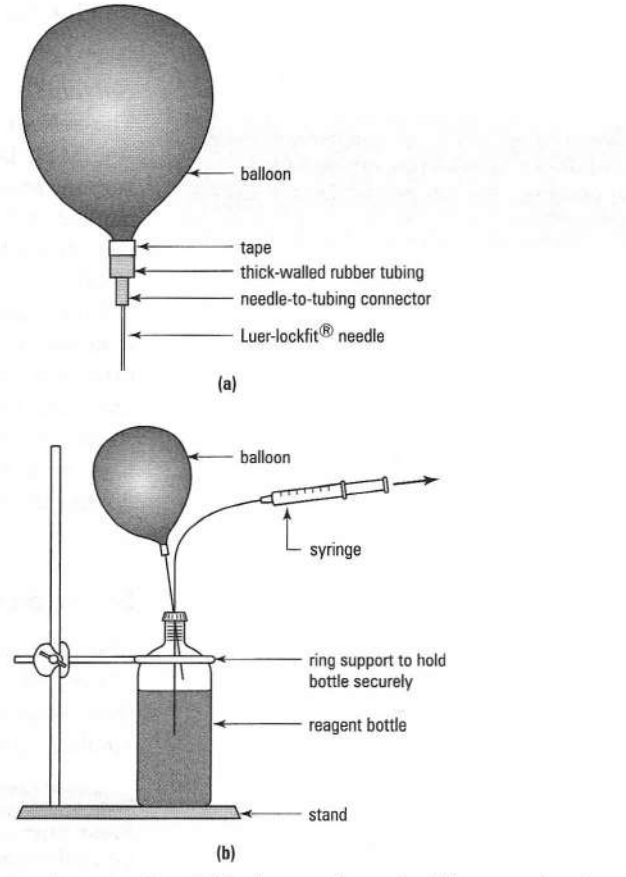


Fig. 18.5 Inert atmosphere transfers: (a) balloon and needle; (b) preserving the inert atmosphere while removing reagent.

Working with viscous liquids – attempting to draw the solution up a fine needle creates a strong vacuum in the syringe, which may result in air being pulled into the syringe via the small gap between the syringe barrel and the piston. Use a larger diameter needle.

Fig. 18.6 Needle with non-coring tip for piercing septa.

Syringe needles and cannulae

Stainless steel Luer lock syringe needles come in various lengths and diameters. The length of needle you will need depends on the size of the vessel from which you wish to withdraw the liquid; the diameter required depends on the size of the syringe – you should not use a large-diameter needle with a small-volume syringe – and the viscosity of the solution or liquid. Needle diameters are expressed in 'gauge': the higher the gauge, the narrower the needle diameter. For most inert atmosphere work you should use a needle with a 'non-coring' or 'deflecting' tip (Fig. 18.6), which ensures that a piece of the septum is not trapped in the needle when you push it through. Cannulae are long, flexible, double-ended needles made from stainless steel or inert plastics, which are used to transfer large volumes of reagents or solvents from one vessel to another under inert gas pressure (Fig. 18.7).

A generic method for transferring an air-sensitive reagent, by syringe, from a bottle to a pressure-equalizing dropping funnel is described in Box 18.1.

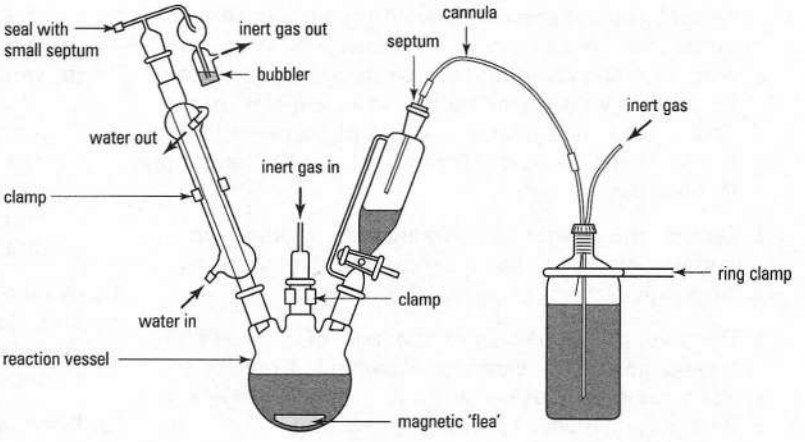


Fig. 18.7 Transfer of air-sensitive reagents using the double-ended needle technique.

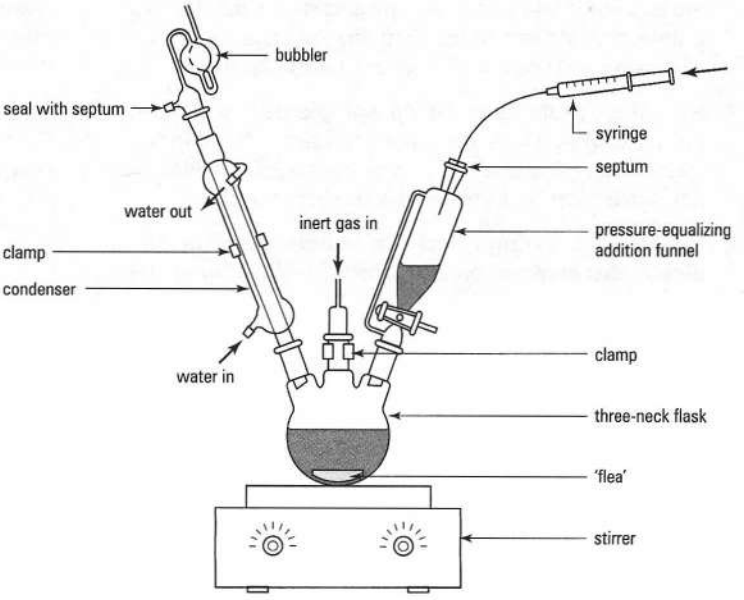


Fig. 18.8 Transferring an air-sensitive reagent to a pressure-equalizing dropping funnel.

Box 18.1 How to transfer an air-sensitive reagent using a syringe

1. **Assemble the apparatus (Fig. 18.8) while hot and allow to cool while nitrogen is flowing through it** and then add all the chemicals and solvents as required.
2. **Assemble the syringe and needle, making sure that the needle is locked in place** having first checked that it is air-tight.
3. **Draw a few millilitres of solvent into the syringe,** invert it and squirt the solvent and any air into the waste solvent bottle. Repeat this step three times to ensure that syringe and needle contain only solvent vapour.
4. **Using the syringe, inject a few millilitres of the dry solvent into the pressure-equalizing dropping funnel,** either via the septum or by quickly removing the stopper while diverting the gas flow through funnel by putting your finger tip over the 'bubbler' outlet. This ensures that there is an inert atmosphere in the funnel. Replace the stopper and release the gas flow through the 'bubbler'.
5. **Clamp the bottle of air-sensitive reagent to a support stand** so that it cannot fall over while you manipulate the syringe needles.
6. **Remove the needle from the end of a needle-to-tubing connector carrying a balloon.** Connect the inert gas supply using clean, dry rubber tubing and inflate the balloon. Turn off the gas supply, twist the neck of the balloon to stop the gas escaping, reattach the needle, ensuring it is locked in place, and release the neck of the balloon. Dip the end of the needle into a little dry solvent to confirm that nitrogen is being released from the needle – check for bubbles.
7. **Hold the needle near the tip and pierce the septum on the bottle.** Make sure that the needle tip is in the space above the liquid. Support the needle connector and balloon by a clamp on the support stand.
8. **Holding the syringe and the needle near the tip, pierce the septum on the bottle.** Still holding the needle, ease the syringe needle into the space above the liquid.
9. **Draw up some gas into the syringe** and expel it back into the bottle. Repeat three times and then ease the syringe needle into the liquid.
10. **Draw up the required volume of solution into the syringe and carefully ease the needle out of the bottle.** Make sure that you do not press the syringe piston and squirt the reagent out of the syringe!
11. **Inject the solution into the pressure-equalizing dropping funnel, either by:**
    (a) holding the syringe needle near the tip, piercing the septum on the pressure-equalizing dropping funnel and injecting the reagent; or by
    (b) putting your finger over the 'bubbler' outlet and removing the stopper from the pressure-equalizing dropping funnel. You can now inject the solution into the funnel, protected by the nitrogen and solvent vapour coming out of the funnel neck. Replace the stopper and release the gas flow through the 'bubbler'.
12. **Draw a few millilitres of dry solvent into the syringe and inject them into the pressure-equalizing dropping funnel.** This rinses any residual reagent from the syringe.
13. **Draw some methanol or another solvent which reacts gently with the reagent to destroy the air-sensitive reagent and squirt it into an excess of water.** To clean the syringe assembly, draw water into the syringe several times and then disassemble it, wash well with propanone (acetone) and water, and then dry in the oven.
14. **Remove the needle–balloon assembly from the reagent bottle,** cover the holes in the septum with a little hydrocarbon grease and screw the bottle cap in place. Place the needle in the oven after washing with propanone (acetone) and then water.

Resources for laboratory techniques

Books

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Videos

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Classical techniques

19 Qualitative techniques for inorganic analysis

Qualitative techniques are used to identify cations and anions in aqueous solution by simple reactions, mostly involving the production of a precipitate, evolution of gas or a visual colour change. It is important to make observations accurately and to interpret them in a step-wise manner.

The following basic equipment is required to carry out qualitative analysis:

- Test tubes – in which the reactions are performed.
- Cork or rubber stoppers – for the protection of the contents of test tubes from contamination and for safe storage.
- Test tube rack – this allows test tubes to be stored upright when not in use.
- Test tube holder – this allows individual test tubes to be heated safely.
- A glass rod - this has several functions including the stirring, transfer of solutions, and the break-up of precipitates.
- Watch-glasses these have several functions including the covering of beakers to prevent contamination and as a receptacle for solutions.
- A wash bottle containing distilled water.
- Spatula – for transferring small quantities of solids.
- Pasteur pipettes – for transferring liquids.
- Micro-Bunsen burner – for heating solutions to boiling and for evaporating solutions.
- Evaporation crucible – this is used as a container for solutions when complete evaporation of liquid is required, leaving a solid product.
- Crucible tongs – for removing the crucible from the heat source.
- Centrifuge – for separating precipitates from solution.
- Heated water bath.

Wash bottle – always keep a wash bottle of distilled water handy.

Table 19.1 Typical reagents for qualitative analysis

2 M NH_4OH	Conc. HCl
2 M NaOH	0.1 M HNO_3
2 M $AgNO_3$	2 M HNO_3
2 M CH_3COOH	Conc. HNO_3
2 M $BaCl_2$	2 M H_2SO_4
0.1 M HCl	Conc. H_2SO_4
2 M HCl	

Reagents

At the start of your experimental work always check that the appropriate reagents are readily available (a list of commonly used reagents is given in Table 19.1). Note that it is essential to use distilled water in all qualitative analysis. Tap water contains ions such as Ca^{2+}, Mg^{2+}, Fe^{2+}, Fe^{3+}, SO_4^{2-} and Cl^- and its use could lead to 'false-positive' results.

Testing for anions and cations

Specific literature containing tests for the determination of anions and cations can be found in the resources section (p. 160). In general, however, the following tips are useful when carrying out qualitative analysis:

Spatula – never place the spatula in the test solution, it may lead to false-positive tests for iron and chromium.

- Always work tidily to prevent cross-contamination of samples.
- Ensure that all glassware has been cleaned thoroughly in detergent and then rinsed twice with distilled water. Invert the test tubes to drain; never dry the inner surface with towelling or tissue.
- Label test tubes at the start – it may prove difficult to remember what you have done later on.

Qualitative tests for cations and anions

An unknown solution was tested as follows:

Test	Observation	Conclusion
2 drops of dilute HCl, boil, then add 1 drop $BaCl_2$ solution	White precipitate	Sulphate (SO_4^{2-}) present

Test performed on unknown solution | Report of the observations made | Conclusion drawn from the observation

Fig. 19.1 Recording your observations in qualitative analysis.

A 'clear' solution is transparent; a 'colourless' solution has no colour.

- Always test solutions with a known composition before you attempt to analyse solutions with an unknown content. This allows you to gain the necessary experience in solution manipulation, observation skills and the interpretation of results.
- The colour of solutions and/or precipitates has to be interpreted from written or oral information. Interpretation of colour can be subjective, so you will need to gain sufficient experience using solutions of known content to establish how a particular colour appears to you.
- Establish a protocol for recording of observations after carrying out different tests (Fig. 19.1).
- Reagents should be added from Pasteur pipettes held with the tip just above the mouth of the test tube. Never put Pasteur pipettes inside test tubes as this can lead to contamination of the reagents.
- Effective mixing of the test solution and added reagents is essential. This can be achieved by holding the test tube between the thumb and index finger of one hand and 'flicking' the tube with the index finger of your other hand. Alternatively, solutions can be mixed by bubbling air from a Pasteur pipette held at the bottom of the test tube.
- Evolved gas can be drawn up into a Pasteur pipette and then bubbled through a test solution, e.g. CO_2 can be drawn into a Pasteur pipette and then 'blown' out through lime water ($Ca(OH)_2$ solution) giving a milky-white solution.
- Solutions can be tested for pH using litmus paper. Never place litmus paper directly into the test solution. Instead, dip a glass rod into the solution, remove, touch the wet glass rod onto the litmus paper and note the colour. Acidic solutions change blue litmus paper to red; alkaline solutions change red litmus paper to blue. Alternatively, universal indicator paper can be used. In this case, the orange paper turns 'reddish' with acidic solutions and 'bluish' with alkaline solutions. By comparing any change in colour with a chart (supplied with the universal indicator paper), the pH of the solution can be estimated.

Centrifugation of solutions

Centrifugation causes particulate material to accumulate at the bottom of the test tube. The procedure for centrifuging your sample is described in Box 19.1. The speed and time of the run will depend on the centrifuge available, but will typically be in the range 5000–10 000 rpm for 5–10 min, respectively. Always allow the centrifuge to stop in its own time, as abruptly halting the centrifuge will disturb light precipitates. After centrifugation, hold the test tube at an angle so that it is easy to remove the liquid component (or centrifugate) with a Pasteur pipette (Fig. 19.2). You will find that it is difficult to remove all the centrifugate from the precipitate, and to maximize the transfer of centrifugate it is necessary to wash the precipitate. This is carried out as follows:

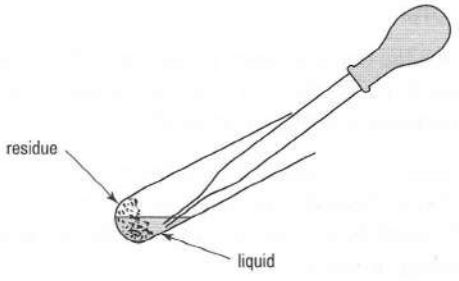


Fig. 19.2 Separation of liquid from a residue using a pipette.

- Add a small quantity of distilled water to the precipitate.
- Use a glass rod to break up the precipitate and mix thoroughly.
- Recentrifuge the mixture.
- Transfer the liquid obtained to the original centrifugate and store this solution for further analysis.
- Repeat the washing process, but this time discard the centrifugate, and retain the precipitate for further tests.

Box 19.1 How to use a low-speed bench centrifuge

1. **Choose the appropriate test tube size**, with stoppers where necessary. Most low-speed machines have four-place or six-place rotors. Use the correct number of samples to fill the rotor assembly whenever possible.
2. **Fill the tubes to the appropriate level:** do not overfill, or the sample may spill during centrifugation.
3. **Ensure that the rotor is balanced during use.** To achieve this prepare identical test tubes and place these diametrically opposite each other in the rotor assembly. However, for low-speed work, where you are using small amounts of particulate matter in aqueous solution it is sufficient to counterbalance a sample with a second test tube filled with water.
4. **If you are using centrifuges with swing-out rotors,** check that each holder/bucket is correctly positioned in its locating slots on the rotor and that it is able to swing freely. All buckets must be in position on a swing-out rotor, even if they do not contain sample tubes buckets are an integral part of the rotor assembly.
5. **Load the sample test tubes into the centrifuge.** Make sure that the outside of the centrifuge tubes, the sample holders and sample chambers are dry: any liquid present will cause an imbalance during centrifugation (as well as potentially causing corrosive damage to the rotor). Balanced tubes must be placed opposite each other, use a simple code if necessary, to prevent errors.
6. **Bring the centrifuge up to operating speed** by gentle acceleration. Do not exceed the maximum speed for the rotor and tubes used. If the centrifuge vibrates at any time during use, switch it off and find the source of the problem.
7. **On completion of the run, allow** the rotor to stop spinning, release the lid, and remove all test tubes. If any sample has spilled, make sure you clean it up thoroughly.
8. **Finally,** close the lid (to prevent the entry of dust) and return all controls to zero.

Heating test tubes and other containers

Safety note Take care when heating unknown solutions. As well as the risk of burns, some reactions can be violent.

Safety note Never point a test tube towards yourself or, for that matter, towards anyone else while evaporation is being carried out.

Safety note Never look down into a test tube (even with safety glasses on – there is still a risk of hot solution suddenly being ejected into your face). Always view coloured products through the wall of the test tube.

Safety note Noxious fumes can be given off in some instances. Be careful not to breathe them in. Always work in a well-ventilated room or a fume cupboard.

Beware – hot glass looks exactly like cold glass.

It is often necessary to heat a solution in a test tube, either to cause precipitation or to dissolve a precipitate. You can carry out this heating effectively and safely by partially immersing the test tube containing the mixture in a simmering boiling-water bath (remember to use a test tube holder!).

It is possible to reduce the volume of the solution in the test tube, i.e. to pre-concentrate the sample, by evaporation. Two different methods can be employed.

1. Transfer the solution to a small evaporating dish. Place the evaporating dish on a wire gauze located on a tripod stand, and apply heat using a micro-Bunsen burner. Note that the volumes of solutions in qualitative analysis are often small, and excessive heating might result in hardening of any residue, making it unusable.
2. Alternatively, evaporate the solution directly in a test tube by gentle heating over a micro-Bunsen burner. Remember to use a test tube holder. Position the test tube at an angle with the tip of the Bunsen burner flame positioned at the upper surface of the liquid. Place a glass rod inside the test tube and rotate constantly. This acts to disperse bubbles of steam that are given off. Extreme caution is required with this method of evaporation, as the steam bubbles can cause the solution to 'bump' out of the test tube. 'Bumping' can result in hot (and maybe toxic) substances being ejected over a suprisingly large distance. To prevent this it is normal to add anti-bumping granules (p. 31).

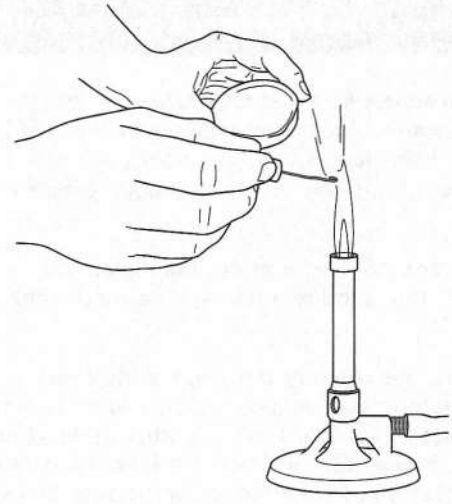


Fig. 19.3 Holding a nichrome wire in a flame test.

Table 19.2 Flame tests for cations

Cation	Flame colour
Barium	Apple-green
Calcium	Brick-red**
Copper	Green
Potassium	Lilac*
Sodium	Intense yellow
Strontium	Crimson**
Lead, arsenic, antimony and bismuth	Dull blue

* The colour is often obliterated by trace impurites of sodium present (sodium gives an intense yellow colour). You can overcome this by viewing the colour through cobalt-blue glass which allows the lilac colouration from potassium to be seen.
** Viewing through cobalt-blue glass also allows calcium and strontium to be distinguished. In this case, calcium is light green in colour while strontium appears purple.

Flame tests

Simple flame tests can be carried out on solid samples. Place a little of the solid on a watch-glass and moisten with a drop of concentrated hydrochloric acid. The purpose of the hydrochloric acid is to produce metal chlorides which are volatile at the temperature of the Bunsen burner.

Pre-clean a platinum or nichrome wire by holding it in the hottest part of the Bunsen flame (just above the central blue cone) until there is no coloured flame from the wire. Cool, then dip the cleaned wire into the moistened solid sample. Place the wire at the edge of the Bunsen flame (Fig. 19.3) and record the colour of the flame from the sample (see Table 19.2).

20 Gravimetry

Gravimetric analysis is the process of converting an element into a definitive compound, isolating this compound from other constituents in a sample and then weighing the compound (Box 20.1). The weight of the element can then be calculated from the formula of the compound and the relative atomic masses of the elements involved. You need to be able to weigh accurately, by difference, a substance to four decimal places (see p. 23).

KEY POINT **The essential component of gravimetric analysis is the transformation of the element of interest into a pure stable solid compound, suitable for weighing.**

The most common approach for isolating the element is by precipitation from a solution where it is present in ionic form (see p. 50). Ideally, the constituent under investigation is precipitated out of solution as a water-insoluble compound, so that no losses occur when the precipitate is separated by filtration, washed free of soluble impurities and then weighed.

Box 20.1 How to carry out gravimetric analysis

Suppose you wanted to analyse the amount of metal in an alloy. For example, you might want to determine the nickel content, as a w/w percentage (p. 47), in a particular sample of steel.

1. **Select an appropriate solvent for your sample**: in this example, you could dissolve the steel in aqua regia, a combination of concentrated nitric acid and concentrated hydrochloric acid in the volume ratio of 1:3 respectively.
2. **Choose an appropriate precipitant and carry out the precipitation reaction**. Here, an alcoholic solution of dimethylglyoxime could be used to precipitate nickel from a hot solution of aqua regia, by adding a slight excess of aqueous ammonia solution, forming a red precipitate of nickel dimethylglyoximate (Fig. 20.3).
3. **Filter the precipitate through a pre-weighed Gooch crucible.** This should have been previously dried in an oven at 120°C and stored in a desiccator until required.
4. **Wash the precipitate**: in this example, the nickel dimethylglyoximate can be washed with cold water until qualitative testing shows that the wash solution is free of chloride ions.
5. **Dry the precipitate in an oven**, for example, at 120 °C, and allow to cool in a desiccator.
6. **Determine the weight of the precipitated compound.** Suppose in this example that the nickel had been precipitated from steel (2.0980 g) using dimethylglyoxime (H_2DMG), giving a precipitate of nickel dimethylglyoximate ($Ni(HDMG)_2$) weighing 0.2370 g.
7. **Write out the equation for the reaction and perform the calculation.** In this example, you need to check the relative atomic masses of the elements involved. The relative molecular mass of nickel dimethylglyoximate is 288.91 g mol^{-1} (for structure see Fig. 20.3; A_r, H = 1.01; O = 16.00; C = 12.01; N = 14.01; Ni = 58.69). The equation for the reaction can be summarized as:

$$Ni^{2+} + 2H_2DMG = Ni(HDMG)_2 + 2H^+$$

According to the above equation, for each mole of Ni in the steel sample, 1 mole of precipitate will be formed. Therefore, 0.2370 g of precipitate corresponds to:

0.2370 g $Ni(HDMG)_2$ ÷ 288.95 g mol^{-1} $Ni(HDMG)_2$ = 8.20×10^{-4} mol $Ni(HDMG)_2$

The amount of nickel in the steel sample must therefore be:

8.2×10^{-4} mol $Ni(HDMG)_2 \times 58.69$ g mol^{-1} Ni = 0.0481 g Ni

The percentage weight of nickel in the steel sample is therefore:

0.0481 g Ni ÷ 2.0980 g sample × 100 = 2.29% w/w

Table 20.1 Common precipitants

Precipitant	Ion(s) of interest	Possible interferents
Dimethylglyoxime	Ni^{2+}	Pd^{2+}, Pt^{2+}, Bi^{3+} and Au^{3+}
Cupferron	Sn^{4+}	Cu^{2+} and Pb^{2+}
8-Hydroxyquinoline (oxine)	Al^{3+}	Many metals

Fig. 20.1 Structures of common precipitants.

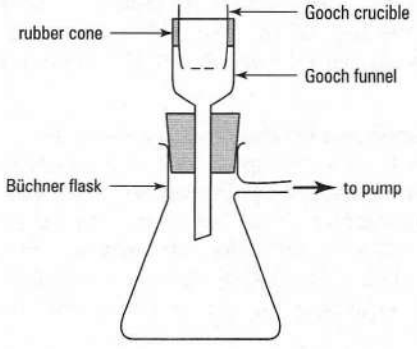


Fig. 20.2 Experimental arrangement for filtration of precipitate.

Filtering your precipitate – remember that the particle size of your precipitate must be such that it is not lost during the filtering process.

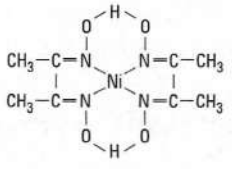


Fig. 20.3 Structure of nickel-dimethylglyoxime complex.

Precipitation

Inorganic ions can be separated from mixtures using organic reagents (precipitants), with which they form sparingly soluble, often coloured, compounds. The precipitants usually have high molecular weights, so a small quantity of the ion will produce a large amount of precipitate. Ideally, the precipitant should be specific for a particular ion, though this is rarely so. Examples of common precipitants and their target ions are shown in Table 20.1 and their structures are shown in Fig. 20.1.

Dimethylglyoxime is only slightly soluble in water ($0.40\,g\,L^{-1}$) and it is therefore used as a 1% w/v solution in ethanol. Cupferron, the ammonium salt of *N*-nitroso-*N*-phenylhydroxylamine, is used as a 5–10% w/v solution in hydrochloric acid or sulphuric acid. Oxine (8-hydroxyquinoline) is almost insoluble in water and is used as either a 2% or a 5% w/v solution in $2\,mol\,L^{-1}$ acetic acid.

When precipitating a compound:

- Mix your reagents slowly, with continuous stirring, to encourage the growth of large crystals of the compound.
- Improve the precipitation process by heating your solutions: ideally, one or both solutions should be heated to just below boiling point.
- Wash your precipitate with a dilute electrolyte solution, to remove any other constituents (it is essential to remove any impurities). Choose a solution that does not interact with the precipitate, and that is volatile at the drying temperature to be used.
- Use the minimum quantity of wash solution as no precipitate is absolutely insoluble. While suitable wash solutions include dilute electrolytes, e.g. ammonium salts, ammonia solution or acids, pure water is rarely used, as it may dissolve the precipitate.
- Test your filtered wash solution for impurities using simple qualitative tests (Chapter 19). Continue until your final washing solution contains no trace of other constituents.
- It is best to wash repeatedly with several small amounts of solution, allowing the precipitate to drain between washings.

Filtration

To carry out this procedure, you will need to assemble a Gooch crucible and funnel on a Büchner flask, clamped for stability using a retort stand (Fig. 20.2). The glass Gooch crucible has a porous disk of sintered ground glass, typically of pore diameter 20–30 μm, which is satisfactory for moderately sized precipitates. Fit the crucible into a Gooch funnel using a rubber cone, put the funnel into a one-holed rubber bung and then into a Büchner flask. The tip of the funnel must project below the side arm of the flask to prevent loss of filtrate down the side arm (see p. 28). Then, connect the Büchner flask to a water pump. Pour your precipitate suspension into the Gooch crucible, using a glass rod (p. 18) to direct the liquid into the centre of the sintered base. The lower end of the glass rod should be close to, but not touching, the sintered-glass base. Never overfill the crucible. The precipitate remaining in the bottom of the beaker should be rinsed out with the filtrate solution: disconnect the pump and pour the filtrate back into the beaker containing the precipitate. The pump should be disconnected by pulling off the vacuum tube from the Büchner flask. On no account turn off the water pump while doing this. You may need to rinse the beaker several times, to collect all of the precipitate in the crucible.

21 Procedures in volumetric analysis

Volumetric analysis, also known as titrimetric analysis, is a quantitative technique used to determine the amount of a particular substance in a solution of unknown composition.

This requires:

- A standard solution, which is a solution of a compound of accurately known concentration, that reacts with the substance to be analysed.
- The test solution, containing an unknown concentration of the substance to be analysed.
- Some means of detecting the end-point of the reaction between the standard and test solutions, e.g. a chemical indicator or, in the case of potentiometric titrations, a pH electrode (see Chapter 34). Some reactions exhibit a colour change at the end-point without the addition of an indicator.

Definition

A stoichiometric titration is one with a known reaction path, for which a chemical reaction can be written, and having no alternative or side reactions.

The volume of standard solution that reacts with the substance in the test solution is accurately measured. This volume, together with a knowledge of concentration of the standard solution and the stoichiometric relationship between the reactants, is used to calculate the amount of substance present in the test solution. Specific examples of the different types of calculations involved are shown in Chapters 22 to 25.

Classification of reactions in volumetric analysis

There are four main types of reaction

1. Acid–base or neutralization reactions, where free bases are reacted with a standard acid (or vice versa). These reactions involve the combination of hydrogen and hydroxide ions to form water.
2. Complex formation reactions, in which the reactants are combined to form a soluble ion or compound. The most important reagent for formation of such complexes is ethylenediamine tetra-acetic acid, EDTA (as the disodium salt).
3. Precipitation reactions, involving the combination of reactants to form a precipitate.
4. Oxidation–reduction reactions, i.e. reactions involving a gain (reduction) or loss (oxidation) of electrons. The standard solutions used here are either oxidizing agents (e.g. potassium permanganate) or reducing agents (e.g. iron (II) compounds).

What can be measured by titration?

- The concentration of an unknown substance e.g. 0.900 mol L^{-1}.
- Percentage purity, e.g. 56%.
- Water of crystallization, e.g. $(NH_4)_2SO_4.nH_2O$.
- Percentage of a metal in a salt, e.g. 12% Fe in a salt.
- Water hardness, e.g. determination of the concentration of calcium and magnesium ions.

Examples of the types of calculations used in volumetric analysis are shown in Box 21.1.

Box 21.1 Types of calculations used in volumetric analysis – titrations

In titrations you react a solution of a known concentration with a solution of an unknown concentration.

If you know the mole ratio of the two reacting chemicals in solution, you can calculate the amount (the number of moles and thus the number of grams) of the solute in the solution of unknown concentration.

Let's look at the reaction between NaOH and HCl:

$$HCl + NaOH = NaCl + H_2O$$

Since the equation is balanced we know that 1 mol (36.5 g) of HCl will react with 1 mol (40 g) of NaOH. We know that a 1.0 M solution of HCl contains 1 mol of HCl in 1000 mL (1 litre) of water. Then:

1000 mL of 1.0 M HCl solution is equivalent to 1.0 mol of NaOH
is equivalent to 1000 mL of 1.0 M NaOH solution
is equivalent to 40 g of NaOH
is equivalent to 23 g of Na^+ ions
is equivalent to 17 g of OH^- ions

Similarly for the reaction between potassium hydroxide and sulphuric acid:

$$H_2SO_4 + 2KOH = K_2SO_4 + 2H_2O$$

Since *1 mol* of H_2SO_4 reacts with *2 mol* of KOH, then:

1000 mL of 1.0 M H_2SO_4 solution is equivalent to 2.0 mol of KOH
is equivalent to 2 × 1000 mL of 1.0 M KOH solution
is equivalent to 2 × 56 g of KOH
is equivalent to 2 × 39 g of K^+ ions
is equivalent to 2 × 17 g of OH^- ions

To work out the results of titrations you *must* always:

- Work out the balanced equation to find out the ratio of moles reacting.
- Decide what you are trying to calculate.

Example: 25.00 mL of sodium hydroxide solution were titrated by 24.00 mL of 0.1 M HCl solution. Calculate the concentration of the sodium hydroxide solution.

- $HCl + NaOH = NaCl + H_2O$
- Concentration of NaOH, i.e. moles of NaOH in 1000 mL, since concentration is mol L^{-1}.

Now:

1000 mL of 1.0 M HCl solution is equivalent to 1.0 mol of NaOH

but the concentration of HCl is only 0.1 M:

1000 mL of *0.1 M* HCl solution is equivalent to 0.1 × 1.0 mol of NaOH

but only 24.00 mL of HCl solution were used:

1.0 mL of 0.1 M HCl solution is equivalent to $\frac{1.0 \times 1.0 \times 1.0}{1000}$ *mol* of NaOH

and

24 mL of 0.1 M HCl solution is equivalent to $\frac{24 \times 1.0 \times 0.1 \times 1.0}{1000}$ *mol* of NaOH

$$= 2.4 \times 10^{-3} \text{ mol of NaOH}$$

but 25.00 mL of NaOH solution were used:

25.00 mL of NaOH solution contains 2.4×10^{-3} mol of NaOH

Box 21.1 (continued)

Then

1.0 mL of NaOH solution contains $\frac{2.4 \times 10^{-3}}{25}$ mol of NaOH

and

1000 mL of NaOH solution contains $\frac{1000 \times 2.4 \times 10^{-3}}{25}$ mol of NaOH

$= 0.096$ mol of NaOH

Therefore concentration of NaOH solution is *0.096 mol L^{-1}*.

Using this set of equations you can calculate directly the mass of NaOH per litre, the mass or moles of sodium ions and the mass or moles of hydroxide ions.

Note: The expression $[C_1]V_1 = [C_2]V_2$ was not used, even though it is applicable in this case.

Problems arise when $[C_1]V_1 = [C_2]V_2$ is used for reactions which are not *1 : 1*, e.g.:

$$H_2SO_4 + 2KOH = K_2SO_4 + 2H_2O$$

or

$$2MnO_4^- + 16H^+ + 5C_2O_4^{2-} = 2Mn^{2+} + 10CO_2 + 8H_2O$$

or

$$IO_3^- + 5I^- + 6H^+ = 3I_2 + 3H_2O$$

Definition

A primary standard should be easy to obtain in a pure form. It should be unaffected in air during weighing, be capable of being tested for impurities and be readily soluble under the conditions used. Finally, the reaction with the standard solution should be stoichiometric and instantaneous.

Titrations

The process of adding the standard solution to the test solution is called a titration, and is carried out using a burette (see below). The point at which the reaction between the standard solution and the test substance is just complete is called the equivalence point or the theoretical (or stoichiometric) end-point. This is normally detected by a visible change, either of the standard solution itself or, more commonly, by the addition of an indicator.

Standard solutions

A standard solution can be prepared by weighing out the appropriate amount of a pure reagent and making up the solution to a particular volume, as described on p. 24. The concentration of a standard solution is expressed in terms of molarity (p. 19). A substance used in a primary standard should fulfil the following criteria:

- It should be obtainable in high purity (>99.9%).
- It should remain unaltered in air during weighing (i.e. it should not be hygroscopic).
- It must not decompose when dried by heating or vacuum.
- It should be capable of being tested for impurities.
- It should be readily soluble in an appropriate solvent.
- It must react with the test substance stoichiometrically and rapidly.

Preparing a standard solution

The molarity of a solution is the concentration of the solution expressed as mol L^{-1}. If x g of a substance of molecular weight M_r is dissolved in y mL of distilled water, the moles of substance dissolved $= \frac{x}{M_r} = m$. Therefore,

molarity (mol L^{-1}) $= \frac{m \times 1000}{y}$ [21.1]

Primary standards prepared from solid compounds should be weighed out using the 'weighing by difference' method as described in Chapter 4, and accurately made up to volume using a volumetric flask. Complete transfer of the substance from the weighing vessel to the volumetric flask is best achieved by inserting a funnel into the neck of the flask (Fig. 21.1). As much of the solid as possible should be transferred *via* the funnel. The funnel should be washed with distilled water prior to removal. Distilled water is then added to the flask, with occasional swirling to help to dissolve the solid. This is continued until the meniscus is about 1 cm below the volume mark. At this point a stopper is inserted and the flask is inverted several times to ensure the solid is completely dissolved. Finally, using a Pasteur pipette, distilled water is added up to the volume mark. The solution should be thoroughly mixed before use.

If the solid is not readily soluble in cold water it may be possible to dissolve it by stirring in warm water in a beaker. After allowing the solution to cool to room temperature, it can be transferred to the volumetric flask using a glass rod and filter funnel (Fig. 21.1) followed by several rinses of the glass rod/filter funnel. Finally, the solution is made up to the mark with distilled water.

Filling a burette

For accurate readings – always remember to position your burette vertically.

- Clamp a clean 50.00 mL burette (p. 10) in a laboratory stand. Place a beaker on a white tile immediately below the outlet of the burette (Fig. 21.2).
- Place a small filter funnel on top of the burette and, with the tap open, carefully pour in the standard solution (or titrant) until it starts to drain into the beaker.
- Close the burette tap, and fill the burette with the standard solution until the meniscus is about 1–2 cm above the zero mark. Remove the funnel.
- Open the tap and allow the solution to drain until the meniscus falls to the zero mark. The burette is then ready for the titration.

Note that to avoid contamination the solution in the beaker should be discarded, rather than recycled.

Using a pipette

Titrand – this is the solution of unknown composition in the conical flask. The titrant is the standard solution in the burette.

Safety note Never mouth pipette.

A clean 25.00 mL pipette (p. 10) is required together with a suitable pipette filler. Various designs of pipette filler are available. The most common type is based on a rubber-bulb suction device. It is best to evaluate a range of pipette fillers, if available in the laboratory, for ease of use and performance. The pipetting procedure is as follows:

- Pour the solution of unknown composition (the titrand) into a beaker. Never place the pipette in the volumetric flask containing the solution as this can lead to contamination of the solution from the external surface of the pipette.
- Using your pipette filler, draw the titrand to just beyond the graduation mark (Fig. 21.3a). Remove the pipette filler, and invert the pipette to allow the solution to drain out. This ensures that the titrand used subsequently will be undiluted and uncontaminated by any residue or liquids in the pipette.

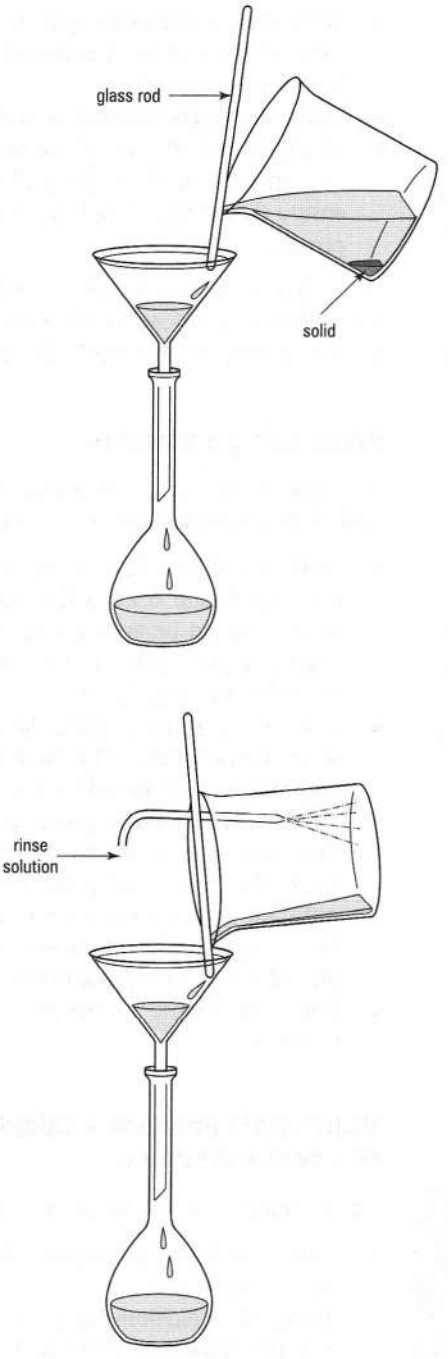


Fig. 21.1 Quantitative transfer of a solid to a volumetric flask.

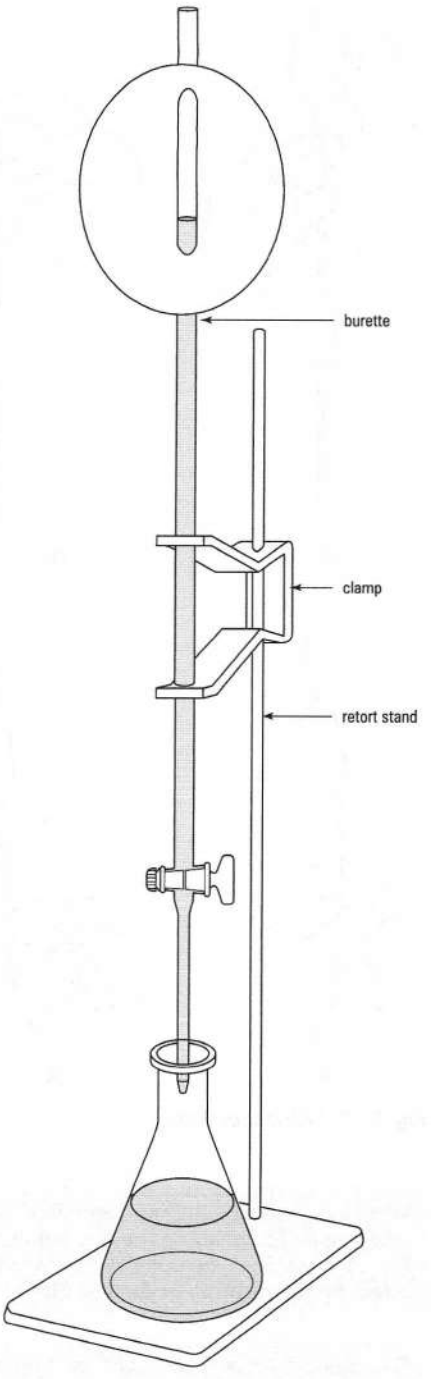


Fig. 21.2 Apparatus for a titration.

- Refill the pipette until the meniscus of the titrand is above the graduation mark (Fig. 21.3a). Remove the pipette filler and block the hole at the top of the pipette with the index finger of your right hand (if right-handed; Fig. 21.3b). Carefully raise the pipette to eye level, and allow the titrand to drain out into a beaker by lifting your finger slightly from the top of the pipette. Continue until the bottom of the meniscus is on the graduation mark.

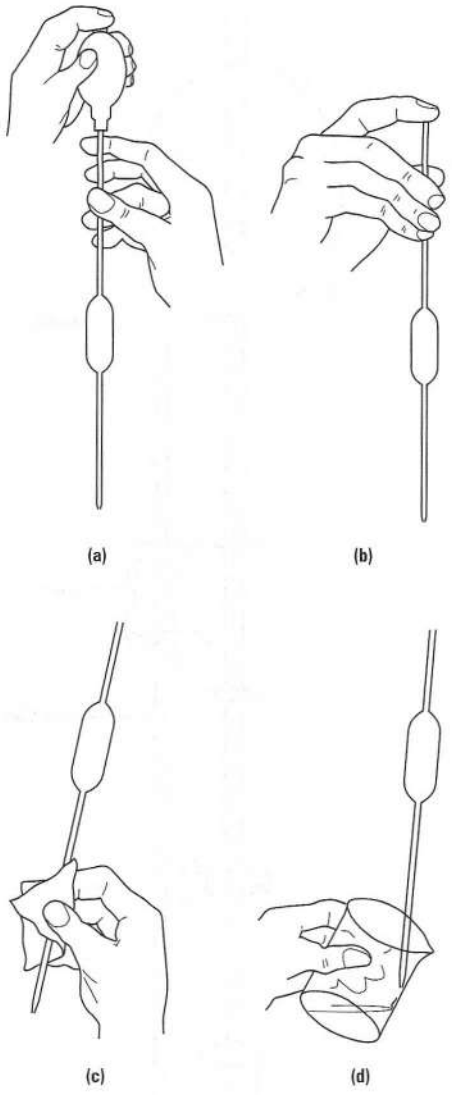


Fig. 21.3 Using a pipette.

Washing the test solution – any distilled water used for washing the test solution from the walls of the conical flask has no effect on the titration or the calculation.

Reading a burette – your eye-line should be level with the bottom of the meniscus. Then, record the volume used to one decimal place.

Rough titration – always carry out an initial rough titration to determine the approximate volume of titrant required for the end-point. This allows you to anticipate the end-point in subsequent titrations to determine the accurate volume.

- Wipe the outside of the pipette with a tissue (Fig. 21.3c). Be careful not to touch the point of the pipette with the tissue otherwise solution will be lost by capillary action.
- Allow the pipette's contents to drain into a conical flask.
- Finally, touch the end of the pipette on the wall of the flask (Fig. 21.3d), and rinse the inside of the neck of the flask with distilled water. This will ensure that exactly 25.00 mL of the test solution has been delivered by the pipette.

Note that it is normal for a small quantity of solution to remain in the pipette tip. This volume is taken into account when pipettes are calibrated, so do not attempt to 'blow out' this liquid into the conical flask.

Performing a titration

Add one or two drops of indicator to the titrand contained in the conical flask. For a right-handed person the process is as follows:

- Hold the conical flask containing the titrand and the indicator in your right hand, and control the tap of the burette with your left hand. The burette should be arranged so that the tap is on the opposite side of the burette to your palm. In this way, your left hand also supports the body of the burette (Fig. 21.4).
- Add the titrant by opening the tap, and simultaneously swirl the contents of the conical flask. This may take a bit of practice, so do not worry if you cannot do it straight away. The titrant can be added quickly at first, but as the end-point approaches, additions should be made drop-wise.
- The end-point is indicated when the appropriate colour change takes place. When the end-point is reached, one drop of titrant should be sufficient to cause the colour change. You should note the volume used for the titration to the nearest 0.1–0.05 mL. This is done by reading off the volume of titrant used (Fig. 21.5).
- Refill the burette to the zero mark using a funnel ready for the next titration.

Volumetric analysis – calculating the concentration of a test substance

The calculation should be carried out in a logical order as follows:

1. Write the balanced equation for the reaction between the standard and the test substance.
2. From the stoichiometry of the reaction, determine how many moles of the test substance react with 1 mole of the standard substance. For example, in the reaction between an H_2SO_4 standard solution and an NaOH test solution:

$$H_2SO_4 + 2NaOH \rightarrow Na_2SO_4 + 2H_2O \qquad [21.2]$$

   Therefore 2 moles of NaOH react with 1 mole of H_2SO_4.
3. Calculate the number of moles of standard substance used to reach the end-point of the reaction. This can be determined from knowledge of the concentration of the standard solution (mol L^{-1}) and the volume of titrant used (mL). Remember to take care with units – in this instance division by a factor of 1000 is required to convert mL to L:

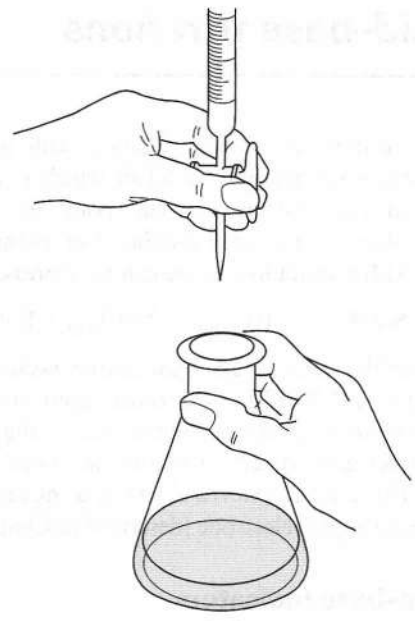


Fig. 21.4 Performing a titration.

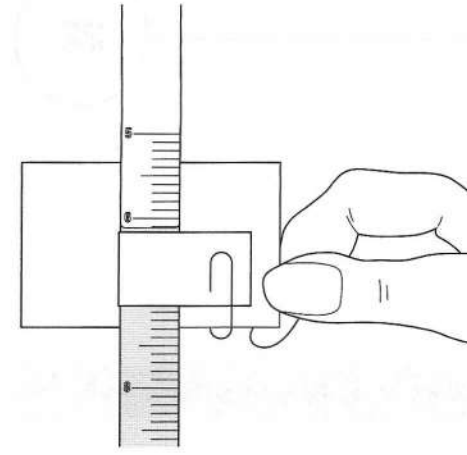


Fig. 21.5 Reading a burette. Place a white card below the level of the meniscus. This allows an accurate reading to be made.

For consistency of results – always perform two or three titrations (or until consistent results are obtained, i.e. titre values within 0.1–0.5 mL).

$$\text{Number of moles} = \frac{\text{concentration (mol L}^{-1}) \times \text{volume (mL)}}{1000}$$

4. The number of moles of test substance present in the titrand is then obtained from knowledge of the equivalences. In the example given above (point 2) the number of moles of test substance is twice the number of moles of standard substance. Therefore, if X moles of H_2SO_4 are used (as calculated in point 3), 2X moles of NaOH were present in the initial volume of test solution.
5. Finally, the concentration of the test solution can be calculated using the formula:

$$\text{Concentration of test solution (mol L}^{-1}) = \frac{1000 \times \text{amount of test substance (mol)}}{\text{initial volume of test solution (mL)}}$$

Again, the factor of 1000 is used to convert mL to L.

22 Acid–base titrations

The titration of an acid solution with a standard solution of alkali will determine the amount of alkali which is equivalent to the amount of acid present (or vice versa). The point at which this occurs is called the equivalence point or end-point. For example, the titration of hydrochloric acid with sodium hydroxide can be expressed as follows:

$$NaOH_{(aq)} + HCl_{(aq)} \rightarrow NaCl_{(aq)} + H_2O_{(l)} \quad [22.1]$$

If both the acid and alkali are strong electrolytes, the resultant solution will be neutral (pH 7). If on the other hand either the acid or alkali is a weak electrolyte the resultant solution will be slightly alkaline or acidic, respectively. In either case, detection of the end-point requires accurate measurement of pH. This can be achieved either by using an indicator dye, or by measuring the pH with a glass electrode (described in Chapter 7).

Safety note Never pipette by mouth.

Acid–base indicators

Typical acid–base indicators are organic dyes that change colour at or near the equivalence or end-point. They have the following characteristics:

- They show pH-dependent colour changes.
- The colour change occurs within a fairly narrow pH range (approximately 2 pH units).
- The pH at which a colour change occurs varies from one indicator to another, and it is possible to select an indicator which exhibits a distinct colour change at a pH close to the equivalence or end-point.

Selected common indicators together with their pH ranges and colour changes are shown in Table 22.1. Examples for thymol blue and phenolphthalein are shown in Fig. 22.1.

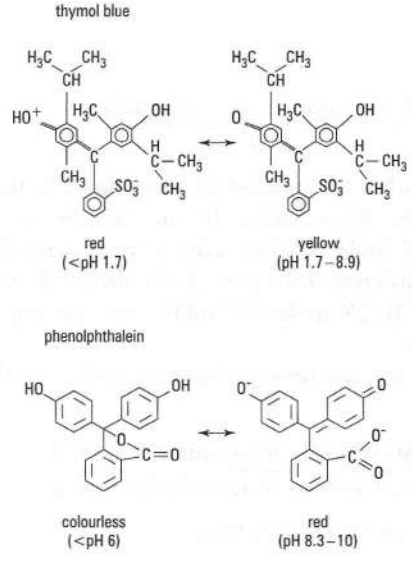


Figure 22.1 Examples of indicators used in acid-base titrations: thymol blue and phenolphthalein

Table 22.1 Colour changes and pH range of selected indicators

Indicator	pH range	Colour in acid solution	Colour in alkaline solution
Thymol blue	1.2–6.8	Red	Yellow
Methyl orange	2.8–4.0	Red	Yellow
Methyl red	4.3–6.1	Red	Yellow
Phenol red	6.8–8.2	Yellow	Red
Phenolphthalein	8.3–10.0	Colourless	Pink/red

Neutralization curves

A plot of pH against the volume of alkali added (mL) is known as a neutralization or titration curve (Fig. 22.2). The curve is generated by a 'potentiometric titration' in which pH is measured after each addition of alkali (or acid). The significant feature of the curve is the very sharp and sudden change in pH near to the equivalence point of the titration. For a strong acid and alkali this will occur at pH 7. If either the acid or base concentration is unknown, a preliminary titration is necessary to find the approximate equivalence point followed by a more accurate titration as described on p. 146. The ideal pH range for an indicator is 4.5–9.5.

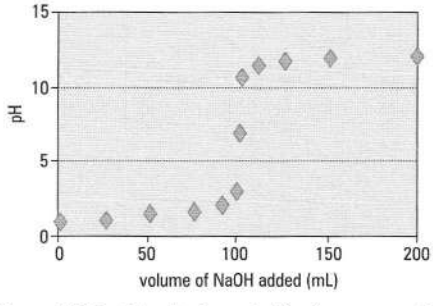


Figure 22.2 A typical neutralization curve: 0.1 M HCl with 0.1 M NaOH.

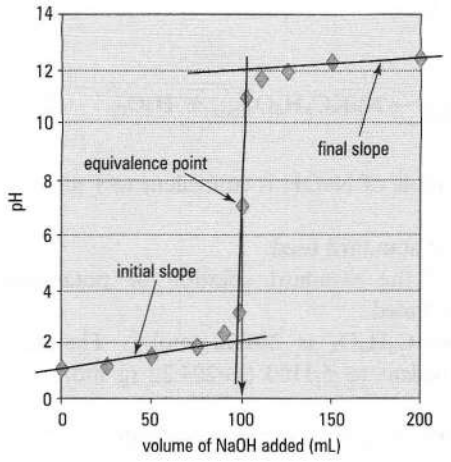


Figure 22.3 Determination of the equivalence point.

Subscripts – 'aq' is used to represent aqueous, 'l' the liquid state.

Determination of the equivalence point

From the neutralization curve (Fig. 22.2), the initial and final slopes are drawn (Fig. 22.3) and a parallel line is drawn such that the mid-point is on the curve. This is the equivalence point, producing a titration value of x mL.

Example calculations

Standardization of a sodium hydroxide solution

What is the molarity of a solution of sodium hydroxide, 25.0 mL of which requires 21.0 mL of a standard solution of hydrochloric acid of concentration 0.100 mol L^{-1} for neutralization?

Following the sequence in Box 21.1:

1. Write the equation:

$$NaOH_{(aq)} + HCl_{(aq)} \rightarrow NaCl_{(aq)} + H_2O_{(l)} \qquad (22.2)$$

2. Determine the equivalences.
   Equation [22.2] shows that 1 mole of NaOH requires 1 mole of HCl for neutralization, i.e. 1 mole of NaOH is equivalent to 1 mole of HCl.
3. Calculate the number of moles of standard used.

$$\text{Number of moles of HCl} = \frac{\text{concentration (mol L}^{-1}) \times \text{volume (mL)}}{1000}$$

$$= \frac{0.100 \text{ mol L}^{-1} \times 21.0 \text{ mL}}{1000}$$

$$= 2.1 \times 10^{-3} \text{ mol}$$

4. Calculate the number of moles of NaOH in the initial volume of test solution.
   As indicated in point 2 above, 1 mole of NaOH is equivalent to 1 mole of HCl. Therefore:

$$\text{no. of moles of NaOH in initial volume} = \text{no. of moles of HCl used}$$

$$= 2.1 \times 10^{-3} \text{ mol}$$

5. Determine the concentration of the NaOH solution.

$$\text{Concentration of test solution (mol L}^{-1}) = \frac{1000 \times \text{amount of test substance}}{\text{initial volume of test solution (mL)}}$$

$$= \frac{1000 \times 2.1 \times 10^{-3} \text{(mol)}}{25 \text{ (mL)}}$$

$$= 0.084 \text{ mol L}^{-1}$$

Standardization of a sodium hydroxide solution using potassium hydrogen phthalate as a primary standard (p. 143)

An accurately weighed amount (5.1100 g) of potassium hydrogen phthalate ($KHC_8H_4O_4$) was dissolved in distilled water (250.00 mL). This solution (25.00 mL) required sodium hydroxide solution (23.50 mL) to reach equivalence. What is the molarity of the sodium hydroxide solution? (Note that, in this case, 25.00 mL of the standard solution was used as the titrand, whereas the test solution (NaOH) was the titrant.)

Following the sequence in Box 21.1:

1. Write the equation.

$$NaOH_{(aq)} + KHC_8H_4O_{4(aq)} \rightarrow NaKC_8H_4O_{4(aq)} + H_2O_{(l)} \qquad [22.3]$$

2. Determine the equivalences.

   Equation [22.3] shows that 1 mole of NaOH is equivalent to 1 mole of $KHC_8H_4O_4$.

3. Calculate the number of moles of standard used.

   Firstly, the concentration of the standard solution of potassium hydrogen phthalate must be calculated.

   The molecular weight of $KHC_8H_4O_4$ is $204.22\,g\,mol^{-1}$. Therefore 5.1100 g of $KHC_8H_4O_4$ is equivalent to 5.1100 (g)/204.22 ($g\,mol^{-1}$) = 0.025 mol.

   We can now calculate the concentration of the standard solution:

$$\text{Concentration of standard solution } (mol\,L^{-1}) = \frac{1000 \times \text{amount of } KHC_8H_4O_4\,(mol)}{\text{volume of standard solution (mL)}}$$

$$= \frac{1000 \times 0.025\,(mol)}{250\,(mL)}$$

$$= 0.100\,mol\,L^{-1}$$

   The number of moles of standard used in the titration is as follows.

$$\text{Number of moles of } KHC_8H_4O_4 = \frac{\text{concentration } (mol\,L^{-1}) \times \text{volume (mL)}}{1000}$$

$$= \frac{0.100\,mol\,L^{-1} \times 25.0\,mL}{1000}$$

$$= 2.5 \times 10^{-3}\,mol$$

4. Calculate the number of moles of NaOH used to reach equivalence.

   As indicated in point 2 above, 1 mole of NaOH is equivalent to 1 mole of $KHC_8H_4O_4$. Therefore:

$$\text{No. of moles of NaOH used} = \text{no. of moles of } KHC_8H_4O_4 \text{ in the titrand}$$

$$= 2.5 \times 10^{-3}\,mol$$

5. Determine the concentration of the NaOH solution:

$$\text{Concentration of NaOH solution } (mol\,L^{-1}) = \frac{1000 \times \text{amount of NaOH (mol)}}{\text{volume of NaOH solution used (mL)}}$$

$$= \frac{1000 \times 2.5 \times 10^{-3}\,(mol)}{23.50\,(mL)}$$

$$= 0.106\,mol\,L^{-1}$$

23 Complexometric titrations

Complexometric titrations are mainly used to determine the concentration of cations in solution. The method is based on the competition between a metal ion (for example) and two ligands, one of which acts as an indicator and the other is a component of a standard solution.

Some knowledge of the principles of metal–ligand binding is required in order to understand this method.

Types of ligand

Ligands are chemical species that co-ordinate with metal ions to form a complex. They are classified on the basis of the number of points of attachment to the central ion.

- Monodentate ligand – here the ligand is bound to the central ion at only one point, e.g. H_2O, NH_3.
- Bidentate ligand – this has two points of attachment to the central ion, e.g. ethylenediamine (en) (Fig. 23.1).
- Multidentate ligand – these have several points of attachment, e.g. ethylenediaminetetra-acetic acid (EDTA), which is a hexadentate ligand (six points of attachment) (Fig. 23.2).

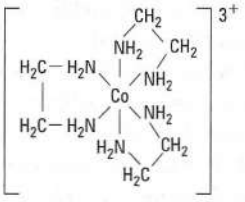
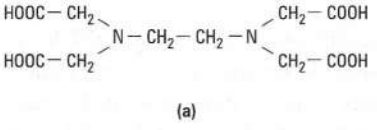
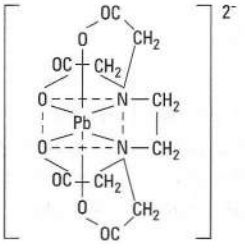


Fig. 23.1 Structure of $[Co(en)_3]3^+$. It is a six-co-ordinate octahedral complex of ethylenediamine (en) with cobalt (III). The complex has three five-membered rings.



(a)



(b)

Fig. 23.2 Structure of EDTA. (a) EDTA contains two donor N atoms and four donor O atoms. It can therefore form a hexadentate complex (b) with a metal ion, e.g. Pb^{2+}.

The basis of a complexometric titration involving EDTA

The metal ion under investigation is bound to an indicator in solution (under strict pH control). This solution is then titrated against a standard solution of EDTA. This can be expressed in the form of an equation:

$$\text{Metal–indicator} + \text{EDTA} \rightarrow \text{metal–EDTA} + \text{indicator} \qquad [23.1]$$

For example, if the indicator being used was solochrome black, the metal–indicator solution would be red while the colour of the free indicator would be blue (in the pH range 7–11). The reaction takes place if the EDTA displaces the indicator from the metal–indicator complex. Therefore the metal–EDTA complex must be more stable thermodynamically than the metal–indicator complex.

Stability of complexes

The thermodynamic stability of a species is an indication of the extent to which that species will be formed (under certain conditions and provided that it is allowed to reach equilibrium).

As an example consider the general case of a metal, M, in solution together with a monodentate ligand, L. It is possible to describe this system in terms of step-wise equilibria:

$$M + L = ML \qquad K_1 = [ML]/[M][L] \qquad [23.2]$$

$$ML + L = ML_2 \qquad K_2 = [ML_2]/[ML][L] \qquad [23.3]$$

Or, in general terms:

$$ML_{(n-1)} + L = ML_n \qquad K_n = [ML_n]/[ML_{(n-1)}][L] \qquad [23.4]$$

where K_1, K_2, ... K_n are step-wise stability constants.

An alternative approach for expressing the equilibria might be as follows:

$$M + L = ML \qquad \beta_1 = [ML]/[M][L] \qquad [23.5]$$

$$M + L_2 = ML_2 \qquad \beta_2 = [ML_2]/[M][L]^2 \qquad [23.6]$$

Or, in general terms:

$$M + L_n = ML_n \qquad \beta_n = [ML_n]/[M][L]_n \qquad [23.7]$$

where $\beta_1, \beta_2, \ldots, \beta_n$ are the overall stability constants and are related to the step-wise stability constants as follows:

$$\beta_n = K_1 \times K_2 \times K_n \qquad [23.8]$$

Factors influencing the stability of complexes

The stability of a complex is related to the ability of the metal ion to complex with a given ligand, and to the characteristics of the ligand.

End-points can be determined more easily when a single complex is formed rather than when the complex is formed in a step-wise fashion. This can be achieved by using the aminopolycarboxylic acid, EDTA (Fig. 23.2).

In equations, EDTA can be expressed as H_4Y. The disodium salt Na_2H_2Y is frequently used as a source of the complex-forming ion, H_2Y^{2-}. Thus the typical reaction of EDTA with a metal ion can be written in the following form:

$$M^{2+} + H_2Y^{2-} \rightarrow MY^{2-} + 2H^+ \qquad [23.9]$$

The reaction of a metal ion with EDTA is always in the ratio 1:1. The stability constants of selected metal–EDTA complexes are given in Table 23.1.

Table 23.1 Stability constants of selected metal–EDTA complexes (expressed as log *K*)*

Ion	log k	Ion	log k
Mg^{2+}	8.7	Ni^{2+}	18.6
Ca^{2+}	10.7	Cu^{2+}	18.8
Fe^{2+}	14.3	Hg^{2+}	21.9
Co^{2+}	16.3	Sc^{3+}	23.1
Al^{3+}	16.3	Cr^{3+}	24.0
Cd^{2+}	16.6	In^{3+}	24.9

*Ionic strength of solution was 0.1 at 20 °C. Adapted from *Vogel's Textbook of Quantitative Inorganic Analysis*, 4th Edn, J. Bassett, R.C. Denney, G.H. Jeffery and J. Mendham, Longman Scientific and Technical, Harlow, (1978) p. 264.

The detection of the end-point in titrations involving EDTA is most commonly achieved using a metal-ion indicator, i.e. a compound that changes its colour when it complexes with a particular metal ion. The structures of selected metal-ion indicators are shown in Fig. 23.3 and the properties of a variety of metal-ion indicators are given in Table 23.2.

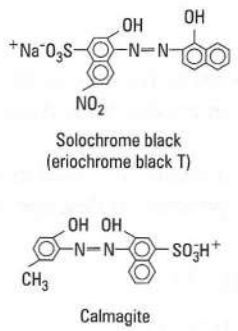


Fig. 23.3 Examples of metal-ion indicators: solochrome black and calmagite.

Types of complexometric titration

Direct titration

In this case, the metal ion is titrated with a standard solution of EDTA. The solution containing the metal ion is buffered to an appropriate pH at which the stability constant of the metal–EDTA complex is large. The free indicator has a different colour from that of the metal–indicator complex.

Back titration

In certain circumstances a particular metal ion cannot be titrated directly. This includes situations where:

- The metal ion precipitates in the absence of EDTA.
- The metal ion reacts too slowly with EDTA.
- The metal ion forms an inert complex.
- No suitable indicator is available.

In these cases a back titration is required. This involves addition of a known excess of EDTA to the metal ion (buffered to an appropriate pH). Then, the excess EDTA is titrated with a standard solution of a different metal ion. The choice of a second metal ion is important as it must not displace the analyte metal ion from its EDTA complex.

Table 23.2 Properties of selected indicators

Indicator	Colour of free indicator	Colour of metal-ion complex
Murexide		
< pH 9 (H_4In^-)	Red–violet	orange (Cu^{2+}), yellow (Ni^{2+} and Co^{2+}) and red (Ca^{2+})
pH 9–11 (H_3In^{2-})	Violet	
> pH 11 (H_2In^{3-})	Blue	
Solochrome black		
< pH 5 (H_2In^-)	Red	In pH range 7–11 colour change is blue–red (Mg, Mn, Zn, Cd, Hg, Pb, Cu, Al, Fe, Ti, Co, Ni and Pt metals)
pH 7–11 (HIn^{2-})	Blue	
> pH 11.5 (In^{3-})	Orange	
Calmagite		
<pH 5 (H_2In^-)	Red	Same colour change as solochrome black but clearer and sharper
pH 7–9 (HIn^{2-})	Blue	
>pH 11.4 (In^{3-})	Red–orange	
Pyrocatechol violet		
< pH 1.5 (H_4In)	Red	In pH range 2–6, yellow to blue (Bi and Th); pH 7 violet to blue (Cu^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+} and Co^{2+})
pH 2–6 (H_3In^-)	Yellow	
pH 7 (H_2In^{2-})	Violet	
>pH 10 (In^{4-})	Blue	

Practical considerations

- pH adjustment is critical in EDTA titrations. The pH is monitored with a pH meter or pH test paper.
- The metal ion under investigation should ideally be approximately 0.25 mM in a volume of 50–150 mL of solution. Dilution of the metal ion may be necessary to avoid end-point detection problems.
- Do not add excess indicator, as too intense a colour can lead to problems, e.g. masking of the colour change.
- It is sometimes difficult to detect the end-point because the colour change can be slow to develop. Stirring is recommended to assist colour transformation.
- The use of metal-ion indicators to indicate the end-point of complexometric titrations is based on a specific colour change. Some individuals may find it difficult to detect a particular colour change (e.g. those with colour blindness). Alternative approaches for end-point detection are available based on a colorimeter/spectrophotometer (devices for measuring colour, see Chapter 26) or electrochemical detection (see Chapter 34).

For a metal-ion indicator to be useful it must be less stable than the corresponding metal–EDTA complex.

Example calculation

A solution of Ni^{2+} (25.00 mL) was titrated with 0.1036 mol L^{-1} EDTA at pH 5 and required 20.25 mL for the metal–indicator to change colour. What is the concentration (g L^{-1}) of Ni^{2+}? The atomic weight of nickel is 58.71 g mol^{-1}.

1. Write the balanced equation for the reaction between the standard and the test substance

$$Ni^{2+} + H_2Y^{2-} \rightarrow NiY^{2-} + 2H^+ \qquad [23.10]$$

2. Determine the equivalences of the reacting species:

1 mole of EDTA is equivalent to 1 mole of Ni^{2+}

3. Calculate the number of moles of standard substance (EDTA) used to reach the end-point of the reaction:

$$\text{moles of EDTA} = \text{volume (L)} \times \text{molarity (mol L}^{-1}\text{)}$$
$$= 20.25 \times 10^{-3}\,\text{L} \times 0.1036\,\text{mol L}^{-1}$$
$$= 2.098 \times 10^{-3}\,\text{mol}$$

4. Calculate the corresponding number of moles of Ni^{2+} present in the 25 mL of nickel solution.
   Since 1 mole of EDTA is equivalent to 1 mole of Ni^{2+}:

$$\text{moles of } Ni^{2+} = 2.098 \times 10^{-3}\,\text{mol}$$

5. Calculate the concentration of the Ni^{2+} solution:

$$\text{concentration of } Ni^{2+} \text{ solution (mol L}^{-1}\text{)} = \frac{1000 \times 2.098 \times 10^{-3}\,\text{(mol)}}{25\,\text{(mL)}}$$
$$= 83.92 \times 10^{-3}\,\text{mol L}^{-1}$$

   In this instance, the Ni^{2+} concentration is required in $g\,L^{-1}$.
   The molecular weight of nickel is $58.71\,g\,mol^{-1}$:

$$\text{concentration of } Ni^{2+} \text{ solution (g L}^{-1}\text{)} = \text{molecular weight (g mol}^{-1}\text{)} \times \text{molarity (mol L}^{-1}\text{)}$$
$$= 58.71 \times 83.92 \times 10^{-3}$$
$$= 4.927\,\text{g L}^{-1}$$

24 Redox titrations

All reduction–oxidation reactions involve a transfer of electrons. The oxidizing agent accepts electrons, and the reducing agent donates electrons. To establish the reaction for a redox titration it is necessary to determine the 'half-equation' for both the oxidizing agent and the reducing agent. By adding the two 'half-equations' it is possible to determine the overall equation for the titration. The basic theory of electrochemistry is described in Chapter 34.

One of the most common oxidants is potassium permanganate which in acidic solution can undergo the following reaction:

$$MnO_4^- + 8H^+ + 5e^- = Mn^{2+} + 4H_2O \qquad [24.1]$$

Unfortunately, potassium permanganate is not obtainable in high enough purity and can undergo decomposition by exposure to sunlight. Therefore it cannot be used as a primary standard (p. 143). However, it can be used in redox titrations provided it is standardized with sodium oxalate (which is available in high purity). The redox reaction involving oxalate is as follows:

$$C_2O_4^{2-} = 2CO_2 + 2e^- \qquad [24.2]$$

The overall reaction between permanganate and oxalate can be obtained by balancing the electrons on each side of the equation. This can be achieved by multiplying eqn [24.1] by 2 and eqn [24.2] by 5, and then combining them as follows:

$$2MnO_4^- + 16H^+ + 5C_2O_4^{2-} = 2Mn^{2+} + 10CO_2 + 8H_2O \qquad [24.3]$$

Another common method for the standardization of potassium permanganate is to use iron (II):

$$Fe^{2+} = Fe^{3+} + e^- \qquad [24.4]$$

The combined equation is obtained by multiplying eqn [24.4] by 5 and adding to eqn [24.1]:

$$MnO_4^- + 8H^+ + 5Fe^{2+} = Mn^{2+} + 5Fe^{3+} + 4H_2O \qquad [24.5]$$

Potassium permanganate has a major advantage when used for titrations in that it can act as its own indicator.

A list of other common oxidizing agents and reducing agents is given in Table 24.1.

Table 24.1 Common oxidizing and reducing agents used in redox titrations

Oxidizing agents		Reducing agents	
Ce^{4+}	Ceric	AsO_3^{3-}	Arsenite
$Cr_2O_7^{2-}$	Dichromate	Fe^{2+}	Ferrous
H_2O_2	Hydrogen peroxide	NH_2OH	Hydroxylamine
IO_3^-	Iodate	Sn^{2+}	Stannous
MnO_4^-	Permanganate	$S_2O_3^{2-}$	Thiosulphate

Example calculation

Standardization of potassium permanganate with a primary standard, sodium oxalate

An accurately weighed portion of sodium oxalate (0.1550 g) was dissolved in dilute sulphuric acid (250 mL). Whilst maintaining the temperature of the solution above 70 °C, it was titrated to equivalence with potassium permanganate solution (18.5 mL). What is the molarity of potassium permanganate?

1. Write the balanced equation for the reaction between the standard and the test substance (using the two half-equations [24.1] and [24.2]):

$$2MnO_4^- + 16H^+ + 5C_2O_4^{2-} = 2Mn^{2+} + 10CO_2 + 8H_2O \qquad [24.3]$$

2. Determine the equivalences of the reacting species:

2 moles of MnO_4^- are equivalent to 5 moles of $C_2O_4^{2-}$.

3. Calculate the number of moles of standard substance (sodium oxalate) used to reach the end-point of the reaction.

The molecular weight of sodium oxalate is 134 g mol^{-1}. Therefore 0.1550 g of sodium oxalate is equivalent to:

$$\frac{0.1550\,(\text{g})}{134\,(\text{g mol}^{-1})} = 1.157 \times 10^{-3}\,\text{mol}$$

4. Calculate the corresponding number of moles of potassium permanganate present in the volume of titrant added.

From the equation:

5 moles of $Na_2C_2O_4$ = 2 moles of $KMnO_4$

Therefore,

$$\text{moles of } KMnO_4 \text{ used in the titration} = \tfrac{2}{5} \times 1.157 \times 10^{-3}\,\text{mol}$$
$$= 4.628 \times 10^{-4}\,\text{mol}$$

5. Calculate the concentration of the $KMnO_4$ solution:

$$\text{Concentration of } KMnO_4 \text{ solution (mol L}^{-1}) = \frac{1000 \times \text{amount of } KMnO_4\,(\text{mol})}{\text{Volume of } KMnO_4 \text{ solution used (mL)}}$$
$$= \frac{1000 \times 4.628 \times 10^{-4}\,(\text{mol})}{18.5\,(\text{mL})}$$
$$= 0.025\,\text{mol L}^{-1}$$

25 Precipitation titrations

Definition

Argentimetric is derived from the Latin *argentum*, which means silver.

Definition

To be exact, the *p* function should be defined in terms of activities instead of concentrations.

Titration curves – plots of concentration of titrand against volume of titrant used.

Precipitation is the term used to describe the process whereby a substance leaves solution rapidly, forming either a crystalline solid or amorphous solid (the precipitate). In the case of a precipitation titration, this process occurs when the analyte forms a precipitate with the titrant. The most common types of precipitation titrations use silver nitrate as the titrant. They are often referred to as argentimetric titrations.

Titration curves used in precipitation reactions usually use a concentration-dependent variable called the '*p* function' rather than the concentration itself. The *p* function for a species X is defined as follows:

$$pX = -log_{10}\,[X] \qquad [25.1]$$

For example, in the titration of 100 mL of 0.1 mol L^{-1} NaCl with 0.1 mol L^{-1} $AgNO_3$ the initial concentration of $[Cl^-]$ is 0.1 mol L^{-1}, so by using eqn [25.1] the *p* function is 1 or $pCl^- = 1$.

When 25 mL of 0.1 mol L^{-1} $AgNO_3$ has been added, 75 mL of NaCl remains in a total volume of 125 mL. Therefore, the concentration of the chloride ion is given by

$$[Cl^-] = \frac{75\,mL \times 0.1\,M}{125\,mL} = 6 \times 10^{-2}\,mol\,L^{-1} \qquad [25.2]$$

and $pCl^- = 1.22$. (Note that the solubility product, K_s, of AgCl is 1.1×10^{-10}, see p. 50.)

Therefore:

$$[Ag^+] \times [Cl^-] = K_s = 1.1 \times 10^{-10} \qquad [25.3]$$

or

$$pAg^+ + pCl^- = 9.96 = pAgCl \qquad [25.4]$$

It was found above that $pCl^- = 1.22$, hence $pAg^+ = 9.96 - 1.22 = 8.74$. In a similar manner, the pAg^+ values can be calculated.

At the equivalence point $[Ag^+] = [Cl^-]$. Therefore:

$$[Ag^+] = [Cl^-] = \sqrt{K_s} = \sqrt{1.1 \times 10^{-10}} = 1.05 \times 10^{-5} \qquad [25.5]$$

$$pAg^+ = -\log(1.05 \times 10^{-5}) = 4.98 \qquad [25.6]$$

Beyond the equivalence point the situation changes. For 100.1 mL $AgNO_3$ solution:

$$[Ag^+] = \frac{0.1\,mL \times 0.1\,M}{200.1\,mL} = 5 \times 10^{-5}\,mol\,L^{-1} \qquad [25.7]$$

or $pAg^+ = 4.3$. Therefore,

$$pCl^- = pAgCl - pAg^+ = 9.96 - 4.3 = 5.66$$

Values calculated in this way up to the addition of 150 mL of 0.1 M $AgNO_3$ are given in Table 25.1 and the titration curve in Fig. 25.1.

Table 25.1 Titration of 100 mL of 0.1 M NaCl with 0.1 M $AgNO_3$. (Note that K_s, for AgCl $= 1.1 \times 10^{-10}$)

0.1 M $AgNO_3$ (mL)	pCl^-	pAg^-
0	0.1	0.0
25	1.2	8.7
50	1.5	8.5
90	2.3	7.7
95	2.6	7.4
98	3.0	7.0
99	3.3	6.7
99.5	3.6	6.4
99.8	4.0	6.0
99.9	4.3	5.7
100	5.0	5.0
100.1	5.7	4.3
100.2	6.0	4.0
100.5	6.4	3.6
101	6.7	3.3
102	7.0	3.0
105	7.4	2.6
110	7.7	2.3
120	8.0	2.0
130	8.1	1.9
140	8.2	1.8
150	8.3	1.7

Adapted from *Vogel's Textbook of Quantitative Inorganic Analysis*, 4th Edn, J. Bassett, R.C. Denney, G.H. Jeffery and J. Mendham, Longman Scientific and Technical, Harlow (1978), p. 280.

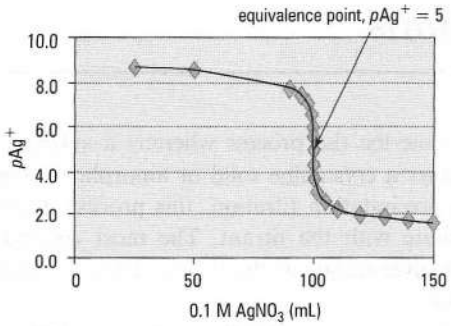


Fig. 25.1 Precipitation titration curve. Initial and final slopes are drawn (see Fig. 22.3) and a parallel line is drawn such that the mid-point is on the curve. This is the equivalence point.

In acid media (pH < 6), the concentration of CrO_4^{2-} is lowered by the following reaction: $CrO_4^{2-} + 2H^+$ $2HCrO_4^-$ $Cr_2O_7^{2-} + H_2O$. In alkaline media (pH >10), $Ag(OH)_{(s)}$ may precipitate.

In all argentimetric titrations strong light (including daylight) should be avoided as it can lead to decomposition of the silver salts.

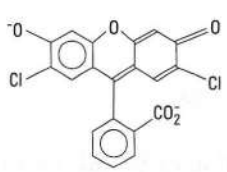


Fig. 25.2 Structure of dichlorofluorescein.

End-point determination

Three techniques are commonly used to determine the end-point in precipitation titrations. They are:

1. potentiometric methods;
2. chemical indicator methods;
3. light-scatterring methods, exemplified by turbidimetry or nephelometry.

Only indicator methods will be discussed further. Three types of indicator methods can be applied to determine the end-point of an Ag^+ and a Cl^- titration. These are:

1. Mohr titration, which involves the formation of a coloured precipitate by reaction with the indicator. For example, in the determination of chloride concentration with silver nitrate a small amount of potassium chromate solution is added as an indicator. This results in the formation of a red silver chromate (Ag_2CrO_4) precipitate at the end-point:

$$2Ag^+ + CrO_4^{2-} \rightarrow \underset{\text{(red)}}{Ag_2CrO_{4(s)}} \qquad [25.8]$$

   In this case, the precipitate may form slightly after the end-point, but this error can usually be neglected. Also, the titration should be done in neutral or slighly alkaline solution (pH 6.5–9) otherwise silver chromate might not be formed.
2. Volhard titration, which involves the formation of a soluble coloured compound. This approach is exemplified by the quantitative analysis of chlorides, bromides and iodides by back titration. In this case, the halide is titrated with silver:

$$Ag^+ + Cl^- \rightarrow AgCl_{(s)} \qquad [25.9]$$

   Excess silver ions are then titrated with standard potassium thiocyanate solution in the presence of an iron (III) salt:

$$Ag^+ + SCN^- \rightarrow AgSCN_{(s)} \qquad [25.10]$$

   When all the Ag^+ has been reacted, the SCN^- reacts with Fe^{3+} to form a red complex, indicating the end-point:

$$Fe^{3+} + SCN^- \rightarrow \underset{\text{(red)}}{FeSCN^{2+}} \qquad [25.11]$$

   A problem with the determination of chloride by this approach is that the end-point coloration slowly fades, as AgCl is more soluble than AgSCN. As a consequence the AgCl slowly dissolves to be replaced by the $FeSCN^{2+}$. Two approaches are possible to prevent this secondary reaction from taking place. The most common method is to filter off the AgCl and titrate only the Ag^+ left in solution. Alternatively, add a few millilitres of an immiscible liquid (e.g. nitrobenzene) to the titrand prior to the back titration. The nitrobenzene acts to 'coat' the AgCl precipitate, thereby isolating it from the SCN^-.
3. Fajans titration, which involves the adsorption of a coloured indicator onto the precipitate at the end-point, resulting in a colour change. During this adsorption process a change occurs in the indicator resulting in a change of colour. The indicators used for this are often anionic dyes,

e.g. fluorescein or eosin. The most common indicator for AgCl is dichlorofluorescein (Fig. 25.2) (this is greenish yellow in solution but changes colour to pink when it is adsorbed on AgCl).

Selected examples of precipitation titrations are shown in Table 25.2.

Table 25.2 Selected applications of precipitation titrations

Analyte	Comments
Cl^-, Br^-	Mohr method: Ag_2CrO_4 used as end-point
Br^-, I^-, AsO_4^-	Volhard method: precipitate removal is unnecessary
Cl^-, CN^-, CO_3^{2-}	Volhard method: precipitate removal is required
Cl^-, Br^-, I^-, SCN^-	Fajans method: titration with Ag^+. Detection with fluorescein, dichlorofluorescein and eosin
F^-	Titration with $Th(NO_3)_4$ to produce ThF_4. End-point detection with alizarin red S

Adapted from: *Quantitative chemical analysis*, 4th Edn, D.C. Harris, W.H. Freeman, New York (1995), p. 176.

Resources for classical techniques

General books

Main supplementary text:

Mendham, J., Denney, R.C., Barnes, J.D. and Thomas, M.J.K. (2000) *Vogel's Textbook of Quantitative Chemical Analysis*, 6th Edn, Prentice Hall, Harlow, Essex.

Other useful sources (chronological order):

Bennett, S.W. and O'Neale, K. (1999) *Progressive development of practical skills in chemistry. A guide to early undergraduate experimental work*, Royal Society of Chemistry, Cambridge.

Crawford, K. and Heaton, A. (1999) *Problem Solving in Analytical Chemistry*, Royal Society of Chemistry, Cambridge.

Day R.A. and Underwood, A.L. (1991) *Quantitative Analysis*, 6th Edn, Prentice Hall, Harlow, Essex.

Rubinson, J.F. and Rubinson, K.A. (1998) *Contemporary Chemical Analysis*, Prentice Hall, Harlow, Essex.

Skoog, D.A., West, D.M. and Holler, F.J. (1996) *Fundamentals of Analytical Chemistry*, 7th Edn, Saunders College Publishing, Orlando, Florida.

Specific books on qualitative analysis

Main supplementary text:

Svehla, G. (1989) *Vogel's Qualitative Inorganic Analysis*, Longman, Harlow, Essex.

Other useful sources (chronological order):

Whitten, K.W., Davis, R.E. and Peck, M.L. (2000) *General Chemistry with Qualitative Analysis*, 6th Edn, Saunders College Publishing, Orlando, Florida.

Hardcastle, W.A. *Qualitative Analysis. A guide to best practice*, Royal Society of Chemistry, Cambridge.

Videos

Basic Laboratory Skills, LGC, Royal Society of Chemistry, Cambridge (1998).

Further Laboratory Skills LGC, Royal Society of Chemistry, Cambridge (1998).

CD-ROMs

Chemistry Video Consortium, Practical Laboratory Chemistry, Educational Media Film and Video Ltd, Harrow, Essex, UK – Inorganic analysis (gravimetric analysis).

Chemistry Video Consortium, Practical Laboratory Chemistry, Educational Media Film and Video Ltd, Harrow, Essex, UK – Volumetric techniques (using a balance, using a pipette, using a burette and making-up solutions).

Chemistry Video Consortium, Practical Laboratory Chemistry, Educational Media Film and Video Ltd, Harrow, Essex, UK – Volumetric analyses methods (doing a titration, some common end-points and potentiometric titrations).

Instrumental techniques

26 Basic spectroscopy

Spectroscopic techniques can be used to:

- identify compounds tentatively, by determining their absorption or emission spectra;
- quantify substances, either singly or in the presence of other compounds, by measuring the signal strength at an appropriate wavelength;
- determine molecular structure;
- follow reactions, by measuring the disappearance of a substance, or the appearance of a product as a function of time.

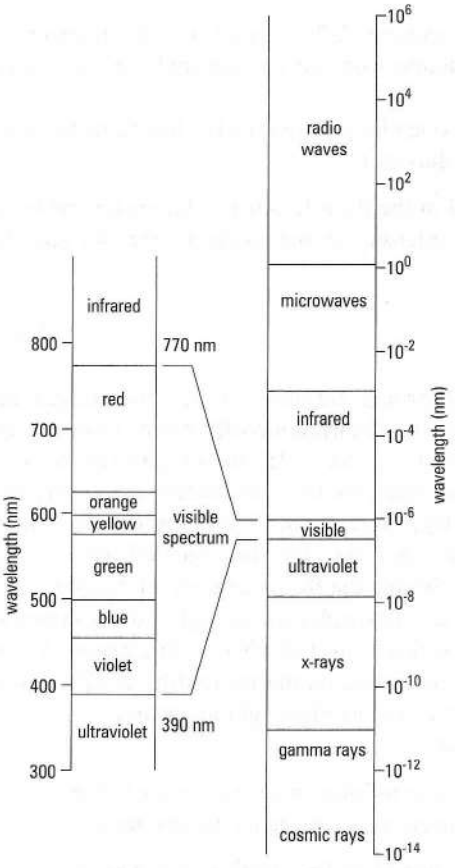


Fig. 26.1 The electromagnetic spectrum.

Light is most strictly defined as that part of the spectrum of electromagnetic radiation detected by the human eye. However, the term is also applied to radiation just outside that visible range, e.g. ultraviolet (UV) and infrared (IR) 'light'. Electromagnetic radiation is emitted by the sun and by other sources (e.g. an incandescent lamp) and the electromagnetic spectrum is a broad band of radiation, ranging from cosmic rays to radio waves (Fig. 26.1). Most chemical experiments involve measurements within the UV, visible and IR regions (generally, within the wavelength range 200–1000 nm).

Radiation has the characteristics of a particle and of a vibrating wave, travelling in discrete particulate units, or 'packets', termed photons. A quantum is the amount of energy contained within a single photon (it is important not to confuse these two terms, although they are sometimes used interchangeably in the literature). In some circumstances, it is appropriate to measure light in terms of the number of photons, usually expressed directly in moles (6.02×10^{23} photons = 1 mol). Alternatively, the energy content (power) may be measured (e.g. in $W\,m^{-2}$). Radiation also behaves as a vibrating electrical and magnetic field moving in a particular direction, with the magnetic and electrical components vibrating perpendicular to one another and perpendicular to the direction of travel. The wave nature of radiation gives rise to the concepts of wavelength (λ, usually measured in nm), frequency (ν, measured in s^{-1}, but often recorded in hertz, Hz), speed (c, the speed of electromagnetic radiation, which is $3 \times 10^{8}\,m\,s^{-1}$ in a vacuum), and direction. In other words, radiation is a vector quantity, where:

$$c = \lambda\nu \qquad [26.1]$$

Sometimes, it is necessary to rearrange the equation such that:

$$\nu = \frac{c}{\lambda} \qquad [26.2]$$

Introduction to spectroscopy

The absorption and emission of electromagnetic radiation of specific energy (wavelength) is a characteristic feature of many molecules, involving the movement of electrons between different energy states, in accordance with the laws of quantum mechanics. Electrons in atoms or molecules are distributed at various energy levels, but are mainly at the lowest energy level, usually termed the ground state. When exposed to energy (e.g. from electromagnetic radiation), electrons may be excited to higher energy levels (excited states), with the associated absorption of energy at specific wavelengths giving rise to an absorption spectrum. One quantum of energy is absorbed for a single electron transition from the ground state to an excited state. On the other hand, when an electron returns to its ground state, one quantum of energy is released; this may be dissipated to the surrounding molecules (as heat) or may give rise to an emission spectrum. The energy change (ΔE) for an electron moving between two energy states, E_1 and E_2, is given by the equation:

$$\Delta E = E_1 - E_2 = h\nu \qquad [26.3]$$

where h is the Planck constant (p. 72) and ν is the frequency of the electromagnetic radiation (expressed in Hz or s^{-1}). By substituting for

frequency in [26.3] it is possible to rearrange this equation to give the expression:

$$\Delta E = \frac{hc}{\lambda} \qquad [26.4]$$

UV/visible spectrophotometry

This is a widely used technique for measuring the absorption of radiation in the visible and UV regions of the spectrum. A spectrophotometer is an instrument designed to allow precise measurement at a particular wavelength, while a colorimeter is a simpler instrument, using filters to measure broader wavebands (e.g. light in the green, red or blue regions of the visible spectrum).

Principles of light absorption

Two fundamental principles govern the absorption of light passing through a solution:

1. The absorption of light is exponentially related to the number of molecules of the absorbing solute that are encountered, i.e. the solute concentration [C].
2. The absorption of light is exponentially related to the length of the light path through the absorbing solution, l.

These two principles are combined in the Beer–Lambert relationship, which is usually expressed in terms of the intensity of the incident light (I_0) and the emergent light (I):

$$\log_{10}\left(\frac{I_0}{I}\right) = \varepsilon[C]l \qquad [26.5]$$

where ε is a constant for the absorbing substance at the wavelength the measurement is made and is termed the absorption coefficient or absorptivity, [C] is expressed as either mol L^{-1} or g L^{-1} (see p. 45) and l is given in cm. This relationship is extremely useful, since most spectrophotometers are constructed to give a direct measurement of $\log_{10}(I_0/I)$, termed the absorbance (A), or extinction (E), of a solution (older texts may use the outdated term optical density). Note that for substances obeying the Beer–Lambert relationship, A is linearly related to [C]. Absorbance at a particular wavelength is often shown as a subscript, e.g. A_{550} represents the absorbance at 550 nm. The proportion of light passing through the solution is known as the transmittance (T), and is calculated as the ratio of the emergent and incident light intensities.

Some instruments have two scales:

1. An exponential scale from zero to infinity, measuring absorbance.
2. A linear scale from 0 to 100, measuring (per cent) transmittance.

For most practical purposes, the Beer–Lambert relationship will apply and you should use the absorbance scale.

Definition

Absorbance (A) – this is given by:

$A = \log_{10}(I_0/I)$

Definition

Transmittance (T) – this is usually expressed as a percentage, where:

$T = (I/I_0) \times 100(\%)$

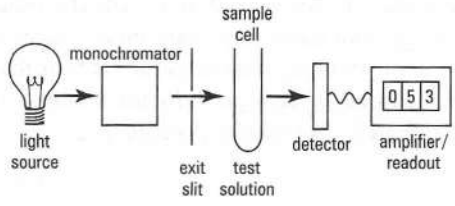


Fig. 26.2 Components of a UV/visible spectrophotometer.

UV/visible spectrophotometer

The principal components of a UV/visible spectrophotometer are shown in Fig. 26.2. High-intensity tungsten bulbs are used as the light source in basic instruments, capable of operating in the visible region (i.e. 400–700 nm). Deuterium lamps are used for UV spectrophotometry (200–400 nm); these

All spectroscopic equipment is costly and equipment must be used under guidance from a demonstrator or technician. All equipment in this section has an inherent hazard due to its use of mains electricity.

lamps are fitted with quartz envelopes, since glass does not transmit UV radiation.

The spectrophotometer is a major improvement over the simple colorimeter since it uses a diffraction grating to produce a parallel beam of monochromatic light from the (polychromatic) light source. In practice the light emerging from such a monochromator is not of a single wavelength, but is a narrow band of wavelengths. This bandwidth is an important characteristic, since it determines the wavelengths used in absorption measurements – the bandwidth of basic spectrophotometers is around 5–10 nm while research instruments have bandwidths of less than 1 nm.

Bandwidth is affected by the width of the exit slit (the slit width), since the bandwidth will be reduced by decreasing the slit width. To obtain accurate data at a particular wavelength setting, the narrowest possible slit width should be used. However, decreasing the slit width also reduces the amount of light reaching the detector, decreasing the signal-to-noise ratio. The extent to which the slit width can be reduced depends upon the sensitivity and stability of the detection/amplification system and the presence of stray light.

Most UV/visible spectrophotometers are designed to take cells (cuvettes) with an optical path length of 10 mm. Disposable plastic cells are suitable for routine work in the visible range using aqueous and alcohol-based solvents, while glass cells must be used for most other organic solvents. Glass cells are manufactured to more exacting standards, so you should use optically matched glass cells for accurate work, especially at low absorbances (< 0.1), where any differences in the optical properties of cells for reference and test samples will be pronounced. Glass and plastic absorb UV light, so quartz cells must be used at wavelengths below 300 nm.

Using plastic disposable cells – these are adequate for work in the near-UV region, e.g. for Job's plot studies, as well as the visible range.

Before taking a measurement, make sure that cells are clean, unscratched, dry on the outside, filled to the correct level and in the correct position in their sample holders. Unwanted material can accumulate on the inside faces of glass/quartz cells, so remove any deposits using acetone on a cotton bud, or soak overnight in 1 mol L^{-1} nitric acid. Corrosive and hazardous solutions must be used in cells with tightly fitting lids or Teflon® stoppers, to prevent damage to the instrument and to reduce the risk of accidental spillage.

Handling cells – Never handle the cells by the non-polished sides.

Examples The molar absorptivity of phenol is 6.20×10^3 L mol^{-1} cm^{-1} at 210 nm. For a test solution giving an absorbance of 0.21 in a cell with a light path of 5 mm, using eqn [26.5] this is equal to a concentration of:

$0.21 = 6.20 \times 10^3 \times 0.5 \times [C]$

$[C] = 0.000\,067\,7$ mol L^{-1} (or 67.7 μmol L^{-1})

Safety note Never balance full cells on the instrument.

Basic instruments use photocells similar to those used in simple colorimeters or photodiode detectors. In many cases, a different photocell must be used at wavelengths above and below 550–600 nm, owing to differences in the sensitivity of such detectors over the visible waveband. The detectors used in more sophisticated instruments, give increased sensitivity and stability when compared with photocells.

Digital displays are increasingly used in preference to needle-type meters, as they are not prone to parallax errors and misreading of the absorbance scale. Some digital instruments can be calibrated to give a direct readout of the concentration of the test substance.

Measuring absorbances in colorimetric analysis – if any final solution has an absorbance that is too high to be read with accuracy on your spectrophotometer (i.e. $A > 2$), it is bad practice to dilute the solution so that it can be measured. This dilutes both the sample molecules and the colour reagents to an equal extent. Instead, you should dilute the original sample and re-assay.

Types of UV/visible spectrophotometer

Basic instruments are single-beam spectrophotometers in which there is only one light path. The instrument is set to zero absorbance using a blank solution, which is then replaced by the test solution, to obtain an absorbance reading. An alternative approach is used in double-beam spectrophotometers, where the light beam from the monochromator is split into two separate beams, one beam passing through the test solution and the other through a reference blank. Absorbance is then measured by an electronic circuit which compares the outputs from the reference (blank) and sample cells. Double-

Plotting calibration curves in quantitative analysis – do not force your calibration line to pass through zero if clearly it does not. There is no reason to assume that the zero value is any more accurate than any other reading you have made.

beam spectrophotometry reduces measurement errors caused by fluctuations in output from the light source or changes in the sensitivity of the detection system, since reference and test solutions are measured at the same time (Box 26.1). Recording spectrophotometers are double-beam instruments, designed for use with a chart recorder or computer, either by recording the difference in absorbance between reference and test solutions across a predetermined waveband to give an absorption spectrum, or by recording the change in absorbance at a particular wavelength as a function of time (e.g. in a kinetic determination.

Quantitative spectrophotometric analysis

A single (purified) substance in solution can be quantified using the Beer–Lambert relationship (eqn [26.5]), provided its absorptivity is known at a particular wavelength (usually at the absorption maximum for the substance, since this will give the greatest sensitivity). The molar absorptivity is the absorbance given by a solution with a concentration of $1\,\text{mol}\,\text{L}^{-1}$

Box 26.1 Using a UV/visible spectrophotometer

1. **Switch on and select the correct lamp** for your measurements (e.g. deuterium for UV, tungsten for visible light).
2. **Allow up to 15 min for the lamp to warm up** and for the instrument to stabilize before use.
3. **Select the appropriate wavelength:** on older instruments a dial is used to adjust the monochromator, while newer machines have microprocessor-controlled wavelength selection.
4. **Select the appropriate detector:** some instruments choose the correct detector automatically (on the basis of the specified wavelength), while others have manual selection.
5. **Choose the correct slit width** (if available): this may be specified in the protocol you are following, or may be chosen on the manufacturer's recommendations.
6. **Insert appropriate reference blank(s):** single-beam instruments use a single cell, while double-beam instruments use two cells (a matched pair for accurate work). The reference blank should match the test solution in all respects apart from the substance under test, i.e. they should contain all reagents apart from this substance. Make sure that the cells are positioned correctly, with their polished (transparent) faces in the light path, and that they are accurately located in the cell holder(s).
7. **Check/adjust the 0% transmittance:** most instruments have a control which allows you to zero the detector output in the absence of any light (termed dark current correction). Some micro-processor-controlled instruments carry out this step automatically.
8. **Set the absorbance reading to zero:** usually via a dial, or digital readout.
9. **Analyse your samples:** replace the appropriate reference blank with a test sample, allow the absorbance reading to stabilize (5–10 s) and read the absorbance value from the meter/readout device. For absorbance readings greater than one (i.e. $< 10\%$ transmission), the signal-to-noise ratio is too low for accurate results. Your analysis may require a calibration curve or you may be able to use the Beer–Lambert relationship (eqn [26.5]) to determine the concentration of test substance in your samples.
10. **Check the scale zero at regular intervals** using a reference blank, e.g. after every 10 samples.
11. **Check the reproducibility of the instrument:** measure the absorbance of a single solution several times during your analysis. It should give the same value.

    Problems (and solutions): Inaccurate/unstable readings are most often due to incorrect use of cells, e.g. dirt, fingerprints or test solution on outside of cell (wipe the polished faces using a soft tissue before insertion into the cell holder), condensation (if cold solutions are not allowed to reach room temperature before use), air bubbles (which scatter light and increase the absorbance; tap gently to remove), insufficient solution (causing refraction of light at the meniscus), particulate material in the solution (check for 'cloudiness' in the solution and centrifuge before use, where necessary) or incorrect positioning in light path (locate in correct position).

($= 1\,\text{kmol}\,\text{m}^{-3}$) of the compound in a light path of 1 cm. The appropriate value may be available from tabulated spectral data (e.g. Anon., 1963), or it can be determined experimentally by measuring the absorbance of known concentrations of the substance (Box 26.1) and plotting a standard curve. This should confirm that the relationship is linear over the desired concentration range and the slope of the line will give the molar absorptivity.

Fluorescence

With most molecules, after electrons are raised to a higher energy level by absorption of electromagnetic radiation, they soon fall back to the ground state by radiationless transfer of energy (heat) to the solvent. However, with some molecules, the events shown in Fig. 26.3 may occur, i.e. electrons may lose only part of their energy by non-radiant routes and the rest may be emitted as electromagnetic radiation, a phenomenon known as fluorescence. Since not all of the energy that was absorbed is emitted (due to non-radiant loss), the wavelength of the fluorescent light is longer than the absorbed light (longer wavelength = lower energy). Thus, a fluorescent molecule has both an absorption spectrum and an emission spectrum.

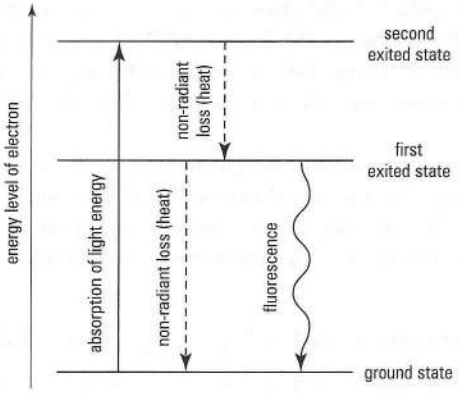


Fig. 26.3 Energy levels and energy transitions in fluorescence.

Fluorescence spectrophotometry

The principal components of a fluorescence spectrophotometer (fluorimeter) are shown in Fig. 26.4. The instrument contains two monochromators, one to select the excitation wavelength and the other to monitor the light emitted, usually at 90° to the incident beam (though light is actually emitted in all directions). As an example, the wavelengths used to measure the highly fluorescent compound naphthalene are 270 nm (excitation) and 340 nm (emission). Some examples of molecules with intrinsic fluorescence are given in Table 26.1.

Table 26.1 Examples of compounds with intrinsic fluorescence

Drugs	Aspirin, morphine, barbiturates, propanalol, ampicillin, tetracyclines
Vitamins	Riboflavin, vitamins A, B6 and E, nicotinamide
Pollutants	Naphthalene, anthracene, benzopyrene

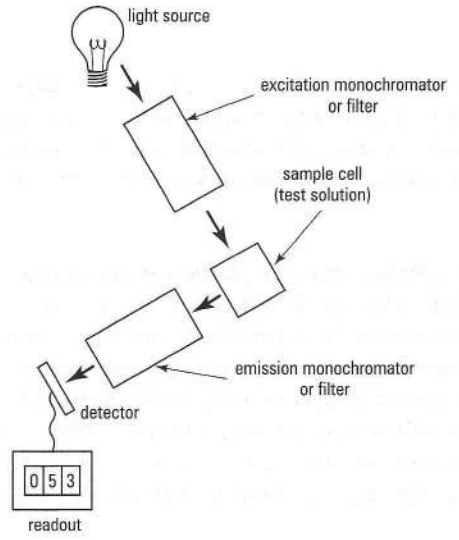


Fig. 26.4 Components of a fluorimeter (fluorescence spectrophotometer). Note that sample cells for fluorimetry must have clear sides all round.

Compared with UV/visible spectrophotometry, fluorescence spectroscopy has certain advantages, including:

- Enhanced sensitivity (up to 1000-fold), since the emitted light is detected against a background of zero, in contrast to spectrophotometry where small changes in signal are measured against a large 'background' (see eqn [26.5]).
- Increased specificity, because not one, but two, specific wavelengths are required for a particular compound.

However, there are also certain drawbacks:

- Not all compounds show intrinsic fluorescence, limiting its application. However, some non-fluorescent compounds may be coupled to fluorescent dyes, or fluorophores (e.g. alcohol ethoxylates may be coupled to naphthoyl chloride).
- The light emitted can be less than expected owing to quenching, i.e. when substances in the sample (e.g. oxygen) either interfere with energy transfer, or absorb the emitted light (in some instances, the sample molecules may self-quench if they are present at high concentration).

The sensitivity of fluorescence has made it invaluable in techniques in which specific chemicals, e.g. polycyclic aromatic hydrocarbons and alcohol ethoxylates, are linked to a fluorescent dye for detection in high-performance liquid chromatography (p. 218).

Phosphorescence and luminescence

A phenomenon related to fluorescence is phosphorescence, which is the emission of light following intersystem crossing between electron orbitals (e.g. between excited singlet and triplet states). Light emission in phosphorescence usually continues after the exciting energy is no longer applied and, since more energy is lost in intersystem crossing, the emission wavelengths are generally longer than with fluorescence. Phosphorescence has limited applications in chemical sciences.

Luminescence (or chemiluminescence) is another phenomenon in which light is emitted, but here the energy for the initial excitation of electrons is provided by a chemical reaction rather than by electromagnetic radiation. An example is the action of the enzyme luciferase, extracted from fireflies, which catalyses the following reaction:

$$\text{luciferin} + \text{ATP} + O_2 \rightarrow \text{oxyluciferin} + \text{AMP} + PP_i + CO_2 + \text{light} \quad [26.6]$$

The light produced is either yellow–green (560 nm) or red (620 nm). This system can be used in biomolecular analysis of ATP, e.g. to determine ATP concentration in a biological sample. Measurement can be performed using the photomultiplier tubes of a scintillation counter (p. 237) to detect the emitted light, with calibration of the output using a series of standards of known ATP content.

Atomic spectroscopy

Atoms of certain metals will absorb and emit radiation of specific wavelengths when heated in a flame, in direct proportion to the number of atoms present. Atomic spectrophotometric techniques measure the absorption or emission of particular wavelengths of UV and visible light, to identify and quantify such metals.

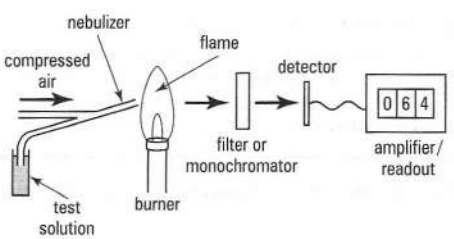


Fig. 26.5 Components of a flame photometer.

Safety note In atomic spectroscopy, the use of high pressure gas sources, e.g. cylinders, can be particularly hazardous. Always consult a demonstrator or technician before use.

Flame atomic emission spectrophotometry (or flame photometry)

The principal components of a flame photometer are shown in Fig. 26.5. A liquid sample is converted into an aerosol in a nebulizer (atomizer) before being introduced into the flame, where a small proportion (typically less than 1 in 10 000) of the atoms will be raised to a higher energy level, releasing this energy as light of a specific wavelength, which is passed through a filter to a photocell detector. Flame photometry can be used to measure the alkali metal ions K^+, Na^+ and Ca^{2+} in, for example, biological fluids and water samples (Box 26.2).

Atomic absorption spectroscopy

This technique is applicable to a broad range of metal ions, including those of Pb, Cu, Zn, etc. It relies on the absorption of light of a specific wavelength by atoms dispersed in a flame. The appropriate wavelength is provided by a hollow cathode lamp, coated with the element to be analysed, focused through the flame and onto the detector. When the sample is introduced into the flame, it will decrease the light detected in

Box 26.2 Using a flame photometer

1. **Allow time for the instrument to stabilize.** Switch on the instrument, light the flame and wait at least 5 min before analysing your solutions.
2. **Check for impurities in your reagents.** For example, if you are measuring Na^+ in an acid digest of some material, e.g. soil, check the Na^+ content of a reagent blank, containing everything except the soil, processed in exactly the same way as the samples. Subtract this value from your sample values to obtain the true Na^+ content.
3. **Quantify your samples using a calibration curve** (p. 251). Calibration standards should cover the expected concentration range for the test solutions – your calibration curve may be non-linear (especially at concentrations above 1 mmol L^{-1}, i.e. 1 mol m^{-3}).
4. **Analyse all solutions in duplicate,** so that repeatability can be assessed.
5. **Check your calibration.** Make repeated measurements of a standard solution of known concentration after every six or seven samples, to confirm that the instrument calibration is still valid.
6. **Consider the possibility of interference.** Other metal atoms may emit light which is detected by the photocell, since the filters cover a wider waveband than the emission line of a particular element. This can be a serious problem if you are trying to measure low concentrations of a particular metal in the presence of high concentrations of other metals (e.g. Na^+ in sea water), or other substances which form complexes with the test metal, suppressing the signal (e.g. phosphate).

direct proportion to the amount of metal present. Practical advantages over flame photometry include:

- improved sensitivity;
- increased precision;
- decreased interference.

The technique can be used with or without a flame. In the flameless technique several variations are possible, including a graphite furnace or cold vapour, all of which are more sensitive than flame photometry. Further details on atomic absorption spectroscopy are given in Chapter 27.

Safety note Caution is needed when using strong (concentrated) acids: always work in a fume cupboard, wear gloves to protect your hands from 'acid burns', and always rinse affected areas with large amounts of water.

27 Atomic spectroscopy

All spectroscopic equipment is costly. The use of such equipment must always be done under guidance from a demonstrator or technician. All equipment in this section has an inherent risk due to its use of mains electricity.

Safety note In atomic spectroscopy, the use of high-pressure gas sources, e.g. cylinders, can be particularly hazardous. Always consult a demonstrator or technician before use.

Atomic spectroscopy is a quantitative technique used for the determination of metals in samples. Atomic spectroscopy is characterized by two main techniques: atomic absorption spectroscopy and atomic emission spectroscopy. Atomic absorption spectroscopy (AAS) is normally carried out with a flame (FAAS), although other devices can be used. Atomic emission spectroscopy (AES) is typified by the use of a flame photometer (p. 168) or an inductively coupled plasma. The flame photometer is normally used for elements in groups I and II of the Periodic Table only, i.e. alkali and alkali earth metals.

In both AAS and AES the substance to be analysed must be in solution. In order to do quantitative analysis, i.e. determine how much of the metal is present, the preparation of analytical standard solutions is necessary. While the concentration range over which the technique can be used may be different, for various instruments, the principles associated with the preparation of analytical standard solutions are the same (Boxes 27.1–27.5).

Atomic Absorption Spectroscopy

The components of an atomic absorption spectrometer are a radiation source, an atomization cell, a sample introduction system, a method of wavelength selection and a detector (Fig. 27.2).

Sample/standard dilutions – all dilutions should be done using appropriate glassware or plastic ware. Typically, this involves the use of grade A pipettes for the transfer of known volumes of liquids and grade A volumetric flasks for subsequent dilutions.

Radiation source

The main radiation source for AAS is the hollow-cathode lamp (HCL). The HCL (Fig. 27.3) emits radiation characteristic of a particular element. The choice of HCL for AAS is simple. For example, if you are analysing for lead, you will need a lead-coated HCL. It is normal to pre-warm the HCL for about 10 min prior to use. This can be done either by using a separate pre-

Box 27.1 How to prepare a 1000 $\mu g\,mL^{-1}$ stock solution of a metal ion from a metal salt

Stock solutions can be prepared directly from reagent-grade chemicals. It is important to use only reagent-grade chemicals of the highest purity e.g. AnalaR®. This includes the water to be used – distilled and deionized – MilliQ water. (Note: many reagents (solids and liquids) contain metallic impurities in trace amounts. While you can minimize this risk of contamination by using the highest-purity reagents, it is essential to run 'reagent blanks', especially for elemental determinations trace levels.)

1. **Determine the M_r of the metal salt**. For example, the M_r of $Pb(NO_3)_2 = 331.20\,g\,mol^{-1}$.
2. **Determine the A_r of the metal.** The A_r for Pb is $207.19\,g\,mol^{-1}$.
3. **Ratio the M_r to A_r:**

$$\frac{331.20}{207.19} = 1.5985\,g \text{ of } Pb(NO_3)_2 \text{ in 1 litre}$$

4. **Accurately weigh out (p. 19) the metal salt.** In this case, weigh 1.5985 g of $Pb(NO_3)_2$.
5. **Quantitatively transfer the metal salt to a pre-cleaned 100 mL beaker** and dissolve in 1% v/v HNO_3 (AnalaR® or equivalent).
6. **Quantitatively transfer the dissolved metal salt to a 1 L volumetric flask** and make up to the graduation mark with 1% v/v HNO_3 (AnalaR® or equivalent).

Often, a certified stock standard with a single or multielement composition can be purchased, usually at a concentration (per element) of $1000\,mg\,L^{-1}$ ($1000\,\mu g\,mL^{-1}$).

Box 27.2 How to prepare a set of five calibration solutions in the concentration range 0–10 $\mu g\,mL^{-1}$ ($mg\,L^{-1}$)

Assuming that we are starting with a 1000 $\mu g\,mL^{-1}$ stock solution of a particular metal, e.g. lead, then you will need the following: six 100.00 mL grade A volumetric flasks; two 100 mL beakers; and, a graduated pipette (0–10.00 mL).

1. **Ensure that all the glassware is clean** (see p. 13).
2. **Transfer ≈ 15 mL of the stock solution into one of the pre-cleaned beakers.**
3. **Quantitatively transfer 10.00 mL of the stock solution** into a 100.00 mL volumetric flask. Then, dilute to 100.00 mL with 1 % v/v HNO_3 (high purity).
4. **What is the concentration of this new solution?** Remember that we started with an initial 1000 $\mu g\,mL^{-1}$ Pb stock solution.

$$\frac{1000\mu g}{mL} \times 10\,mL \equiv 10\,000\,\mu g\,Pb$$

10 000 μg Pb was placed in a 100.00 mL volumetric flask, so:

$$\frac{10\,000\mu g}{100\,mL} \equiv 100\,\mu g\,mL^{-1}\ Pb$$

You now have a 100 $\mu g\,mL^{-1}$ 'working' stock solution of Pb.

5. **Transfer ≈ 15 mL of the working stock solution into the other pre-cleaned beaker.**
6. **Then, quantitatively transfer 2.00 mL of the solution into a 100.00 mL volumetric flask** and dilute to 100.00 mL with 1 % v/v HNO_3 (high purity). Label the flask as the 2 $\mu g\,mL^{-1}$ Pb calibration solution.
7. **Similarly transfer 0, 4.00, 6.00, 8.00 and 10.00 mL volumes into separate volumetric flasks** and dilute to 100.00 mL with the nitric acid and label as 0, 4, 6, 8 and 10 $\mu g\,mL^{-1}$ Pb calibration solutions.
8. **Take the 0, 2, 4, 6, 8 and 10 $\mu g\,mL^{-1}$ Pb calibration solutions** for FAAS analysis.

Safety note Caution is needed when using strong (concentrated) acids. When using concentrated acids always work in a fume cupboard. Wear gloves to protect your hands from 'acid burns'. Always rinse affected areas with copious amounts of water.

heater unit, capable of warming up several HCLs simultaneously, or by inserting the HCL in the AAS instrument and switching on the current. The lamp is typically operated at an electric current between 2 and 30 mA.

Atomization cell

Several types of atomization cell are available: flame, graphite furnace, hydride generation and cold vapour. Flame is the most common. In the pre-mixed laminar flame, the fuel and oxidant gases are mixed before they enter the burner (the ignition site) in an expansion chamber. The more commonly used flame in FAAS is the air–acetylene flame (temperature, 2500 K), while the nitrous oxide-acetylene flame (temperature, 3150 K) is used for refractory elements, e.g. Al. Both are formed in a slot burner positioned in the light path of the HCL (Fig. 27.4).

In the graphite furnace atomizer, a small volume of sample (5–100 μL) is introduced onto the inner surface of a graphite tube (or onto a platform placed within the tube) through a small opening (Fig. 27.5). The graphite tube is arranged so that light from the HCL passes directly through the centre. Passing an electric current through the tube allows the operator to program a heating cycle, with several stages (Fig. 27.6) including the elimination of water from the sample (drying), removal of the sample matrix (ashing), atomization of the analyte (analysis), and removal of extraneous material (cleaning). An internal gas flow of inert gas (N_2 or Ar) during the drying and ashing stages removes any extraneous material.

Hydride generation is a sample introduction technique exclusively for elements that form volatile hydrides (e.g. As, Se, Sn). An acidified sample solution is reacted with sodium borohydride solution, liberating the gaseous hydride in a gas–liquid separator. The generated hydride is then transported to

Box 27.3 How to analyse a sample using the method of standard additions in FAAS

The method of standard additions is used when the sample matrix may cause difficulties, e.g. chemical interferences, in sample concentration determination. Standard additions allows any adverse effects to be overcome by incorporating a known amount of the sample in the calibration solutions.

1. **Prepare a 1000 μg mL^{-1} stock solution** (see Box 27.1).
2. **Then, prepare a 100 μg mL^{-1} working stock solution** (see Box 27.2).
3. **You will also need to have prepared the sample.** If the sample is a solid you will need to digest the sample (see Box 27.7).
4. **An estimate of the metal concentration in the sample is required prior to carrying out standard additions** so that the linear relationship between signal (absorbance) and concentration is maintained.
5. **You can then prepare the standard addition solutions.** This is most easily done as in Table 27.1.
6. **Analyse the samples using FAAS.**
7. **Plot the graph.** The graphical output should appear as shown in Fig. 27.1.
8. **The graph should contain several features:** it must have a linear response (signal against concentration); it does not pass through the origin; and extrapolation of the graph is required until it intersects the *x*-axis, e.g. 3.2 μg mL^{-1}.
9. **Determine the concentration of the metal in the original sample.** This can be done by taking into account the dilutions involved in the standard additions method and any dilutions used to prepare the sample (see dilution factor, Box 27.5).

Table 27.1 Standard additions solutions

Volumetric flask (100.00 mL capacity)	Volume of 100 μg mL^{-1} working stock solution (mL)	Volume of aqueous sample solution (mL)
1	0	10
2	1	10
3	3	10
4	5	10
5	7	10

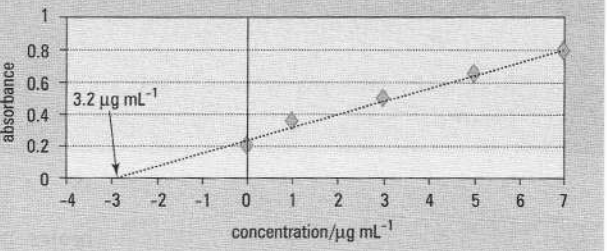


Fig. 27.1 Standard additions graph.

an atomization cell using a carrier gas. The atomization cell is normally an electrically heated or flame-heated quartz tube. Using arsenic as an example it is possible to write the following equation for the generation of arsine (AsH_3):

$$3BH_4^- + 3H^+ + 4H_3AsO_3 \rightarrow 3H_3BO_3 + 4AsH_3 + 3H_2O \quad [27.1]$$

Cold vapour generation is the term exclusively reserved for mercury. Mercury in a sample is reduced to elemental mercury by tin (II) chloride (eqn 27.2):

$$Sn^{2+} + Hg^{2+} \rightarrow Sn^{4+} + Hg^0 \quad [27.2]$$

and the mercury vapour produced is transported to an atomization cell by a carrier gas. The atomization cell consists of a long-path glass absorption cell located in the path of the HCL. Mercury is monitored at a wavelength of 253.7 nm.

Sample introduction into the flame

Samples are almost exclusively introduced into flames as liquids. Solid samples need to be converted to aqueous solutions using methods such as decomposition (see p. 177). Once in the aqueous form the sample is introduced into the flame using a nebulizer/expansion chamber.

The pneumatic concentric nebulizer (see also p. 176) consists of a stainless steel tube through which a Pt/Ir capillary tube is located. The aqueous

Box 27.4 Sample size and certified reference materials

If a linear calibration graph (plot of concentration against absorbance) has been prepared for lead in FAAS with the concentrations 0, 2, 4, 6, 8 and 10 $\mu g\,mL^{-1}$, the absorbance for the digested sample needs to fall within the linear portion of the graph. This is controlled by two (non-instrumental) factors: the weight of the sample digested and the final volume that the digested sample is made up to. As the volume of the digested sample is limited by the availability of volumetric flasks (10.00, 25.00, 50.00, 100.00 or 250.00 mL are most commonly used, with the 50.00 and 100.00 mL volumetric flasks the most common) it is often easier to alter the sample size. In order to have a representative sample, a minimum sample size is often recommended. For example, if using a certified reference material (CRM) the supplier will recommend a minimum sample size to ensure homogeneity, e.g. not < 0.5 g for a powdered solid steel or alloy sample, or not < 1.0 g for a powdered biological sample such as citrus leaves. Often the maximum sample size is limited by the cost of the CRM. If using a 'real' sample then it is best to take a larger sample size, since a CRM has usually been tested and prepared to a high specification with respect to drying, milling and shelf-life time. A typical minimum sample size for a soil might be 5.00 g. It is important if using 'real' samples to consider the following additional factors:

- Sampling – how it is to be done? How will a representative sample be arrived at?
- Storage of sample – what containers will be used for storage of sample? Be aware of contamination for the storage container and from the implements used to sample and transfer the sample.
- Lifetime of stored sample – how long will the sample remain stable? Is preservation of the sample necessary?

Note: CRMs can be obtained from appropriate suppliers, e.g. Laboratory of the Government Chemist in the UK or the National Institute for Science and Technology in the USA. In addition to the CRM, a certificate is provided that contains information on the concentration of various metals within the sample as well as their variation (normally quoted as one standard deviation either side of the mean value), e.g. $2.5 \pm 0.3\,\mu g\,g^{-1}$ Pb. CRMs are used to test the accuracy of a new method or to enable a quality control scheme to be operated by a commercial laboratory. In practical work they are useful to assess student performance in terms of his/her ability to prepare and analyse a sample.

Box 27.5 Analysis of a sample: dilution factor

A sample was weighed (0.4998 g) and digested in concentrated nitric acid (20 mL). After cooling, the digested sample was quantitatively transferred into a 100.00 mL volumetric flask and made up with ultrapure water and then analysed for lead by FAAS. Let us suppose that the absorbance obtained corresponds to a concentration of 3.4 $\mu g\,mL^{-1}$. What is the concentration of lead in the original sample?

The method of calculation is most appropriately done as follows:

- Calculate the dilution factor. This can be done if the final volume of the sample and its original weight are known. In this case 100 mL and 0.4998 g.
- You then multiply the concentration from the graph with the dilution factor:

$$\frac{3.4\,\mu g}{mL} \times \frac{100\,mL}{0.4998\,g} = 680\,\mu g\,g^{-1}$$

Note:

- The volume of acid used is irrelevant in the calculation – only the final volume in the volumetric flask matters.
- The units cancel (mL on top line cancels with mL on the bottom line) leaving you with units of $\mu g\,g^{-1}$ ($\mu g/g$).
- Alternatively, the units can be expressed in $mg\,kg^{-1}$ (mg/kg), i.e. $680\,mg\,kg^{-1}$ or % w/w, i.e. 0.068% w/w (see p. 46). ($10\,000\,\mu g\,g^{-1} \equiv 1\%$ w/w or for aqueous samples $10\,000\,\mu g\,mL^{-1} \equiv 1\%$ w/v).

sample is drawn up through the capillary tube by the action of the oxidant gas (air) escaping through the exit orifice that exists between the outside of the capillary tube and the inside of the stainless steel tube. The action of the escaping air and aqueous sample is sufficient to form a coarse aerosol in a process termed the Venturi effect. The typical uptake rate of the nebulizer is between 3 and 6 $mL\,min^{-1}$.

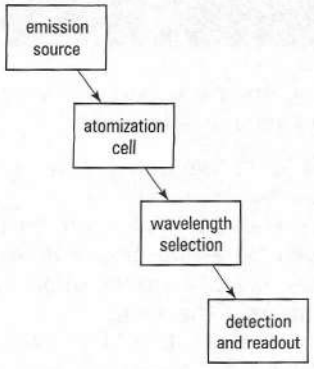


Fig. 27.2 Components of an atomic absorption spectrometer.

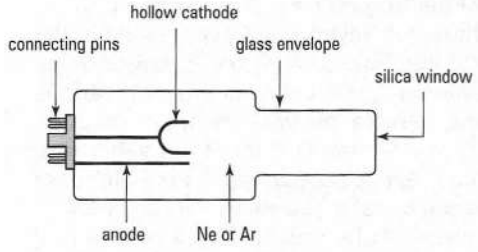


Fig. 27.3 Components of a hollow-cathode lamp (HCL).

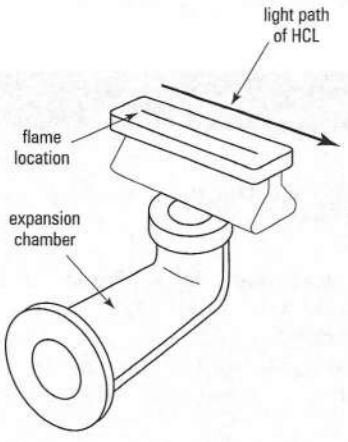


Fig. 27.4 Components of a slot burner for FAAP.

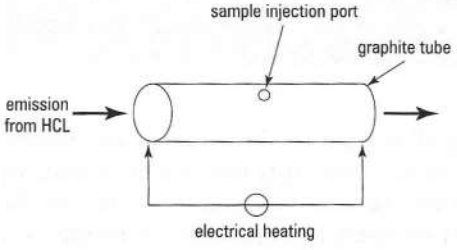


Fig. 27.5 Schematic diagram of a graphite furnace atomizer.

The expansion chamber (Fig. 27.7) has two functions. The first is concerned with aerosol generation the objectives of which are:

- to convert the aqueous sample solution into a coarse aerosol using the oxidant gas;
- to disperse the coarse aerosol further into a fine aerosol, by interaction with baffles located within the chamber;
- to condense any residual aerosol particles, which then go to waste.

The second function involves the safe pre-mixing of the oxidant and fuel gases before they are introduced into the laminar flow burner.

Wavelength selection and detection

As AAS is used to monitor one metal at a time, the spectrometer used is termed a monochromator. Two optical arrangements are possible; single and double beam. The latter is preferred as it corrects for fluctuations in the HCL caused by warm-up, drift and source noise, thus leading to improved precision in the absorbance measurement. A schematic diagram of the optical arrangement is shown in Fig. 27.8. The attenuation of the HCL radiation by the atomic vapour is detected by a photomultiplier tube (PMT), a device for proportionally converting photons of light to electric current.

Background correction methods

One of the main practical problems with the use of AAS is the occurrence of molecular species that coincide with the atomic signal. One approach to remove this molecular absorbance is by the use of background correction methods. Several approaches are possible, but the most common is based on the use of a continuum source, D_2. In the atomization cell (e.g. flame) absorption is possible from both atomic species and from molecular species (unwanted interference). By measuring the absorption that occurs from the radiation source (HCL) and comparing it with the absorbance that occurs from the continuum source (D_2) a corrected absorption signal can be obtained. This is because the atomic species of interest absorb the specific radiation associated with the HCL source, whereas the absorption of radiation by the continuum source for the same atomic species will be negligible.

Interferences in the flame

Interferences in the flame can be classified into four categories: chemical, ionization, physical and spectral.

Chemical interferences occur when the analyte forms a thermally stable compound with a molecular or ionic species present in the sample solution. Examples include the suppression of alkaline earth metals due to the presence of phosphate, silicate or aluminate in the sample solution in the air–acetylene flame. The most well-known example of this is the absorption signal suppression that occurs for Ca at 422.7 nm owing to increasing amounts of phosphate. This signal suppression is due to the formation of calcium pyrophosphate, a thermally stable compound in the flame.

Ionization interferences occur most commonly for alkali and alkaline earth metals. The low ionization potential of these metals can lead to their ionization in the relatively hot environment of the flame. If this occurs, no absorption signal is detected, since FAAS is a technique for measuring atoms not ions. This process can be prevented by the addition of an ionization suppressor or 'buffer', e.g. an alkali metal such as Cs. Addition of excess Cs

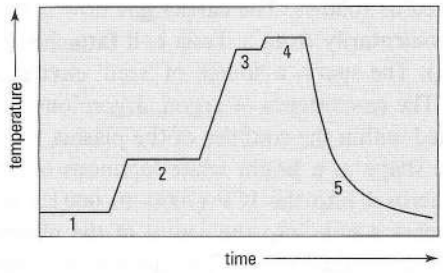


Fig. 27.6 Heating cycle for a graphite furnace atomizer. 1. drying; 2. ashing; 3. analysis; 4. cleaning and 5. cooling

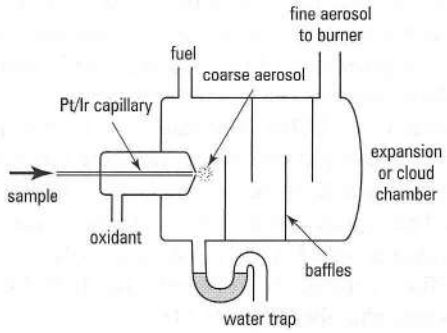


Fig. 27.7 Schematic diagram of a nebulizer – expansion chamber for FAAS.

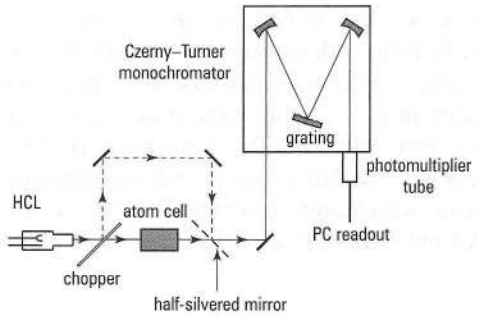


Fig. 27.8 Schematic diagram of the optical arrangement for AAS.

Make sure distilled water is available to aspirate into the flame once it is ignited.

Contamination risk – wipe the outside of the aspirator tube with a clean tissue in between samples/standards to prevent contamination.

Safety note Once the flame is ignited it should not be left unattended.

leads to its ionization in the flame in preference to the metal of interest, e.g. Na. This process is termed the 'mass action' effect.

Physical interferences are due to the effects of the sample solution on aerosol formation within the spray chamber. The formation of an aerosol is dependent upon the surface tension, density and viscosity of the sample solution. This type of interference can be controlled by the matrix matching of sample and standard solutions, i.e. add the same sample components to the standard solution, but without the metal of interest. If this is not possible, it is then necessary to use the method of standard additions (Box 27.3).

Spectral interferences are uncommon in AAS owing to the selectivity of the technique. However, some interferences may occur, e.g. the resonance line of Cu occurs at 324.754 nm and has a line coincidence from Eu at 324.753 nm. Unless the Eu is 1000 times in excess, however, it is unlikely to cause any problems for Cu determination. In addition to atomic spectral overlap, molecular band absorption can cause problems, e.g. calcium hydroxide has an absorption band on the Ba wavelength of 553.55 nm while Pb at 217.0 nm has molecular absorption from NaCl. Molecular band absorption can be corrected for using background correction techniques (see p. 174). The operation of a flame atomic absorption spectrometer is described in Box 27.6.

Atomic Emission Spectroscopy

The main components of an atomic emission spectrometer are an atomization and ionization cell, a method of sample introduction, the spectrometer and detector. In contrast to AAS, no radiation source is required.

Flame photometry (see also p. 168) is almost exclusively used for the determination of alkali metals because of their low excitation potential (e.g. sodium 5.14 eV and potassium 4.34 eV). This simplifies the instrumentation required and allows a cooler flame (air–propane, air–butane or air–natural gas) to be used in conjunction with a simpler spectrometer (interference filter). The use of an interference filter allows a large excess of light to be viewed by the detector. Thus, the expensive photomultiplier tube is not required and a cheaper detector can be used, e.g. a photodiode or photoemissive detector. The sample is introduced using a pneumatic nebulizer as described for FAAS (p. 172). Flame photometry is therefore a simple, robust and inexpensive technique for the determination of potassium (766.5 nm) or sodium (589.0 nm) in clinical or environmental samples. The technique suffers from the same type of interferences as in FAAS. The operation of a flame photometer is described in Box 26.2.

Inductively coupled plasma

A radio frequency inductively coupled plasma (ICP) is formed within the confines of three concentric glass tubes or plasma torch (Fig. 27.9). Each concentric glass tube has a tangentially arranged entry point through which argon gas enters the intermediate (plasma) and external (coolant) tubes. The inner tube consists of a capillary tube through which the aerosol is introduced from the sample introduction system. Located around the plasma torch is a coil of water-cooled copper tubing. Power input to the ICP is achieved through this copper, load or induction coil, typically in the range 0.5–1.5 kW at a frequency of 27 or 40 MHz.

Be prepared – ensure all standards, blanks and samples are readily and easily accessible prior to ignition.

Safety note Check that the drain is full of water prior to use.

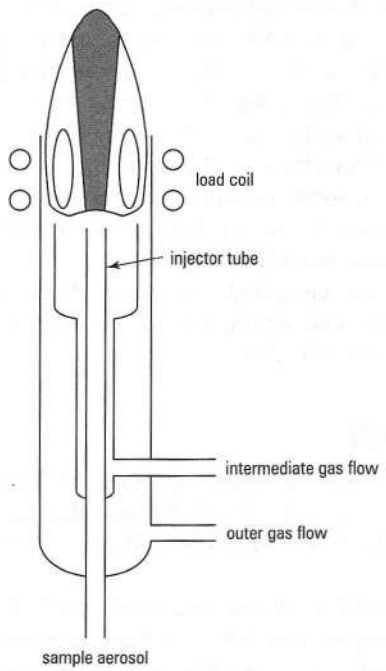


Fig. 27.9 Schematic diagram of an inductively coupled plasma.

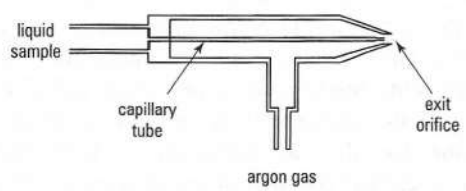


Fig. 27.10 Schematic diagram of a pneumatic nebulizer.

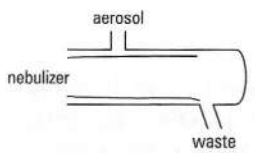


Fig. 27.11 Schematic diagram of a spray chamber.

Initiation of the plasma is achieved as follows. The carrier gas flow is first switched off and a spark added momentarily from a Tesla coil (attached to the outer edge of the plasma torch). The spark, a source of 'seed' electrons, causes ionization of the argon gas. The co-existence of argon, argon ions and electrons constitutes a plasma located within the confines of the plasma torch but protruding from the top in the shape of a bright white luminous bullet. In order to introduce the sample aerosol into the ICP (7000–10 000 K) the carrier gas is switched on and punches a hole into the centre of the plasma creating the characteristic doughnut or toroidal shape. The emitted radiation is viewed laterally (side-on) through the luminous plasma.

Sample introduction

The most common method of liquid sample introduction in ICP–AES is the nebulizer. The nebulizer operates in the same manner as that used for FAAS but there are differences in its construction material and manufactured tolerance (the nebulizer for ICP–AES generates a finer aerosol, but is more inefficient). The pneumatic nebulizer consists of a concentric glass tube through which a capillary tube passes (Fig. 27.10). The sample is drawn up through the capillary by the action of the argon carrier gas escaping through the exit orifice that exists between the outside of the capillary tube and the inside of the glass concentric tube. The typical uptake rate of the nebulizer is between 0.5 and 4 mL min^{-1}. In common with FAAS, a means to reduce the coarse aerosol generated to a fine aerosol is required. In ICP–AES terminology this device is called a spray chamber (Fig. 27.11).

Spectrometers

The nature of the ICP is such that all elemental information from the sample is contained within it. The only limitation is whether it is possible to observe all the elemental information at the same time or one element at once. This limitation is associated not with the ICP but with the type of spectrometer used to view the emitted radiation. A monochromator allows measurement of one wavelength, corresponding to one element at a time, while a polychromator allows multiwavelength or multielement detection. The former can perform sequential multielement analysis, while the latter carries out simultaneous multielement analysis. The typical wavelength coverage required for a spectrometer is between 167 nm (Al) and 852 nm (Cs).

Detectors

The most common detector for AES is the photomultiplier tube (see p. 174). An alternative approach for the detection of multielement (multiwavelength) information is the charged-coupled device (CCD). A CCD is essentially an array of closely spaced metal–insulator–semiconductor diodes formed on a wafer of semiconductor material. Incident light striking the CCD is converted into an electrical signal.

Interferences in ICP–AES

Interferences for AES can be classified into two main categories, spectral and matrix interferences. Spectral interference can occur as a result of an interfering emission line from either another element or the argon source gas, impurities within or entrained into the source, e.g. molecular species such as N_2. Such interferences can be eliminated or reduced either by increasing the resolution of the spectrometer or by selecting an alternative spectral emission line.

Box 27.6 How to operate a flame atomic absorption spectrometer

You should only operate an FAAS system under direct supervision. The instrument should be located under a fume extraction hood. The spectrometer requires approximately 20 min to warm up before switching the gases on and using the instrument.

1. **Adjust the operating wavelength and slit width of the monochromator.** This is done by consulting standard operating conditions, e.g. for lead see Table 27.2.
2. **Decide what wavelength is to be used for the analysis.** For lead the maximum sensitivity is achieved by selecting 217.0 nm.
3. **Adjust the wavelength selector to the appropriate wavelength.**
4. **Adjust the gain control** until the energy meter reading reaches a maximum.
5. **Adjust the wavelength selector** for maximum signal reading. You are now ready to ignite the air–acetylene flame.
6. **Turn on the fume extraction hood.** This allows toxic gases to be safely removed from the laboratory environment.
7. **Turn on the air supply** such that the oxidant flow meter is at the desired setting.
8. **Turn on the acetylene supply** such that the fuel flow meter is at the desired setting.

Table 27. 2 Standard operating conditions for lead

Wavelength (nm)	Slit (nm)	Characteristic concentration ($mg\,L^{-1}$)
283.3	0.7	0.45
217.0	0.7	0.19
205.3	0.7	5.4
202.2	0.7	7.1
261.4	0.7	11.0
368.3	0.7	27.0
364.0	0.7	67.0

Note: recommended flame: air-acetylene, oxidizing (lean, blue).

9. **Press the ignite button (or flame button).** The flame should light instantaneously with a 'pop'.
10. **After establishing the flame,** insert the aspirator tube into distilled water. Allow the flame to stabilize for up to 1 minute by aspirating distilled water, prior to analysis.
11. **After completing your analysis,** shut off the acetylene first (by closing the cylinder valve) and vent the acetylene gas line while the air is still on. Then, shut off the air compressor and allow the air line to vent.
12. **Finally, switch off the fume extraction hood.**

Matrix interferences are often associated with the sample introduction process. For example, pneumatic nebulization can be affected by the dissolved-solids content of the aqueous sample, which affects the uptake rate of the nebulizer and hence the sensitivity of the assay. Matrix effects in the plasma source typically involve the presence of easily ionizable elements (EIEs), e.g. alkali metals, within the plasma source.

Decomposition techniques for solid inorganic samples

Conversion of a solid matrix into a liquid matrix involves the decomposition of the sample. One of the major problems in preparing solid samples for trace element analysis is the potential risk of contamination. Contamination can arise from several sources: the grade of reagents used; the vessels used for digestion and the subsequent dilution of the sample; and human involvement.

In order to minimize the risk of contamination you should take the following measures:

- Use the highest purity of reagents and acids, including the water used for sample dilution.
- Use sample blanks in the analytical procedure, to identify the base level of impurity in the reagents.

- Soak sample vessels in an acid leaching bath (e.g. 10% v/v nitric acid) for at least 24 hours, followed by rinsing in copious amounts of ultrapure water.
- Store cleaned volumetric flasks with their stoppers inserted; cover beakers with Clingfilm® or store upside down to protect from dust.
- In addition to the wearing of a laboratory coat and safety glasses, it may be necessary to wear 'contaminant'-free gloves and a close-fitting hat.

Decomposition involves the liberation of the analyte (metal) of interest from an interfering matrix using a reagent (mineral/oxidizing acids or fusion flux) and/or heat. An important aspect in the decomposition of an unknown sample is the sample size (Box 27.4). You need to consider two aspects. Firstly, the dilution factor required to convert the solid sample to an aqueous solution (Box 27.5), and, secondly, the sensitivity of the analytical instrument, e.g. FAAS.

Acid digestion

This involves the use of mineral or oxidizing acids and an external heat source to decompose the sample matrix. The choice of an individual acid or combination of acids depends upon the nature of the matrix to be decomposed. For example, the digestion of a matrix containing silica, SiO_2 (e.g. a geological sample), requires the use of hydrofluoric acid (HF). A summary of the most common acids used for digestion and their application is shown in Table 27.3.

Once you have chosen an appropriate acid, place your sample into an appropriate vessel for the decomposition stage. Typical vessels include an open glass beaker or boiling tube for conventional heating or for microwave heating, a PTFE or Teflon® PFA (perfluoroalkoxyvinylether) vessel. A typical microwave system operates at 2.45 GHz with up to 14 sample vessels arranged on a rotating carousel; commercial systems have additional features such as: a PTFE-lined cavity; a safety vent (if the pressure inside a vessel is excessive the vent will open, allowing the contents to go to waste); and an ability to measure both the temperature and pressure inside the digestion vessels. The procedure for acid digestion of a sample is shown in Box 27.7.

Table 27. 3 Common acids* used for digestion

Acid(s)	Boiling point (°C)	Comments
Hydrochloric acid (HCl)	110	Useful for salts of carbonates, some oxides and some sulphides. A weak reducing agent; not generally used to dissolve organic matter
Hydrofluoric acid (HF)	112	For digestion of silica-based materials only. Cannot be used with glass containers (use plasticware). In addition to laboratory coat and safety glasses, extra safety precautions are needed, e.g. gloves. In case of spillages, calcium gluconate gel is required for treatment of skin contact sites and should be available during use; evacuate to hospital immediately if skin is exposed to liquid HF
Nitric acid (HNO_3)	122	Useful for the digestion of metals, alloys and biological samples. Oxidizing attack on many samples not dissolved by HCl; liberates trace metals as the soluble nitrate salt
Sulphuric acid (H_2SO_4)	338	Useful for releasing a volatile product; good oxidizing properties for ores, metals, alloys, oxides and hydroxides. Often used in combination with HNO_3. Note: Sulphuric acid must never be used in PTFE vessels (melting point 327 °C)
Hydrochloric/nitric acids (HCl/HNO_3)	–	A 3 : 1 v/v mixture of HCl and HNO_3 is called aqua regia. It forms a reactive intermediate, NOCl. Useful for digesting metals, alloys, sulphides and other ores

*All concentrated acids should be used only in a fume cupboard.

Box 27.7 How to acid-digest a sample using a hot plate

1. **Accurately weigh your sample into a beaker (100 mL).** For digestion of a powdered metal sample 0.5000 g is appropriate (for details on how to weigh accurately see p. 24).
2. **Add 20 mL of concentrated acid(s)** (see Table 27.3).
3. **Cover the beaker with a watch glass.** This is done to prevent the loss of sample and to minimize the risk of contamination.
4. **Place the beaker on a pre-heated hot plate.**
5. **Reflux the sample for approx. 30 mins to 1 hour;** depending on the nature of the sample a coloured, clear solution should result.
6. **Remove the beaker from the heat and allow to cool.** This may take several minutes. Retain the watch-glass cover during this stage to reduce airborne contamination.
7. **Wash the watch-glass cover** into the beaker to 'capture' any splashes of solution.
8. **Dilute the digested sample** with deionized, distilled water.
9. **Quantitatively transfer the diluted, digested sample** to a 100.00 mL volumetric flask (see p. 18). Make up to the graduation mark with de-ionized, distilled water.
10. **Prepare a sample blank using the same procedure,** i.e. perform all of the above tasks, but without adding the actual sample.
11. **Prepare samples in at least duplicate.** For statistical work on the results, at least seven sample digests and two sample blanks are recommended.

Other methods of sample decomposition

The use of acid(s) and heat is probably the most common approach to the decomposition of samples. However, several alternatives exist including dry ashing and fusion.

Dry ashing involves heating the sample in air in a muffle furnace at 400–800 °C to destroy the sample matrix, e.g. soil. After decomposition, the sample residue is dissolved in acid and quantitatively transferred to a volumetric flask prior to analysis. The method may lead to the loss of volatile elements, e.g. Hg, As.

Some substances, such as silicates and oxides, are not always destroyed by the direct action of acid and heat. In these situations an alternative approach is required. Fusion involves the addition of a 10-fold excess of a suitable reagent (e.g. lithium metaborate or tetraborate) to a finely ground sample. The mixture is placed in a metal crucible, e.g. Pt, and then heated in a muffle furnace at 900–1000 °C. After heating (from several minutes to several hours) a clear 'melt' should result, indicating completeness of the decomposition. After cooling, the melt is dissolved in HF (Table 27.3). This process can lead to a higher risk of contamination.

28 Infrared spectroscopy

Identifying compounds – the combination of techniques described in this and the following chapters can often provide sufficient information to identify a compound with a low probability of error.

Definitions

Spectroscopy – any technique involving the production and subsequent recording of a spectrum of electromagnetic radiation, usually in terms of wavelength or energy.

Spectrometry – any technique involving the measurement of a spectrum, e.g. of electromagnetic radiation, molecular masses, etc.

Interpreting spectra – the spectrum produced in UV–vis, IR and NMR spectroscopy is a plot of wavelength or frequency or energy (*x*-axis) against absorption of energy (*y*-axis). Convention puts high frequency (high energy, short wavelength) at the left-hand side of the spectrum.

In addition to ultraviolet–visible (UV–vis) spectroscopy (p. 164), there are three other essential techniques that you will encounter during your laboratory course. They are:

1. *Infrared (IR) spectroscopy*: this is concerned with the energy changes involved in the stretching and bending of covalent bonds in molecules.
2. *Nuclear magnetic resonance (NMR) spectroscopy*: this involves the absorption of energy by specific atomic nuclei in magnetic fields and is probably the most powerful tool available for the structural determination of molecules (Chapter 29).
3. *Mass spectrometry (MS)*: this is based on the fragmentation of compounds into smaller units. The resulting positive ions are then separated according to their mass-to-charge ratio (m/z) (Chapter 30).

As with UV–vis spectroscopy, IR and NMR spectroscopy are based on the interaction of electromagnetic radiation with molecules, whereas MS is different in that it relies on high-energy particles (electrons or ions) to break up the molecules. The relationship between the various types of spectroscopy and the electromagnetic spectrum is shown in Table 28.1.

Infrared spectroscopy

A covalent bond between two atoms can be crudely modelled as a spring connecting two masses and the frequency of vibration of the spring is defined by Hooke's law (eqn [28.1]), which relates the frequency of the vibration (ν) to the strength of the spring, expressed as the force constant (k), and to the masses (m_1 and m_2) on the ends of the spring (defined as the reduced mass $\mu = (m_1 \times m_2) \div (m_1 + m_2)$).

$$\nu = \frac{1}{2\pi}\sqrt{\frac{k}{\mu}} \qquad [28.1]$$

In simple terms, this means that:

- the stretching vibration of a bond between two atoms will increase in frequency (energy) if on changing from a single bond to a double bond and then to a triple bond between the same two atoms (masses), i.e. the spring gets stronger. For example,

Table 28.1 The electromagnetic spectrum and types of spectroscopy

Type of radiation	Origin	Wavelength	Type of spectroscopy
γ-rays	Atomic nuclei	< 0.1 nm	γ-ray spectroscopy
X-rays	Inner shell electrons	0.01–2.0 nm	X-ray fluorescence (XRF)
Ultraviolet (UV)	Ionization	2.0–200 nm	Vacuum UV spectroscopy
UV/visible	Valency electrons	200–800 nm	UV/visible spectroscopy
Infrared	Molecular vibrations	0.8–300 μm	IR and Raman spectroscopy
Microwaves	Molecular rotations Electron spin	1 mm to 30 cm	Microwave spectroscopy Electron spin resonance (ESR)
Radio waves	Nuclear spin	0.6–10 m	Nuclear magnetic resonance (NMR)

ν for C≡C > ν for C=C > ν for C–C

- as the masses of the atoms on a bond increases, the frequency of the vibration decreases, i.e the effect of reducing the magnitude of μ; for example,

ν for C–H > ν for C–C; ν for C–H > ν for C–D; ν for O–H > ν for S–H

Bonds can also bend, but this movement requires less energy than stretching and thus the bending frequency of a bond is always *lower* than the corresponding stretching frequency. When IR radiation of the same frequency as the bond interacts with the bond it is absorbed and increases the amplitude of vibration of the bond. This absorption is detected by the IR spectrometer and results in a peak in the spectrum. For a vibration to be detected in the IR region the bond must undergo a change in dipole moment when the vibration occurs. Bonds with the greatest change in dipole moment during vibration show the most intense absorption, e.g. C=O and C–O.

IR absorption bands – since the frequency of vibration of a bond is a specific value you would expect to see line spectra on the chart. However, each vibration is associated with several rotational motions and bands (peaks) are seen in the spectrum.

Since bonds between specific atoms have particular frequencies of vibration, IR spectroscopy provides a means of identifying the type of bonds in a molecule, e.g. all alcohols will have an O–H stretching frequency and all compounds containing a carbonyl group will have a C=O stretching frequency. This property, which does not rely on chemical tests, is extremely useful in diagnosing the functional groups within a covalent molecule.

IR spectra

A typical IR spectrum is shown in Fig. 28.1 and you should note the following points:

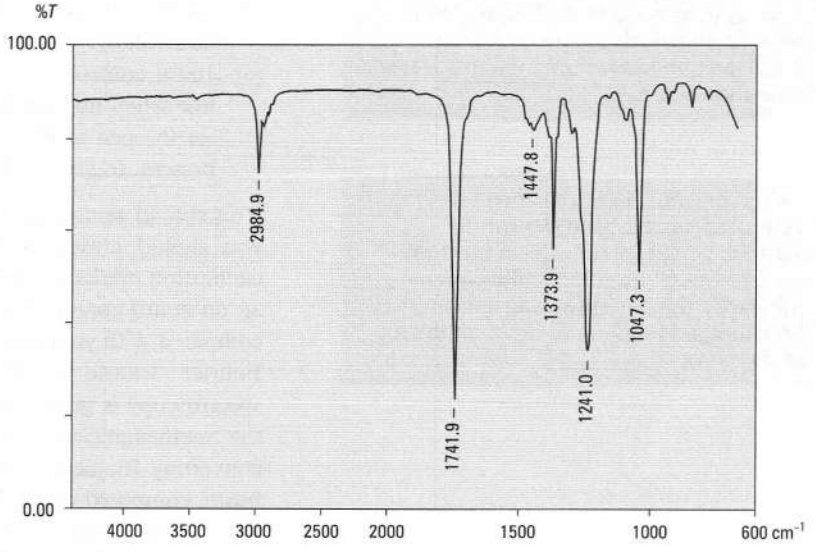


Fig. 28.1 IR spectrum of ethyl ethanoate $CH_3COOCH_2CH_3$ as a liquid film.

The use of wavenumber – this is an old established convention, since high wavenumber = high frequency = high energy = short wavelength. Expression of the IR range, 4000 cm^{-1} to 650 cm^{-1}, is in 'easy' numbers and the high energy is found on the left-hand side of the spectrum. Note that IR spectroscopists often refer to wavenumbers as 'frequencies', e.g. 'the peak of the C=O stretching 'frequency' is at 1720 cm^{-1}'.

- The x-axis, the wavelength of the radiation, is given in wavenumbers ($\bar{\nu}$) and expressed in reciprocal centimetres (cm^{-1}). You may still see some spectra from old instruments using microns (μ, equivalent to the SI unit 'micrometres', μm, at 1×10^{-6} m) for wavelength; the conversion is given by eqn [28.2]:

$$\text{wavenumber } (cm^{-1}) = 1/\text{wavelength (cm)} = 10\,000/\text{wavelength } (\mu m) \quad [28.2]$$

- The y-axis, expressing the amount of radiation absorbed by the molecule, is usually shown as % transmittance (p. 164). When no radiation is absorbed (all is transmitted through the sample) we have 100% transmittance and 0% transmittance implies all radiation is absorbed at a particular wavenumber. Since the y-axis scale goes from 0 to 100% transmittance, the absorption peaks are displayed *down* from the 100% line; this is *opposite* to most other common spectra.
- The cells holding the sample usually display imperfections and are not completely transparent to IR radiation, even when empty. Therefore the base line of the spectrum is rarely set on 100% transmittance and quantitative applications of IR spectroscopy are more complex than for UV–vis (p. 166).

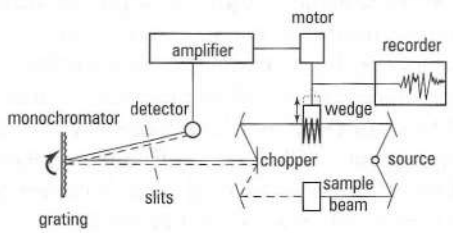


Fig. 28.2 Schematic diagram of a double-beam IR spectrometer.

Using double-beam instruments – you can identify the sample beam by quickly placing your hand in the beam. If the pen records a peak, this is the sample beam, but if the pen moves up, then this is the reference beam.

Using the 100% control – if you use this control to set the base line for the sample, you *must* turn down the 100% control when you remove the sample, otherwise the pen-drive mechanism may be damaged in trying to drive off the top of the chart.

IR spectrometers

There are two general types:

1. Double-beam or dispersive instruments in which the IR radiation from a single source is split into two identical beams. One beam passes through the sample and the other is used as a reference and passes through air or the pure solvent used to dissolve the sample. The difference in intensity of the two beams is detected and recorded as a peak; the principal components of this type of instrument are shown in Fig. 28.2. The important controls on the spectrometer are:

   (a) scan speed: this is the rate at which the chart moves – slower for greater accuracy and sharp peaks;
   (b) wavelength range: the full spectrum or a part of the IR range may be selected;
   (c) 100% control: this is used to set the pen at the 100% transmittance line when no sample is present the base line. It is usual practice to set the pen at 90% transmittance at 4000 cm^{-1} when the sample is present, to give peaks of the maximum deflection.

   You should remember that this is an electromechanical instrument and you should always make sure that you align the chart against the calibration marks on the chart holder. In the more advanced instruments an on-board computer stores a library of standard spectra, which can be compared with your experimental spectrum.
2. Fourier transform IR (FT–IR) spectrometer: the value of IR spectroscopy is greatly enhanced by Fourier transformation, named after the mathematician J.B. Fourier. The FT is a procedure for inter-converting frequency functions and time or distance functions. The IR beam, composed of all the frequencies in the IR range, is passed through the sample and generates interference patterns, which are then transformed electronically into a normal IR spectrum. The advantages of FT–IR are:

   (a) rapid scanning speed – typically four scans can be made per minute, allowing addition of the separate scans to enhance the signal-to-noise ratio and improve the resolution of the spectrum;
   (b) simplicity of operation – the reference is scanned first, stored and then subtracted from the sample spectrum;
   (c) enhanced sensitivity: the facility of spectrum addition from multiple scans permits detection of smaller quantities of chemicals;

Box 28.1 How to run an infrared spectrum of a liquid or solid film, mull or KBr disk

A. Double-beam spectrometer

1. **Ensure that the instrument is switched on** and that it has had a few minutes to warm up.
2. **Make sure that the chart is aligned with the calibration marks on the chart bed or chart drum.** Most spectrometers scan from 4000 cm^{-1} to 650 cm^{-1} and the pen should be at the 4000 cm^{-1} mark.
3. **Adjust the 100% transmittance control to about 90%,** if necessary.
4. **Place the sample cell in the sample beam and adjust the 100% transmittance control to 90%,** or the highest value possible.
6. **Select the scan speed.** You must balance the definition required in the spectrum with the time available for the experiment. For most qualitative applications the fastest setting is satisfactory.
7. **Press the 'scan' or 'start' button to run the spectrum.** The spectrum will be recorded and the spectrometer will automatically align itself at the end of the run. *Do not press* any other buttons while the spectrum is running or the instrument may not realign itself at the end of the run.
8. **Adjust the 100% transmittance control to about 50%,** remove the sample cell from the spectrometer and turn the 100% transmittance control to about 90%.
9. **Enter all of the following data on the spectrum:** name, date, compound and phase (liquid film, Nujol® mull, KBr disk, etc.).

B. FT–IR spectrometer

1. **Make sure that the sample compartment is empty** and close the lid.
2. **Select the number of scans;** usually four is adequate for routine work.
3. **Select 'background' on the on-screen menu,** and scan the background. *Do not press* any other buttons or icons while the spectrum is running.
4. **Place the sample cell in the beam,** close the lid, select 'sample' and scan the sample. *Do not press* any other buttons or icons while the spectrum is running.
5. **Select 'customize',** or a similar function, and enter all the data – name, date, compound, phase (liquid film, Nujol® mull, KBr disk, etc.) – on the spectrum.
6. **Select 'print',** to produce the spectrum from the printer.

Problems with IR spectra

These are usually caused by poor sample preparation and the more common faults are:

1. **The large peaks have tips below the bottom of the chart or the large peaks have 'squared tips' near the bottom of the chart:** the sample is too thick; remove some sample from the cell and rerun the spectrum.
2. **The spectrum is 'weak', i.e. few peaks:** the sample is too thin – add more sample or remake the KBr disk.
3. **The base line cannot be adjusted to 90% transmittance:** the NaCl plates or KBr disk are 'fogged', scratched or dirty – replace or remake the KBr disk.
4. **The pen tries to 'go off' the top of the spectrum:** obviously due to some absorption at 4000 cm^{-1} when you were setting the base line. Repeat baseline set-up but at 80% transmittance and bear in mind that dirty plates, above, can be the cause.

(d) the integral computer system enables the use of libraries of spectra and simplifies spectrum manipulation, such as the subtraction of contaminant or solvent spectra.

The procedures for running IR spectra on double-beam and FT spectrometers are described in Box 28.1.

Sample handling

IR spectra of aqueous solutions – special sample cells made from CaF_2 are available for aqueous solutions, but they are expensive and only used in specific applications.

You can obtain IR spectra of solids, liquids and gases by use of the appropriate sample cell (sample holder). The sample holder must be completely transparent to IR radiation; consequently glass and plastic cells cannot be used. The most common sample cells you will encounter are made from sodium chloride or potassium bromide and you cannot use aqueous

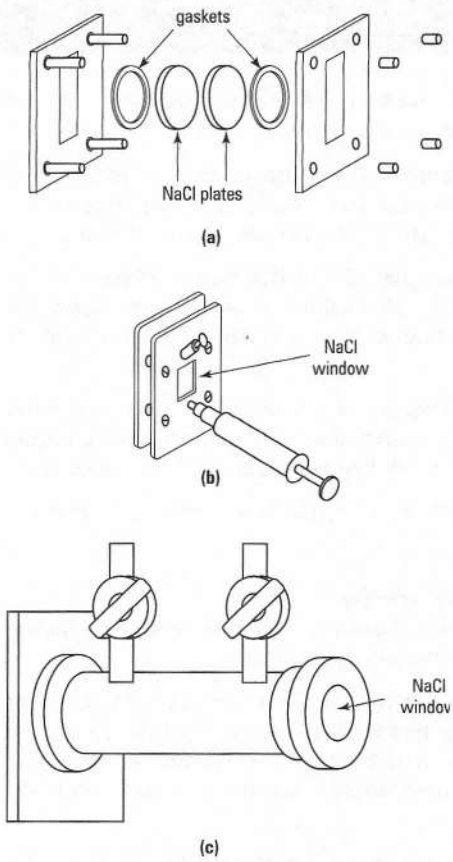


Fig. 28.3 Cells for IR spectroscopy: (a) demountable cell for liquid and solid films and mulls; (b) solution cell; (c) gas cell.

Storing IR sample cells and KBr powder – cells are always stored in desiccators to prevent 'fogging' by absorption of moisture. KBr powder must be dried in the oven, cooled and kept in a desiccator.

Handling NaCl plates and KBr disks – NaCl plates are delicate and easily damaged by scratching, dropping or squeezing. Hold them only by the edges and place them on filter paper or tissue when adding chemicals. KBr disks should be handled using tweezers.

solutions or very wet samples, otherwise the sample cells will dissolve. A typical range of sample cells is shown in Fig. 28.3 and for routine qualitative work you will regularly use NaCl plates and KBr disks to obtain spectra of solids and liquids. Solution cells and gas cells are utilized in more specialized applications and require specific instructions and training.

Liquid samples

The most convenient way to obtain the IR spectrum of a pure, dry liquid is to make a thin liquid film between two NaCl disks (plates). Since the film thickness is unknown, this procedure is not applicable to quantitative work.

Solid samples

If you were to place a fine powder between two NaCl plates, a usable spectrum would not be obtained because the IR radiation would be scattered by diffraction at the edges of the particles and would not pass through to the detector. There are *three* solutions to this problem:

1. *Mulls*: in which the finely ground solid is mixed with a liquid, usually Nujol® (liquid paraffin) or, less frequently, HCB (hexachloro-1,3-butadiene). This mulling liquid does not dissolve the chemical but fills the gaps round the edges of the crystals preventing diffraction and scattering of the IR radiation. Remember that these mulling liquids have their own IR spectrum, which is relatively simple, and can be subtracted either 'mentally' or by the computer. The choice of mulling liquid depends upon the region of the IR spectrum of interest: Nujol® is a simple hydrocarbon containing only C–H and C–C bonds, whereas HCB has no C–H bonds, but has C–Cl, C=C and C–C bonds. Examination of the separate spectra of your unknown compound in each of these mulling agents enables the full spectrum to be analysed.
2. *KBr disks*: here the finely ground solid compound is mixed with anhydrous KBr and squeezed under pressure. The KBr becomes fluid and forms a disk containing the solid compound dispersed evenly within it and suitable for obtaining a spectrum. The advantage of the KBr disk technique is the absence of the spectrum from the mulling liquid, but the disadvantages are the equipment required (Fig. 28.4) and the practice required to obtain suitable transparent disks, which are very delicate and rapidly absorb atmospheric moisture.
3. *Thin solid films*: here a dilute solution of the compound in a low-boiling-point solvent such as dichloromethane or ether is allowed to evaporate on a NaCl plate producing a thin transparent film. This method gives excellent results but is slightly limited by solubility factors.

When you are recording spectra of mulls, KBr disks and thin solid films air is used as the reference and they are suitable for qualitative analysis only. The procedure for the preparation of liquid and solid films and mulls is described in Box 28.2 and that for KBr disks in Box 28.3.

Interpretation of IR spectra

To identify compounds from their IR spectrum you should know at which frequencies the stretching and bending vibrations occur. A detailed analysis can be achieved using the correlation tables found in specialist textbooks. For interpretation, the spectrum is divided into three regions.

is very important and must be understood; it provides one of the most common forms of error when copying formulae. Be sure to understand how your spreadsheet performs these operations.

Naming blocks

When a group of cells (a block) is carrying out a particular function, it is often easier to give the block a name which can then be used in all formulae referring to that block. This powerful feature also allows the spreadsheet to be more readable.

Graphics display

Most spreadsheets now offer a wide range of graphics facilities which are easy to use and this represents an ideal way to examine your data sets rapidly and comprehensively. The quality of the final graphics output (to a printer) is variable but is usually sufficient for initial investigation of your data. Many of the options are business graphics styles but there are usually histogram, bar chart, X–Y plotting, line and area graphics options available. Note that some spreadsheet graphics may not come up to the standards expected for the formal presentation of scientific data (p. 343).

Printing spreadsheets

This is usually a straightforward menu-controlled procedure, made difficult only by the fact that your spreadsheet may be too big to fit on one piece of paper. Try to develop an area of the sheet which contains only the data that you will be printing, i.e. perhaps a summary area. Remember that columns can usually be hidden for printing purposes and you can control whether the printout is in portrait or landscape mode, and for continuous paper or single sheets (depending on printer capabilities). Use a screen preview option, if available, to check your layout before printing. Most spreadsheets are now WYSIWYG (What You See Is What You Get) so that the appearance on the screen is a realistic impression of the printout. A 'print to fit' option is also available in some programs, making the output fit the page dimensions.

Using string functions – these allow you to manipulate text within your spreadsheet and include functions such as 'search and replace' and alphabetical or numerical 'sort'.

Use as a database

Many spreadsheets can be used as databases, using rows and columns to represent the fields and records (see Chapter 48). For many applications in chemistry, the spreadsheet form of database is perfectly adequate and should be seriously considered before using a full-feature database program.

Box 28.2 How to prepare liquid and solid films and mulls

A. Preparing a liquid film

1. **Select a pair of clean NaCl plates from a desiccator,** clean them by wiping with a soft tissue soaked in dichloromethane and place them on the bench on a piece of filter paper or tissue paper to prevent scratching by the bench surface.
2. **Using a glass rod or boiling stick, place a small drop of liquid in the centre of one of the plates.** Do not use a Pasteur pipette, which may scratch the surface of the plate.
3. **Carefully, holding it by the edge, place the other plate on top and see if a thin film spreads between the plates, covering the centres.** Do not press to force the plates together. If there is not enough liquid, carefully separate the plates by lifting at the edge and add another drop of liquid. If there is too much liquid, separate the plates and wipe the liquid from one of them using a soft tissue.

B. Preparing a thin solid film

1. **Dissolve the sample (about 5 mg) in a suitable low-boiling-point solvent (about 0.25 mL),** such as DCM or ether.
2. **Place two drops of the solution onto the centre of a NaCl plate** and allow the solvent to evaporate. Use a Pasteur pipette, but *do not* touch the surface of the plate.
3. **If the resulting thin film of solid does not cover the centre of the plate,** add a little more solution.
4. **Mount the *single* NaCl plate in the spectrometer and run the spectrum.** Note that the NaCl plate can rest on the 'V'-shaped wedge on the sample holder in the spectrometer.

C. Preparing a mull

1. **Grind a small sample of your compound (about 5 mg) using a small agate mortar and pestle for at least 2 minutes.** The powder should be as fine as possible.
2. **Add one drop of mulling agent (Nujol® or HCB) and continue grinding until a smooth paste is formed.** If the mull is too thick, add another drop of mulling agent, or if it is too thin, add a little more solid. Only experience will give you the correct consistency of the mull and the key to a good spectrum is a mull of the correct fluidity.
3. **Transfer the mull to the centre or along the diameter of an NaCl plate,** on a piece of filter paper or tissue paper to prevent scratching by the bench surface, using a small plastic spatula or a boiling stick.
4. **Carefully, holding it by the edge, place the other plate on top and very gently press to ensure that the mull spreads as a thin film between the plates.** If there is not enough liquid, carefully separate the plates by lifting at the edge and add another drop of mull. If there is too much liquid (poor spectrum), separate the plates and wipe the mull from one of them using a tissue.

C. Setting up the cell holder for liquid films and mulls

1. **Place the back-plate of the cell holder on the bench, position the rubber gasket, place the NaCl plates on the gasket and then put the second gasket on top of the plates.** These gaskets are essential to prevent fracture of the plates when you tighten the locking nuts.
2. **Carefully place the cell holder top-plate on the top gasket, drop the locking nuts into place and carefully tighten each in rotation.** These are safety nuts and if you over-tighten them or if the back- and top-plates are not parallel, they will spring loose to prevent the NaCl plates being crushed.
3. **Transfer the cell holder assembly to the spectrometer and make sure it is securely mounted in the cell compartment.**
4. **Clean the plates in the fume cupboard by wiping them with a tissue soaked in DCM,** stand them on filter paper or tissue paper to allow the solvent to evaporate and put them in the desiccator. Allow the DCM to evaporate from the tissue swab and dispose of it in the chemical waste.

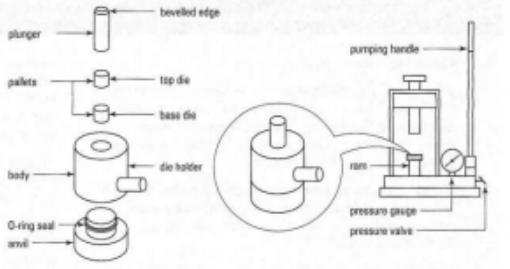


Fig. 28.4 Equipment for preparation of a KBr disk.

Region 1 *(4000–2000 cm^{-1})*: this region contains the high frequency vibrations such as C–H, N–H and O–H stretching, together with C=C and C≡N stretching vibrations.

Region 2 *(2000–1500 cm^{-1})*: this is known as the 'functional group region' and includes the stretching frequencies for C=C, C=O, C=N, N=O and N–H bending vibrations.

Region 3 *(1500–650 cm^{-1})*: this region contains stretching bands for C–O, C–N, C–Hal and the C–H bending vibrations. It is known as the 'fingerprint region' because it also contains complex low-energy vibrations resulting from the overall molecular structure and these are unique to each different molecule. Fig. 28.5 shows the spectra of 1-propanol and 1-butanol, both of which show almost identical peaks for the O–H, C–H and C–O stretching frequencies and the C–H bending frequencies, but the spectra are different in the number and intensity of the peaks between 1500 and 650 cm^{-1}, resulting from the presence of the additional CH_2 in 1-butanol. Conversely, these highly specific bands in the 'fingerprint' region are useful for identification of molecules by comparison with authentic spectra via a database.

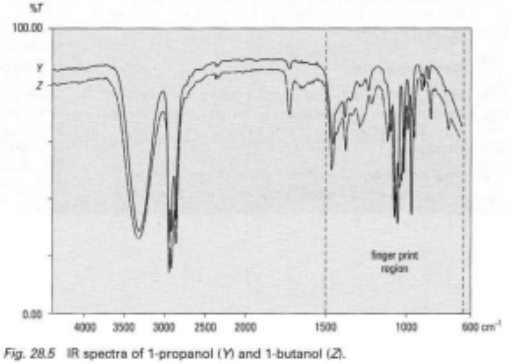


Fig. 28.5 IR spectra of 1-propanol (*Y*) and 1-butanol (*Z*).

Box 28.3 How to prepare a KBr disk

1. **Take spectroscopic-grade KBr powder from the oven** and allow it to cool in a desiccator.
2. **Grind your compound (1–2 mg) in an agate mortar for 2 minutes, then add the KBr (0.2 g) and continue grinding to a fine powder.** Put the KBr powder back into the oven.
3. **Obtain a 'disk kit'** and make sure that:
   (a) it is complete – comprising a plunger, two dies (base and top), a die holder and an anvil, as shown in Fig. 28.4;
   (b) the components are for the same device – they are not interchangeable with another disk kit – and should be numbered.
4. **Press the die holder onto the anvil ensuring a proper fit.**
5. **Lower the base die, numbered-side down, into the die holder** and make sure it slides into a depth of about 50 mm.
6. **Pour the compound/KBr powder mixture, about one-third to one-half of the amount prepared, into the die holder** and tap gently to produce an even layer on the base die.
7. **Lower the top die, numbered-side up, on top of the KBr mixture and make sure it slides down onto the powder.**
8. **Slide the plunger, with the bevelled edge at the top, into the die holder** ensuring that it is touching the top die and press down gently so that the dies slide to the bottom, ensuring that you do not then push off the anvil.
9. **Place the assembled disk kit in the hydraulic press** and tighten the top screw so that it touches the top of the plunger.
10. **Connect the anvil to a source of vacuum,** e.g. a rotary vacuum pump.
11. **Close the hydraulic release valve on the side of the press and gently pump the handle** until the pressure gauge reads between 8 and 10 tons and leave for 30 seconds.
12. **Open the hydraulic release valve gently** and, when the pressure has fallen to zero, disconnect the vacuum from the anvil.
13. **Loosen the top screw** and remove the disk kit from the press.
14. **Turn the disk kit upside down and carefully pull off the anvil.** Make sure that the plunger does not slide out by supporting it in the palm of your hand.
15. **Gently push the plunger and the base die will emerge from the die holder.** Take off the base die leaving the KBr disk exposed.
16. **Carefully slide the KBr disk into the special disk holder** using a microspatula.
17. **Run the IR spectrum immediately,** because the disk will begin to cloud over as it absorbs atmospheric moisture.
18. **Clean the disk kit components with a tissue and check that all parts are present.**
19. **If the dies or the plunger stick in the die holder, tell your instructor.**

A simple correlation chart indicating the three regions of the spectrum and their associated bond vibrations is shown in Fig. 28.6. You can obtain most diagnostic information from spectral regions 1 and 2, since these are the simplest regions containing the peaks related to specific functional groups, while region 3 is normally used for confirmation of your findings. Another important aspect of the IR spectrum is the relative intensities of the commonly found peaks and you should become familiar with peak sizes. A chart indicating the positions, general shapes and relative intensities of commonly found peaks is shown in Fig. 28.7. When you are attempting to interpret an IR spectrum you should use the approach described in Box 28.4.

If you are studying complexes formed from metals and organic ligands, the metal–ligand stretching vibration will occur below 600 cm^{-1} and special IR spectrometers are used to observe this region. However, changes in the IR spectrum of the organic ligand on complexation can be detected in the normal 4000–650 cm^{-1} range.

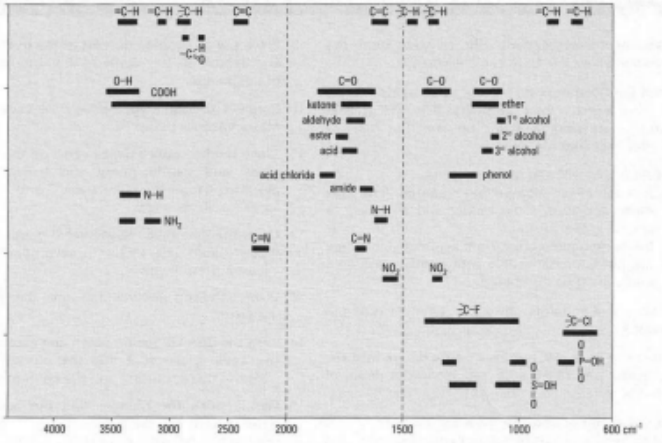


Fig. 28.6 Simplified correlation chart of functional group absorptions.

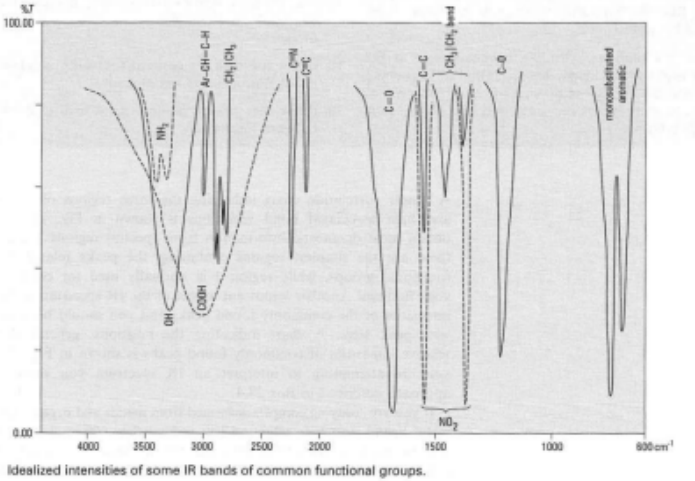


Fig. 28.7 Idealized intensities of some IR bands of common functional groups.

Box 28.4 How to interpret an IR spectrum

1. **Note the conditions under which the spectrum was obtained**, which should be written on the spectrum as 'phase'. If it is a solution or a mull, you will need to identify and 'subtract' the spectrum of the mulling agent or solvent.
2. **Consider carefully the reaction you have carried out.** You should know, from the correlation table, the functional groups and peaks in the starting materials and those expected in the product.
3. **Remember that the absence of peaks may be as useful in interpretation as the presence of peaks.**
4. ***Do not attempt to identify all the peaks***, just those which are relevant to your interpretation. Go for the large peaks first.
5. **Many sharp peaks of medium to strong intensity** throughout the spectrum generally indicate an aromatic compound.
6. **Examine region 1 (4000–2000 cm^{-1}).** It is useful to draw a line on the chart at 3000 cm^{-1}: just above the line (3000–3100 cm^{-1}) you will find the stretching frequencies for C_{sp^2}–H and C_{sp^3}–H indicating unsaturation, whilst just below (2980–2800 cm^{-1}) you will find the C_{sp^3}–H stretching frequencies for CH_3, CH_2 and CH in saturated systems. Other bands for O–H, N–H, C≡C and C≡N are obvious.
7. **Examine region 2 (2000–1500 cm^{-1}).** Here you will find C=O stretch, usually the most intense band in the spectrum; C=C and C=N stretches, less intense and sharper; N=O stretch (from NO_2) intense and sharp and with a twin band in region 3; N–H bending vibrations – do not confuse with C=O.
8. **Examine region 3 (1500–650 cm^{-1}).** The large bands here are C–O, C–N, C–Cl, S=O, P=O, N=O (twin from region 2) stretches and C–H 'breathing' bands (900–700 cm^{-1}), which indicate the number and position of substituents on a benzene ring. Medium-intensity peaks of importance include the CH_3 and CH_2 bands at 1460 cm^{-1} and 1370 cm^{-1} from the carbon skeleton which are also found in Nujol®.
9. **Tabulate your results and make the appropriate deductions**, after consulting the detailed correlation table. Remember to correlate the spectroscopic data with the chemical data.

29 Nuclear magnetic resonance spectroscopy

Electromagnetic radiation (typically at radio frequencies of 60–600 MHz) is used to identify compounds in a process known as nuclear magnetic resonance (NMR) spectroscopy. This is possible because of differences in the magnetic states of atomic nuclei, involving very small transitions in energy levels. The atomic nuclei of the isotopes of many elements possess a magnetic moment. When these magnetic moments interact with a uniform external magnetic field, they behave like tiny compass needles and align themselves in a direction 'with' or 'against' the field. The two orientations, characteristic of nuclei with a nuclear spin quantum number $I = \frac{1}{2}$, have two different energies: the orientation aligned 'with' the field has a lower energy than that aligned 'against' the field (Fig. 29.1).

Typical magnetic nuclei of general use to chemists and biochemists are ^{1}H, ^{13}C, ^{19}F and ^{31}P, all of which have nuclear spin quantum numbers $I = \frac{1}{2}$. The energy difference between the two levels (ΔE) corresponds to a precise electromagnetic frequency (ν), according to similar quantum principles for the excitation of electrons (p. 163). When a sample containing an isotope with a magnetic nucleus is placed in a magnetic field and exposed to an appropriate radio frequency, transitions between the energy levels of magnetic nuclei will occur when the energy gap and applied frequency are in *resonance* (i.e. when they are matched exactly in energy). Differences in energy levels, and hence resonance frequencies (ν_0), depend upon the magnitude of the applied magnetic field (B_0) and the magnetogyric ratio (γ), according to the equation:

$$\nu_0 = \gamma B_0/2\pi \qquad [29.1]$$

For a given value of the applied field (B_0), nuclei of different elements have different values of the magnetogyric ratio (γ) and will give rise to resonance at various radio frequencies. The principal components of an NMR spectrometer are shown in Fig. 29.2.

Nuclear spin quantum numbers and NMR – other common nuclei with non-zero quantum numbers are ^{14}N, ^{2}D ($I = 1$) and ^{11}B and ^{35}Cl ($I = \frac{3}{2}$). Their NMR spectra are not used on a *routine* basis.

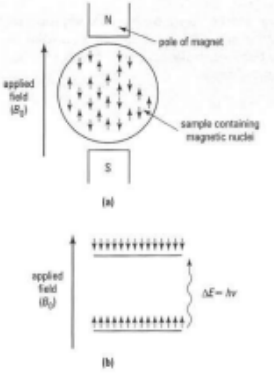


Fig. 29.1 Effect of an applied magnetic field, B_0, on magnetic nuclei. (a) Nuclei in magnetic field have one of two orientations – either with the field or against the field (in the absence of an applied field, the nuclei would have random orientation). (b) Energy diagram for magnetic nuclei in applied magnetic field.

Example For an external magnetic field of 2.5 T (tesla), ΔE for ^{1}H is 6.6×10^{-26} J and since $\Delta E = h\nu$, the corresponding frequency (ν) is 100 MHz; for ^{13}C in the same field, ΔE is 1.7×10^{-26} J, and ν is 25 MHz.

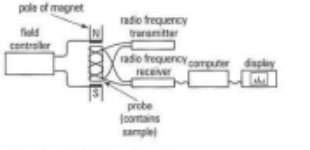


Fig. 29.2 Components of an NMR spectrometer.

For magnetic nuclei in a given molecule, an NMR spectrum is generated because, in the presence of the applied field, different nuclei of the same atoms experience small, different, local magnetic fields depending on the arrangement of electrons, i.e. in the chemical bonds, in their vicinity. The effective field at the nucleus can be expressed as:

$$B = B_0(1 - \sigma) \qquad [29.2]$$

where σ (the shielding constant) expresses the contribution of the small secondary field generated by the nearby electrons. The magnitude of σ

depends on the electronic environment of a nucleus, so nuclei of the same isotope give rise to small different resonance frequencies according to the equation:

$$\nu_0 = \gamma B_0(1 - \sigma)/2\pi \qquad [29.3]$$

KEY POINT **The variation of resonance frequencies with surrounding electron density is crucial to the usefulness of the NMR technique. If it did not occur, *all nuclei* of a single isotope would come into resonance at the same combination of magnetic field and radio frequency and only *one peak* would be observed in the spectrum.**

Chemical shift

Measuring chemical shifts – ppm is *not* a concentration term in NMR but is used to reflect the small frequency changes that occur relative to the reference standard, measured in proportional terms.

The separation of resonance frequencies resulting from the different electronic environments of the nucleus of the isotope is called the *chemical shift*. It is expressed in dimensionless terms, as parts per million (ppm), against an internal standard, usually tetramethylsilane (TMS). By convention, the chemical shift is positive if the sample nucleus is less shielded (lower electron density in the surrounding bonds) than the nucleus in the reference and negative if it is more shielded (greater electron density in the surrounding bonds). The chemical shift scale (δ) for a nucleus is defined as:

$$\delta = [(\nu_{sample} - \nu_{reference}) \times 10^6]/(\nu_{reference}) \qquad [29.4]$$

This means that the chemical shift of a specific nucleus in a molecule is at the same δ value, no matter what the operating frequency of the NMR spectrometer.

An NMR spectrum is a plot of chemical shift (δ) as the x-axis against absorption of energy (resonance) as the y-axis. On the right-hand side of the spectrum at $\delta = 0$ ppm there may be a small peak, which is the reference (TMS). A typical ^{1}H–NMR spectrum is shown in Fig. 29.3.

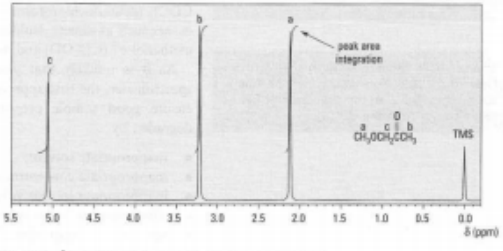


Fig. 29.3 ^{1}H–NMR spectrum of 1-methoxypropanone.

NMR spectrometers

These can operate at different radio frequencies and magnetic fields and are usually referred to in terms of radio frequency, e.g. 60 MHz, 270 MHz and 500 MHz spectrometers. Spectrometers operating above 100 MHz require expensive superconducting magnets to generate the high magnetic fields. In routine laboratory work 60 MHz and 90 MHz instruments are common but 270 MHz machines are becoming more affordable. Increasing the operating

frequency of the spectrometer effectively increases the resolution of the chemical shifts of the nuclei under examination. For example, the difference in frequency between 0 and 1δ is 60 Hz in a 60 MHz spectrometer but 270 Hz for a 270 MHz instrument.

Spectrometers can be divided into two types:

1. *Continuous-wave (CW) spectrometers*, which use a permanent magnet or an electromagnet, usually operating at 60 or 90 MHz. In practice the radio frequency is held constant and small electromagnets on the faces of the main magnet (sweep coils) vary the magnetic field over the chemical shift range. The spectrometer sweeps through the spectroscopic region plotting resonances (absorption peaks) on a chart recorder (cf. dispersion IR spectrometers). CW spectrometers are usually dedicated to observation of a specific nucleus such as ^{1}H.
2. *Fourier transform (FT) spectrometers*, using superconducting magnets containing liquid nitrogen and liquid helium for cooling. Here the magnetic field is held constant and the sample is irradiated with a radio frequency pulse containing all the radio frequencies over the chemical shift region of the nucleus being examined, cf. FT–IR (p. 182). Computer control allows rapid repeat scans to accumulate spectra, presenting the data as a standard CW-type spectrum via FT processing. Simple variation of the radio frequencies permits observation of different nuclei (multinuclear NMR spectrometers). Thus an FT–NMR spectrometer can be used for obtaining ^{1}H, ^{13}C, ^{19}F, ^{15}N and ^{31}P NMR spectra.

Sample handling

Using deuterated solvents – these are expensive and should not be wasted. $CDCl_3$ is 100 times more expensive than spectroscopic-grade $CHCl_3$ and the others are at least 10–15 times more expensive than $CDCl_3$.

Using $CDCl_3$ – when using this solvent a peak in the spectrum at $\delta = 7.26$ ppm is seen. This is due to the presence of $CHCl_3$ as an impurity in the $CDCl_3$.

The majority of NMR spectra are obtained from samples in solution and therefore the solvent should preferably not contain atoms of the nuclei being observed (except in the case of ^{13}C–NMR). The most common solvents are those in which the hydrogen atoms have been replaced by deuterium, which is not observed under the conditions under which the spectrum is obtained. $CDCl_3$ (deuteriochloroform, chloroform-*d*) is often the solvent of choice, but others such as dimethylsulphoxide-d^6, $[(CD_3)_2SO]$, propanone-d^6 $[(CD_3)_2CO]$, methanol-d^4 (CD_3OD) and deuterium oxide (D_2O) are in common use.

As it is unlikely that you will be allowed 'hands-on' use of an NMR spectrometer, the best approach you can take to obtain a good spectrum is to ensure good sample preparation. The quality of an NMR spectrum is degraded by:

- inappropriate solvent;
- inappropriate concentration of solute;
- inappropriate solvent volume;
- solid particles in the solution;
- water in the sample (inefficient drying);
- paramagnetic compounds.

Sample preparation for NMR spectroscopy is described in Box 29.1.

Interpreting NMR spectra

As a matter of routine in your laboratory work you will be required to interpret ^{1}H–NMR spectra (also known as proton spectra). ^{13}C–NMR spectra are becoming more common, while ^{19}F and ^{31}P spectra may be obtained in specialized experiments. Therefore you should concentrate on the interpretation of ^{1}H and ^{13}C spectra in the first instance.

Box 29.1 How to prepare a sample for NMR spectroscopy

1. **Make sure that your compound is free from water and solvent (p. 39).**
2. **Test the solubility of your compound in cold CH_2Cl_2.** If it is soluble you can use $CDCl_3$ as the solvent for the NMR experiment. If it is insoluble, consult your instructor for the availability of other deuterated solvents.
3. **Dissolve your compound $CDCl_3$ (about 2 mL) in a clean, dry sample tube.** Use about 10 mg of sample for CW-NMR or 5 mg of sample for FT-NMR. Check to see if the solvent contains TMS; if it does not, consult your instructor.
4. **Make a simple filter** in a new Pasteur pipette to remove insoluble material and water (Fig. 29.4). Check that your compound does not react with cotton wool and neutral alumina (alcohols and acids are strongly adsorbed on neutral alumina). If it does, replace the cotton wool with glasswool and do not use alumina. You *must* wear gloves when handling glasswool.
5. **Put the filter into a suitable clean, dry NMR tube** and, using a clean, dry Pasteur pipette, filter the solution into the NMR tube.
6. **Fill the NMR tube to the appropriate level:** between 30 and 50 mm in height is sufficient.
7. **Cap the NMR tube with the correct-size tube cap,** making sure that it is correctly fitted to prevent oscillation when the tube is spinning in the spectrometer. Make sure that the cap is fitted correctly so that it will not fall off when the tube is in the spectrometer.
8. **Wipe the outside of the tube with a clean, dry tissue** to make sure that the spectrometer will not be contaminated. Cleaning the spectrometer probe is a very difficult task.

^{1}H-NMR spectra – most of the spectra shown in this chapter do not extend over the normal spectral range $\delta = 0$–10 ppm. They are expanded to show the details of coupling patterns.

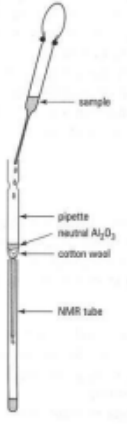


Fig. 29.4 Filtration of solutions for NMR.

^{1}H–NMR spectra

These normally cover the range between $\delta = 0$ and 10 ppm but the range is increased to $\delta = 15$ ppm when acidic protons are present in the molecule. The ^{1}H–NMR spectrum of a molecule gives three key pieces of information about the structure of a molecule:

1. Chemical shift (δ): the peak positions indicate the chemical (magnetic) environment of the protons, i.e. different protons in the molecule have different chemical shifts.
2. Integration: the relative size of peak area indicates how many protons have the δ value shown.
3. Coupling: the fine structure on each peak (coupling) indicates the number of protons on adjacent atoms.

These three features make ^{1}H-NMR a powerful tool in structure determination and there are two extreme approaches to it:

1. Prediction of the spectrum of the expected compound from theoretical knowledge and then comparison with the spectrum obtained. You should recognize 'patterns' (e.g. triplet and quartet for an ethyl group; a singlet of peak area six for two identical methyl groups), which were present in the starting materials, but the δ_H values may have changed in the 'new' molecule. There are computer programs, such as g-NMR®, which will simulate the NMR spectrum from a structural formula.
2. Interpretation of the spectrum from correlation tables, but this is very difficult for the inexperienced.

In practice a combination of the two approaches is used with cross-referencing and checking the proposed structure with tabulated δ_H values and reference spectra until a satisfactory answer is found.

Proton chemical shifts – only hydrogen atoms bonded to carbon will be considered in this simplified treatment.

Interpreting NMR spectra: changes of δ – the terms used to indicate the movement of a particular peak with change in its chemical (magnetic) environment are: *upfield* – towards $\delta = 0$ ppm; *downfield* – towards $\delta = 10$ ppm; *shielded* – increased electron density near the proton; *deshielded* – decreased electron density near the proton.

Table 29.1 Chemical shifts of methyl protons

Compound	Chemical shift (ppm)
$(CH_3)_4Si$	0.00
CH_3R	0.90
CH_3I	2.16
CH_3Br	2.65
CH_3Cl	3.10
CH_3OR	3.30
CH_3F	4.26

Integration of coupled peaks – the area under the singlet, doublet, triplet, quartet, etc., is still that of the type of hydrogen being considered. For example, if the peak for the three protons of a *methyl group* is split into a triplet by an adjacent methylene group, the area of the triplet is *three*.

KEY POINT **Always make sure that your predicted structure is consistent with the spectrum.**

Factors affecting chemical shift (δ_H)

The δ values of protons can be predicted to a general approximation from knowledge of the effects which produce variations in chemical shift.

1. The hybridization of the carbon atom to which the hydrogen atom is attached:
   (a) sp^3 hybridized carbon: peaks occur between $\delta = 0.9$ and 1.5 ppm in simple hydrocarbon systems. The peaks move downfield with change of structure from CH_3 to CH_2 to CH.
   (b) sp hybridized carbon: peaks occur at about $\delta = 1.5$–3.5 ppm in alkynes.
   (c) sp^2 hybridized carbon: in alkenes the resonances occur around $\delta = 4$–8 ppm and the C–H peaks of aromatic rings are found between $\delta = 6$ and 9 ppm. The large downfield shifts of these C_{sp2}–H nuclei result from deshielding of the protons by fields set up by circulation of the π-electrons in the magnetic field. The proton of the aldehyde group (CHO) is particularly deshielded by this effect and is found at $\delta = 9$–10 ppm.
2. Electron attraction or electron release by substituent atoms attached to the carbon atom. Electron attracting atoms, such as N, O, Hal attached to the carbon, attract electron density from the C–H bonds and thus deshield the proton. This results in movement of the chemical shift to higher δ values (Table 29.1). Conversely, electron-releasing groups produce additional shielding of the C–H bonds resulting in upfield shifts of δ values.
3. All the protons in benzene are identical and occur at $\delta = 7.27$ ppm. In substituted aromatic compounds, the overall electron-attracting or releasing effect of the substituent(s) alters the δ values of the remaining ring protons making them non-equivalent. The *ortho* protons are affected most.
4. For protons attached to atoms other than carbon: the chemical shifts of protons attached to oxygen increase with increasing acidity of the O–H group; thus $\delta = 1$–6 ppm for alcohols, 4–12 ppm for phenols and 10–14 ppm for carboxylic acids. Hydrogens bound to nitrogen (1° and 2° amines) are found at $\delta = 3$–8 ppm. The approximate chemical shift regions are shown in Fig. 29.5.

Integration of peak areas

The area of each peak gives the relative number of protons and is produced directly on the spectrum (Fig. 29.6). On CW–NMR spectrometers the height of the peak area integration line must be measured using a ruler, whereas on FT–NMR machines the area is calculated and displayed as a number. You must remember that:

- The areas are *ratios*, not absolute values, and you must find a peak attributable to a specific group to obtain a reference area, e.g. a single peak at $\delta = 1.0$ ppm is likely to be a CH_3 group and thus the area displayed or measured is equal to three protons.
- You must ensure that you include integrations from all the fine-structure (coupling) peaks in the peak area.

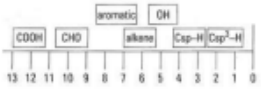


Fig. 29.5 Approximate chemical shift positions in the ^{1}H–NMR spectrum.

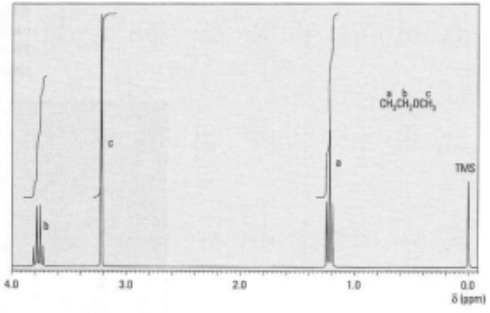


Fig. 29.6 ^{1}H-NMR spectrum of methoxyethane.

- Do not expect the peak area integrations to be exact whole numbers, e.g. an area of 2.8 is probably three protons (CH_3), 5.1 is probably five protons (e.g. a C_6H_5 group), but 1.5 is probably a CH_3 and all the peak area integrations must be doubled.

Coupling (spin–spin splitting)

Many ^{1}H–NMR signals do not consist of a single line but are usually associated with several lines (splitting patterns). Protons giving multiline signals are said to be *coupled*. This coupling arises from the magnetic influence of protons on one atom with those on an adjacent atom(s). Thus information about the nature of adjacent protons can be determined and fed into the structural elucidation problem. To a simple first approximation the following three general points are useful in the interpretation of coupling patterns:

1. Aliphatic systems: if *adjacent* carbon atoms have *different types* of protons (a and b), then the protons will couple. If a proton is coupled to n (n = 1, 2, 3, 4, 5, etc.) other protons on an adjacent carbon atom, the number of lines observed is $n+1$, as shown in the examples below.

   $CH_3CH_2OCH_3$ (a b c)

   Protons a are coupled to two protons b: $n = 2$; therefore the peak for protons a is split into three lines (a triplet).

   Protons b are coupled to three protons a: $n = 3$; therefore the peak for protons b is split into four lines (a quartet).

   Protons c have no adjacent protons and therefore are not coupled and give a single line (singlet) (Fig. 29.6).

   $CH_3CHBrCH_2Br$ (a b c)

   Protons a are coupled to one proton b: $n = 1$; therefore the peak for protons a is split into two lines (doublet)

   Protons b are coupled to three protons a and two protons c: $n = 5$; therefore the peak for protons b is split into six lines (sextet).

   Protons c are coupled to one proton b: $n = 1$; therefore the peak for protons c is split into two lines (doublet).

   Protons a and protons c are not adjacent and do not couple (Fig. 29.7).

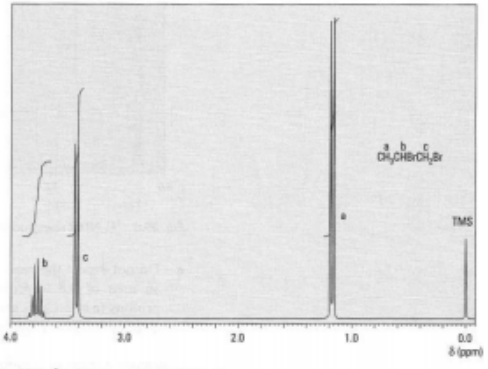


Fig. 29.7 ^{1}H-NMR spectrum of 1,2-dibromopropane.

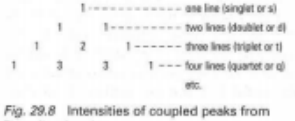


Fig. 29.8 Intensities of coupled peaks from Pascal's triangle.

The intensity of each peak in the resulting singlet, doublet, triplet, quartet, etc., is calculated from Pascal's triangle (Fig. 29.8).

The separation between the coupled lines is called the coupling constant, J, and, for aliphatic protons CH, CH_2 and CH_3, it is usually ~ 8 Hz.

KEY POINT **The $(n+1)$ rule only applies in systems where the coupling constant (J) between the protons is the same. Fortunately this is common in aliphatic systems.**

2. Alkene hydrogens: hydrogen atoms on double bonds have different coupling constants depending upon the stereochemistry of the alkene. Alkene hydrogens in the Z (cis) configuration have $J = 5–14$ Hz, whereas those in the E (trans) configuration have $J = 11–19$ Hz (Figs 29.9a and b).
3. Aromatic hydrogens: coupling of hydrogens, which are non-adjacent, is readily observed in aromatic compounds. Different protons *ortho* to each other couple with $J = 7–10$ Hz, while those in a *meta* relationship have $J = 2–3$ Hz. *Para* coupling ($J = 0–1$ Hz) is not usually seen on the spectrum. The types of aromatic compound you are likely to meet most often are:

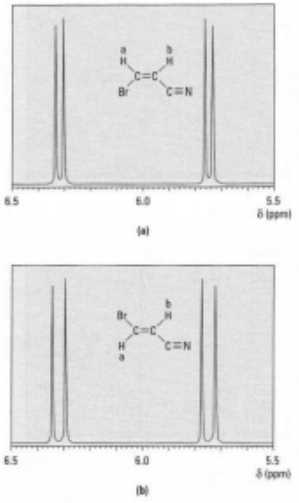


Fig. 29.9 ^{1}H–NMR spectra of: (a) (*Z*)-3-bromopropanonitrile; (b) (*E*)-3-bromopropanonitrile.

(a) Monosubstituted aromatic compounds, in which three basic patterns are found in the aromatic region of the spectrum. If the substituent exerts a weak electronic effect on the ring, the δ values of the ring protons are similar and the protons appear as a single peak of area five (Fig. 29.10a). If the group is strongly electron releasing (OH, NH_2, OCH_3, etc.), the protons appear as complex multiplets (*ortho* and *meta* coupled), below $\delta = 7.27$ ppm of relative areas two to three (Fig. 29.10b). If the group is electron attracting (e.g. NO_2, COOH, etc.), then the complex multiplets have $\delta > 7.27$ ppm (Fig. 29.10c);

(b) *para* disubstituted aromatic compounds, which are of two types. If the substituents are the same, then all the ring protons are identical and a singlet, of relative area four, is seen (Fig. 29.10d). If the substituents are different, then the pairs of hydrogens *ortho* to each substituent are different and *ortho*-couple to give what appears to be pair of doublets, each of relative area two (Fig. 29.10e).

(c) Increasing numbers of substituents, which decrease the number of aromatic hydrogens and the spectrum becomes simpler. Thus the common 1,2,4-trisubstituted pattern (Fig. 29.10f) is recognized easily as an *ortho*-coupled doublet, a *meta*-coupled doublet and a doublet of doublets (coupled *ortho* and *meta*).

The chemical shifts of aromatic protons can be calculated from detailed correlation tables.

^{13}C-NMR spectra

The ^{13}C nucleus has $I = \frac{1}{2}$, like ^{1}H, and the ^{13}C–NMR spectrum of a compound can be observed using a different radio frequency range (in the same magnetic field) to that for ^{1}H. The ^{13}C spectrum will give peaks for each different type of carbon atom in a molecule but the properties of the ^{13}C nucleus give some important and useful differences in the spectrum obtained:

- The natural abundance of ^{13}C is only 1.1% compared with 98.9% for ^{12}C – in any molecule no two adjacent atoms are likely to be ^{13}C and therefore coupling between ^{13}C nuclei will not be seen, giving a very simple spectrum.
- In a sample of a compound, which contains many molecules, the ^{13}C isotope is randomly distributed and all the different carbon atoms in a sample of a compound will be seen in the ^{13}C–NMR spectrum.
- The sensitivity of the ^{13}C nucleus is low and this, together with its low natural abundance, means that FT–NMR is the only practical system to produce a spectrum by accumulation of spectra by repetitions. Larger sample size in bigger NMR tubes also assist in solving the sensitivity/abundance problem.
- The chemical shift range for ^{13}C is greater ($\delta_C = 0$–250 ppm) than for ^{1}H ($\delta_H = 0$–15 ppm) giving greater spectral dispersion, i.e. the peaks for carbons with very slight differences in chemical shifts are separated and do not overlap.
- ^{13}C nuclei will couple with the ^{1}H nuclei to which they are directly bonded, e.g. CH_3 will appear as a quartet, CH_2 as a triplet, CH as a doublet, but C with no hydrogen atoms attached will appear as a singlet. This introduction of complexity in the ^{13}C–NMR spectrum is removed by broadband decoupling (see p. 198).

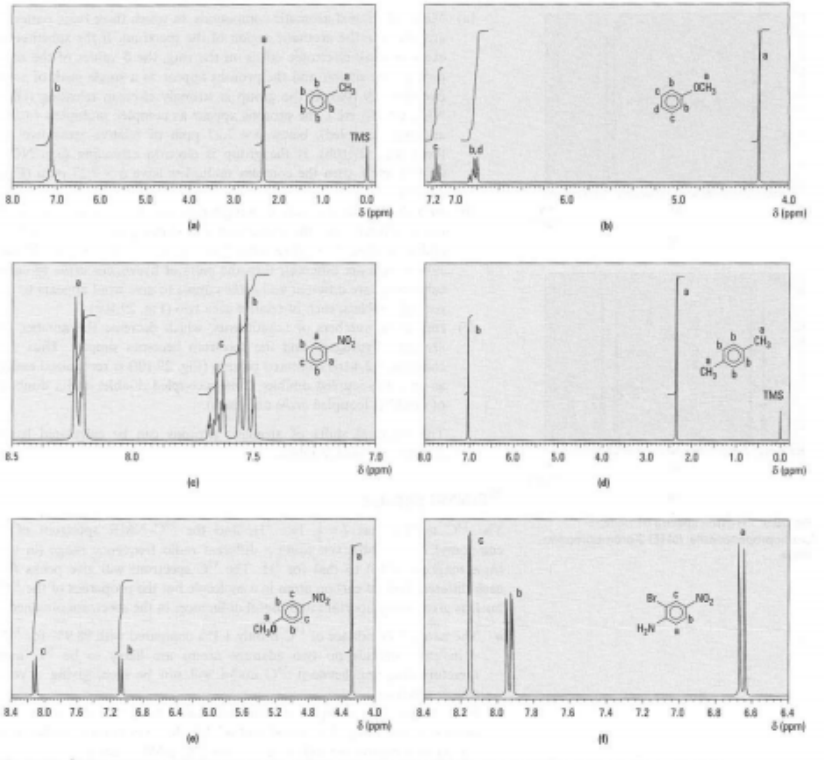


Fig. 29.10 ^{1}H–NMR spectra of (a) methylbenzene; (b) methoxybenzene; (c) nitrobenzene; (d) 1,4-dimethylbenzene; (e) 4-methoxynitrobenzene; (f) 4-amino-3-bromonitrobenzene (NH_2 protons not shown).

- The peak areas of the different carbon atoms are *not* related to the number of carbon atoms having the same chemical shift, as in the case for ^{1}H–NMR spectra.

Interpretation of ^{13}C–NMR spectra – the spectrum is that of all the *carbon atoms* in the molecule. It is easy to forget that the peaks for carbon atoms carrying no hydrogen atoms are present.

Interpreting ^{13}C–NMR spectra

Normally you will be given two ^{13}C–NMR spectra (Fig. 29.11). The upper spectrum, which is more complex (more lines) is called the *off-resonance decoupled* spectrum and shows the ^{13}C–^{1}H coupling to enable you to determine which carbon signals are CH_3, CH_2, CH and C. Then overlapping of peaks may make the identification of different carbon atoms difficult. The

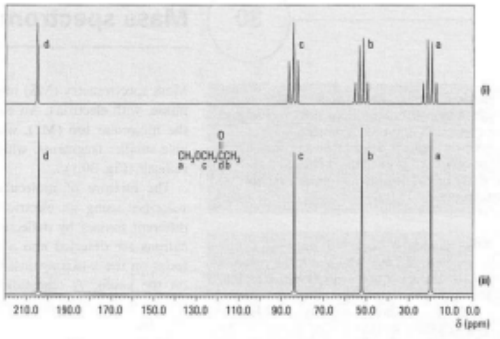


Fig. 29.11 ^{13}C–NMR spectra of 1-methoxypropanone: (i) off-resonance decoupled; (ii) broadband decoupled.

lower spectrum is a *broadband decoupled* spectrum in which the molecule is irradiated with a second radio frequency range for the protons in the molecule and effectively removes all the ^{13}C–^{1}H couplings from the spectrum. The resulting simplicity of the spectrum makes identification of the different types of carbon in the molecule relatively easy.

The chemical shifts of ^{13}C atoms (δ_C) vary in the same manner as those of protons (Fig. 29.12):

1. δ_C moves downfield as the hybridization of the carbon atom changes from sp^3 (0–50 ppm) to sp (75–105 ppm) to sp^2 (100–140 ppm);
2. for sp^3 hybridized carbon: δ_C moves further downfield with the change from CH_3 to CH_2 to CH to C;
3. for sp^2 hybridized carbon: aromatic carbons occur further downfield ($\delta_C = 115–145$ ppm) than alkene carbon atoms ($\delta_C = 100–140$ ppm);
4. bonding more electronegative atoms to carbon deshields the carbon atom and moves the peaks downfield, e.g CH_3–C ($\delta_C \sim 6$ ppm) and CH_3–O ($\delta_C \sim 55$ ppm), C=C ($\delta_C \sim 123$ ppm) and C=O ($\delta_C \sim 205$ ppm).

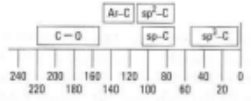


Fig. 29.12 Approximate chemical shift positions in ^{13}C–NMR.

30 Mass spectrometry

Understanding mass spectrometry – since this technique does not involve the production and measurement of electromagnetic spectra and is not based on quantum principles, it should not really be referred to as a spectroscopic technique.

Mass-to-charge ratios – in the overwhelming majority of simple cases the ion detected is a monopositive cation; thus $z = 1$ and the peaks seen on a low-resolution spectrometer equate to the mass of the ion.

Determination of exact molecular mass – high-resolution instruments enable the molecular formula of a compound to be determined by summation of the masses of the individual isotopes of atoms, e.g. both ethane and methanal have integral mass values of 30, but the accurate values are 30.046 950 and 30.010 565 respectively.

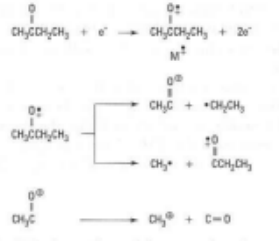


Fig. 30.1 Formation and fragmentation of a molecular ion (M^+).

Mass spectrometry (MS) involves the bombardment of molecules, in the gas phase, with electrons. An electron is lost from the molecule to give a cation, the molecular ion (M^+), which then breaks down in characteristic ways to give smaller fragments, which are cations, neutral molecules and uncharged radicals (Fig. 30.1).

The mixture of molecular ion and fragments is accelerated to specific velocities using an electric field and then separated on the basis of their different masses by deflection in a magnetic or electrostatic field. Only the cations are detected and a mass spectrum is a plot of mass-to-charge ratio (m/z) on the x-axis against the number of ions (relative abundance, RA, %) on the y-axis. A schematic of the components of a mass spectrometer is shown in Fig. 30.2 and an example of a line-graph-type mass spectrum in Fig. 30.3.

There are many types of mass spectrometer, from high-resolution double-focusing instruments, which can distinguish molecular and fragment masses to six decimal places, to 'bench-top' machines with a quadrupole mass detector which can resolve masses up to about $m/z = 500$, but only in whole-number differences. Routinely you are most likely to encounter data from 'bench-top' instruments and therefore only this type of spectrum will be considered.

Sample handling

For low-resolution spectra obtainable from a 'bench-top' MS, samples should be presented in the same form and quantity as demanded for gas chromatographic analysis (p. 211). For high-resolution spectra contamination of any sort must be avoided and samples (typically less than 500 μg) should be submitted in glass sample tubes with screwcaps containing an aluminium-foil insert. MS is so sensitive that the plasticizers from plastic tubes or plastic push-on caps will be detected, as will contaminating grease from ground-glass joints and taps.

Mass spectra

The standard low-resolution mass spectrum (Fig. 30.3) is computer generated, which allows easy comparison with known spectra in a computer database for identification. The peak at the highest mass number is the molecular ion (M^+), the mass of the molecule minus an electron. The peak at RA = 100%, the base peak, is the most abundant fragment in the spectrum and the computer automatically scales the spectrum to give the most abundant ion as 100%. The mass spectrum of a compound gives the following information about its chemical structure:

- molecular ion mass, which includes information on the number of nitrogen atoms and the presence of chlorine and bromine atoms (see below), – which is not easily obtained from IR and NMR spectra;
- the most stable major fragment (base peak), which can be correlated to the structure of the molecule;
- other important fragment ions, which may give information on the structure;

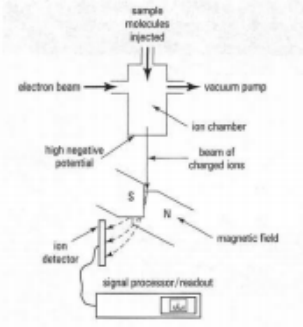


Fig. 30.2 Components of an electron-impact mass spectrometer.

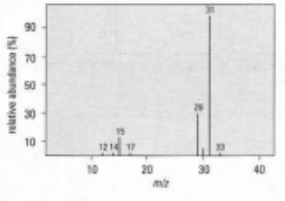


Fig. 30.3 Mass spectrum for methanol; *m/z* = mass-to-charge ratio.

Identification of isotope peaks – the natural abundance of ^{13}C is 1.1%. For a molecule containing *n* carbon atoms the probability is that $1.1 \times n$% of these atoms will be ^{13}C. Thus the mass spectrum of hexane (six carbons) gives a molecular ion (M^+) at $m/z = 86$ and a peak at $m/z = 87$ ($M + 1$) which is 6.6% the intensity of the M^+ peak.

- the detailed fragmentation pattern, which can be used to confirm a structure by reference to a library database, cf. the 'fingerprint' region in IR spectrometry (p. 186).

Molecular ions

The *m/z* value of the molecular ion is the summation of all the atomic masses in the molecule, *including the naturally occurring isotopes*. For organic molecules you will find a small peak ($M + 1$) above the apparent molecular ion mass (M^+) value due to the presence of ^{13}C. The importance of isotope peaks is the detection of chlorine and bromine in molecules since these two elements have large natural abundances of isotopes, e.g. $^{35}Cl : {}^{37}Cl = 3:1$ and $^{79}Br : {}^{81}Br = 1:1$. The mass spectra produced by molecules containing these atoms are very distinctive with peaks at M + 2 and even M + 4 and M + 6 depending on how many chlorine or bromine atoms are present. The identification of the number and type of halogen atoms is illustrated in Box 30.1.

Since the low-resolution mass spectrum produces integer values for *m/z*, the mass of M^+ indicates the number of nitrogen atoms in the molecule. If *m/z* for M^+ is an *odd integer*, there is an *odd number* of N atoms in the molecule and, if the value is an *even number*, then there is an *even number* of N atoms.

Base peak

The molecular ion M^+ fragments into cations, radicals, radical cations and neutral molecules of which only the positively charged species are detected. There are several possible fragmentations for each M^+ but the base peak represents the most *energetically favoured* process with the *m/z* value of the base peak representing the mass of the most *abundant* (and therefore most stable) positively charged species. The fragmentation of M^+ into the base peak follows the simplified rules outlined in Box 30.2, and for a more detailed interpretation you should consult the correlation tables to be found in the specialist texts referred to at the end of the section.

Fragmentation patterns

The mass spectrum of a molecule is unique and can be stored in a computer. A match of the spectrum with those in the computer library is made in terms of molecular weight and the 10 most abundant peaks and a selection of possibilities will be presented. At this point you need to correlate all the information obtained from the spectroscopic techniques described in Chapters 26, 28, 29 and 30 together with the chemistry of the molecule to attempt to identify the structure of the molecule.

When you attempt to interpret the mass spectrum remember that:

- Only the base peak is almost certain to be derived from the molecular ion.
- Some lesser peaks may result from alternative fragmentation pathways, but these may be useful in assigning structural features.
- MS is often used to confirm information from IR and NMR spectra; interpretation of the mass spectrum alone is very difficult, except for the simplest molecules.

Interfacing mass spectrometry

Bench-top mass spectrometers are to be found interfaced with other analytical instruments such as gas chromatographs (p. 215) and high-

Box 30.1 How to identify the number of bromine or chlorine atoms in a molecule from the molecular ion

1. Since Cl and Br have isotopes two mass numbers apart, their presence in a molecule will produce peaks at *m/z* values above M^+, which are two mass numbers apart, i.e. $M + 2$, $M + 4$, etc.

2. The expression for the number and intensities of these peaks is given by the expansion of the formula:

   $(a + b)^n$

   where *a* and *b* are the ratio of the two atom isotopes, and *n* is the number of atoms.

   Example 1: If the molecule contains one chlorine atom then:

   $(a + b)^n = (3 + 1)^1 = 3 + 1$

   Thus the mass spectrum of CH_3Cl would show M^+ at $m/z = 50$ ($CH_3{}^{35}Cl$ and $M + 2$ at $m/z = 52$ ($CH_3{}^{37}Cl$) and the heights of these two peaks will be in the approximate ratio 3 : 1 (Fig. 30.4a).

   Example 2: If the molecule contains two chlorine atoms then:

   $(a + b)^n = a^2 + 2ab + b^2 = (3 + 1)^2 = 9 + 6 + 1$

   Thus the mass spectrum of CH_2Cl_2 would show M^+ at $m/z = 84$ ($CH_2{}^{35}Cl_2$), $M + 2$ at $m/z = 86$ ($CH_2{}^{35}Cl{}^{37}Cl$) and $M + 4$ at $m/z = 88$ ($CH_2{}^{37}Cl_2$) and the heights of these peaks will be the approximate ratio 9 : 6 : 1 (Fig. 30.4b).

   Example 3: If the molecule contains one bromine atom then:

   $(a + b)^n = (1 + 1)^1 = 1 + 1$

   Thus the mass spectrum of CH_3CH_2Br would show M^+ at $m/z = 108$ ($CH_3CH_2{}^{79}Br$) and $M + 2$ at $m/z = 110$ ($CH_3CH_2{}^{81}Br$) and the heights of these peaks will be in the approximate ratio 1 : 1 (Fig. 30.4c).

   Example 4: If the molecule contains three bromine atoms then:

   $(a + b)^3 = a^3 + 3a^2b + 3ab^2 + b^3 =$
   $(1 + 1)^3 = 1 + 3 + 3 + 1$

   Thus the mass spectrum of $CHBr_3$ would show M^+ at $m/z = 250$ ($CH^{79}Br_3$), $M + 2$ at $m/z = 252$ ($CH^{79}Br_2{}^{81}Br$), $M + 4$ at $m/z = 254$ ($CH^{79}Br^{81}Br_2$) and $M + 6$ at $m/z = 256$ ($CH^{81}Br_3$) and the heights of the peaks will be in the approximate ratio 1 : 3 : 3 : 1 (Fig. 30.4d).

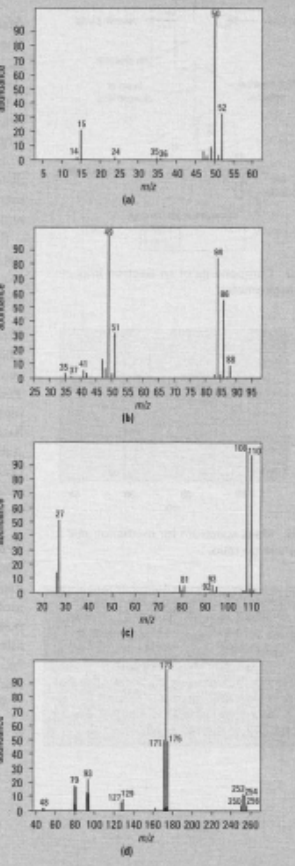
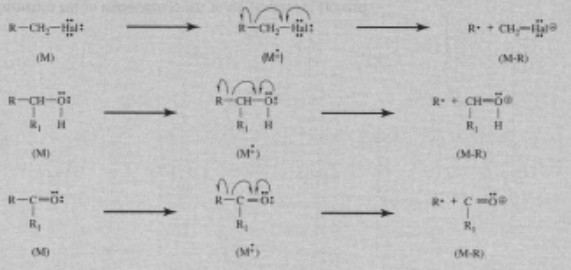


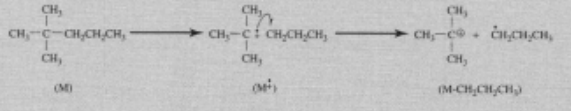
Fig. 30.4 Mass spectra of: (a) CH_3Cl; (b) CH_2Cl_2; (c) CH_3CH_2Br; (d) $CHBr_3$.

Box 30.2 Idealized fragmentation processes for the molecular ion (M^+)

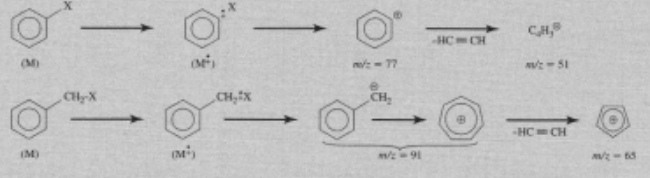
1. **α-Cleavage**: this involves breaking the 'next but one bond' to a hetero-atom (N, O, Hal, etc.) in the functional group of a molecule. The following examples illustrate the general principles:



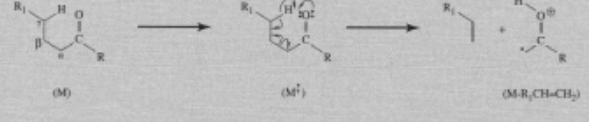
2. **σ-Bonds in alkanes**: C–C bonds break in preference to C–H bonds and the most stable carbocation will be formed as the base peak. For example, 2,2-dimethylpentane will give the stable $(CH_3)_3C^+$ cation as the base peak instead of the less stable propyl cation $CH_3CH_2CH_2^+$.



3. **Aromatic compounds**: simple aromatics cleave to give a phenyl cation, $m/z = 77$, as the base peak which then loses ethyne to give $m/z = 51$. Aromatics with CH_2 next to the ring give the stable tropylium cation $m/z = 91$, and then lose ethyne to $m/z = 65$.



4. **β-Cleavage or McLafferty rearrangement**: this is applied to molecules with a carbonyl group. If there is a hydrogen atom on the carbon atom four away from the carbonyl oxygen (γ carbon atom), a rearrangement of the molecular ion occurs and a neutral alkene is lost from M^+. This process occurs concurrently with the α-cleavage:



performance liquid chromatographs (p. 222) giving rise to the 'hyphenated techniques', GC–MS and LC–MS, respectively.

In both techniques the separated compounds from the chromatograph are sampled automatically and the mass spectrum of each compound is recorded. Comparison of the mass spectra with a library in an 'on-board' database permits identification of the components of the mixture.

31 Chromatography – introduction

Chromatography is often a three-way compromise between:

1. separation of analytes;
2. time of analysis;
3. volume of eluent.

Selecting a separation method – it is often best to select a technique that involves direct interaction between the substance(s) and the stationary phase (e.g. ion-exchange or affinity chromatography), owing to their increased capacity and resolution compared with other methods (e.g. partition or gel permeation chromatography) where the analytes are not bound to the stationary phase.

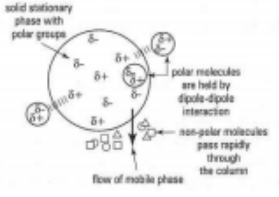


Fig. 31.1 Adsorption chromatography (polar stationary phase).

Chromatography is used to separate the individual components of a mixture on the basis of differences in their physical characteristics, e.g. molecular size, shape, charge, volatility, solubility and/or adsorption properties. The essential components of a chromatographic system are:

- A stationary phase, where a solid, a gel or an immobilized liquid is held by a support matrix.
- A chromatographic bed: the stationary phase may be packed into a glass or metal column, spread as a thin layer on a sheet of glass or plastic, or adsorbed on cellulose fibres (paper).
- A mobile phase, either a liquid or a gas which acts as a solvent, carrying the sample through the stationary phase and eluting from the chromatographic bed.
- A delivery system to pass the mobile phase through the chromatographic bed.
- A detection system to visualize the test substances.

The individual substances in the mixture interact with the stationary phase to different extents, as they are carried through the system, enabling separation to be achieved.

KEY POINT **In a chromatographic system, those substances which interact strongly with the stationary phase will be retarded to the greatest extent, while those which show little interaction will pass through with minimal delay, leading to differences in distances travelled or elution times.**

Chromatography is sub-divided according to the mechanism of interaction of the solute with the stationary phase.

Adsorption chromatography

This is a form of solid–liquid chromatography. The stationary phase is a porous, finely divided solid which adsorbs molecules of the mixture on its surface by dipole–dipole interactions, hydrogen bonding and/or van der Waals' interactions (Fig. 31.1). The range of adsorbents is limited to polystyrene-based resins for non-polar molecules and silica, aluminium oxide and calcium phosphate for polar molecules. Most adsorbents must be activated by heating to 110–120 °C before use, since their adsorptive capacity is significantly decreased if water is adsorbed on the surface. Adsorption chromatography can be carried out in column (p. 217) or thin-layer (p. 216) form, using a wide range of organic solvents.

Partition chromatography

This is based on the partitioning of a substance between two liquid phases, in this instance the stationary and mobile phases. Substances which are more soluble in the mobile phase will pass rapidly through the system while those which favour the stationary phase will be retarded (Fig. 31.2). In normal phase partition chromatography the stationary phase is a polar solvent, usually water, supported by a solid matrix (e.g. cellulose fibres in paper chromatography) and the mobile phase is an immiscible, non-polar organic solvent. For reversed-phase partition chromatography the stationary phase is

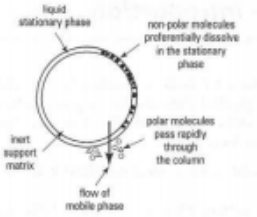


Fig. 31.2 Liquid–liquid partition chromatography, e.g. reversed-phase HPLC.

Maximizing resolution in IEC – keep your columns as short as possible. Once the sample components have been separated, they should be eluted as quickly as possible from the column in order to avoid band broadening resulting from diffusion of sample ions in the mobile phase.

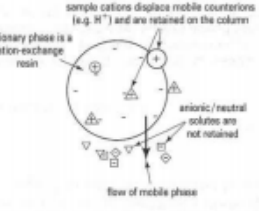


Fig. 31.3 Ion-exchange chromatography (cation exchanger).

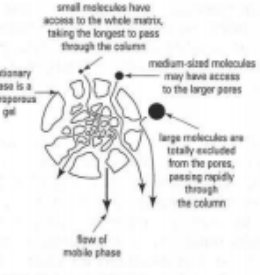


Fig. 31.4 Gel permeation chromatography.

a non-polar solvent (e.g. a C_{18} hydrocarbon, such as octadecylsilane) which is chemically bonded to a porous support matrix (e.g. silica), while the mobile phase can be chosen from a wide range of polar solvents, usually water or an aqueous buffered solution containing one or more organic solvents, e.g. acetonitrile. Solutes interact with the stationary phase through non-polar interactions and so the least polar solutes elute last from the column. Solute retention and separation are controlled by changing the composition of the mobile phase (e.g. % v/v acetonitrile). Reverse-phase high-performance liquid chromatography (RPHPLC, p. 218) is used to separate a broad range of non-polar, polar and ionic molecules, including environmental compounds (e.g. phenols) and pharmaceutical compounds (e.g. steroids).

Ion-exchange chromatography (IEC)

Here, separations are carried out using a column packed with a porous matrix which has a large number of ionized groups on its surfaces, i.e. the stationary phase is an ion-exchange resin. The groups may be cation or anion exchangers, depending upon their affinity for positive or negative ions. The net charge on a particular resin depends on the pK_a of the ionizable groups and the pH of the solution, in accordance with the Henderson–Hasselbalch equation (p. 58).

For most practical applications, you should select the ion-exchange resin and buffer pH so that the test substances are strongly bound by electrostatic attraction to the ion-exchange resin on passage through the system, while the other components of the sample are rapidly eluted (Fig. 31.3). You can then elute the bound components by raising the salt concentration of the mobile phase, either stepwise or as a continuous gradient, so that exchange of ions of the same charge occurs at oppositely charged sites on the stationary phase. Weakly bound sample molecules will elute first, while more strongly bound molecules will elute at a higher concentration.

Computer-controlled gradient formers are available: if two or more components cannot be resolved using a linear salt gradient, an adapted gradient can be used in which the rate of change in salt concentration is decreased over the range where these components are expected to elute. IEC can be used to separate mixtures of a wide range of anionic and cationic compounds. Electrophoresis (Chapter 33) is an alternative means of separating charged molecules.

Gel permeation chromatography (GPC) or gel filtration

Here, the stationary phase is in the form of beads of a cross-linked gel containing pores of a discrete size (Fig. 31.4). The size of the pores is controlled so that at the molecular level, the pores act as 'gates' that will exclude large molecules and admit smaller ones (Table 31.1). However, this gating effect is not an all-or-nothing phenomenon: molecules of intermediate size partly enter the pores. A column packed with such beads will have within it two effective volumes that are potentially available to sample molecules in the mobile phase, i.e. V_o, the volume surrounding the beads and V_{ip}, the volume within the pores. If a sample is placed at the top of such a column, the mobile phase will carry the sample components down the column, but at different rates according to their molecular size. A very large molecule will have access to all of V_o but to none of V_{ip}, and will therefore elute in the minimum possible volume (the 'void volume', or V_o, equivalent to V_o). A very small molecule will have access to all of V_o and all of V_{ip}, and therefore it has to pass through the total liquid volume of the column (V_t, equivalent to

Using a gel permeation system – keep your sample volume as small as possible, in order to minimize band broadening due to dilution of the sample during passage through the column.

Table 31.1 Fractionation ranges of selected GPC media

M_r	Medium
50–1 000	Sephadex G15, Biogel P-2
1 000–5 000	Sephadex G-25
1 500–30 000	Sephadex G-50, Biogel P-10
4 000–150 000	Sephadex G-100
5 000–250 000	Sephadex G-200
20 000–1 500 000	Sephacryl S 300
60 000–20 000 000	Sepharose 4B

Examples Biospecific molecules used in affinity chromatography include:

- enzymes and inhibitors/cofactors/substrates;
- hormones and receptor proteins;
- antibodies and antigens;
- complementary base sequences in DNA and RNA.

Elution of substances from an affinity system – make sure that your elution conditions do not affect the interaction between the ligand and the stationary phase, or you may elute the ligand from the column.

Remember that you cannot quantify a particular analyte without first identifying it: the presence of a single peak on a chromatogram does not prove that a single type of analyte is present.

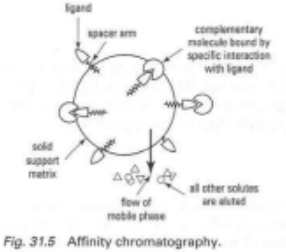


Fig. 31.5 Affinity chromatography.

$V_i + V_{ii}$) before it emerges. Molecules of intermediate size have access to all of V_i but only part of V_{ii}, and will elute at a volume between V_0 and V_t, in order of decreasing size depending on their access to V_{ii}.

Cross-linked dextrans (e.g. Sephadex®), agarose (e.g. Sepharose®) and polyacrylamide (e.g. Bio-gel®) can be used to separate mixtures of macromolecules, particularly enzymes, antibodies and other globular proteins. Selectivity in GPC is solely dependent on the stationary phase, with the mobile phase being used solely to transport the sample components through the column. Thus, it is possible to estimate the molecular mass of a sample component by calibrating a given column using molecules of known molecular mass and similar shape. A plot of elution volume (V_e) against $\log_{10}$ molecular mass is approximately linear. A further application of GPC is the general separation of components of low molecular mass and high molecular mass, e.g. 'desalting' a protein extract by passage through a Sephadex® G-25 column is faster and more efficient than dialysis.

Affinity chromatography

Affinity chromatography allows biomolecules to be purified on the basis of their biological specificity rather than by differences in physico-chemical properties, and a high degree of purification (more than 1000-fold) can be expected. It is especially useful for isolating small quantities of material from large amounts of contaminating substances. The technique involves the immobilization of a complementary binding substance (the ligand) onto a solid matrix in such a way that the specific binding affinity of the ligand is preserved. When a biological sample is applied to a column packed with this affinity support matrix, the molecule of interest will bind specifically to the ligand, while contaminating substances will be washed through with the buffer (Fig. 31.5). Elution of the desired molecule can be achieved by changing the pH or ionic strength of the buffer, to weaken the non-covalent interactions between the molecule and the ligand, or by addition of other substances that have greater affinity for the ligand.

The chromatogram

A plot of the detector response present at the column outlet as a function of time is called a chromatogram (Fig. 31.6). The time from injection of the sample until the peak elutes from the column is called the retention time, t_r. The amount of compound present for a given peak can be quantified by measuring the peak height or area (most useful) and comparing it with the response for a known amount of the same compound.

The aim of any chromatographic system is to resolve a number of components in a sample mixture, i.e. to ensure that individual peaks do not overlap or coincide. To achieve this you need to consider several important factors: capacity factor, separation factor or selectivity, column efficiency and asymmetry factor.

Capacity factor, k' This is a more useful measure of peak retention that retention time, as it is independent of column length and flow rate. To calculate k' you need to measure column dead time, t_0. This is the time it takes an unretained component to pass through the column without any interaction with the stationary phase. It is the time taken from the point of sample injection until the first disturbance in the base line caused by the

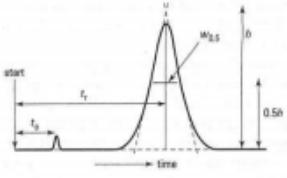


Fig. 31.6 Peak characteristics in a chromatographic separation, i.e. a chromatogram. For symbols, see eqns [31.1] and [31.3].

unretained component. The capacity factor for other components can then be calculated according to the following equation:

$$k' = \frac{t_r - t_0}{t_0} \qquad [31.1]$$

Separation factor, α The separation factor, or selectivity, identifies when the peaks elute relative to each other. It is defined for two peaks as the ratio of the capacity factors ($k'_2 > k'_1$):

$$\alpha = \frac{k'_2}{k'_1} = \frac{t_{r,2} - t_0}{t_{r,1} - t_0} \qquad [31.2]$$

where $t_{r,1}$ and $t_{r,2}$ are the retention times of peak 1 and peak 2, respectively. If two peaks are present the separation factor must be greater than one to achieve an effective separation.

Column efficiency (plate number), N An additional parameter used to characterize a separation system is the plate number, N. It represents, in general terms, the narrowness of the peak and is often calculated as follows:

$$N = 5.54\left(\frac{t_r}{w_{0.5}}\right)^2 \qquad [31.3]$$

where t_r is the retention time of the peak and $w_{0.5}$ is its width at one-half of its height (Fig. 31.6).

For a compound emerging from a column of length L, the number of theoretical plates, N, can be expressed as:

$$N = \frac{L}{H} \qquad [31.4]$$

where H is the plate height (or height equivalent to a theoretical plate). In general, chromatographic columns with larger values of N give the narrowest peaks and generally better separation.

Learning from experience – if you are unable to separate your molecule using a particular method, do not regard this as a failure, but instead think about what this tells you about either the substance(s) or the sample.

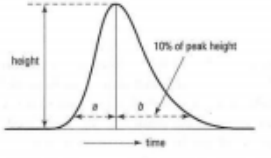


Fig. 31.7 Peak asymmetry.

Asymmetry factor, A_s The plate number, N, assumes that the peak shape is Gaussian, but in practice this is rare. It is more likely that the peak is asymmetrical, i.e. it 'tails'. This is quantified using the asymmetry factor, A_s, calculated as shown in Fig. 31.7.

A vertical line is drawn between the peak maximum and the base line. At 10% of the peak height, the width of the peak to the leading edge and the trailing edge is measured (a and b in Fig. 31.7). The asymmetry factor is then calculated as follows:

$$A_s = \frac{b}{a} \qquad [31.5]$$

In general, A_s values between 0.9 and 1.2 are acceptable. If $A_s > 1$ peak tailing is in evidence; if $A_s < 1$ peak fronting is evident. The practical impact of peak tailing or fronting is that adjacent peaks are not as well separated as they would be if they were symmetrical, leading to difficulties in peak quantitation.

Resolution

It is often important to be able to separate a large number of compounds. A visual inspection of the chromatogram (Fig. 31.8) will usually indicate whether the separation is appropriate. It is desirable that the valley between

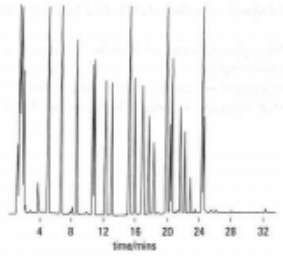


Fig. 31.8 A multicomponent chromatogram. Separation of many compounds, some well resolved, e.g. peaks at 12–13 mins, and others that are not, e.g. peaks at 24–25 mins.

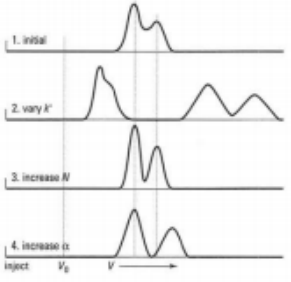


Fig. 31.9 Influence of k', α and N on resolution.

adjoining peaks returns to the base line and resolution is a quantitative measure of the separation. The influence of k', α and N on resolution, R, is shown in the following expression:

$$R = \frac{\sqrt{N}}{4} \times \frac{k'}{k'+1} \times \frac{\alpha - 1}{\alpha} \qquad [31.6]$$

Three conditions must be satisfied in order to achieve some degree of resolution:

1. Peaks have to be retained on the column ($k' > 0$).
2. Peaks have to be separated from each other ($\alpha > 1$).
3. The column must develop some minimum value of N.

These different effects and their influence on resolution are shown in Fig. 31.9, using high-performance liquid chromatography (HPLC) as an example (see Chapter 32).

1. Initial conditions result in inadequate separation of the two components.
2. Effect of varying the capacity factor, k': from an initial mobile phase of 50% methanol : 50% water (v/v) two scenarios are possibile. Firstly, on the left-hand side, the influence of increasing the percentage of organic solvent (70% methanol : 30% water) allows a faster throughput, but the peaks are unresolved. Secondly, on the right-hand side, the percentage organic solvent is reduced (30% methanol : 70% water) allowing the components to remain in the system for a longer time, giving some separation, but causing peak broadening. This is the easiest change to make and will affect resolution. As a guide, a two- to three-fold change in k' will result for each 10% change in mobile phase composition.
3. Effect of increasing the plate number, N: reducing the particle size of the HPLC packing from 5 μm to 3 μm allows a more efficient separation. It should be noted that the retention times of the peaks are not altered (from the initial chromatogram) provided the stationary phase is not altered. Alternatively N can be increased by placing columns in series with one another. However, you should note from eqn [31.6] that R has a square-root dependence on N, i.e. a four-fold increase in N is required to double R.
4. Increase separation factor, α: resolved peaks can be obtained by changing the mobile phase (e.g. methanol to acetonitrile) or the column stationary phase (e.g. C_{18} to C_8). Unfortunately this is the least predictable approach.

Detectors

After separating the components of the mixture it is necessary to detect them. A description of a range of detectors suitable for chromatography is described in Chapter 32. As chromatography is often used as a quantitative technique it is essential to be familiar with the following terms:

- *Universal detector*: this responds to all compounds eluting from the column, irrespective of their composition.
- *Selective/specific detector*: this responds to certain elements or functional groups. This is a useful approach if the components of the mixture are known.

- *Sensitivity*: the ratio of detector signal to sample size (or detector response per amount of sample).
- *Minimum detectable level (MDL)*: the amount of sample in which the peak height is at least twice the noise height.
- *Linear dynamic range*: the concentration range of the sample that is detectable and where the detector response is linear (between the MDL and detector saturation).

32 Gas and liquid chromatography

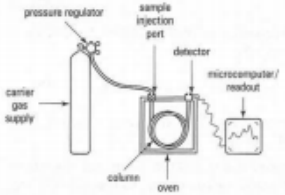


Fig. 32.1 Components of a GC system.

Types of capillary columns

- Wall-coated open tubular (WCOT) column: liquid stationary phase on inside wall of column.
- Support-coated open tubular (SCOT) column: liquid stationary phase coated on solid support attached to inside wall of column.
- Porous layer open tubular (PLOT): solid stationary phase on inside wall of column.

Applications of gas–liquid chromatography – GLC is used to separate volatile, non-polar compounds: substances with polar groups must be converted to less polar derivatives prior to analysis, in order to prevent adsorption on the column, resulting in poor resolution and peak tailing.

Analysing compounds by GC in the split mode – make sure no hazardous materials enter the laboratory atmosphere through the split vent. A charcoal split-vent trap may be required to eliminate potential hazards.

Gas chromatography

In gas chromatography (GC), a gaseous solute (or the vapour from a volatile liquid) is carried by the gaseous mobile phase. In gas–liquid partition chromatography, the stationary phase is a non-volatile liquid coated on the inside of the column or on a fine support. In gas–solid adsorption chromatography, solid particles that adsorb the solute act as the stationary phase.

The typical components of a gas chromatograph are shown in Fig. 32.1. A volatile liquid is injected through a septum into a heated port, which volatilizes the sample. A gaseous mobile phase carries the sample through the heated column, and the separated components are detected and recorded. Two types of columns are available: packed and capillary. Open tubular capillary columns offer higher resolution, shorter analysis time and greater sensitivity than packed columns, but have lower capacity for the sample.

Sample injection

Samples are injected onto the 'top' of the column, through a sample injection port containing a gas-tight septum. The two common sample injection methods for capillary GC are:

1. Split/splitless injector: in the split mode only a portion of the injected sample (typically, 1 part in 50) reaches the column. The rest is vented to waste. A split injector is used for concentrated samples ($> 0.1\,mg\,mL^{-1}$ for FID; see p. 215). In the splitless mode all the sample volume injected passes through to the column. It is used, in this mode, for trace samples ($< 0.1\,mg\,mL^{-1}$ for FID).
2. Cold on-column injector: all the sample is injected onto the column. It is used for thermally unstable compounds and high-boiling solvents.

In both cases a syringe (1 μL) is used to inject the sample. Examples of each type of injection system are shown in Fig. 32.2. The procedure for injection of a sample is shown in Fig. 32.3. In Fig. 32.3a the syringe (see p. 213) is filled with the sample/standard solution (typically 0.5 μL). Then the outside of the syringe is wiped clean with a tissue (Fig. 32.3b). The syringe is placed into the injector of the gas chromatograph (Fig. 32.3c) and, finally, the plunger on the syringe is depressed to inject the sample (Fig. 32.3d). The procedure for the preparation of a series of calibration solutions is shown in Box 32.1.

The column

Modern GC uses capillary columns (internal diameter 0.1–0.5 mm) up to 60 m in length. The stationary phase is generally a cross-linked silicone polymer, coated as a thin film on the inner wall of the fused silica (SiO_2) capillary: at normal operating temperatures, this behaves in a similar manner to a liquid film, but is far more robust. Common stationary phases for GC are shown in Fig. 32.4. The mobile phase ('carrier gas') is usually nitrogen or helium. Selective separation is achieved as a result of the differential partitioning of individual compounds between the carrier gas and silicone polymer phases. The separation of most organic molecules is influenced by

Box. 32.1 How to prepare a set of five calibration solutions in the concentration range 0–10 μg mL^{-1} (mg L^{-1})

Assuming that we are starting with a 1000 μg mL^{-1} stock solution of a particular organic compound, e.g. 2-chlorophenol, then you will need the following: 6 × 10.00 mL grade A volumetric flasks and a syringe (0–100.00 μL).

1. **Ensure that all the glassware is clean** (see p. 13).
2. **Add ≈ 9 mL of organic solvent,** e.g. dichloromethane, to a 10.00 mL with dichloromethane.
3. **Quantitatively transfer 20.00 μL of the stock solution** into the 10.00 mL volumetric flask. Inject the solution from the syringe below the surface of the dichloromethane. Then, dilute to 10.00 mL with dichloromethane.
4. **What is the concentration of this new solution?** Remember that we started with an initial 1000 μg mL^{-1} 2-chlorophenol stock solution.

$$\frac{1000\,\mu g}{mL} \times 20 \times 10^{-3}\,mL = 20\,\mu g\ 2\text{-chlorophenol} \qquad [32.1]$$

so 20 μg 2-chlorophenol was placed in the 10.00 mL volumetric flask. So,

$$\frac{20\,\mu g}{10\,mL} = 2\,\mu g\,mL^{-1}\ 2\text{-chlorophenol} \qquad [32.2]$$

You now have a 2 μg mL^{-1} calibration solution of 2-chlorophenol.

5. **Similarly transfer 0, 40.00, 60.00, 80.00 and 100.00 μL volumes into separate volumetric flasks** and dilute to 10.00 mL with dichloromethane and label as 0, 4, 6, 8 and 10 μg mL^{-1} 2-chlorophenol calibration solutions.
6. **Take the 0, 2, 4, 6, 8 and 10 μg mL^{-1} 2-chlorophenol calibration solutions** to the chromatograph for analysis.

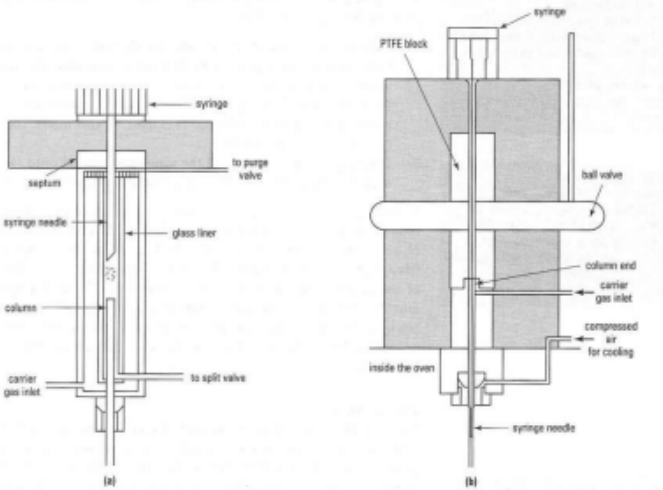
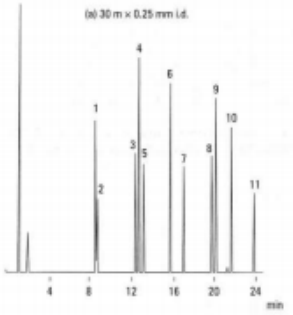


Fig. 32.2 Sample introduction in GC: (a) split/splitless injector; (b) on-column injector.

the temperature of the column, which may be constant during the analysis ('isothermal' – usually 50–250 °C) or, more commonly, may increase in a pre-programmed manner (e.g. from 50 °C to 250 °C at 10 °C per minute).



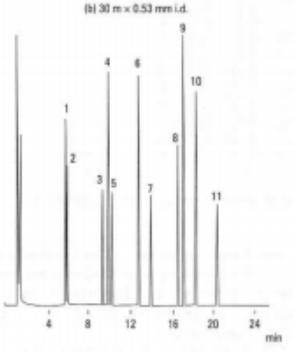


Fig. 32.5 Influence of GC column internal diameter on separation: 1. phenol; 2. 2-chlorophenol; 3. 2-nitrophenol; 4. 2,4-dimethylphenol; 5. 2,4-dichlorophenol; 6. 4-chloro-3-methylphenol; 7. 2,4,6-trichlorophenol; 8. 2,4-dinitrophenol; 9. 4-nitrophenol; 10. 2-methyl-4,6-dinitrophenol; 11. pentachlorophenol.

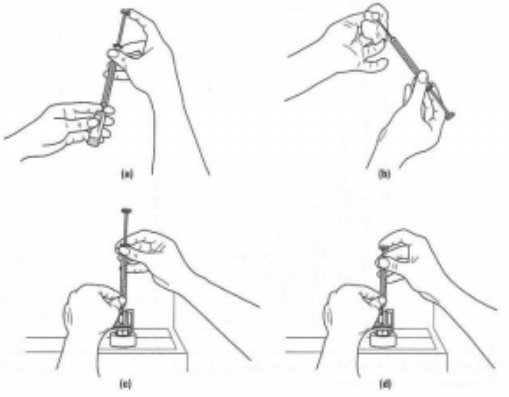


Fig. 32.3 Sample injection in GC. (a) Fill the syringe, (b) wipe clean the outside of the syringe needle, (c) place the syringe needle into the injector and (d) depress the plunger on the syringe to inject the sample.

100% dimethylsiloxane:
the least polar bonded phase. Used for boiling point separations (solvents, petroleum products, etc.).
Typical names: DB-1, HP-1, Rtx-1

95% dimethylsiloxane–5% diphenylpolysiloxane:
a non-polar phase. Used for separation of environmental samples, e.g. polycyclic aromatic hydrocarbons.
Typical names: DB-5, HP-5, Rtx-5

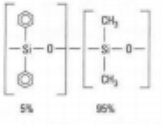


Fig. 32.4 Common stationary phases for capillary GC.

Selecting an appropriate column for capillary GC is a difficult task and one which is usually left to the technician. However, it is important to be aware of some general issues and what influence they can have on the separation. The column internal diameter can affect both resolution and speed of analysis. Smaller internal diameters columns (0.25 mm i.d.) can provide good resolution of early eluting peaks (Fig. 32.5a). However, the problem is that the analysis times of the eluting components may be longer and that the linear dynamic range (see p. 210) may be restricted. In contrast,

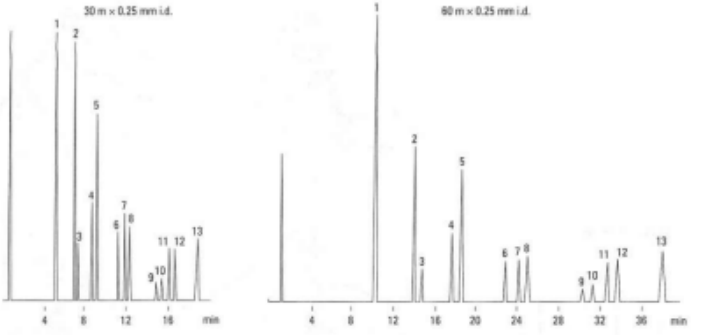


Fig. 32.6 Influence of column length on analysis time. Analysis of phenols under isothermal conditions: 1. phenol; 2. *o*-cresol; 3. 2,6-xylenol; 4. *p*-cresol; 5. *n*-cresol; 6. *o*-ethylphenol; 7. 2,4-xylenol; 8. 2,5-xylenol; 9. 2,3-xylenol; 10. *p*-ethylphenol; 11. *m*-ethylphenol; 12. 3,5-xylenol; 13. 3,4-xylenol.

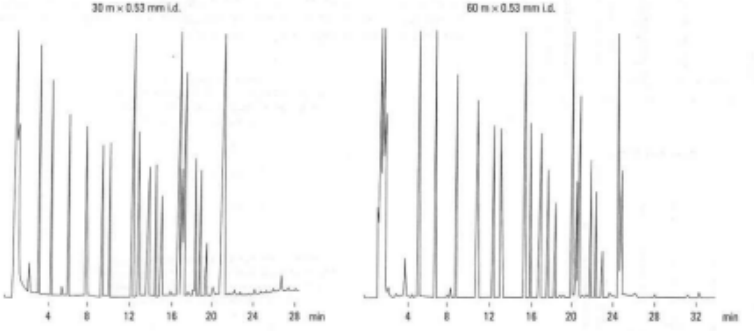


Fig. 32.7 Influence of column length on analysis time. Analysis of bacterial acid methyl esters under temperature-programmed conditions.

Solvent selection in GC – choose a solvent with a boiling point at least 20 °C below that of the first eluting compound. Thicker stationary-phase films provide longer retention and higher peak capacity.

larger internal diameter columns (0.53 mm i.d.) provide less resolution for early eluting compounds (Fig. 32.5b), but this is reflected in shorter analysis times and a greater linear dynamic range (see p. 210). This type of column may provide sufficient resolution for the analysis of complex mixtures. Fig. 32.5 illustrates the effects of column internal diameter.

Another important column effect is the length of the column and the influence this can have on the resolution of eluting components. It was previously shown (p. 208) that resolution was influenced by k', α and N. Substituting eqn [31.4] into eqn [31.6] produces the following equation:

$$R = \frac{1}{4}\sqrt{\frac{L}{h}} \times \frac{k'}{k'+1} \times \frac{\alpha - 1}{\alpha} \qquad [32.3]$$

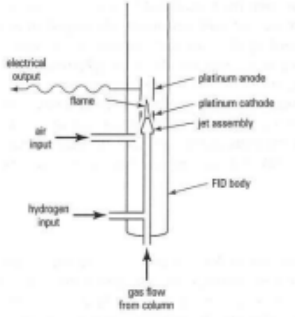


Fig. 32.8 Components of a flame ionization detector (FID).

Detectors – the most appropriate detector depends on the type of chromatography and the application: ideally, the detector should show high sensitivity, a low detection limit and minimal noise or drift. These terms are defined in Chapter 31.

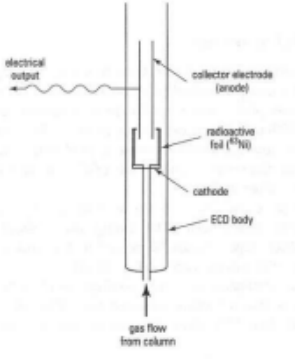


Fig. 32.9 Components of an electron capture detector (ECD).

The importance of this equation can be shown by considering the influence on resolution, R, of column length, L. Under isothermal analysis conditions, i.e. the same column temperature, the retention of eluting compounds is more dependent upon column length. For example, doubling the column length doubles the analysis times and increases the resolution by 41%. This is shown in Fig. 32.6 for the analysis of phenols. In contrast, under temperature-programmed analysis, e.g. 130 °C to 250 °C at 4 °C min^{-1}, the retention time of eluting components is more dependent on temperature. For example, doubling the column length has minimal effect on analysis times. This is shown in Fig. 32.7 for the analysis of bacterial acid methyl esters.

GC detectors

The output from the GC column is monitored by a detector. The most commonly used detectors for GC analysis of organic molecules are as follows.

The flame ionization detector (FID) is particularly useful for the analysis of a broad range of organic molecules. It involves passing the exit gas stream from the column through a hydrogen flame that has a potential of more than 100 V applied across it (Fig. 32.8). Most organic compounds, on passage through this flame, produce ions and electrons that create a small current across the electrodes, and this is amplified for measurement purposes. The FID is very sensitive (typically down to ≈ 0.1 pg), with a linear response over a wide concentration range. One drawback is that the sample is destroyed during analysis.

The thermal conductivity detector (TCD) is based on changes in the thermal conductivity of the gas stream brought about by the presence of separated sample molecules. The detector elements are two electrically heated platinum wires, one in a chamber through which only the carrier gas flows (the reference detector cell), and the other in a chamber that takes the gas flow from the column (the sample detector cell). In the presence of a constant gas flow, the temperature of the wires (and therefore their electrical resistance) is dependent on the thermal conductivity of the gas. Analytes in the gas stream are detected by temperature-dependent changes in resistance based on the thermal conductivity of each separated molecule; the size of the signal is directly related to concentration of the analyte.

The advantages of the TCD include its applicability to a wide range of organic and inorganic molecules and its non-destructive nature, since the sample can be collected for further study. Its major limitation is its low sensitivity (down to ≈ 10 ng), compared with other systems.

The electron capture detector (ECD) is highly sensitive (Fig. 32.9) and is useful for the detection of certain compounds with electronegative functional groups, e.g. halogens, peroxides and quinones. The gas stream from the column passes over a β-emitter (p. 235 such as ^{63}Ni, which provides electrons that cause ionization of the carrier gas (e.g. nitrogen). When carrier gas alone is passing the β-emitter, its ionization results in a constant current flowing between two electrodes placed in the gas flow. However, when electron-capturing sample molecules are present in the gas flow, a decrease in current is detected. An example of the application of the ECD is in detecting and quantifying chlorinated pesticides.

Mass spectrometry (see also p. 200) used in conjunction with GC provides a powerful tool for identifying the components of complex mixtures, e.g. environmental pollutants, synthetic products, etc. (Fig. 32.10). The procedure requires computer control of the instrument and for data storage/analysis.

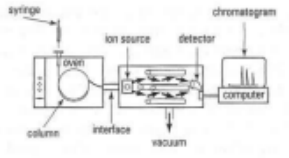


Fig. 32.10 Schematic diagram of a GCMS instrument.

Compounds eluting from the column are bombarded by electrons (electron impact, EI, mode) causing fragmentation and production of charged species. These charged species are separated by the mass spectrometer on the basis of their mass-to-charge ratio. Ions passing through the mass spectrometer are detected by an electron multiplier tube. The mass spectrometer can be used in two modes: total ion and selected ion monitoring. In the former mode, the complete mass spectrum of each of the components of the mixture eluting from the column is recorded. In the latter mode, only ions of specified mass-to-charge ratios are detected. Selected ion monitoring offers increased sensitivity and selectivity.

Liquid chromatography

The basic chromatographic system comprises a stationary phase (adsorbant), usually alumina, silica gel or cellulose, through which a mobile phase travels (elutes). Separation of a mixture of compounds is achieved by a combination of the differing 'adsorption' and solubility characteristics of the components on the stationary phase and in the mobile phase respectively.

Liquid chromatography is used both as an analytical method to determine the complexity of mixtures and the purity of compounds, and as a preparative system for the separation of mixtures. Liquid chromatography is divided into two general types:

1. Thin-layer chromatography (TLC): in which a glass or plastic plate is coated with a thin layer of the stationary phase and the mobile phase *ascends* the plate by capillary action. TLC is essentially an analytical tool and preparative TLC has been largely superseded by flash chromatography.
2. Column chromatography: in which the stationary phase is packed into a glass column and the mobile phase is passed *down* the column, either by gravity (gravity chromatography) or under low pressure from a pump or nitrogen cylinder (flash chromatography). These are the preparative systems.

Thin-layer chromatography

The essential components of a TLC system are:

- The stationary phase comprising the layer of adsorbant on a solid backing – the chromatoplate. Aluminium or plastic-backed chromatoplates are now the norm having replaced glass plates, which needed to be prepared 'in-house'. The chromatoplates (20 cm × 20 cm) can be cut down to the more useful size (2 cm × 5 cm) for analytical work, using a guillotine. The adsorbant often contains a fluorescent compound (ZnS) to enable visualization of the compounds after elution.
- The development tank: for plates of (2 cm × 5 cm) a clean, dry beaker (100 mL) covered with a watch-glass is ideal. The eluting solvent should be about 3 mm deep and filter paper should be placed in the tank to saturate the tank atmosphere with solvent vapour (Fig. 32.11).
- The application system: a micropipette or a microsyringe to place the solution of the mixture on the chromatoplate. Micropipettes (Fig. 32.12) are the more common and Box 32.2 gives the instructions for their preparation.
- The eluent: finding the eluent, which will give the best separation of the components of the mixture, is by experiment – you may need to try

Using TLC plates – do not touch the surface of the TLC plate with your fingers. Hold them by the edges to prevent contamination.

Using TLC plates – a plate (2 cm × 5 cm) will hold *three* sample 'spots'.

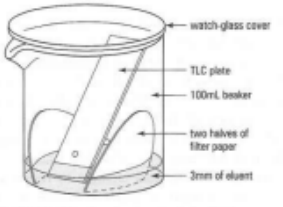


Fig. 32.11 The developing tank for TLC.

Solutions of the mixture – the mixture must be applied to the plate as a solution. If the solvent for the solution is not the same as the eluent to be used, you must evaporate the solvent from the plate, before placing it in the eluent.

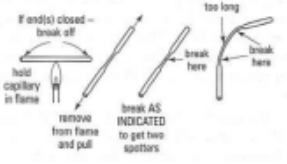


Fig. 32.12 How to make micropipettes.

Box 32.2 How to make micropipettes for TLC

1. **Heat the middle of an open-ended melting point tube at the tip of the hot flame of a microburner until it begins to sag.** If the melting point tube is sealed at one or both ends, carefully break off the sealed end(s) wearing gloves for protection.
2. **Quickly remove the tube from the flame and pull gently, forming a short capillary.** Do not pull the tube while it is in the flame.
3. **Allow the tube to cool and then break in the centre of the capillary.** You now have two micropipettes. If the capillary is too long, break it near to each end and *immediately* dispose of the fine waste glass into the broken-glass bin. *Do not leave* the waste glass on the laboratory bench.
4. **Make at least 10 micropipettes** and store them in a plastic-capped sample tube for future use.

Finding an eluent – a medium-polarity solvent such as dichloromethane (DCM) is generally a good starting point.

Table 32.1 The elutropic series of solvents for chromatography

Non-polar	Light petroleum (b.pt. 40–60 °C)
	Cyclohexane
	Toluene
	Dichloromethane
	Diethylether (ether)
	Ethyl ethanoate (ethyl acetate)
	Propanone (acetone)
	Ethanoic acid (acetic acid)
Polar	Methanol

Safety note Great care must be taken when using UV light. *Do not* look directly at the UV source and wear gloves if you put your hands into the UV cabinet.

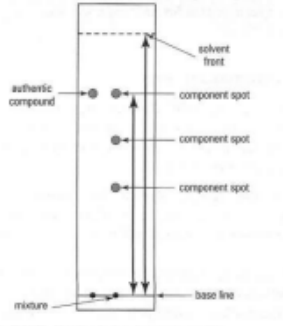


Fig. 32.13 A thin-layer chromatogram.

several solvents of differing polarity (Table 32.1) or mixtures of solvents to find the best eluent.

- A visualization system to be able to see colourless separated components on the chromatogram. If the plate contains a fluorescer, it can be viewed under UV light ($\lambda = 254$ nm) in a special box or cabinet. The ZnS in the stationary phase fluoresces green, whereas the 'spots' of separated compounds appear dark. Alternatively, the plate can be placed in a sealed jar containing a few iodine crystals. The iodine vapour stains the plate light brown and the 'spots' dark brown.

You can express the movement of an individual compound up the TLC plate in terms of its R_f (relative frontal mobility) value, where:

$$R_f = \text{distance moved by compound/distance moved by solvent} \quad [31.4]$$

The R_f value is a constant for a particular substance and eluent system on a specific stationary phase, but variations in chromatographic conditions adsorbant, eluent (in particular solvent mixtures), temperature and atmosphere make the application of R_f values to absolute identification rather problematical. Usually an authentic sample is run alongside the unknowns in the mixture (Fig. 32.13) or on top of the mixture – 'double spotting' – as shown in Fig. 32.14, to enable identification. The general procedure for running a TLC plate is described in Box 32.3.

Column chromatography

This is used for the preparative scale separation of mixtures of compounds. There are many variations in detail of equipment and technique such as column type, column packing, sample application and fraction collection, many of which are a matter of personal choice and apparatus available. Typical arrangements are shown in Fig. 32.15 and for a detailed description of all these variations you should consult the specialist texts such as Errington (1997, p. 163), Harwood *et al.* (2000, p. 175) and Furniss *et al.* (1989, p. 209).

Gravity chromatography is used to separate the components of a mixture which have a difference in R_f value of at least 0.3. Flash chromatography, because of the smaller size of the adsorbant particles, is more effective separating mixtures components of $\Delta R_f = 0.15$ and is also faster.

KEY POINT **Before attempting a preparative mixture separation by column chromatography, you must always analyse the mixture by TLC to establish the stationary phase and solvent parameters for effective separation and to determine the R_f values of the components.**

Box 32.3 How to run a thin-layer chromatogram

1. **Prepare the TLC development tank using a clean, dry beaker, as shown in Fig. 32.11,** and allow it to equilibriate for 10 minutes.
2. **Prepare the plastic-backed TLC plate** by drawing a fine line (the base line) in *pencil* about 1 cm above the bottom. Take care not to scrape off any of the stationary phase. Put three pencil dots on the base line – one in the centre and the others equidistant on either side, but no closer than 3 mm to the edges of the plate.
3. **Dissolve the mixture (1–3 mg) in two or three drops of a suitable volatile solvent** (dichloromethane or ether are the most common), in a small sample tube.
4. **Dip the tip of a micropipette into the solution** and capillary action will draw the solution into the pipette.
5. **Touch the tip of the micropipette onto one of the pencil spots on the base line.** Capillary action will draw the solution from the micropipette onto the plate as a spot. Do not allow the spot be more than 2 mm in diameter. Put the micropipette back into the sample tube containing the mixture.
6. **Spot other samples** (e.g. reference compounds) onto the plate as appropriate, *using a new micropipette* in each case.
7. **Evaporate the solvent from the plate** by waving it in the air, holding the plate by the edges.
8. **Lower the plate into the developing tank,** holding it by the edges and make sure that *the eluent does not* cover the base line. If it does, discard the plate, prepare another and empty some eluent from the tank.
9. **Put the lid on the tank and allow the eluent to rise up the plate** to about 1 cm from the top of the plate.
10. **Remove the chromatoplate from the** tank and quickly mark the height reached by the eluent, using a pencil.
11. **Wave the chromatoplate in the air** to evaporate the eluent from the plate.
12. **Visualize the chromatogram by either placing the dry chromatoplate in the UV cabinet and using a pencil to draw round the spots (*remember to wear gloves when your hands are in the UV cabinet*) or putting the plate in the iodine jar until the dark spots develop.**

Problems with thin-layer chromatography

Overloading the chromatoplate with sample. **TLC is an extremely sensitive technique and it is easy to put too much sample on the plate. The sample solution must not be too concentrated and you must not repeat applications of solutions on the same spot. The result is non-separation of the mixture and a 'smear' up the plate. Dilute the solution or don't put so much on the plate.**

Putting the spots of sample too close together. **The separated spots 'bleed' into each other and you can't tell from which sample they originate.**

Putting the spots too close to the edge of the plate. **This results in inaccurate R_f values (spots travel faster up the edge of the plate) and 'bleeding'.**

Contamination, which produces unexpected spots. **Make sure all apparatus is clean, solvents are clean and use a fresh micropipette for each application.**

Safety note Iodine vapour is toxic and the iodine tank should be stored in the fume cupboard.

UV visualization – if you use an eluent such as toluene, which absorbs in the UV region, you must allow all the eluent to evaporate or you will see only a dark plate.

High-performance liquid chromatography

High-performance liquid chromatography (HPLC) uses high pressure to force the mobile phase through a closed column packed with micrometre-sized particles. This allows rapid separation of complex mixtures. Several operating modes of HPLC are possible (see also p. 205). These are:

- Normal phase (NPHPLC): the sample should be soluble in a hydrophobic solvent, e.g. hexane, and should be non-ionic. The mobile phase is non-polar while the stationary phase is polar, e.g. silica, cyano, amino.
- Reversed phase (RPHPLC): the sample should be soluble in water or a polar organic solvent, e.g. methanol, and should be non-ionic. The mobile phase is polar while the stationary phase is non-polar, e.g. C18 (ODS), C_8 (octyl), phenyl.

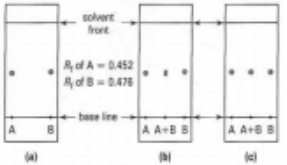


Fig. 32.14 Double-spotting technique: (a) compounds A and B with close R_f values; (b) figure '8' of double spot shows that A and B are different; (c) single spot for double spot shows that A and B could be the same.

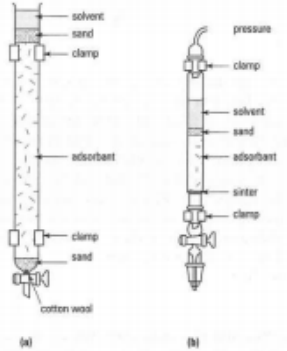


Fig. 32.15 Column chromatography: (a) gravity chromatography; (b) flash chromatography.

HPLC is a versatile form of chromatography, used with a wide variety of stationary and mobile phases, to separate individual compounds of a particular class of molecules on the basis of size, polarity, solubility or adsorption characteristics.

Preparing samples for HPLC – filter all samples through either a 0.2 μm or a 0.45 μm filter prior to injection.

- Size exclusion chromotography (SEC): this is used when the major difference between compounds in a mixture is their molecular weight. It is normally used for compounds with molecular weights greater than 2000. The mobile phase should be a strong solvent for the sample. Aqueous SEC is called gel filtration chromatography (GFC) and is used for separation of proteins and other biomolecules, while organic SEC is called gel permeation chromatography (GPC) and is used for the separation of polymers.
- Ion exchange chromotography (IEC): it is used when compounds are ionic, or potentially ionic, e.g. anions, cations, organic acids and bases, amino acids, catecholamines, peptides. The mobile phase is typically a buffer and the choice of pH is critical. Two types can be differentiated: SAX (*s*trong-*a*nion e*x*change) and SCX (*s*trong-*c*ation e*x*change).

The essential components of an HPLC system are a solvent delivery system, a method of sample introduction, a column, a detector and an associated readout device (Fig. 32.16).

Solvent delivery system

This should fulfil certain requirements:

- It should be chemically inert.
- It should be capable of delivering a wide flow-rate range.
- It should be able to withstand high pressures.
- It should be able to deliver high flow-rate precision.
- It should have a low internal volume.
- It should provide minimum flow pulsation.

Although several systems are available that meet these requirements, the most common is the reciprocating or piston pump. The choice of solvent delivery system depends on the type of separation to be performed:

- Isocratic separation: a single solvent (or solvent mixture) is used throughout the analysis.
- Gradient elution separation: the composition of the mobile phase is altered using a microprocessor-controlled gradient programmer, which mixes appropriate amounts of two different solvents to produce the required gradient.

The main advantages of gradient HPLC are that you can control mobile-phase composition. This allows you to resolve closely related compounds and provide faster elution of strongly retained compounds thereby producing reduced analysis times and faster method development time. However, these advantages have to be compared with some disadvantages, such as the initial higher cost of the equipment compared with an isocratic system. Also, after each gradient run, a re-equilibration of the system is required to return to the initial mobile-phase conditions.

Sample introduction

The most common method of sample introduction in HPLC is via a rotary valve, e.g. a Rheodyne® valve. A schematic diagram of a rotary valve is shown in Fig. 32.17. In the load position, the sample is introduced via a syringe to fill an external loop of volume 5, 10 or 20 μL. While this occurs, the mobile phase passes through the valve to the column. In the inject position, the valve is rotated so that the mobile phase is diverted through the

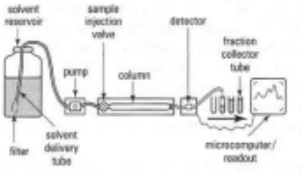


Fig. 32.16 Components of an HPLC system.

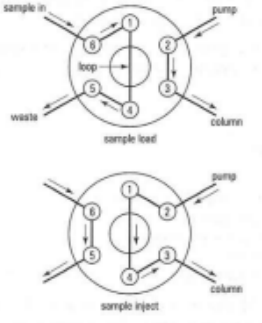


Fig. 32.17 Schematic diagram of a rotary valve.

Always use the highest purity solvents.

Safety note Always dispose of organic solvent waste in accordance with laboratory procedure (never down the sink).

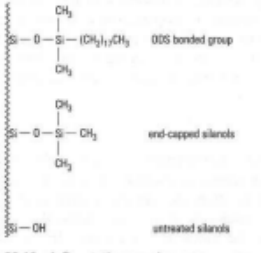


Fig. 32.19 A C_{18} stationary phase.

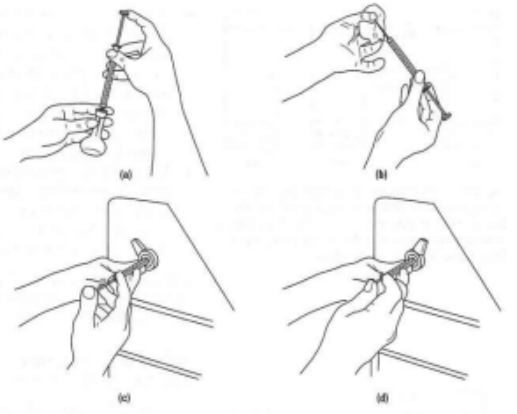


Fig. 32.18 Sample injection in HPLC.

sample loop, thereby introducing a reproducible volume of the sample into the mobile phase. The procedure for injection of a sample is shown in Fig. 32.18. In Fig. 32.18a the syringe (see p. 11) is filled with the sample/standard solution (typically 1 mL). Then the outside of the syringe is wiped clean with a tissue (Fig. 32.18b). The syringe is placed into the Rheodyne® injector of the chromatograph while in the 'load' position (Fig. 32.18c) and the plunger on the syringe is depressed to fill the sample loop. Finally, the position of the Rheodyne® valve is switched to the 'inject' position to introduce the sample into the chromatograph (Fig. 32.18d) and then the syringe is removed from the injection valve. The procedure for the preparation of a series of calibration solutions is shown in Box 32.1.

The column

This is usually made of stainless steel, and all components, valves, etc., are manufactured from materials which can withstand the high pressures involved. The most common form of liquid chromatography is reversed phase HPLC. In RPHPLC the most common column packing material consists of C_{18} or octadecylsilane (ODS). A chemically bonded stationary phase is shown in Fig. 32.19. However, some of the surface silanol groups remain unaffected. These unreacted groups lead to undesirable chromatographic effects, such as peak tailing (p. 208). One approach to remove the unreacted silanol groups is end capping. In this way, the silanol group is reacted with a small silylating group, e.g. trimethylchlorosilane. An alternative approach to nullify the action of the silanol groups is to add triethylamine to the mobile phase, which modifies the silica surface while in use.

HPLC detectors

Most HPLC systems are linked to a continuous monitoring detector of high sensitivity, e.g. phenols may be detected spectrophotometrically by

Using silica-based HPLC columns – these are limited to a pH range of 2–8 (preferably 3–7). At low pH the bonded phase may be removed; at high pH the silica particles may be dissolved.

UV cutoff for organic solvents:

Hexane	195 nm
Acetonitrile	190 nm
Methanol	205 nm
Water	190 nm

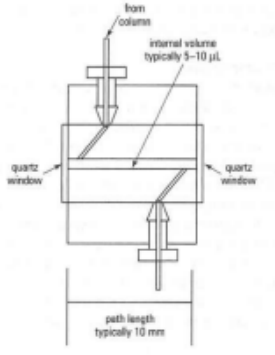


Fig. 32.20 UV detector cell for HPLC.

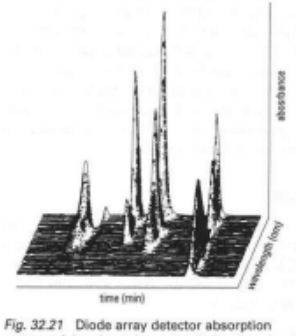


Fig. 32.21 Diode array detector absorption spectra of the eluent from an HPLC separation of a mixture of four steroids, taken every 15 seconds.

monitoring the absorbance of the eluent at 280 nm as it passes through a flow cell. Other detectors can be used to measure changes in fluorescence, current or potential, as described below. Most detection systems are non-destructive, which means that you can collect eluent with an automatic fraction collector for further study.

UV/visible detectors are widely used and have the advantages of versatility, sensitivity and stability. Such detectors are of two types: fixed wavelength and variable wavelength. Fixed-wavelength detectors are simple to use, with low operating costs. They usually contain a mercury lamp as a light source, emitting at several wavelengths between 254 nm and 578 nm; a particular wavelength is selected using suitable cutoff filters. The most frequently used wavelengths for analysis of organic molecules are 254 nm and 280 nm. Variable wavelength detectors use a deuterium lamp and a continuously adjustable monochromator for wavelengths of 190–600 nm. For both types of detector, sensitivity is in the absorbance range 0.001–1.0 (down to ≈ 1 ng), with noise levels as low as 4×10^{-5}. Note that sensitivity is partly influenced by the path length of the flow cell (typically 10 mm), see Fig. 32.20. Monitoring at short-wavelength UV (e.g. below 240 nm) may give increased sensitivity but decreased specificity, since many organic molecules absorb in this range. Additional problems with short-wavelength UV detection include instrument instability, giving a variable base line, and absorption by components of the mobile phase (e.g. organic solvents, which often absorb at < 210 nm).

An important development in chromatographic monitoring is diode array detection (DAD). The incident light comprises the whole spectrum of light from the source, which is passed through a diffraction grating and the diffracted light detected by an array of photodiodes. Typical DAD can measure the absorbance of each sample component at 1–10 nm intervals over the range 190–600 nm. This gives an absorbance spectrum for each eluting substance which may be used to identify the compound and give some indication as to its purity. An example of a three-dimensional diode array spectrum is shown in Fig. 32.21.

Many aromatic organic molecules, including some polycyclic aromatic hydrocarbons, show natural fluorescence (Table 26.1), or can be made to fluoresce by pre-column or post-column derivatization with a fluorophore. Fluorescence detection is more sensitive than UV/visible detection, and may allow analysis in the picogram (10^{-12} g) range. A fluorescence detector consists of a light source (e.g. a xenon lamp), a diffraction grating to supply light at the excitation wavelength, and a photomultiplier to monitor the emitted light (usually arranged to be at right angles to the excitation beam). The use of instruments with a laser light source can give an extremely narrow excitation waveband, and increased sensitivity and specificity.

Electrochemical detectors offer very high sensitivity and specificity, with the possibility of detection of femtogram amounts of electroactive compounds such as catecholamines, vitamins, thiols, purines, ascorbate and uric acid. The two main types of detector, amperometric and coulometric, operate on similar principles, i.e. by measuring the change in current or potential as sample components pass between two electrodes within the flow cell. One of these electrodes acts as a reference (or counter) electrode (e.g. calomel electrode), while the other – the working electrode – is held at a voltage that is high enough to cause either oxidation or reduction of sample molecules. In the oxidative mode, the working electrode is usually glassy carbon, while in reductive mode a mercury electrode is used. In either case, a current flow between the electrodes is induced and detected.

Overcoming interference with fluorescence detectors – use a dual flow cell to offset background fluorescence due to components of the mobile phase.

Maximizing sensitivity with fluorescence detectors – the concentration of other sample components, e.g. pigments, must not be so high that they cause quenching of fluorescence.

Optimizing electrochemical detection – the mobile phase must be free of any compounds that might give a response; all constituents must be of the highest purity.

Interpreting chromatograms – never assume that a single peak is a guarantee of purity: there may be more than one compound with the same chromatographic characteristics.

Problems with peaks – non-symmetrical peaks may result from column overloading, co-elution of solutes, poor packing of the stationary phase, or interactions between the substance and the support material.

Mass spectrometry (p. 204) used in conjunction with chromatographic methods can provide a powerful tool for identifying the components of complex mixtures, e.g. pharmaceuticals. One drawback is the limited capacity of the mass spectrometer – due to its vacuum requirements – compared with the volume of material leaving the chromatography column. Similarly, in HPLC, devices have been developed for solving the problem of large solvent volumes, e.g. by splitting the eluent from the column so only a small fraction reaches the mass spectrometer.

The computer-generated outputs from the mass spectrometer are similar to chromatograms obtained from other methods, and show peaks corresponding to the elution of particular components. However, it is then possible to select an individual peak and obtain a mass spectrum for the component in that peak to aid in its identification (p. 200). This has helped to identify hundreds of components present in a single sample, including flavour molecules in food, drug metabolites and water pollutants.

Recording and interpreting chromatograms

For analytical purposes, the detector output is usually connected to a computer-based data acquisition and analysis system. This consists of a personal computer (PC) with data acquisition hardware to convert an analogue detector signal to digital format, plus software to control the data acquisition process, store the signal information and display the resulting chromatogram. The software will also detect peaks and calculate their retention times and sizes (areas) for quantitative analysis. The software often incorporates functions to control the chromatographic equipment, enabling automatic operation. In sophisticated systems, the detector output may be compared with that from a 'library' of chromatograms for known compounds, to suggest possible identities of unknown sample peaks.

In simpler chromatographic systems, you may need to use a chart recorder for detector output. Two important settings must be considered before using a chart recorder:

1. The base-line reading – this should be set only after a suitable quantity of mobile phase has passed through the column (prior to injection of the sample) and stability is established. The chart recorder is usually set a little above the edge of the chart paper grid, to allow for base-line drift.
2. The detector range – this must be set to ensure that the largest peaks do not go off the top of the chart. Adjustment may be based on the expected quantity of analyte, or by a trial-and-error process. Use the maximum sensitivity that gives intact peaks. If peaks are still too large on the minimum sensitivity, you may need to reduce the amount of sample used, or prepare and analyse a diluted sample.

Interpreting chromatograms

Make sure you know the direction of the horizontal axis of the chromatogram (usually, either volume or time) – it may run from right to left or vice versa – and make a note of the detector sensitivity on the vertical axis. Ideally, the base line should be 'flat' between peaks, but it may drift up or down owing to a number of factors including:

- changes in the composition of the mobile phase (e.g. in gradient elution, p. 219);
- tailing of material from previous peaks;

Avoiding problems with air bubbles in liquid chromatography – always ensure that buffers are effectively degassed by vacuum treatment before use, and regularly clean the flow cell of the detector.

Degassing your mobile phase solvent – this is an important step and the best approach is to prepare the solvent composition (e.g. 50:50 v/v methanol:water, for isocratic RPHPLC) and then filter through a 0.22 μm porosity filter using a Büchner flask arrangement (p. 28).

- carry-over of material from previous samples; this can be avoided by efficient cleaning of columns between runs – allow sufficient time for the previous sample to pass through the column before you introduce the next sample;
- loss of the stationary phase from the column (column 'bleed'), caused by extreme elution conditions;
- air bubbles (in liquid chromatography); if the buffers used in the mobile phase are not effectively degassed, air bubbles may build up in the flow cell of the detector, leading to a gradual upward drift of the base line, followed by a sharp fall when the accumulated air is released. Small air bubbles that do not become trapped may give spurious small peaks as they pass through the detector.

A peak close to the origin may be due to non-retained sample molecules, flowing at the same rate as the mobile phase, or to artefacts, e.g. air (GC) or solvent (HPLC) in the sample. Whatever its origin, this peak can be used to measure the void volume and dead time of the column (p. 207). No peaks from genuine sample components should appear before this type of peak.

Peaks can be denoted on the basis of their elution volume (used mainly in liquid chromatography) or their retention times (mainly in GC). If the peaks are not narrow and symmetrical, they may contain more than one component. Where peaks are more curved on the trailing side compared with the leading side (peak tailing, p. 208), this may indicate too great an association between the component and the stationary phase, or overloading of the column.

Optimizing chromatographic separations

In an ideal chromatographic analysis the sample molecules will be completely separated, and detection of components will result in a series of discrete individual peaks corresponding to each type of molecule. However, to minimize the possibility of overlapping peaks, or of peaks composed of more than one substance, it is important to maximize the separation efficiency of the technique, which depends on:

- the selectivity, as measured by the relative retention times of the two components (p. 208), or by the volume of the mobile phase between the peak maxima of the two components after they have passed through the column; this depends on the ability of the chromatographic method to separate two components with similar properties;
- the band-broadening properties of the chromatographic system, which influence the width of the peaks; these are mainly due to the effects of diffusion.

The resolution of two adjacent components can be defined in terms of k', α and N, using eqn [31.6]. In practical terms, good resolution is achieved when there is a large 'distance' (either time or volume) between peak maxima, and the peaks are as narrow as possible. The resolution of components is also affected by the relative amount of each substance: for systems showing low resolution, it can be difficult to resolve small amounts of a particular component in the presence of larger amounts of a second component. If you cannot obtain the desired results from a poorly resolved chromatogram, other chromatographic conditions, or even different methods, should be tried in an attempt to improve resolution. For liquid chromatography, changes in the following factors may improve resolution:

- Stationary-phase particle size – the smaller the particle, the greater the area available for partitioning between the mobile phase and the stationary phase. This partly accounts for the high resolution observed with HPLC compared with low-pressure methods.
- The slope of the salt gradient in eluting IEC columns, e.g. using computer-controlled adapted gradients.
- In low-pressure liquid chromatography, the flow rate of the mobile phase must be optimized because this influences two band-broadening effects which are dependent on diffusion of sample molecules: (i) the flow rate must be slow enough to allow effective partitioning between the mobile phase and the stationary phase: and (ii) it must be fast enough to ensure that there is minimal diffusion along the column once the molecules have been separated. To allow for these opposing influences, a compromise flow rate must be used.
- If you prepare your own columns, they must be packed correctly, with no channels present that might result in uneven flow and eddy diffusion.

Quantitative analysis

Most detectors and chemical assay systems give a linear response with increasing amounts of the test substance over a given 'working range'. Alternative ways of converting the measured response to an amount of substance are:

Quantifying molecules – note that quantitative analysis often requires assumptions about the identity of separated components and that further techniques may be required to provide information about the nature of the molecules present, e.g. mass spectrometry (see Chapter 30).

When using external standardization, samples and standards should be analysed more than once, to confirm the reproducibility of the technique.

When using an internal standard, you should add an internal standard to the sample at the first stage in the extraction procedure, so that any loss or degradation of test substance during purification is accompanied by an equivalent change in the internal standard, as long as the extraction characteristics of the internal standard and the test substance are very similar.

- External standardization: this is applicable where the sample volume is sufficiently precise to give reproducible results (e.g. HPLC). You measure the peak areas (or heights) of known amounts of the substance to give a calibration factor or calibration curve which can be used to calculate the amount of test substance in the sample.
- Internal standardization: where you add a known amount of a reference substance (not originally present in the sample) to the sample, to give an additional peak in the elution profile. You determine the response of the detector to the test and reference substances by analysing a standard containing known amounts of both substances, to provide a response factor (r), where:

$$r = \frac{\text{peak area (or height) of test substance}}{\text{peak area (or height) of reference substance}} \qquad [32.5]$$

Use this response factor to quantify the amount of test substance (Q_t) in a sample containing a known amount of the reference substance (Q_r), from the relationship:

$$Q_t = \frac{\text{[peak area (or height) of test substance]}}{\text{[peak area (or height) of reference substance]}} \times \frac{Q_r}{r} \qquad [32.6]$$

Internal standardization should be the method of choice wherever possible, since it is unaffected by small variations in sample volume (e.g. for GC microsyringe injection). The internal standard should be chemically similar to the test substance(s) and must give a peak that is distinct from all other substances in the sample. An additional advantage of an internal standard which is chemically related to the test substance is that it may show up problems due to changes in detector response, incomplete derivatization, etc. A disadvantage is that it may be difficult to fit an internal standard peak into a complex chromatogram.

33 Electrophoresis

Electrophoresis is a separation technique based on the movement of charged molecules in an electric field. Dissimilar molecules move at different rates and the components of a mixture will be separated when an electric field is applied. It is a widely used technique, particularly for the analysis of complex mixtures or for the verification of purity (homogeneity) of isolated molecules.

While electrophoresis is mostly used for the separation of charged macromolecules, techniques are available for high-resolution separations, e.g. capillary electrophoresis, of small molecules such as amino acids, anions and catecholamines.

The electrophoretic mobility of a charged molecule depends on:

- Net charge – negatively charged molecules (anions) migrate towards the anode (+), while positively charged molecules (cations) migrate towards the cathode (−); highly charged molecules move faster towards the electrode of opposite charge than those with lesser charge.
- Size – frictional resistance exerted on molecules moving in a solution means that smaller molecules migrate faster than large molecules.
- Shape – the effect of friction also means that the shape of the molecule will affect mobility, e.g. globular proteins compared with fibrous proteins, linear surfactants compared with micellular surfactants.
- Electrical field strength – mobility increases with increasing field strength (voltage), but there are practical limitations to using high voltages, especially due to heating effects.

The combined influence of net charge and size means that mobility is determined by the charge-to-density or the charge-to-mass ratio, according to the formula:

$$\mu = \frac{qE}{r} \qquad [33.1]$$

where q is the net charge on the molecule, r is the molecular radius and E is the field strength.

Most types of electrophoresis using supporting media are simple to carry out and the apparatus can be easily constructed, although inexpensive equipment is commercially available. High-resolution techniques such as two-dimensional electrophoresis and capillary electrophoresis require more sophisticated equipment, both for separation and analysis (see later).

Simple electrophoretic separations can be performed either vertically (Fig. 33.1) or horizontally (Fig. 33.2). The electrodes are normally made of platinum wire, each in its own buffer compartment. In vertical electrophoresis, the buffer solution forms the electrical contact between the electrodes and the supporting medium in which the sample separation takes place. In horizontal electrophoresis electrical contact can be made by buffer-soaked paper 'wicks' dipping in the buffer reservoir and laid upon the supporting medium. The buffer reservoir normally contains a divider acting as a barrier to diffusion (but not to electrical current), so that localized pH changes which occur in the region of the electrodes (as a result of electrolysis, p. 232) are not transmitted to the supporting medium or the sample. Individual samples are spotted onto a solid supporting medium containing

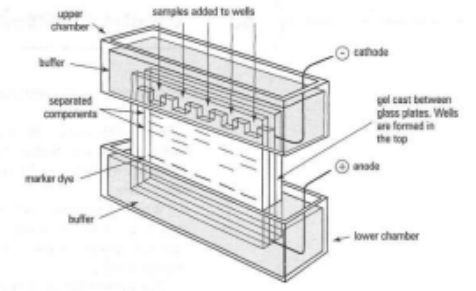


Fig. 33.1 Apparatus for vertical slab electrophoresis (components move downwards from wells, through the gel matrix).

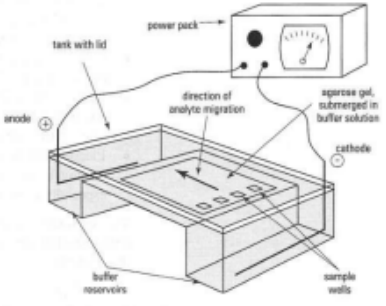


Fig. 33.2 Agarose gel electrophoresis.

buffer or are applied to 'wells' formed in the supporting medium. The power pack used for most types of electrophoresis should be capable of delivering approximately 500 V and approximately 100 mA.

The supporting medium

The effects of convection currents (resulting from the heating effect of the applied electric field) and the diffusion of molecules within the buffer solution can be minimized by carrying out the electrophoresis in a porous supporting medium. This contains buffer electrolytes and the sample is added in a discrete location or zone. When the electric field is applied, individual sample molecules remain in sharp zones as they migrate at different rates. After separation, post-electrophoretic diffusion of selected molecules (e.g. proteins) can be avoided by 'fixing' them in position on the supporting medium, e.g. using trichloracetic acid (TCA).

The heat generated during electrophoresis is proportional to the square of the applied current and to the electrical resistance of the medium: even when a supporting medium is used, heat production will lead to zone broadening

Definition

Ohm's law:

$V = IR$

where V = voltage, I = current and R = resistance.

by increasing the rate of diffusion of sample components and buffer ions. Heat denaturation of certain sample types may also occur, e.g. proteins. Another problem is that heat will reduce buffer viscosity, leading to a decrease in resistance. If the electrophoresis is run at constant voltage, Ohm's law dictates that as resistance falls, the current will increase, leading to further heat production. This can be avoided by using a power pack that provides constant power. In practice, most electrophoresis equipment incorporates a cooling device; even so, distortions of an electrophoretic zone from the ideal 'sharp, linear band' can often be explained by inefficient heat dissipation.

Supporting media can be sub-divided into:

- Inert media – these provide physical support and minimize convection; separation is based on charge density only (e.g. cellulose acetate).
- Porous media – these introduce molecular sieving as an additional effect: their pore size is of the same order as the size of molecules being separated, restricting the movement of larger molecules relative to smaller ones. Thus, separation depends on both the charge density and the size of the molecule.

Definition

Electro-osmotic flow – the osmotically driven mass flow of water resulting from the movement of ions in an electrophoretic system.

With some supporting media, e.g. cellulose acetate, a phenomenon called electro-endosmosis or electro-osmotic flow (EOF) occurs. This is due to the presence of negatively charged groups on the surface of the supporting medium, attracting cations in the electrophoresis buffer solution and creating an electrical double layer. The cations are hydrated (surrounded by water molecules) and when the electric field is applied, they are attracted towards the cathode, creating a flow of solvent that opposes the direction of migration of anionic molecules towards the anode. The EOF can be so great that weakly anionic molecules may be carried towards the cathode.

Where necessary, EOF can be avoided by using supporting media such as agarose or polyacrylamide, but it is not always a hindrance to electrophoretic separation. Indeed, the phenomenon of EOF is used in the high-resolution technique of capillary electrophoresis.

Definitions

Electrophoretic mobility – the rate of migration of a particular type of molecule in response to an applied electrical field.

Understanding electrophoresis – this is, in essence, an incomplete form of electrolysis, since the applied electric field is switched off well before sample molecules reach the electrodes.

Capillary electrophoresis

The technique of capillary electrophoresis (CE) combines the high resolving power of electrophoresis with the speed and versatility of HPLC (p. 218). The technique largely overcomes the major problem of carrying out electrophoresis without a supporting medium, i.e. poor resolution due to convection currents and diffusion. A capillary tube has a high surface-area-to-volume ratio, and consequently the heat generated as a result of the applied electric current is rapidly dissipated. A further advantage is that very small sample volumes (5–10 nL) can be analysed. The versatility of CE is demonstrated by its use in the separation of a range of molecules, e.g. amino acids, proteins, nucleic acids, drugs, vitamins, organic acids and inorganic ions; CE can even separate neutral species, e.g. steroids, aromatic hydrocarbons.

The components of a typical CE apparatus are shown in Fig. 33.3. The capillary is made of fused silica and externally coated with a polymer for mechanical strength. The internal diameter is usually 25–50 μm, a compromise between efficient heat dissipation and the need for a light path that is not too short for detection using UV/visible spectrophotometry. A gap in the polymer coating provides a window for detection purposes. Samples

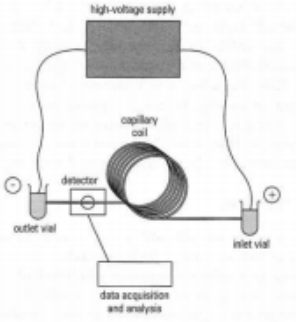


Fig. 33.3 Components of a capillary electrophoresis system.

are injected into the capillary by a variety of means, e.g. electrophoretic loading or displacement. In the former, the inlet end of the capillary is immersed in the sample and a pulse of high voltage is applied. The displacement method involves forcing the sample into the capillary, either by applying pressure in the sample vial using an inert gas, or by introducing a vacuum at the outlet. The detectors used in CE are similar to those used in chromatography (p. 220), e.g. UV/visible spectrophotometric systems. Fluorescence detection is more sensitive, but this may require sample derivitization. Electrochemical and conductivity detection are also used in some applications, e.g. conductivity detection of inorganic cations such as Na^+, K^+.

EOF, described above, is essential to the most commonly used types of CE. The existence of EOF in the capillary is the result of the net negative charge on the fused silica surface at pH values over 3.0. The resulting solvent flow towards the cathode is greater than the attraction of anions towards the anode, so they will flow towards the cathode (note that the detector is situated at the cathodic end of the capillary). The greater the net negative charge on an anion, the greater is its resistance to the EOF and the lower its mobility. Separated components migrate towards the cathode in the order: (1) cations, (2) neutral species, (3) anions.

Capillary zone electrophoresis (CZE)

This is the most widely used form of CE, and is based on electrophoresis in free solution and EOF, as discussed above. Separations are due to the charge-to-mass ratio of the sample components, and the technique can be used for almost any type of charged molecule, and is especially useful for pharmaceutical compound separation and confirmation of purity.

Micellar electrokinetic chromatography (MEKC)

This technique involves the principles of both electrophoresis and chromatography. Its main strength is that it can be used for the separation of neutral molecules as well as charged ones. This is achieved by including surfactants (e.g. SDS, Triton X-100) in the electrophoresis buffer at concentrations that promote the formation of spherical micelles, with a hydrophobic interior and a charged, hydrophilic surface. When an electric field is applied, these micelles will tend to migrate with or against the EOF depending on their surface charge. Anionic surfactants like SDS are attracted by the anode, but if the pH of the buffer is high enough to ensure that the EOF is faster than the migration velocity of the micelles, the net migration is in the direction of the EOF, i.e. towards the cathode. During this migration, sample components partition between the buffer and the micelles (acting as a pseudo-stationary phase); this may involve both hydrophobic and electrostatic interactions. For neutral species it is only the partitioning effect that is involved in separation; the more hydrophobic a sample molecule, the more it will interact with the micelle, and the longer will be its migration time, since the micelle resists the EOF. The versatility of MEKC enables it to be used for separations of molecules as diverse as amino acids and polycyclic hydrocarbons.

34 Electroanalytical techniques

Electrochemical methods are used to quantify a broad range of different molecules, including ions, gases, metabolites and drugs.

Definitions

Oxidation – loss of electrons by an atom or molecule (or gain of O atoms, loss of H atoms, increase in positive charge, or decrease in negative charge).

Reduction – gain of electrons by an atom or molecule (or loss of O atoms, gain of H atoms, decrease in positive charge or increase in negative charge).

KEY POINT **The basis of all electrochemical analysis is the transfer of electrons from one atom or molecule to another atom or molecule in an obligately coupled oxidation–reduction reaction (a redox reaction).**

It is convenient to separate such redox reactions into two half-reactions and, by convention, each is written as:

$$\text{oxidized form} + \text{electron(s)}\ (ne^-) \underset{oxidation}{\overset{reduction}{\rightleftharpoons}} \text{reduced form} \qquad [34.1]$$

You should note that the half-reaction is reversible: by applying suitable conditions, reduction *or* oxidation can take place. As an example, a simple redox reaction occurs when metallic zinc (Zn) is placed in a solution containing copper ions (Cu^{2+}), as follows:

$$Cu^{2+} + Zn \rightarrow Cu + Zn^{2+} \qquad [34.2]$$

The half-reactions are (i) $Cu^{2+} + 2e^- \rightarrow Cu$ and (ii) $Zn^{2+} + 2e^- \rightarrow Zn$. The oxidizing power of (i) is greater than that of (ii), so in a coupled system, the latter half-reaction proceeds in the opposite direction to that shown above, i.e. as $Zn - 2e^- \rightarrow Zn^{2+}$. When Zn and Cu electrodes are placed in separate solutions containing their ions, and connected electrically (Fig. 34.1), electrons will flow from the Zn electrode to Cu^{2+} via the Cu electrode owing to the difference in oxidizing power of the two half-reactions.

By convention, the electrode potential of any half-reaction is expressed relative to that of a standard hydrogen electrode (half-reaction $2H^+ + 2e^- \rightarrow H_2$) and is called the standard electrode potential, E°. Table 34.1 shows the values of E° for selected half-reactions. With any pair of half-reactions from this series, electrons will flow from that having the lowest electrode potential to that of the highest. E° is determined at pH = 0. It is often more appropriate to express standard electrode potentials at pH 7 for biological systems, and the symbol $E^{\circ\prime}$ is used: in all circumstances, it is important that the pH is clearly stated.

The arrangement shown in Fig. 34.1 represents a simple galvanic cell where two electrodes serve as the interfaces between a chemical system and an electrical system. For analytical purposes, the magnitude of the potential (voltage) or the current produced by an electrochemical cell is related to the concentration (strictly the activity, *a*, p. 48) of a particular chemical species. Electrochemical methods offer the following advantages:

- excellent detection limits, and wide operating range (10^{-1} to 10^{-8} mol L^{-1});
- measurements may be made on very small volumes (μL) allowing small amounts (pmol) of sample to be measured in some cases;
- miniature electrochemical sensors can be used for certain *in vivo* measurements, e.g. pH, glucose, oxygen content.

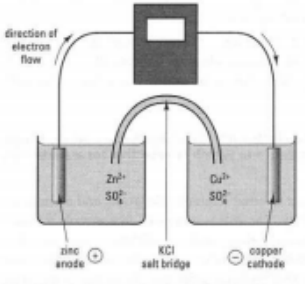


Fig. 34.1 A simple galvanic electrochemical cell. The KCl salt bridge allows migration of ions between the two compartments but prevents mixing of the two solutions.

Definition

Galvanic cell – an electrochemical cell in which reactions occur spontaneously at the electrodes when they are connected externally by a conductor, producing electrical energy.

Table 34.1 Standard electrode potentials* (E°) for selected half-reactions

Half-reaction	E° at 25 °C (V)
$Cl_2 + 2e^- \rightleftharpoons 2Cl^-$	+1.36
$O_2 + 4H^+ + 4e^- \rightleftharpoons 2H_2O$	+1.23
$Br_2 + 2e^- \rightleftharpoons 2Br^-$	+1.09
$Ag^+ + e^- \rightleftharpoons Ag$	+0.80
$Fe^{3+} + e^- \rightleftharpoons Fe^{2+}$	+0.77
$I_3^- + 2e^- \rightleftharpoons 3I^-$	+0.54
$Cu^{2+} + 2e^- \rightleftharpoons Cu$	+0.34
$Hg_2Cl_2 + 2e^- \rightleftharpoons 2Hg + 2Cl^-$	+0.27
$AgCl + e^- \rightleftharpoons Ag + Cl^-$	+0.22
$Ag(S_2O_3)_2^{3-} + e^- \rightleftharpoons Ag + 2S_2O_3^{2-}$	+0.01
$2H^+ + 2e^- \rightleftharpoons H_2$	+0.00
$AgI + e^- \rightleftharpoons Ag + I^-$	−0.15
$PbSO_4 + 2e^- \rightleftharpoons Pb + SO_4^{2-}$	−0.35
$Cd^{2+} + 2e^- \rightleftharpoons Cd$	−0.40
$Zn^{2+} + 2e^- \rightleftharpoons Zn$	−0.76

*From Milazzo *et al.* (1978).

Using a calomel electrode – always ensure that the KCl solution is saturated by checking that KCl crystals are present.

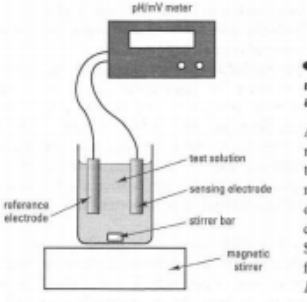


Fig. 34.2 Components of a potentiometric cell.

Using ISEs, including pH electrodes – standards and samples must be measured at the same temperature, as the Nernst equation shows that the measured potential is temperature dependent.

Potentiometry and ion-selective electrodes

Operating principles

These systems involve galvanic cells (p. 229) and are based on measurement of the potential (voltage) difference between two electrodes in solution when no net current flows between them: no net electrochemical reaction occurs and measurements are made under equilibrium conditions. These systems include methods for measuring pH, ions, and gases such as CO_2 and NH_3. A typical potentiometric cell is shown in Fig. 34.2. It contains two electrodes:

1. a 'sensing' electrode, the half-cell potential of which responds to changes in the activity (concentration) of the substance to be measured; the most common type of indicator electrodes are ion-selective electrodes (ISEs);
2. a 'reference' electrode, the potential of which does not change, forming the second half of the cell.

To assay a particular analyte, the potential difference between these electrodes is measured by an mV meter (e.g. a standard pH meter).

Reference electrodes for potentiometry are of three main types:

1. The standard hydrogen electrode, which is the reference half-cell electrode, defined as 0.0 V at all temperatures, against which values of E° are expressed. H_2 gas at 1 atmosphere pressure is bubbled over a platinum electrode immersed in an acid solution with an activity of unity. This electrode is rarely used for analytical work, since it is unstable and other reference electrodes are easier to construct and use.
2. The calomel electrode (Fig. 34.3), which consists of a paste of mercury covered by a coat of calomel (Hg_2Cl_2), immersed in a saturated solution of KCl. The half-reaction $Hg_2Cl_2 + 2e^- \rightarrow 2Hg + 2Cl^-$ gives a stable standard electrode potential of +0.24 V.
3. The silver/silver chloride electrode. This is a silver wire coated with AgCl and immersed in a solution of constant chloride concentration. The half-reaction $AgCl + e^- \rightarrow Ag + Cl^-$ gives a stable, standard electrode potential of +0.20 V.

KEY POINT **Ion-selective electrodes (ISEs) are based on measurement of a potential across a membrane which is selective for a particular analyte.**

An ISE consists of a membrane, an internal reference electrode, and an internal reference electrolyte of fixed activity. The ISE is immersed in a sample solution that contains the analyte of interest, along with a reference electrode. The membrane is chosen to have a specific affinity for a particular ion, and if activity of this ion in the sample differs from that in the reference electrolyte, a potential develops across the membrane that is dependent on the ratio of these activities. Since the potentials of the two reference electrodes (internal and external) are fixed, and the internal electrolyte is of constant activity, the measured potential, E, is dependent on the membrane potential and is given by the Nernst equation:

$$E = K + 2.303\frac{RT}{zF}\log[a] \qquad [34.3]$$

where K represents a constant potential which is dependent on the reference electrode, z represents the net charge on the analyte, $[a]$ the activity of analyte in the sample and all other symbols and constants have their usual meaning (p. 70). For a series of standards of known activity, a plot of E against log $[a]$ should be linear over the working range of the electrode, with a slope of $2.303\,RT/zF$ (0.059 V at 25 °C). Although ISEs strictly measure *activity*, the

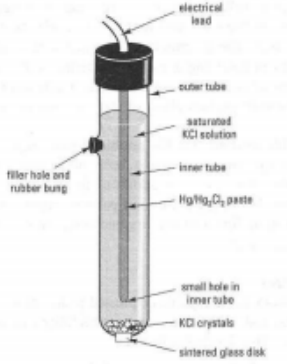


Fig. 34.3 A calomel reference electrode.

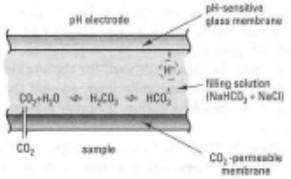


Fig. 34.4 Underlying principles of a gas-sensing electrode.

Using CO_2 electrodes – applications include measurement of blood PCO_2 and in enzyme studies where CO_2 is utilized or released: calibration of the electrode is accomplished using 5% v/v and 10% v/v mixtures of CO_2 in an inert gas equilibrated against the measuring solution.

potential differences can be approximated to concentration as long as (i) the analyte is in dilute solution (p. 48), (ii) the ionic strength of the calibration standards matches that of the sample, e.g. by adding appropriate amounts of a high ionic strength solution to the standards, and (iii) the effect of binding to sample macromolecules (e.g. proteins, nucleic acids) is minimal. Potentiometric measurements on undiluted biological fluids, e.g. K^+ and Na^+ levels in plasma, tissue fluids or urine, are likely to give lower values than flame emission spectrophotometry, since the latter procedure measures total ion levels, rather than just those in aqueous solution.

All of the various types of membrane used in ISEs operate by incorporating the ion to be analysed into the membrane, with the accompanying establishment of a membrane potential. The scope of electrochemical analysis has been extended to measuring gases and non-ionic compounds by combining ISEs with gas-permeable membranes, enzymes, and even immobilized bacteria or tissues.

Glass membrane electrodes

The most widely used ISE is the glass membrane electrode for pH measurement (p. 57). The membrane is thin glass (50 μm wall thickness) made of silica which contains some Na^+. When the membrane is soaked in water, a thin hydrated layer is formed on the surface in which negative oxide groups ($Si\text{-}O^-$) in the glass act as ion-exchange sites. If the electrode is placed in an acid solution, H^+ exchanges with Na^+ in the hydrated layer, producing an external surface potential: in alkaline solution, H^+ moves out of the membrane in exchange for Na^+. Since the inner surface potential is kept constant by exposure to a fixed activity of H^+, a consistent, accurate potentiometric response is observed over a wide pH range. Glass electrodes for other cations (e.g. Na^+, NH_4^+) have been developed by changing the composition of the glass, so that it is predominantly sensitive to the particular analyte, though the specificity of such electrodes is not absolute. The operating principles and maintenance of such electrodes are broadly similar to those for pH electrodes (p. 60).

Gas-sensing glass electrodes

Here, an ISE in contact with a thin external layer of aqueous electrolyte (the 'filling solution') is kept close to the glass membrane by an additional, outer membrane that is selectively permeable to the gas of interest. The arrangement for a CO_2 electrode is shown in Fig. 34.4: in this case the outer membrane is made of CO_2-permeable silicone rubber. When CO_2 gas in the sample selectively diffuses across the membrane and dissolves in the filling solution (in this case an aqueous $NaHCO_3$/NaCl mixture), a change in pH occurs owing to the shift in the equilibrium:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \qquad [34.4]$$

The pH change is 'sensed' by the internal ion-selective pH electrode, and its response is proportional to the partial pressure of CO_2 of the solution (PCO_2). A similar principle operates in the NH_3 electrode, where a Teflon® membrane is used, and the filling solution is NH_4Cl.

Liquid and polymer membrane electrodes

In these types of ISEs, the liquid is a water-insoluble viscous solvent containing a soluble ionophore, i.e. an organic ion exchanger, or a neutral carrier molecule, that is specific for the analyte of interest. When this liquid is

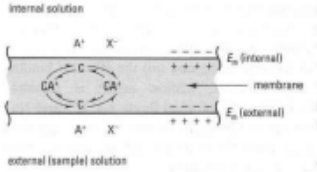


Fig. 34.5 Underlying principles of a liquid membrane ion-selective electrode. A^+ = analyte; C = neutral carrier ionophore; E_m = surface potential; membrane potential = E_m(internal) – E_m(external).

Definition

Ionophore – a compound that enhances membrane permeability to a specific ion: an ionophore may be incorporated into an ISE to detect that ion.

Definitions

Electrolytic cell – an electrochemical cell in which reactions are driven by the application of an external voltage greater than the spontaneous potential of the cell.

Electrolysis – a non-spontaneous chemical reaction resulting from the application of a potential to an electrode.

soaked into a thin membrane such as cellulose acetate, it becomes effectively immobilized. The arrangement of analyte (A^+) and ionophore in relation to this membrane is shown in Fig. 34.5. The potential on the inner surface of the membrane is kept constant by maintaining a constant activity of A^+ in the internal solution, so the potential change measured is that which results from A^+ in the sample interacting with the ionophore in the outer surface of the membrane.

A relevant example of a suitable ionophore is the antibiotic valinomycin, which specifically binds K^+. Other ionophores have been developed for measurement of, for example, NH_4^+, Ca^{2+}, Cl^-. In addition, electrodes have been developed for organic species by using specific ion-pairing reagents in the membrane that interact with ionic forms of the organic compound, e.g. with drugs such as 5,5-diphenylhydantoin.

Solid-state membrane electrodes

These contain membranes made from single crystals or pressed pellets of salts of the analyte. The membrane material must show some permeability to ions and must be virtually insoluble in water. Examples include:

- the fluoride electrode, which uses LaF_3 impregnated with Eu^{2+} (the latter to increase permeability to F^-). A membrane potential is set up when F^- in the sample solution enters spaces in the crystal lattice;
- the chloride electrode, which uses a pressed pellet membrane of Ag_2S and AgCl.

Voltammetric methods

Voltammetric methods are based on measurements made using an electrochemical cell in which electrolysis is occurring. Voltammetry, sometimes also called amperometry, involves the use of a potential applied between two electrodes (the working electrode and the reference electrode) to cause oxidation or reduction of an electroactive analyte. The loss or gain of electrons at an electrode surface causes current to flow, and the size of the current (usually measured in mA or μA) is directly proportional to the concentration of the electroactive analyte. The materials used for the working electrode must be good conductors and electrochemically inert, so that they simply transfer electrons to and from species in solution. Suitable materials include Pt, Au, Hg and glassy carbon.

Two widely used devices that operate on the voltammetric principle are the oxygen electrode and the glucose electrode. These are sometimes referred to as amperometric sensors.

Oxygen electrodes

The Clark (Rank) oxygen electrode

These instruments measure oxygen in solution using the polarographic principle, i.e. by monitoring the current flowing between two electrodes when a voltage is applied. The most widespread electrode is the Clark type (Fig. 34.6), manufactured by Rank Bros, Cambridge, UK, which is suitable for measuring O_2 concentrations in cell, organelle and enzyme suspensions. Pt and Au electrodes are in contact with a solution of electrolyte (normally saturated KCl). The electrodes are separated from the medium by a Teflon® membrane, permeable to O_2. When a potential is applied across the electrodes,

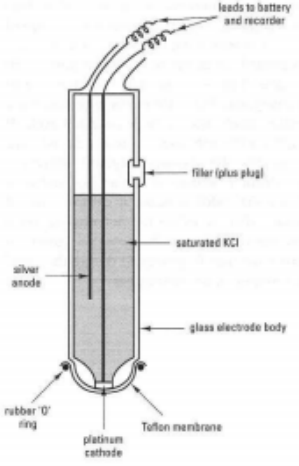


Fig. 34.7 A Clark-type oxygen probe.

Definition

Biosensor – a device for measuring a substance, combining the selectivity of a biological reaction with that of a sensing electrode.

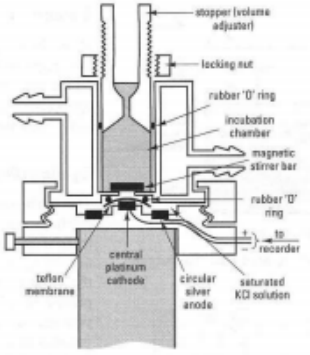


Fig. 34.6 Transverse section through a Clark (Rank) oxygen electrode.

this generates a current proportional to the O_2 concentration. The reactions can be summed up as:

$4Ag \rightarrow 4Ag^+ + 4e^-$	(at silver anode)
$O_2 + 2e^- + 2H^+ \rightleftharpoons H_2O_2$	(in electrolyte solution; O_2 replenished by diffusion from test solution)
$H_2O_2 + 2e^- + 2H^+ \rightarrow 2H_2O$	(at platinum cathode)

Oxygen probes

Clark-type oxygen electrodes are also available in probe form for immersion in the test solution (Fig. 34.7) e.g. for field studies, allowing direct measurement of oxygen status *in situ*, in contrast to chemical assays. The main point to note is that the solution must be stirred during measurement, to replenish the oxygen consumed by the electrode ('boundary layer' effect).

The glucose electrode

The glucose electrode is a simple type of biosensor, whose basic design is shown in Fig. 34.8. It consists of a Pt electrode, overlaid by two membranes. Sandwiched between these membranes is a layer of the immobilized enzyme glucose oxidase. The outer membrane is glucose permeable and allows glucose in the sample to diffuse through to the glucose oxidase layer, where it is converted to gluconic acid and H_2O_2. The inner membrane is selectively permeable to H_2O_2, which is oxidized to O_2 at the surface of the Pt electrode. The current arising from this release of electrons is proportional to the glucose concentration in the sample within the range 10^{-7} to 10^{-3} mol L^{-1}.

Electrochemical detectors used in chromatography operate by voltammetric principles and currents are produced as the mobile phase flows over electrodes set at a fixed potential: to achieve maximum sensitivity, this potential must be set at a level that allows electrochemical reactions to occur in all analytes of interest.

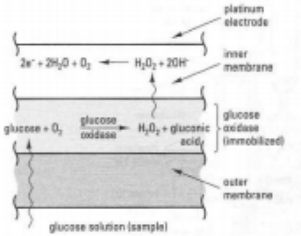


Fig. 34.8 Underlying principles of a glucose electrode.

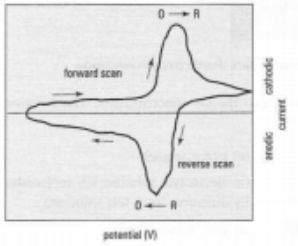


Fig. 34.9 A typical cyclic voltammogram for a reversible $O + ne^- \leftrightarrow R$ redox process.

Coulometric methods

Here, the charge required to electrolyse a sample completely is measured: the time required to titrate an analyte is measured at constant current and related to the amount of analyte using Faraday's law. There are few biochemical applications of this technique, though it is sometimes used for determination of Cl^- in serum and body fluids.

Cyclic voltammetry

The technique provides qualitative information about electrochemical reactions, e.g. the redox behaviour of compounds and the kinetics of electron transfer reactions. In practice, a triangular potential waveform is applied linearly to the working electrode in a unstirred solution. After a few seconds, the ramp is reversed and the potential is returned to its initial value. The process may be repeated several times. The resulting plot of current versus potential is termed a cyclic voltammogram. Fig. 34.9 shows a typical cyclic voltammogram for a reversible redox couple after a single potential cycle. It is assumed that the oxidized form, O, is the only species present at the start. Therefore, the first scan is towards the (more) negative direction, commencing at a value were no reduction occurs. As the applied potential approaches the characteristic E° for the redox process, a catholic current starts to increase, up to a maximum. After exceeding the potential at which the reduction process takes place, the direction of the potential current is reversed. During this stage, reduced molecules R, generated during the initial process, are reoxidized back to O, resulting in an anodic peak.

35 Radioactive isotopes and their uses

Examples $^{12}_{6}C$, $^{13}_{6}C$ and $^{14}_{6}C$ are three of the isotopes of carbon. About 98.9% of naturally occurring carbon is in the stable $^{12}_{6}C$ form. $^{13}_{6}C$ is also a stable isotope but it only occurs at 1.1% natural abundance. Trace amounts of radioactive $^{14}_{6}C$ are found naturally; this is a negatron-emitting radioisotope (see Table 35.2).

The isotopes of a particular element have the same number of protons in the nucleus but a different number of neutrons, giving them the same proton number (atomic number) but a different nucleon number (mass number, i.e. number of protons + number of neutrons). Isotopes may be stable or radioactive. Radioactive isotopes (radioisotopes) disintegrate spontaneously at random to yield radiation and a decay product.

Radioactive decay

There are three forms of radioactivity (Table 35.1) arising from three main types of nuclear decay:

Table 35.1 Types of radioactivity and their properties

Radiation	Range of maximum energies (MeV*)	Penetration range in air (m)	Suitable shielding material
Alpha (α)	4–8	0.025–0.080	Unnecessary
Beta (β)	0.01–3	0.150–16	Plastic (e.g. Perspex®)
Gamma (γ)	0.03–3	1.3–13**	Lead

*Note that 1 MeV = 1.6×10^{-13} J.
**Distance at which radiation intensity is reduced to half.

Examples

^{226}Ra decays to ^{222}Rn by loss of an alpha particle, as follows:

$$^{226}_{88}Ra \rightarrow ^{222}_{86}Rn + ^{4}_{2}He^{2+}$$

^{14}C shows beta decay, as follows:

$$^{14}_{6}C \rightarrow ^{14}_{7}N + \beta^{-}$$

^{22}Na decays by positron emission, as follows:

$$^{22}_{11}Na \rightarrow ^{22}_{10}Ne + \beta^{+}$$

^{55}Fe decays by electron capture and the production of an x-ray, as follows:

$$^{55}_{26}Fe \rightarrow ^{55}_{25}Mn + x$$

The decay of ^{22}Na by positron emission (β^{+}) leads to the production of a γ-ray when the positron is annihilated on collision with an electron.

- Alpha decay involves the loss of a particle equivalent to a helium nucleus. Alpha (α) particles, being large and positively charged, do not penetrate far in living tissue, but they do cause ionization damage and this makes them generally unsuitable for tracer studies.
- Beta decay involves the loss or gain of an electron or its positive counterpart, the positron. There are three sub-types:
  (a) Negatron (β^{-}) emission: loss of an electron from the nucleus when a neutron transforms into a proton. Examples of negatron-emitting isotopes are: ^{3}H, ^{14}C, ^{32}P, ^{45}Ca and ^{60}Co.
  (b) Positron (β^{+}) emission: loss of a positron when a proton transforms into a neutron. This only occurs when sufficient energy is available from the transition and may involve the production of gamma rays when the positron is later annihilated by collision with an electron.
  (c) Electron capture (EC): when a proton 'captures' an electron and transforms into a neutron. This may involve the production of x-rays as electrons 'shuffle' about in the atom (as with ^{125}I) and it frequently involves electron emission.
- Internal transition involves the emission of electromagnetic radiation in the form of gamma (γ) rays from a nucleus in a metastable state and always follows initial alpha or beta decay. Emission of gamma radiation leads to no further change in atomic number or mass.

Note from the above that more than one type of radiation may be emitted when a radioisotope decays. The main radioisotopes used in chemistry and their properties are listed in Table 35.2.

Each radioactive particle or ray carries energy, usually measured in electron volts (eV). The particles or rays emitted by a particular radioisotope exhibit a range of energies, termed an energy spectrum, characterized by the maximum energy of the radiation produced, E_{max} (Table 35.2).

Table 35.2 Properties of selected isotopes

Isotope	Emission(s)	Maximum energy (MeV)	Half-life
^{3}H	β^{-}	0.0186	12.3 years
^{14}C	β^{-}	0.156	5730 years
^{32}P	β^{-}	1.71	14.262 days
^{33}P	β^{-}	0.25	25.34 days
^{64}Cu	β^{-}	0.571	12.701 hours
	β^{+}	0.657	
	γ	0.511	
^{210}Pb	α	3.72	22.3 years
	γ	0.0465	

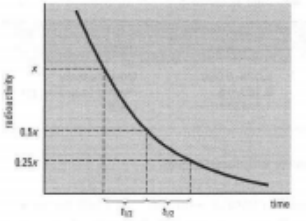


Fig. 35.1 Decay of a radioactive isotope with time. The time taken for the radioactivity to decline from x to $0.5x$ is the same as the time taken for the radioactivity to decline from $0.5x$ to $0.25x$, and so on. This time is the half-life ($t_{1/2}$) of the isotope.

Table 35.3 Relationships between units of radioactivity

1 Bq	= 1 d.p.s.
1 Bq	= 60 d.p.m.
1 Bq	= 27 pCi
1 d.p.s.	= 1 Bq
1 d.p.m.	= 0.0167 Bq
1 Ci	= 37 GBq
1 mCi	= 37 MBq
1 μCi	= 37 kBq
1 Sv	= 100 rem
1 Gy	= 100 rad
1 Gy	≈ 100 roentgen
1 rem	= 0.01 Sv
1 rad	= 0.01 Gy
1 roentgen	≈ 0.01 Gy

The energy spectrum of a particular radioisotope is relevant to the following:

- Safety: isotopes with the highest maximum energies will have the greatest penetrating power, requiring appropriate shielding (Table 35.1).
- Detection: different instruments vary in their ability to detect isotopes with different energies.
- Discrimination: some instruments can distinguish between isotopes, based on the energy spectrum of the radiation produced (p. 237).

The decay of an individual atom (a 'disintegration') occurs at random, but that of a population of atoms occurs in a predictable manner. The radioactivity decays exponentially, having a characteristic half-life ($t_{1/2}$). This is the time taken for the radioactivity to fall from a given value to half that value (Fig. 35.1). The $t_{1/2}$ values of different radioisotopes range from fractions of a second to more than 10^{19} years (see also Table 35.2). If $t_{1/2}$ is very short, as with ^{15}O ($t_{1/2} \approx 2$ min), then it is generally impractical to use the isotope in experiments because you would need to account for the decay during the experiment and counting period.

To calculate the fraction (f) of the original radioactivity left after a particular time (t), use the following relationship:

$$f = e^{x}, \text{ where } x = -0.693t/t_{1/2} \qquad [35.1]$$

Note that the same units must be used for t and $t_{1/2}$ in the above equation.

Measuring radioactivity

The SI unit of radioactivity is the becquerel (Bq), equivalent to one disintegration per second (d.p.s.), but disintegrations per minute (d.p.m.) are also used. The curie (Ci) is a non-SI unit equivalent to the number of disintegrations produced by 1 g of radium (37 GBq). Table 35.3 shows the relationships between these units. In practice, most instruments are not able to detect all of the disintegrations from a particular sample, i.e. their efficiency is less than 100% and the rate of decay may be presented as counts min^{-1} (c.p.m.) or counts s^{-1} (c.p.s.). Most modern instruments correct for background radiation and inefficiencies in counting, converting count data to d.p.m. Alternatively, the results may be presented as the measured count rate, although this is only valid where the efficiency of counting does not vary greatly among samples.

KEY POINT **The specific activity is a measure of the quantity of radioactivity present in a known amount of the substance:**

$$\text{specific activity} = \frac{\text{radioactivity (Bq, Ci, d.p.m., etc.)}}{\text{amount of substance (mol, g, etc.)}} \quad [35.2]$$

This is an important concept in practical work involving radioisotopes, since it allows interconversion of disintegrations (activity) and amount of substance (see Box 35.1).

Two SI units refer to doses of radioactivity and these are used when calculating exposure levels for a particular source. The sievert (Sv) is the amount of radioactivity giving a dose in man equivalent to 1 gray (Gy) of *x*-rays: 1 Gy = an energy absorption of 1 J kg^{-1}. The dose received in most biological experiments is a negligible fraction of the maximum permitted exposure limit. Conversion factors from older units are given in Table 35.3.

The most important methods of measuring radioactivity for chemical purposes are described below.

The Geiger–Müller (G–M) tube

This operates by detecting radiation when it ionizes gas between a pair of electrodes across which a voltage has been applied. You should use a hand-held Geiger–Müller tube for routine checking for contamination. It is not possible to detect low-energy β^- and α particles as they are not able to penetrate the window of the tube. In addition, γ-rays (of medium to high energy) pass through the filling gas causing little ionization, and hence have low efficiency.

The scintillation counter

This operates by detecting the scintillations (fluorescence 'flashes') produced when radiation interacts with certain chemicals called fluors. In solid (or external) scintillation counters (often referred to as 'gamma counters') the radioactivity causes scintillations in a crystal of fluorescent material held close to the sample. This method is only suitable for radioisotopes producing penetrating radiation.

Liquid scintillation counters are mainly used for detecting beta decay. The sample is dissolved in a suitable solvent containing the fluor(s) – the 'scintillation cocktail'. The radiation first interacts with the solvent, and the energy from this interaction is passed to the fluors which produce detectable light. The scintillations are measured by photomultiplier tubes which turn the light pulses into electronic pulses, the magnitude of which is directly related to the energy of the original radioactive event. The spectrum of electronic pulses is thus related to the energy spectrum of the radioisotope.

Correcting for quenching – find out how your instrument corrects for quenching and check the quench indication parameter (QIP) on the printout, which measures the extent of quenching of each sample. Large differences in the QIP would indicate that quenching is variable among samples and might give you cause for concern.

Modern liquid scintillation counters use a series of electronic 'windows' to split the pulse spectrum into two or three components. This may allow more than one isotope to be detected in a single sample, provided their energy spectra are sufficiently different (Fig. 35.2). A complication of this approach is that the energy spectrum can be altered by pigments and chemicals in the sample, which absorb scintillations or interfere with the transfer of energy to the fluor; this is known as quenching (Fig. 35.2). Most instruments have computer-operated quench correction facilities (based on measurements of standards of known activity and energy spectrum) which correct for such changes in counting efficiency.

Box 35.1 How to determine the specific activity of an experimental solution

Suppose you need to make up a certain volume of an experimental solution, to contain a particular amount of radioactivity. For example, 50 mL of a mannitol solution at a concentration of 25 mmol L^{-1}, to contain 5 Bq μL^{-1} – using a manufacturer's stock solution of ^{14}C-labelled mannitol (specific activity = 0.1 Ci $mmol^{-1}$).

1. **Calculate the total amount of radioactivity in the experimental solution**, in this example 5×1000 (to convert μL to mL) $\times$ 50 (50 mL required) $= 2.5 \times 10^5$ Bq (i.e. 250 kBq).
2. **Establish the volume of stock radioisotope solution required**: for example, a manufacturer's stock solution of ^{14}C-labelled mannitol contains 50 μCi of radioisotope in 1 mL of 90% v/v ethanol: water. Using Table 35.3, this is equivalent to an activity of $50 \times 37 = 1850$ kBq. So, the volume of solution required is 250/1850 of the stock volume, i.e. 0.135 1 mL (135 μL).
3. **Calculate the amount of non-radioactive substance required** as for any calculation involving concentration (see pp. 17, 146), e.g. 50 mL (0.05 L) of a 25 mmol L^{-1} (0.025 mol L^{-1}) mannitol (relative molecular mass 182.17) will contain $0.05 \times 0.025 \times 182.17 = 0.227\,7$ g.
4. **Check the amount of radioactive isotope to be added**. In most cases, this represents a negligible amount of substance, e.g. in this instance, 250 kBq of stock solution at a specific activity of 14.8×10^6 kBq $mmol^{-1}$ (converted from 0.4 Ci $mmol^{-1}$ using Table 35.3) is equal to 250/14 800 000 = 16.89 nmol, which is equivalent to approximately 3 μg mannitol. This can be ignored in calculating the mannitol concentration of the experimental solution.
5. **Make up the experimental solution** by adding the appropriate amount of non-radioactive substance and the correct volume of stock solution.
6. **Measure the radioactivity in a known volume of the experimental solution**. If you are using an instrument with automatic correction to Bq, your sample should contain the predicted amount of radioactivity, e.g. an accurately dispensed volume of 100 μL of the mannitol solution should give a corrected count of $100 \times 5 = 500$ Bq (or $500 \times 60 = 30\,000$ d.p.m.).
7. **Note the specific activity of the experimental solution**: in this case, 100 μL (1×10^{-4} L) of the mannitol solution at a concentration of 0.025 mol L^{-1} will contain 25×10^{-7} mol (2.5 μmol) mannitol. Dividing the radioactivity in this volume (30 000 d.p.m.) by the amount of substance (eqn [35.2]) gives a specific activity of 30 000/2.5 = 12 000 d.p.m. μmol $^{-1}$, or 12 d.p.m. $nmol^{-1}$. This value can be used:
   - (a) To assess the accuracy of your protocol for preparing the experimental solution: if the measured activity is substantially different from the predicted value, you may have made an error in making up the solution.
   - (b) To determine the counting efficiency of an instrument; by comparing the measured count rate with the value predicted by your calculations.
   - (c) To interconvert activity and amount of substance: the most important practical application of specific activity is the conversion of experimental data from counts (activity) into amounts of substance. This is only possible where the substance has not been metabolized or otherwise converted into another form; for example, a tissue sample incubated above with a measured activity of 245 d.p.m. can be converted to nmol mannitol by dividing by the specific activity, expressed in the correct form. Thus 245/12 = 20.417 nmol mannitol.

Liquid scintillation counting of high-energy beta emitters – beta particles with energies greater than 1 MeV can be counted in water (Čerencov radiation), with no requirement for additional fluors (e.g. ^{32}P).

Many liquid scintillation counters treat the first sample as a 'background', subtracting whatever value is obtained from the subsequent measurements as part of the procedure for converting to d.p.m. If not, you will need to subtract the background count from all other samples. Make sure that you use an appropriate background sample, identical in all respects to your radioactive sample but with no added radioisotope, in the correct position within the machine. Check that the background reading is reasonable (15–30 c.p.m.). Tips for preparing samples for liquid scintillation counting are given in Box 35.2.

Box 35.2 Tips for preparing samples for liquid scintillation counting

Modern scintillation counters are very simple to operate; problems are more likely to be due to inadequate sample preparation than to incorrect operation of the machine. Common pitfalls are the following:

- Incomplete dispersal of the radioactive compound in the scintillation cocktail. This may lead to underestimation of the true amount of radioactivity present:
  - (a) Water-based samples may not mix with the scintillation cocktail – change to an emulsifier-based cocktail. Take care to observe the recommended limits, upper and lower, for amounts of water to be added or the cocktail may not emulsify properly.
  - (b) Solid specimens may absorb disintegrations or scintillations: extract radiochemicals using an intermediate solvent like ethanol (ideally within the scintillation vial) and then add the cocktail.
  - (c) Particulate samples may sediment to the bottom of the scintillation vial – suspend them by forming a gel. This can be done with certain emulsifier-based cocktails by adding a specific amount of water.
- Chemiluminescence. This is where a chemical reacts with the fluors in the scintillation cocktail causing spurious scintillations, a particular risk with solutions containing strong bases or oxidizing agents. Symptoms include very high initial counts which decrease through time. Possible remedies are:
  - (a) Leave the vials at room temperature for a time before counting. Check with a suitable blank that counts have dropped to an acceptable level.
  - (b) Neutralize basic samples with acid (e.g. acetic acid or HCl).
  - (c) Use a scintillation cocktail that resists chemiluminescence such as Hiconicfluor®.
  - (d) Raise the energy of the lower counts detected to about 8 keV – most chemiluminescence pulses are weak (0–7 keV). This approach is not suitable for ^{3}H.

γ-ray spectrometry

This is a method by which a mixture of γ-ray-emitting radionuclides can be resolved quantitatively by pulse-height analysis. It is based on the fact that pulse heights (voltages) produced by a photomultiplier tube are proportional to the amounts of γ-ray energy arriving at the scintillant or a lithium-drifted germanium detector. The lithium-drifted germanium detector, which is abbreviated to Ge(Li) – pronounced 'jelly' provides higher resolution (narrower peaks), essential in the analysis of complex mixtures.

Autoradiography

This is a method where photographic film is exposed to the isotope. It is used mainly to locate radioactive tracers in thin sections of an organism or on chromatography papers and gels, but quantitative work is possible. The radiation interacts with the film in a similar way to light, silver grains being formed in the developed film where the particles or rays have passed through. The radiation must have enough energy to penetrate into the film, but if it has too much energy the grain formation may be too distant from the point where the isotope was located to identify precisely the point of origin (e.g. high-energy beta-emitters). Autoradiography is a relatively specialized method and individual lab protocols should be followed for particular isotopes/applications.

Chemical applications for radioactive isotopes

The main advantages of using radioactive isotopes in chemical experiments are:

- Radioactivity is readily detected. Methods of detection are sufficiently sensitive to measure extremely small amounts of radioactive substances.

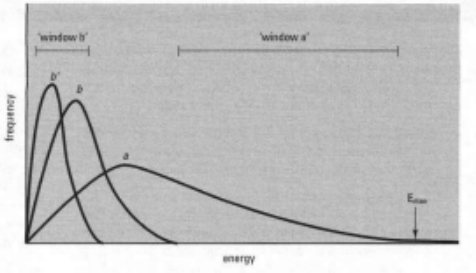


Fig. 35.2 Energy spectra for three radioactive samples, detected using a scintillation counter. Sample *a* is a high-energy beta emitter while *b* contains a low-energy beta emitter, giving a lower spectral range. Sample *b'* contains the same amount of low-energy beta emitter, but with quenching, shifting the spectral distribution to a lower energy band. The counter can be set up to record disintegrations within a selected range (a 'window'). Here, 'window a' could be used to count isotope *a* while 'window b' could give a value of isotope *b*, by applying a correction for the counts due to isotope *a*, based on the results from 'window a'. Dual counting allows experiments to be carried out using two isotopes (double labelling).

Degradation of radiolabelled compounds – you may need to separate individual metabolites before counting, e.g. using chromatography (p. 211), or electro-phoresis (p. 225).

Example Carbon dating – living organisms have essentially the same ratio of ^{14}C to ^{12}C as the atmosphere; however, when an organism dies, its $^{14}C/^{12}C$ falls because the radioactive ^{14}C isotope decays. Since we know the half-life of ^{14}C (5 715 years), a sample's $^{14}C/^{12}C$ ratio will allow us to estimate its age; for example, if the ratio were exactly one-eighth of that in the atmosphere, the sample would be three half-lives old and was formed 17 145 years before present. Such estimates carry an error of the order of 10% and are unreliable for samples older than 50 000 years, for which longer lived isotopes can be used.

- Studies can be carried out in synthetic chemistry using radiolabelled compounds, e.g. ^{3}H or ^{14}C.
- Protocols are relatively simple compared with equivalent methods for instrumental chemical analysis.

The main disadvantages are:

- The 'isotope effect'. Molecules containing different isotopes of the same atom may react at slightly different rates and behave in slightly different ways to the natural isotope. The isotope effect is more extreme the smaller the atom and is most important for ^{3}H-labelled compounds of low molecular mass.
- The possibility of mistaken identity. The presence of radioactivity does not tell you anything about the compound in which radioactivity is present: it could be different from the one in which it was applied, owing to chemical breakdown of a ^{14}C-containing organic compound.

The main types of experiments are:

- Radiolabelled compounds: the use of radiolabelled compounds in synthetic and tracer studies is important as it allows the scientist to locate the labelled atom, i.e. ^{14}C, ^{3}H, in, for example, chemical synthesis and laboratory environmental fate (degradation) studies. If using radiolabelled compounds several issues arise and these include deciding upon the radionuclide itself, its position in the molecule, the specific activity, the solvent and cost.
- Radio-dating: the age of plant or mineral samples can be determined by measuring the amount of a radioisotope in the sample. The age of the specimen can be found using $t_{1/2}$ by assuming how much was originally incorporated.
- Medical uses: in radiotherapy the use of gamma radiation from ^{60}Co to destroy cancerous cells; ^{24}Na can be introduced into the blood stream to follow the flow of blood and identify obstructions; heart disease can be assessed using ^{201}Tl and ^{99}Tc where the former concentrates in healthy heart tissue and the latter concentrates in abnormal heart tissue.
- Assays: radioisotopes are used in several quantitative detection methods of value to chemists. Radioimmunoassay is a quantitative method for measurement of a substance (the analyte) using antibodies which bind specifically to that analyte. Isotope dilution analysis works on the

assumption that introduced radiolabelled molecules will equilibrate with unlabelled molecules present in the sample. The amount of substance initially present can be worked out from the change in specific activity of the radioisotope when it is diluted by the 'cold' material. A method is required whereby the substance can be purified from the sample and sufficient substance must be present for its mass to be measured accurately. Activation analysis is a sensitive technique for the determination of element concentration. It is based upon selectivity inducing radioactivity in some of the atoms of the elements comprising the sample and then selectively measuring the radiations emitted by the radionuclides. After bombardment with suitable nuclear particles, the induced radionuclides are identified or quantitatively measured. Neutron activation analysis is the most common method of analysis.

Working practices when using radioactive isotopes

In the UK, institutions must be registered for work with specific radioisotopes under the Radioactive Substances Act (1960).

In the UK, the Ionizing Radiations Act (1985) provides details of local arrangements for the supervision of radioisotope work.

By law, undergraduate work with radioactive isotopes must be very closely supervised. In practical classes, the protocols will be clearly outlined, but in project work you may have the opportunity to plan and carry out your own experiments, albeit under supervision. Some of the factors that you should take into account, based on the assumption that your department and laboratory are registered for radioisotope use, are discussed below:

- Must you use radioactivity? If not, it may be a legal requirement that you use the alternative method.
- Have you registered for radioactive work? Normal practice is for all users to register with a local Radiation Protection Supervisor. Details of the project may have to be approved by the appropriate administrator(s). You may have to have a short medical examination before you can start work.
- What labelled compound will you use? Radioactive isotopes must be ordered well in advance through your department's Radiation Protection Supervisor. Aspects that need to be considered include:
  (a) The radionuclide. With many organic compounds this will be confined to ^{3}H and ^{14}C (but see Table 35.2). The risk of a significant 'isotope effect' may influence this decision (see above).
  (b) The labelling position. This may be a crucial part of a metabolic study. Specifically labelled compounds are normally more expensive than those that are uniformly ('generally') labelled.
  (c) The specific activity. The upper limit for this is defined by the isotope's half-life, but below this the higher the specific activity, the more expensive the compound.
- Are suitable facilities available? You will need a suitable work area, preferably out of the way of general lab traffic and within a fume cupboard for those cases where volatile radioactive substances are used or may be produced.

Each new experiment should be planned carefully and experimental protocols laid down in advance so you work as safely as possible and do not waste expensive radioactively labelled compounds. In conjunction with your supervisor, decide whether your method of application will introduce enough radioactivity into the system, how you will account for any loss of radioactivity during recovery of the isotope and whether there will be enough activity to count at the end. You should be able to predict approximately the

Carrying out a 'dry run' – consider doing this before working with radioactive compounds, perhaps using a dye to show the movement or dilution of introduced liquids, as this will lessen the risks of accident and improve your technique.

Using Benchkote® – the correct way to use Benchkote® and similar products is with the waxed surface down (to protect the bench or tray surface) and the absorbent surface up (to absorb any spillage). Write the date in the corner when you put down a new piece. Monitor using a G–M tube and replace regularly under normal circumstances. If you are aware of spillage, replace immediately and dispose of correctly.

Fig. 35.3 Tape showing the international symbol for radioactivity.

amount of radioactivity in your samples, based on the specific activity of the isotope used, the expected rate of uptake/exchange and the amount of sample to be counted. Use the isotope's specific activity to estimate whether the non-radioactive ('cold') compound introduced with the radiolabelled ('hot') compound may lead to excessive concentrations being administered. Advice for handling data is given in Box 35.1.

Safety and procedural aspects

Make sure the bench surface is one that can be easily decontaminated by washing (e.g. Formica®) and always use a disposable surfacing material such as Benchkote®. It is good practice to carry out as many operations as possible within a Benchkote®-lined plastic tray so that any spillages are contained. You will need a lab coat to be used exclusively for work with radioactivity, safety spectacles and a supply of thin latex or vinyl disposable gloves. Suitable vessels for liquid waste disposal will be required and special plastic bags for solids – make sure you know beforehand the disposal procedures for liquid and solid wastes. Wash your hands after handling a vessel containing a radioactive solution and again before removing your gloves. Gloves should be placed in the appropriate disposal bag as soon as your experimental procedures are complete.

It is important to comply with the following guidelines:

- Read and obey the local rules for safe usage of radiochemicals.
- Maximize the distance between you and the source as much as possible.
- Minimize the duration of exposure.
- Wear protective clothing (properly fastened lab coat, safety glasses, gloves) at all times.
- Use appropriate shielding at all times (Table 35.1).
- Monitor your working area frequently for contamination.
- Mark all glassware, trays, bench work areas, etc., with tape incorporating the international symbol for radioactivity (Fig. 35.3).
- Keep adequate records of what you have done with a radioisotope – the stock remaining and that disposed of in waste form must agree.
- Store radiolabelled compounds appropriately and return them to storage areas immediately after use.
- Dispose of waste promptly and with due regard for local rules.
- Make the necessary reports about waste disposal etc. to your departmental Radiation Protection Supervisor.
- Clear up after you have finished each experiment.
- Wash thoroughly after using radioactivity.
- Monitor the work area and your body when finished.

36 Thermal analysis

Thermal methods are techniques in which changes in physical and/or chemical properties of a substance are measured as a function of temperature. Several methods of analysis are used:

- Thermogravimetry (TG) is a technique in which a change in the weight of the substance under investigation is monitored with respect to temperature or time.
- Differential thermal analysis (DTA) is a technique for measuring the difference in temperature between the substance under investigation and an inert reference material with respect to temperature or time.
- Differential scanning colorimetry (DSC) is a technique in which the energy necessary to establish a zero temperature difference between the substance under investigation and a reference material is monitored with respect to temperature or time.

When carrying out a thermal analysis procedure it is important to consider and record the following details:

- Sample: a chemical description of the sample, plus its source and any pre-treatment. Also, the purity, chemical composition and formula, if known. Other important items to note are: the particle size, whether the sample has been mixed with a 'binder' (and, if so, what it has been mixed with and in what ratio) and the 'history' of the sample.
- Crucible: the material and design of the sample holder is important. Obviously it is important that the crucible does not react with the sample during heating. In addition, the geometry of the crucible can influence the gas flow.
- Rate of heating: this is very important if you intend to repeat the experiment on a subsequent occasion. Obviously the rate of heating of the sample in the crucible is not instantaneous but depends upon conduction, convection and radiation within the system. Thermal lag is therefore likely to be observed.
- Atmosphere: The nature of the atmosphere surrounding the sample is important in relation to the transfer of heat and the chemistry of the sample reaction. Common sample atmospheres are shown in Table 36.1. In addition, the flow rate of the gas is important: a static system will not remove reaction products from the sample.
- Mass of sample: obviously the amount of sample will have an effect on the heating rate. Also, sample homogeneity may be an issue with very small samples.

Table 36.1 Common sample atmospheres

Gas at 1 atm	Thermal conductivity at 400/K ($mW\,m^{-1}\,K^{-1}$)
Air	33.3
Carbon dioxide	25.1
Helium	190.6
Nitrogen	32.3

Thermogravimetry

The apparatus required for TG analysis is shown in Fig. 36.1. TG is normally carried out on solid samples. Example operating conditions are as follows:

Sample:	calcium oxalate monohydrate
Crucible:	platinum pan
Rate:	$10\,K\,min^{-1}$
Atmosphere:	nitrogen, $20\,mL\,min^{-1}$
Mass:	10.5 mg

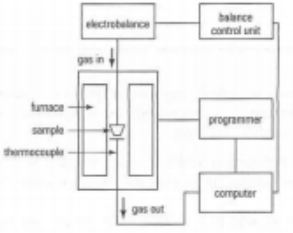


Fig. 36.1 Schematic diagram of a system for thermogravimetry.

Box 36.1 How to interpret a thermal analysis trace

1. **Identify the start position of the trace** (see Fig. 36.2); this is usually indicated by the scale on the trace.
2. **Identify any regions of decomposition**: these are where there is a rapid change in the vertical axis. Three distinct regions of decomposition can be identified in Fig. 36.2: (a) between the start and the first plateau there is a loss of 12.5% (stage 1); (b) between the first and second plateau a loss of 18.75% occurs (stage 2); and (c) between the second plateau and the final residue there is a loss of 29.75% (stage 3).
3. **Determine the M_r of the starting material**. The M_r of $CaC_2O_4.H_2O$ is 146.1.
4. **Using the M_r determine the decomposition loss associated with each region.**

   Stage 1: $146.1 \times 12.5/100 = 18.3$ (18 corresponds to the loss of water)

   $$CaC_2O_4.H_2O_{(s)} \rightarrow CaC_2O_{4(s)} + H_2O_{(v)}$$

   Stage 2: $146.1 \times 18.75/100 = 27.4$ (28.01 corresponds to loss of CO)

   $$CaC_2O_{4(s)} \rightarrow CaCO_{3(s)} + CO_{(g)}$$

   Stage 3: $146.1 \times 29.75/100 = 43.5$ (44.01 corresponds to loss of CO_2)

   $$CaCO_{3(s)} \rightarrow CaO_{(s)} + CO_{2(g)}$$

5. **Determination of the final product**. In this case for $CaC_2O_4.H_2O$ the final residue is CaO ($M_r = 56.08$).
6. **Check the M_r of the original compound**:

   original sample (M_r) = [residue (M_r)/% residue] × 100

   Residue from Fig. 36.2 is 39%. Therefore,

   original sample (M_r) = [56.08/39%] × 100 = 143.8

   Thus the calculated (M_r) of $CaC_2O_4.H_2O$ is 143.8, which is similar to the known M_r of $CaC_2O_4.H_2O$ of $146.1\,g\,mol^{-1}$.
7. **Assess the purity of the original material**. The percentage purity of $CaC_2O_4.H_2O$ is calculated as follows:

   $143.8 \times 100 \times 1/146.1 = 98.43\%$

The result can be expressed as either a TG curve, a plot of changing weight with respect to temperature or time, or a derivative of the curve, i.e. DTG, where the first derivative of the TG curve is plotted with respect to temperature or time. As well as providing information on the thermal decomposition of inorganic compounds, additional information can be deduced, e.g. sample purity and M_r.

The oxalate hydrates of the alkaline earth metals, e.g. calcium, strontium and barium, are all insoluble. If a calcium salt made acidic with ethanoic acid is treated with sodium oxalate solution, a white precipitate of calcium oxalate

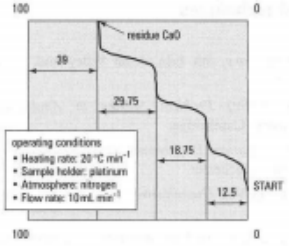


Fig. 36.2 A typical thermal analysis trace for $CaC_2O_4.H_2O$.

monohydrate is formed quantitatively. After washing the precipitate with ethanol it can be analysed. A typical TG curve, for calcium oxalate monohydrate, $CaC_2O_4.H_2O$, is shown in Fig. 36.2. Box 36.1 shows how to interpret a thermal analysis trace for calcium oxalate monohydrate.

Applications

As well as inorganic complexes, thermal analysis is applicable to a wide range of substances, e.g. polymers, drugs, soils and coals. It can also be applied to mixtures of, for example, polymer blends.

Degradation of polymers The effect of heat on polymers varies according to the type of polymer under investigation. In an inert atmosphere, polymeric materials react in two distinct ways: they either depolymerize or carbonize. For example, poly(methyl methacrylate) may degrade back to the monomer.

Soil The composition of soil is complex and varies with location and geology. Three general stages of soil decomposition on heating can be identified:

1. Loss of moisture and simple organic compounds (between room temperature and 150 °C).
2. Ignition of soil organic matter (between 250 and 550 °C).
3. Presence of minerals e.g. carbonates. The process can be complicated by the presence of hydrated minerals e.g. aluminium and iron oxides, and micas (above 550 °C).

Drugs The presence of water in both 'free' and 'bound' states in pharmaceuticals can be identified.

Resources for instrumental techniques

General books

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Crawford, K. and Heaton, A. (1999) *Problem Solving in Analytical Chemistry*, Royal Society of Chemistry, Cambridge.

Currell, G. (2000) *Analytical Instrumentation. Performance and characteristics of quality*, John Wiley and Sons Ltd., Chichester.

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Kellner, R. (1998) *Analytical Chemistry*, John Wiley and Sons Ltd, Chichester.

Mendham, J., Denney, R.C., Barnes, J.D. and Thomas, M.J.K. (2000) *Vogel's Textbook of Quantitative Chemical Analysis*, 6th Edn, Prentice Hall, Harlow, Essex.

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Skoog, D.A., West, D.M. and Holler, F.J. (1996) *Fundamentals of Analytical Chemistry*, 7th Edn, Saunders College Publishing, Orlando, Florida.

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Willard, H.H., Meritt, Jr., L.L., Dean, J.A. and Settle, Jr., F.A. (1988) *Instrumental Methods of Analysis*, 7th Edn, Wadsworth Publishing Co., Belmont, CA.

Specific books on atomic spectroscopy

Cresser, M.S. (1995) *Flame Spectrometry in Environmental Chemical Analysis: A Practical Approach*, The Royal Society of Chemistry, Cambridge.

Dean, J.R. (1997) *Atomic Absorption and Plasma Spectroscopy*, ACOL, John Wiley and Sons Ltd, Chichester.

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Synder, L.R., Kirkland, J.J. and Glajch, J.L. (1997) *Practical HPLC Method Development*, 2nd Edn, John Wiley and Sons Ltd, New York.

Specific book on electroanalytical techniques

Wang, J. (2000) *Analytical Electrochemistry*, 2nd Edn, John Wiley and Sons Ltd, Chichester.

Specific book on radiochemistry

Newton, G.W.A. (1999) *Environmental Radiochemical Analysis*, Royal Society of Chemistry, Cambridge.

Specific book on thermal methods

Hatakeyama T. and Zhenhai, L. (1998) *Handbook of Thermal Analysis*, John Wiley and Sons Ltd, New York.

CD-ROMs

Chemistry Video Consortium, Practical Laboratory Chemistry, Educational Media Film and Video Ltd, Harrow, Essex, UK – Chromatographic techniques (TLC, column, ion exchange and gas chromatography).

Chemistry Video Consortium, Practical Laboratory Chemistry, Educational Media Film and Video Ltd, Harrow, Essex, UK – Electrochemical techniques (using galvanic cells, using conductometric cells, determining standard electrode potentials, determining solubility products, thermodynamic characteristics of cells, conductometric titrations and using an automatic titrator).

Chemistry Video Consortium, Practical Laboratory Chemistry, Educational Media Film and Video Ltd, Harrow, Essex, UK – Flame photometry, AA and TGA measurements (using a flame photometer, using an atomic absorption spectrometer and thermogravimetric analysis).

Chemistry Video Consortium, Practical Laboratory Chemistry, Educational Media Film and Video Ltd, Harrow, Essex, UK – Microscale chromatography (TLC, column chromatography, gas chromatography and preparation of a Grignard reagent).

Chemistry Video Consortium, Practical Laboratory Chemistry, Educational Media Film and Video Ltd, Harrow, Essex, UK – Polarimetry, refractometry and radiochemistry (using a polarimeter, determining the refractive indices of liquids, measuring the rates of radioactive processes and measuring gas phase emission spectra).

Video

Greeves, N., Hodson, L. and Keedall, G. (1998) *Intermediate NMR Spectroscopy*, Royal Society of Chemistry.

Sotware

AAS softbook, Royal Society of Chemistry, Cambridge.

The advanced ICP softbook, Royal Society of Chemistry, Cambridge.

GC application and method development softbook, Royal Society of Chemistry, Cambridge.

GC softbook, Royal Society of Chemistry, Cambridge.

HPLC softbook, Royal Society of Chemistry, Cambridge.

MS softbook, Royal Society of Chemistry, Cambridge.

Analysis and presentation of data

37 Using graphs

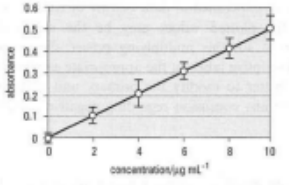

Fig. 37.1 Calibration curve for the determination of lead in soil using FAAS. Vertical bars show standard errors ($n = 3$).

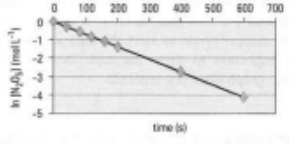

Fig. 37.2 Decomposition of N_2O_5. The first-order rate constant can be determined from the slope of the line.

Selecting a title – it is a common fault to use titles that are grammatically incorrect: a widely applicable format is to state the relationship between the independent and dependent variables within the title, e.g. 'The relationship between absorbance and concentration'.

Remembering which axis is which – a way of remembering the orientation of the *x*-axis is that *x* is a 'cross', and it runs 'across' the page (horizontal axis).

Graphs can be used to show detailed results in an abbreviated form, displaying the maximum amount of information in the minimum space. Graphs and tables present findings in different ways. A graph (figure) gives a visual impression of the content and meaning of your results, while a table provides an accurate numerical record of data values. You must decide whether a graph should be used, e.g. to illustrate a pronounced trend or relationship, or whether a table (Chapter 38) is more appropriate.

A well-constructed graph will combine simplicity, accuracy and clarity. Planning of graphs is needed at the earliest stage in any write-up as your accompanying text will need to be structured so that each graph delivers the appropriate message. Therefore, it is best to decide on the final form for each of your graphs before you write your text. The text, diagrams, graphs and tables in a laboratory write-up or project report should be complementary, each contributing to the overall message. In a formal scientific communication it is rarely necessary to repeat the same data in more than one place (e.g. as a table and as a graph). However, graphical representation of data collected earlier in tabular format may be applicable in laboratory practical reports.

Practical aspects of graph drawing

The following comments apply to graphs drawn for laboratory reports. Figures for publication, or similar formal presentation, are usually prepared according to specific guidelines, provided by the publisher/organizer.

Two examples of graphs are shown (Fig. 37.1 and 37.2). The first is typical in quantitative analytical chemistry (Fig. 37.1), while the second is typical in physical chemistry (Fig. 37.2).

KEY POINT **Graphs should be self-contained – they should include all material necessary to convey the appropriate message without reference to the text. Every graph must have a concise explanatory title to establish the content. If several graphs are used, they should be numbered, so they can be quoted in the text.**

- Consider the layout and scale of the axes carefully. Most graphs are used to illustrate the relationship between two variables (*x* and *y*) and have two axes at right angles (e.g. Figs 37.1 and 37.2). The horizontal axis is known as the abscissa (*x*-axis) and the vertical axis as the ordinate (*y*-axis).
- The axis assigned to each variable must be chosen carefully. Usually the *x*-axis is used for the independent variable (e.g. concentration) while the dependent variable (e.g. concentration response) is plotted on the *y*-axis. When neither variable is determined by the other, or where the variables are interdependent, the axes may be plotted either way round.
- Each axis must have a descriptive label showing what is represented, together with the appropriate units of measurement, separated from the descriptive label by a solidus or 'slash' (/), as in Fig. 37.1, or brackets as in Fig. 37.2.
- Each axis must have a scale with reference marks ('tics') on the axis to show clearly the location of all numbers used.

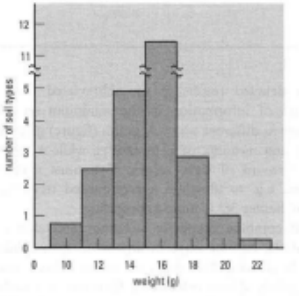

Fig. 37.3 Frequency distribution of weights for a range of different soil types (sample size 24 085); the size class interval is 2 g.

Example For a data set where the smallest number on the log axis is 12 and the largest number is 9 000, three-cycle log-linear paper would be used, covering the range 10–10 000.

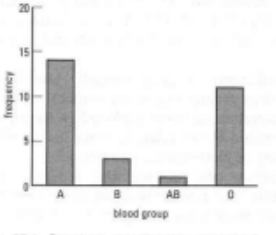

Fig. 37.4 Bar chart, showing the number of students belonging to each ABO blood group ($n = 29$).

- A figure legend should be used to provide explanatory detail, including the symbols used for each data set.

Handling very large or very small numbers

To simplify presentation when your experimental data consist of either very large or very small numbers, the plotted values may be the measured numbers multiplied by a power of 10: this multiplying power should be written immediately before the descriptive label on the appropriate axis (as in Fig. 37.3). However, it is often better to modify the primary unit with an appropriate prefix (p. 70) to avoid any confusion regarding negative powers of 10.

Size

Remember that the purpose of your graph is to communicate information. It must not be too small, so use at least half an A4 page and design your axes and labels to fill the available space without overcrowding any adjacent text. If using graph paper, remember that the white space around the grid is usually too small for effective labelling. The shape of a graph is determined by your choice of scale for the *x*- and *y*-axes which, in turn, is governed by your experimental data. It may be inappropriate to start the axes at zero. In such instances, it is particularly important to show the scale clearly, with scale breaks where necessary, so the graph does not mislead.

Graph paper

In addition to conventional linear (squared) graph paper, you may need the following:

- Probability graph paper. This is useful when one axis is a probability scale.
- Log–linear graph paper. This is appropriate when one of the scales shows a logarithmic progression, e.g. in chemical kinetics. A plot of $\ln k$ against $1/T$ is used to determine the activation energy (E_a), where k is the rate constant and T is the temperature. Log–linear paper is defined by the number of logarithmic divisions covered (usually termed 'cycles') so make sure you use a paper with the appropriate number of cycles for your data. An alternative approach is to plot the log-transformed values on 'normal' graph paper.
- Log–log graph paper. This is appropriate when both scales show a logarithmic progression, e.g. the large linear ranges achievable with some analytical instruments.

Types of graph

Different graphical forms may be used for different purposes, including:

- Plotted curves – used for data where the relationship between two variables can be represented as a continuum.
- Scatter diagrams – used to visualize the relationship between individual data values for two interdependent variables (e.g. Fig. 41.6) often as a preliminary part of a correlation analysis (p. 278).
- Three-dimensional graphs show the inter-relationships of three variables, (e.g. Fig. 32.21).
- Histograms for frequency distributions of continuous variables (e.g. Fig. 37.3).

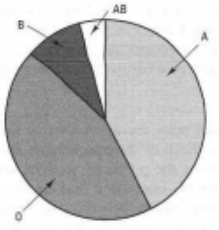

Fig. 37.5 Pie chart: relative abundance of ABO blood groups in man.

- Frequency polygons emphasize the form of a frequency distribution by joining the co-ordinates with straight lines, in contrast to a histogram. This is particularly useful when plotting two or more sets of data values on the same graph.
- Bar charts represent frequency distributions of a discrete qualitative or quantitative variable (e.g. Fig. 37.4).
- Pie charts illustrate portions of a whole (e.g. Fig. 37.5).

The plotted curve

This is the commonest form of graphical representation used in chemistry. The key features are outlined below and in checklist form (Box 37.1).

Data points

Each data point must be shown accurately, so that any reader can determine the exact values of x and y. In addition, the results of each treatment must be readily identifiable. A useful technique is to use a dot for each data point, surrounded by a hollow symbol for each treatment. An alternative is to use symbols only, though the co-ordinates of each point are defined less accurately. Use the same symbol for the same entity if it occurs in several graphs and provide a key to all symbols.

Statistical measures

If you are plotting average values for several replicates and if you have the necessary statistical knowledge, you can calculate the standard error (p. 268), or the 95% confidence limits (p. 278) for each mean value and show these on your graph as a series of vertical bars (see Fig. 37.1). Make it clear in the legend whether the bars refer to standard errors or 95% confidence limits and quote the value of n (the number of replicates per data point). Another approach is to add a least significant difference bar (p. 277) to the graph.

Box 37.1 Checklist for the stages in drawing a graph

The following sequence can be used whenever you need to construct a plotted curve: it will need to be modified for other types of graph.

1. **Collect all of the data values and statistical values** (in tabular form, where appropriate).
2. **Decide on the most suitable form of presentation**: this may include transformation, to convert the data to linear form.
3. **Choose a concise descriptive title**, together with a reference (figure) number and date, where necessary.
4. **Determine which variable is to be plotted on the *x*-axis and which on the *y*-axis.**
5. **Select appropriate scales for both axes** and make sure that the numbers and their location (scale marks) are clearly shown, together with any scale breaks.
6. **Decide on appropriate descriptive labels for both axes**, with SI units of measurement, where appropriate.
7. **Choose the symbols for each set of data points** and decide on the best means of representation for statistical values.
8. **Plot the points** to show the co-ordinates of each value with appropriate symbols.
9. **Draw a trend line for each set of points.**
10. **Write a figure legend**, to include a key which identifies all symbols and statistical values and any descriptive footnotes, as required.

Conveying the correct message – the golden rule is: 'always draw the simplest line that fits the data reasonably well and is consistent with the underlying chemical principles'.

Interpolation

Once you have plotted each point, you must decide whether to link them by straight lines or a smoothed curve. Each of these techniques conveys a different message to your reader. Joining the points by straight lines may seem the simplest option, but may give the impression that errors are very low or non-existent and that the relationship between the variables is complex. Joining points by straight lines is appropriate only in certain graphs, e.g. recording a patient's temperature in a hospital, to emphasize any variation from one time point to the next. However, in most plotted curves the best straight line or curved line should be drawn (according to appropriate mathematical or statistical models, or by eye), to highlight the relationship between the variables – after all, your choice of a plotted curve implies that such a relationship exists! Don't worry if some of your points do not lie on the line: this is caused by errors of measurement and/or sample variation. Most curves drawn by eye should have an equal number of points lying on either side of the line. You may be guided by 95% confidence limits, in which case your curve should pass within these limits wherever possible.

Curved lines can be drawn using a flexible curve, a set of French curves, or freehand. In the last case, turn your paper so that you can draw the curve in a single, sweeping stroke by a pivoting movement at the elbow (for larger curves) or wrist (for smaller ones). Do not try to force your hand to make complex, unnatural movements, as the resulting line will not be smooth.

Extrapolating plotted curves – try to avoid the need to extrapolate by better experimental design.

Extrapolation

Be wary of extrapolation beyond the upper or lower limit of your measured values. This is rarely justifiable and may lead to serious errors. Whenever extrapolation is used, a dotted line ensures that the reader is aware of the uncertainty involved. Any assumptions behind an extrapolated curve should also be stated clearly in your text.

The histogram

In a histogram, each datum is represented by a column with an area proportional to the magnitude of y: in most cases, you should use columns of equal width, so that the height of each column is then directly proportional to y. Shading or stippling may be used to identify individual columns, according to your needs.

While a plotted curve assumes a continuous relationship between the variables by interpolating between individual data points, a histogram involves no such assumptions and is the most appropriate representation if the number of data points is too few to allow a trend line to be drawn. Histograms are also used to represent frequency distributions (p. 265), where the y-axis shows the number of times a particular value of x was obtained (e.g. Fig. 37.3). As in a plotted curve, the x-axis represents a continuous variable which can take any value within a given range, so the scale must be broken down into discrete classes and the scale marks on the x-axis should show either the mid-points (mid-values) of each class (Fig. 37.3), or the boundaries between the classes.

The columns are adjacent to each other in a histogram, in contrast to a bar chart (Fig. 37.4), where the columns are separate because the x-axis of a bar chart represents discrete values.

Interpreting graphs

Using computers to produce graphs – never allow a computer program to dictate size, shape and other aspects of a graph: find out how to alter scales, labels, axes, etc., and make appropriate selections. Draw curves freehand if the program only has the capacity to join the individual points by straight lines.

Whenever you look at graphs drawn by other people, make sure you understand the axes before you look at the relationship. It is all too easy to take in the shape of a graph without first considering the scale of the axes, a fact that some advertisers and politicians exploit when curves are used to misrepresent information. Such graphs are often used in newspapers and on television. Examine them critically – many would not pass the stringent requirements of scientific communication and conclusions drawn from them may be flawed.

38 Presenting data in tables

A table is often the most appropriate way to present numerical data in a concise, accurate and structured form. Laboratory reports and project dissertations should contain tables which have been designed to condense and display results in a meaningful way and to aid numerical comparison. The preparation of tables for recording primary data is discussed on p. 67.

Decide whether you need a table, or whether a graph is more appropriate. Histograms and plotted curves can be used to give a visual impression of the relationships within your data (p. 251). On the other hand, a table gives you the opportunity to make detailed numerical comparisons.

KEY POINT **Always remember that the primary purpose of your table is to communicate information and allow appropriate comparison, not simply to put down the results on paper!**

Preparation of tables

Title

Constructing titles – take care over titles as it is a common mistake in student practical reports to present tables without titles, or to misconstruct the title.

Every table must have a brief descriptive title. If several tables are used, number them consecutively so they can be quoted in your text. The titles within a report should be compared with one another, making sure they are logical and consistent and that they describe accurately the numerical data contained within them.

Structure

Display the components of each table in a way that will help the reader understand your data and grasp the significance of your results. Organize the columns so that each category of like numbers or properties is listed vertically, while each horizontal row shows a different experimental treatment, element, sampling site, etc. (as in Table 38.1). Where appropriate, put control values near the beginning of the table. Columns that need to be compared should be set out alongside each other. If producing by hand, use rulings to sub-divide your table appropriately, but avoid cluttering it up with too many lines. Alternatively, for computer-generated tables, the use of boxes is more appropriate, to avoid confusion.

Saving space in tables – you may be able to omit a column of control data if your results can be expressed as percentages of the corresponding control values.

Table 38.1 Selected properties of elements in the Periodic Table

Element	Symbol	Atomic number	Relative atomic mass**	Atomic radius (pm)	Important oxidation states	Density ($g\,mL^{-1}$)	Compounds in ores
Aluminium	Al	13	26.98	143	+3	2.7	Al_2O_3
Cobalt	Co	27	58.93	125	+2, +3	8.7	CoAsS, $CoAs_2$, CoS
Iron	Fe	26	55.84	126	+2, +3	7.9	Fe_2O_3, Fe_3O_4, $FeCO_3$, FeS_2
Manganese	Mn	25	54.94	126	+2, +3, +4, +7	7.2	MnO_2, Mn_2O_3, Mn_3O_4, mixed oxides
Zinc	Zn	30	65.38	138	+2	7.1	ZnS, ZnO, $ZnCO_3$

* Determined at 28 °C.
** Relative to the atomic mass of ^{12}C (= 12).

Headings and sub-headings

These should identify each set of data and show the units of measurement, where necessary. Make sure that each column is wide enough for the headings and for the longest data value.

Numerical data

Examples Quote the radius of an atom as 0.126 nm or 126 pm, rather than 0.000 000 000 126 m or 0.126×10^{-9} m (or 126×10^{-12} m). However, some texts still use the angstrom, Å. An angstrom is equivalent to 10^{-10} m; hence the radius of an atom would be 1.26 Å, in this example.

Within the table, do not quote values to more significant figures than necessary, as this will imply spurious accuracy. By careful choice of appropriate units for each column you should aim to present numerical data within the range 0 to 1 000. As with graphs, it is less ambiguous to use derived SI units, with the appropriate prefixes, in the headings of columns and rows, rather than quoting multiplying factors as powers of 10. Alternatively, include exponents in the main body of the table (see Table 7.1), to avoid any possible confusion regarding the use of negative powers of 10.

Other notations

Saving further space – in some instances a footnote can be used to replace a whole column of repetitive data.

Avoid using dashes in numerical tables, as their meaning is unclear; enter a zero reading as '0' and use 'NT' for not tested or 'ND' if no data value was obtained, with a footnote to explain each abbreviation. Other footnotes, identified by asterisks, superscripts or other symbols in the table, may be used to provide relevant experimental detail (if not given in the text) and an explanation of column headings and individual results, where appropriate. Footnotes should be as condensed as possible. Table 38.1 provides an example.

Statistics

In tables where the dispersion of each data set is shown by an appropriate statistical parameter, you must state whether this is the (sample) standard deviation, the standard error (of the mean) or the 95% confidence limits and you must give the value of n (the number of replicates). Other descriptive statistics should be quoted with similar detail, and hypothesis-testing statistics should be quoted along with the value of P (the probability). Details of any test used should be given in the legend, or in a footnote.

Text

Sometimes a table can be a useful way of presenting textual information in a condensed form (see example on p. 256).

Box 38.1 Checklist for preparing a table

Every table should have the following components:

1. **A title**, plus a reference number and date where necessary.
2. **Headings for each column and row**, with appropriate units of measurement.
3. **Data values**, quoted to the nearest significant figure and with statistical parameters, according to your requirements.
4. **Footnotes** to explain abbreviations, modifications and individual details.
5. **Rulings to emphasize groupings** and distinguish items from each other.

Using microcomputers and word processing packages – these can be used to prepare high-quality versions of tables for project work (p. 313).

When you have finished compiling your tabulated data, carefully double-check each numerical entry against the original information, to ensure that the final version of your table is free from transcriptional errors. Box 38.1 gives a checklist for the major elements of constructing a table.

39 Hints for solving numerical problems

Chemistry, and in particular physical and analytical chemistry, often requires a numerical or statistical approach. Not only is mathematical modelling an important aid to understanding, but computations are often needed to turn raw data into meaningful information or to compare them with other data sets. Moreover, calculations are part of laboratory routine, perhaps required for making up solutions of known concentration (see p. 170 and below) or for the calibration of an analytical instrument (see p. 171). In research, 'trial' calculations can reveal what input data are required and where errors in their measurement might be amplified in the final result, e.g. flame atomic absorption spectrometer (see Chapter 27).

Table 39.1 Sets of numbers and operations

Sets of numbers	
Whole numbers:	0, 1, 2, 3, ...
Natural numbers:	1, 2, 3, ...
Integers:	... −3, −2, −1, 0, 1, 2, 3, ...
Real numbers:	integers and anything between (e.g. −5, 4.376, 3/16, π, $\sqrt{5}$)
Prime numbers:	subset of natural numbers divisible by 1 and themselves only (i.e. 2, 3, 5, 7, 11, 13, ...)
Rational numbers:	p/q where p (integer) and q (natural) have no common factor (e.g. 3/4)
Fractions:	p/q where p is an integer and q is natural (e.g. −6/8)
Irrational numbers:	real numbers with no exact value (e.g. π)
Infinity:	(symbol ∞) is larger than any number (technically not a number as it does not obey the laws of algebra)
Operations and symbols	
Basic operators:	+, −, × and ÷ will not need explanation; however, / may substitute for ÷, · may substitute for × or this operator may be omitted
Powers:	a^n, i.e. 'a to the power n', means a multiplied by itself n times (e.g. $a^2 = a \times a$ = 'a squared', $a^3 = a \times a \times a$ = 'a cubed'). n is said to be the index or exponent. Note $a^0 = 1$ and $a^1 = a$
Logarithms:	the common logarithm (log) of any number x is the power to which 10 would have to be raised to give x (i.e. the log of 100 is 2; $10^2 = 100$); the antilog of x is 10^x. Note that there is no log for 0, so take this into account when drawing log axes by breaking the axis. Natural or Napierian logarithms (ln) use the base e (= 2.718 28 ...) instead of 10
Reciprocals:	the reciprocal of a real number a is $1/a$ ($a \neq 0$)
Relational operators:	$a > b$ means 'a is greater (more positive) than b', $<$ means less than, $\leqslant$ means less than or equal to, and $\geqslant$ means greater than or equal to
Proportionality:	$a \propto b$ means 'a is proportional to b' (i.e. $a = kb$, where k is a constant). If $a \propto 1/b$, a is inversely proportional to b ($a = k/b$)
Sums:	$\sum x_i$ is shorthand for the sum of all x values from $i = 0$ to $i = n$ (more correctly the range of the sum is specified under the symbol)
Moduli:	$\lvert x \rvert$ signifies modulus of x, i.e. its absolute value (e.g. $\lvert 4 \rvert = \lvert -4 \rvert = 4$)
Factorials:	$x!$ signifies factorial x, the product of all integers from 1 to x (e.g. $3! = 6$). Note $0! = 1! = 1$

KEY POINT **If you have a 'block' about numerical work, practice at problem solving is especially important.**

Practising at problem solving:

- demystifies the procedures involved, which are normally just the elementary mathematical operations of addition, subtraction, multiplication and division (Table 39.1);
- allows you to gain confidence so that you don't become confused when confronted with an unfamiliar or apparently complex form of problem;
- helps you recognize the various forms a problem can take as, for example, in the different forms of titrations (Chapters 21–25 inclusive).

Steps in tackling a numerical problem

The step-by-step approach outlined below may not be the fastest method of arriving at an answer, but most mistakes occur where steps are missing, combined or not made obvious, so a logical approach is often better. Error tracing is distinctly easier when all stages in a calculation are laid out.

Have the right tools ready

Scientific calculators (p. 4) greatly simplify the numerical part of problem solving. However, the seeming infallibility of the calculator may lead you to accept an absurd result which could have arisen because of faulty key-pressing or faulty logic. Make sure you know how to use all the features on your calculator, especially how the memory works; how to introduce a constant multiplier or divider; and how to obtain an exponent (note that the 'exp' button on most calculators gives you 10^x, not 1^x or y^x; so 1×10^6 would be entered as 1 exp 6, *not* 10 exp 6).

A computer spreadsheet may be very useful in repetitive calculations or for 'what if?' case studies (see Chapter 47).

Approach the problem thoughtfully

If the individual steps have been laid out on a worksheet, the 'tactics' will already have been decided. It is more difficult when you have to adopt a strategy on your own, especially if the problem is presented in a descriptive style and it isn't obvious which equations or rules need to be applied.

Table 39.2 Simple algebra – rules for manipulating equations

If $a = b + c$, then $b = a - c$ and $c = a - b$
If $a = b \times c$, then $b = a/c$ and $c = a/b$
If $a = b^n$, then $b = a^{1/n}$ and $c = \log a / \log b$
$a^{1/n} = \sqrt[n]{a}$
$a^{-n} = 1/a^n$
$a^b \times a^c = a^{(b+c)}$ and $a^b / a^c = a^{(b-c)}$
$(a^b)^c = a^{(b \times c)}$
$a \times b = \text{antilog}(\log a + \log b)$

- Read the problem carefully as the text may give clues as to how it should be tackled. Be certain of what is required as an answer before starting.
- Analyse what kind of problem it is, which effectively means deciding which equation(s) or approach will be applicable. If this is not obvious, consider the dimensions/units of the information available and think how they could be fitted to a relevant formula. In examinations, a favourite ploy of examiners is to present a problem such that the familiar form of an equation must be rearranged (see Table 39.2 and Box 39.1). Another is to make you use two or more equations in series. If you are unsure whether a recalled formula is correct, a dimensional analysis can help: write in all the units for the variables and make sure that they cancel out to give the expected answer.
- Check that you have, or can derive, all of the information required to use your chosen equation(s). It is unusual but not unknown for examiners to supply redundant information. So, if you decide not to use some of the information given, be sure why you do not require it.
- Decide on what format and units the answer should be presented in. This is sometimes suggested to you. If the problem requires many changes in

Box 39.1 Example of using the rules of Table 39.2

Problem: if $a = (b - c)/(d + e^n)$, find e

1. Multiply both sides by $(d + e^n)$; formula becomes:
$$a(d + e^n) = (b - c)$$
2. Divide both sides by a; formula becomes:
$$d + e^n = \frac{b - c}{a}$$
3. Subtract d from both sides; formula becomes:
$$e^n = \frac{b - c}{a} - d$$
4. Raise each side to the power $1/n$; formula becomes:
$$e = \left\{\frac{b - c}{a} - d\right\}^{1/n}$$

the prefixes to units, it is a good idea to convert all data to base SI units (multiplied by a power of 10) at the outset.

- If a problem appears complex, break it down into component parts.

Present your answer clearly

The way you present your answer obviously needs to fit the individual problem. Guidelines for presenting an answer include:

Show the steps in your calculations – most markers will only penalize a mistake once and part marks will be given if the remaining operations are performed correctly. This can only be done if those operations are visible!

- Make your assumptions explicit. Most mathematical models of chemical phenomena require that certain criteria are met before they can be legitimately applied (e.g. 'assuming the sample is homogeneous ...'), while some approaches involve approximations which should be clearly stated (e.g. 'to estimate the volume of a tube, it was approximated to a cylinder with radius x and length y ...').
- Explain your strategy for answering, perhaps giving the applicable formula or definitions which suit the approach to be taken. Give details of what the symbols mean (and their units) at this point.
- Rearrange the formula to the required form with the desired unknown on the left-hand side (see Table 39.2).

Units – never write any answer without its unit(s) unless it is truly dimensionless.

- Substitute the relevant values into the right-hand side of the formula, using the units and prefixes as given (it may be convenient to convert values to SI beforehand). Convert prefixes to appropriate powers of 10 as soon as possible.
- Convert to the desired units step by step, i.e. taking each variable in turn.

Rounding off – do not round off numbers until you arrive at the final answer.

- When you have the answer in the desired units, rewrite the left-hand side and underline the answer. Make sure that the result is presented with an appropriate number of significant figures (see p. 67).

Check your answer

Having written out your answer, you should check it methodically, answering the following questions:

- Is the answer of a realistic magnitude? You should be alerted to an error if an answer is absurdly large or small. In repeated calculations, a result standing out from others in the same series should be double-checked.

- Do the units make sense and match up with the answer required? Don't, for example, present a volume in units of m^2.
- Do you get the same answer if you recalculate in a different way? If you have time, recalculate the answer using a different 'route', entering the numbers into your calculator in a different form and/or carrying out the operations in a different order.

Some reminders of basic mathematics

Errors in calculations sometimes appear because of faults in mathematics rather than computational errors. For reference purposes, Tables 39.1 and 39.2 give some basic mathematical principles that may be useful.

Exponents

Example $2^3 = 2 \times 2 \times 2 = 8$

Exponential notation is an alternative way of expressing numbers in the form a^n ('a to the power n'), where a is multiplied by itself n times. The number a is called the base and the number n the exponent (or power or index). The exponent need not be a whole number, and it can be negative if the number being expressed is less than 1. See Table 39.2 for other mathematical relationships involving exponents.

Scientific notation

Example Avogadro's number, $\approx 602\,352\,000\,000\,000\,000\,000\,000$, is more conveniently expressed as $6.023\,52 \times 10^{23}$.

In scientific notation, also known as 'standard form', the base is 10 and the exponent a whole number. To express numbers that are not whole powers of 10, the form $c \times 10^n$ is used, where the coefficient c is normally between 1 and 10. Scientific notation is valuable when you are using very large numbers and wish to avoid suggesting spurious accuracy. Thus if you write 123 000, this suggests that you know the number to ± 0.5, whereas 1.23×10^5 might give a truer indication of measurement accuracy (i.e. implied to be ± 500 in this case). Engineering notation is similar, but treats numbers as powers of 10 in groups of three, i.e. $c \times 10^0, 10^3, 10^6, 10^9$, etc. This corresponds to the SI system of prefixes (p. 70).

A useful property of powers when expressed to the same base is that when multiplying two numbers together, you simply add the powers, while if dividing, you subtract the powers. Thus, suppose you counted eight molecules in a 10^{-7} dilution, there would be 8×10^7 in the same volume of undiluted solution; if you now dilute this 500-fold (5×10^2), then the number present in the same volume would be $8/5 \times 10^{(7-2)} = 1.6 \times 10^5 = 160\,000$.

Logarithms

Example (use to check the correct use of your own calculator)
$102\,963$ as a log $= 5.012\,681$ (to six decimal places)
$10^{5.012681} = 102\,962.96$
(Note loss of accuracy due to loss of decimal places.)

When a number is expressed as a logarithm, this refers to the power n that the base number a must be raised to give that number, e.g. $\log_{10}(1000) = 3$, since $10^3 = 1000$. Any base could be used, but the two most common are 10, when the power is referred to as $\log_{10}$ or simply log, and the constant e (2.718 282), used for mathematical convenience in certain situations, when the power is referred to as $\log_e$ or ln. Where a coefficient would be used in scientific notation, then the log is not a whole number.

To obtain logs, you will need to use the log key on your calculator, or special log tables (now largely redundant). To convert back, use:

- the $\boxed{10^x}$ key, with $x =$ log value;
- the $\boxed{\text{inverse}}$ then the log key; or
- the $\boxed{y^x}$ key, with $y = 10$ and $x =$ log value.

With log tables, you will find complementary antilogarithm tables to do this.

There are many uses of logarithms in chemistry, and in particular physical chemistry, including pH ($= -\log[H^+]$), where $[H^+]$ is expressed in mol L^{-1} (p. 56), and chemical kinetics, e.g. rate constants, where a plot of ln(reactant) against time produces a straight line if the reaction is first order.

Hints for some typical problems

Calculations involving proportions or ratios

The 'unitary method' is a useful way of approaching calculations involving proportions or ratios, such as those required when making up solutions from stocks (see also Chapter 6) or as a subsidiary part of longer calculations.

- If given a value for a multiple, work out the corresponding value for a single item or 'unit'.
- Use this 'unitary value' to calculate the required new value.

Example A lab schedule states that 5 g of a compound of *M*, 220 g mol^{-1} are dissolved in 400 mL of solvent. For writing up your Experimental section, you wish to express this as mol L^{-1}.

1. If there are 5 g in 400 mL, then there are 5/400 g in 1 mL.
2. Hence, 1000 mL will contain $5/400 \times 1000$ L = 12.5 g.
3. 12.5 g = 12.5/220 mol = 0.0568 mol, so [solution] = 56.8 mmol L^{-1} (= 56.8 mol m^{-3}).

Calculations involving series

Series (e.g. used in dilutions, see also p. 20) can be of two main forms:

1. arithmetic, where the *difference* between two successive numbers in the series is a constant, e.g. 2, 4, 6, 8, 10, ...;
2. geometric, where the *ratio* between two successive numbers in the series is a constant, e.g. 1, 10, 100, 1000, 10000, ...

Note that the logs of the numbers in a geometric series will form an arithmetic series (e.g. 0, 1, 2, 3, 4, ... in the above case). Thus, if a quantity y varies with a quantity x such that the rate of change in y is proportional to the value of y (i.e. it varies in an exponential manner), a semi-log plot of such data will form a straight line. This form of relationship is relevant for chemical kinetics and radioactive decay (p. 236).

Statistical calculations

The need for long, complex calculations in statistics has largely been removed because of the widespread use of spreadsheets with statistical functions (Chapter 47) and specialized programs such as Minitab® (p. 315). It is, however, important to understand the principles behind what you are trying to do (see Chapters 40 and 41) and interpret the program's output correctly, either using the 'help' function or a reference manual.

40 Descriptive statistics

The purpose of most practical work is to observe and measure a particular characteristic of a chemical system. However, it would be extremely rare if the same value was obtained every time the characteristic was measured, or with every experimental subject. More commonly, such measurements will show variability, due to measurement error and sampling variation. Such variability can be displayed as a frequency distribution (e.g. Fig. 37.3), where the y axis shows the number of times (frequency, f) each particular value of the measured variable (Y) has been obtained. Descriptive (or summary) statistics quantify aspects of the frequency distribution of a sample (Box 40.1). You can use them to condense a large data set, for presentation in figures or tables. An additional application of descriptive statistics is to provide estimates of the true values of the underlying frequency distribution of the population being sampled, allowing the significance and precision of the experimental observations to be assessed (p. 272).

KEY POINT **The appropriate descriptive statistics to choose will depend on both the type of data, i.e. whether quantitative, ranked or qualitative (see p. 65) and the nature of the underlying frequency distribution.**

In many instances, the normal (Gaussian) distribution best describes the observed pattern, giving a symmetrical, bell-shaped frequency distribution (p. 274) for example; replicate measurements of a particular characteristic (e.g. repeated measurements of the end-point in a titration).

Three important features of a frequency distribution that can be summarized by descriptive statistics are:

1. the sample's location, i.e. its position along a given dimension representing the dependent (measured) variable (Fig. 40.1);
2. the dispersion of the data, i.e. how spread out the values are (Fig. 40.2);
3. the shape of the distribution, i.e. whether symmetrical, skewed, U-shaped, etc. (Fig. 40.3).

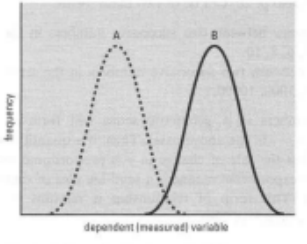

Fig. 40.1 Two distributions with different locations but the same dispersion. The data set labelled B could have been obtained by adding a constant to each datum in the data set labelled A.

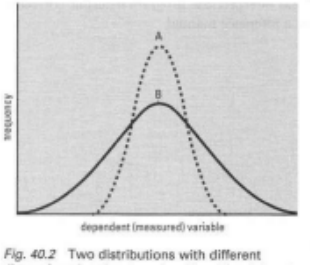

Fig. 40.2 Two distributions with different dispersions but the same location. The data set labelled A covers a relatively narrow range of values of the dependent (measured) variable while that labelled B covers a wider range.

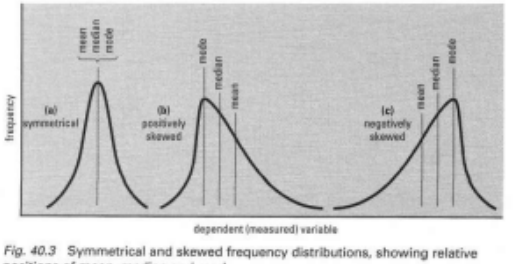

Fig. 40.3 Symmetrical and skewed frequency distributions, showing relative positions of mean, median and mode.

Box 40.1 Calculation of descriptive statistics for a sample of grouped data

Value (Y)	Frequency (f)	Cumulative frequency	fY	fY^2
1	0	0	0	0
2	1	1	2	4
3	2	3	6	18
4	3	6	12	48
5	8	14	40	200
6	5	19	30	180
7	2	21	14	98
8	0	21	0	0
Totals	$21 = \sum f\ (= n)$		$104 = \sum fY$	$548 = \sum fY^2$

In this example, for simplicity and ease of calculation, integer values of Y are used. In many practical exercises, where continuous variables are measured to several significant figures and where the number of data values is small, giving frequencies of 1 for most of the values of Y, it may be simpler to omit the column dealing with frequency and list all the individual values of Y and Y^2 in the appropriate columns. To gauge the underlying frequency distribution of such data sets, you would need to group individual data into broader classes (e.g. all values between 1.0 and 1.9, all values between 2.0 and 2.9, etc.) and then draw a histogram (p. 252).

Statistic	Value*	How calculated
Mean	4.95	$\sum fY/n$, i.e. 104/21
Median	5	Value of the $(n+1)/2$ variate, i.e. the value ranked $(21+1)/2 = 11$th (obtained from the cumulative frequency column)
Mode	5	The most common value (Y value with highest frequency)
Upper extreme	7	Highest Y value in data set
Lower extreme	2	Lowest Y value in data set
Range	5	Difference between upper and lower extremes
Variance (s^2)	1.65	$s^2 = \dfrac{\sum fY^2 - (\sum fY)^2/n}{n-1} = \dfrac{548 - (104)^2/21}{20}$
Standard deviation (s)	1.28	$\sqrt{s^2}$
Standard error (SE)	0.280	$s/\sqrt{n}$
Coefficient of variation (CoV)	25.9%	$100s/\bar{Y}$

*Rounded to appropriate significant figures.

Use of symbols – Y is used in Chapters 40 and 41 to signify the dependent variable in statistical calculations (following the example of Miller and Miller, 2000. Note, however, that some authors use X or x in analogous formulae and many calculators refer to, for example, $\bar{x}$, $\sum x^2$, etc., for their statistical functions.

Measuring location

Here, the objective is to pinpoint the 'centre' of the frequency distribution, i.e. the value about which most of the data are grouped. The chief measures of location are the mean, median and mode.

Mean

The mean (denoted $\bar{Y}$ and also referred to as the arithmetic mean) is the average value of the data. It is obtained from the sum of all the individual data values divided by the number of data values (in symbolic terms, $\sum Y/n$). The mean is a good measure of the centre of symmetrical frequency distributions of qualitative variables. It uses all of the numerical values of the sample and therefore incorporates all of the information content of the data. However, the value of a mean is greatly affected by the presence of outliers. The arithmetic mean is a widely used statistic in chemistry, but there are situations when you should be careful about using it (see Box 40.2 for examples).

Definitions

An outlier – any datum which has a value much smaller or bigger than most of the data.

Rank – the position of a data value when all the data are placed in order of ascending magnitude. If ties occur, an average rank of the tied variates is used. Thus, the rank of the datum 6 in the sequence 1,3,5,6,8,8,10 is 4; the rank of each datum with value 8 is 5.5.

Median

The median is the mid-point of the observations when ranked in increasing order. For odd-sized samples, the median is the middle observation; for even-sized samples it is the mean of the middle pair of observations. For a quantitative variable, the median may represent the location of the main body of data better than the mean when the distribution is asymmetric or when there are outliers.

Box 40.2 Three examples where simple arithmetic means are inappropriate

Mean	n
6	4
7	7
8	1

pH value	[H] (mol L^{-1})
6	1×10^{-6}
7	1×10^{-7}
8	1×10^{-8}
mean	3.7×10^{-7}
$-\log_{10}$ mean	6.43

1. If means of samples are themselves meaned, an error can arise if the samples are of different size. For example, the arithmetic mean of the means in the table shown left is 7, but this does not take account of the different 'reliabilities' of each mean due to their sample sizes. The correct weighted mean is obtained by multiplying each mean by its sample size (n) (a 'weight') and dividing the sum of these values by the total number of observations, i.e. in the case shown, $(24 + 49 + 8)/12 = 6.75$.
2. When making a mean of ratios (e.g. percentages) for several groups of different sizes, the ratio for the combined total of all the groups is *not* the mean of the proportions for the individual groups. For example, if 20 students from a batch of 50 are male, this implies 40% are male. If 60 students from a batch of 120 are male, this implies 50% are male. The mean percentage of males $(50 + 40)/2 = 45\%$ is *not* the percentage of males in the two groups combined, because there are $20 + 60 = 80$ males in a total of 170 students = 47.1% approx.
3. If the measurement scale is not linear, arithmetic means may give a false value. For example, if three media had pH values 6, 7 and 8, the appropriate mean pH is not 7 because the pH scale is logarithmic. The definition of pH is $-\log_{10}[H]$, where [H] is expressed in mol L^{-1} ('molar'); therefore, to obtain the true mean, convert data into [H] values (i.e. put them on a linear scale) by calculating $10^{(-\text{pH value})}$ as shown. Now calculate the mean of these values and convert the answer back into pH units. Thus, the appropriate answer is pH 6.43 rather than 7. Note that a similar procedure is necessary when calculating statistics of dispersion in such cases, so you will find these almost certainly asymmetric about the mean.

Mean values of log-transformed data are encountered in analytical chemistry, e.g. the particle size of the droplets formed by a nebulizer used for flame or inductively coupled plasma (ICP) spectroscopy, where log-transformed values are plotted (Figs 40.4a and b). The use of geometric means in such circumstantces serves to reduce the effects of outliers on the mean.

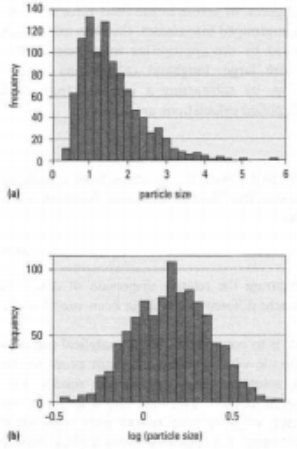

Fig 40.4 (a) An approximately log-transformed distribution: particle size of droplets in flame spectroscopy. (b) The results in (a) plotted against the logarithm of particle size.

Mode

The mode is the most common value in the sample. The mode is easily found from a tabulated frequency distribution as the most frequent value. The mode provides a rapidly and easily found estimate of sample location and is unaffected by outliers. However, the mode is affected by chance variation in the shape of a sample's distribution and it may lie distant from the obvious centre of the distribution. Note that the mode is the only statistic to make sense of qualitative data, e.g. 'the modal (most frequent) technique used in the laboratory is infrared spectroscopy'. The mean, median and mode have the same units as the variable under discussion. However, whether these statistics of location have the same or similar values for a given frequency distribution depends on the symmetry and shape of the distribution. If it is near symmetrical with a single peak, all three will be very similar; if it is skewed or has more than one peak, their values will differ to a greater degree (see Fig. 40.3).

Measuring dispersion

Here, the objective is to quantify the spread of the data about the centre of the distribution. The principal measures of dispersion are the range, variance, standard deviation and coefficient of variation.

Range

The range is the difference between the largest and smallest data values in the sample (the extremes) and has the same units as the measured variable. The range is easy to determine, but is greatly affected by outliers. Its value may also depend on sample size: in general, the larger this is, the greater will be the range. These features make the range a poor measure of dispersion for many practical purposes.

Variance and standard deviation

For symmetrical frequency distributions of quantitative data, an ideal measure of dispersion would take into account each value's deviation from the mean and provide a measure of the average deviation from the mean. Two such statistics are the sample variance, which is the sum of squared deviations from the mean ($\sum(Y-\bar{Y})^2$) divided by $n-1$ (where n is the number of data values), and the sample standard deviation, which is the positive square root of the sample variance.

The sample variance (s^2) has units which are the square of the original units, while the sample standard deviation (s) is expressed in the original units, one reason s is often preferred as a measure of dispersion. Calculating s or s^2 longhand is a tedious job and is best done with the help of a calculator or computer. If you don't have a calculator that calculates s for you, an alternative formula that simplifies calculations is:

$$s = +\sqrt{\frac{\sum Y^2 - (\sum Y)^2/n}{n-1}} \qquad [40.1]$$

Using a calculator for statistics – make sure you understand how to enter individual data values and which buttons will give the sample mean (usually shown as $\bar{X}$ or $\bar{x}$) and sample standard deviation (often shown as σ_{n-1}). In general, you should not use the population standard deviation (usually shown as σ_n).

To calculate s using a calculator:

1. Obtain $\sum Y$, square it, divide by n and store in memory.
2. Square Y values, obtain $\sum Y^2$, subtract memory value from this.
3. Divide this answer by $n-1$.
4. Take the positive square root of this value.

Take care to retain significant figures, or errors in the final value of s will result. If continuous data have been grouped into classes, the class mid-values or their squares must be multiplied by the appropriate frequencies before summation. When data values are large, longhand calculations can be simplified by coding the data, e.g. by subtracting a constant from each datum, and decoding when the simplified calculations are complete.

Coefficient of variation

The coefficient of variation (CoV) is a dimensionless measure of variability relative to location which expresses the sample standard deviation as a percentage of the sample mean, i.e:

$$\text{CoV} = 100s/\bar{Y}(\%) \qquad [40.2]$$

Relative standard deviation (RSD) is sometimes used instead of coefficient of variation.

This statistic is useful when comparing the relative dispersion of data sets with widely differing means or where different units have been used for the same or similar quantities.

A useful application of the CoV is to compare different analytical methods or procedures, so that you can decide which involves the least proportional error – create a standard stock solution, then compare the results from several sub-samples analysed by each method. You may find it useful to use the CoV to compare the precision of your own results with those of a manufacturer, e.g. for an autopipettor (p. 11). The smaller the CoV, the more precise (repeatable) is the apparatus or technique (note: this does not mean that it is necessarily more *accurate*, see p. 65).

Measuring the precision of the sample mean as an estimate of the true value

Understanding statistical symbols – note that sample statistics are given Latin character symbols while population statistics are given Greek symbols.

Most practical exercises are based on a limited number of individual data values (a sample) which are used to make inferences about the population from which they were drawn. For example, the lead content might be measured in blood samples from 100 adult females and used as an estimate of the adult female lead content, with the sample mean ($\bar{Y}$) and sample standard deviation (s) providing estimates of the true values of the underlying population mean (μ) and the population standard deviation (σ). The reliability of the sample mean as an estimate of the true (population) mean can be assessed by calculating the standard error of the sample mean (often abbreviated to standard error or SE), from:

$$\text{SE} = s/\sqrt{n} \qquad [40.3]$$

Strictly, the standard error is an estimate of the standard deviation of the means of n-sized samples from the population. At a practical level, it is clear from eqn [40.3] that the SE is directly affected by sample dispersion and inversely related to sample size. This means that the SE will decrease as the number of data values in the sample increases, giving increased precision.

Example Summary statistics for the sample mean and standard error for the data shown in Box 40.1 would be quoted as $4.95 \pm 0.280\,(n = 21)$.

Summary descriptive statistics for the sample mean are often quoted as $\bar{Y} \pm \text{SE}(n)$, with the SE being given to one significant figure more than the mean. You can use such information to carry out a t-test between two samples (Box 41.1); the SE is also useful because it allows calculation of confidence limits for the sample mean (p. 278).

Describing the 'shape' of frequency distributions

Frequency distributions may differ in the following characteristics:

- number of peaks;
- skewness or asymmetry;
- kurtosis or pointedness.

The shape of a frequency distribution of a small sample is affected by chance variation and may not be a fair reflection of the underlying population frequency distribution: check this by comparing repeated samples from the same population or by increasing the sample size. If the original shape were due to random events, it should not appear consistently in repeated samples and should become less obvious as sample size increases.

Genuinely bimodal or polymodal distributions may result from the combination of two or more unlimited distributions, indicating that more than one underlying population is being sampled (Fig. 40.5). An example of a bimodal distribution is the height of adult humans (females and males combined).

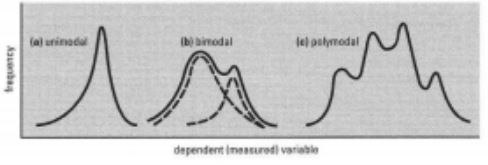

Fig. 40.5 Frequency distributions with different numbers of peaks. A unimodal distribution (a) may be symmetrical or asymmetrical. The dashed lines in (b) indicate how a bimodal distribution could arise from a combination of two underlying unimodal distributions. Note here how the term 'bimodal' is applied to any distribution with two major peaks – their frequencies do not have to be exactly the same.

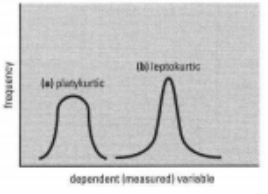

Fig 40.6 Examples of the two types of kurtosis.

A distribution is skewed if it is not symmetrical, a symptom being that the mean, median and mode are not equal (Fig. 40.3). Positive skewness is where the longer 'tail' of the distribution occurs for higher values of the measured variable; negative skewness where the longer tail occurs for lower values. Skewed distributions frequently occur in the study of chemical measurements, owing to the presence of occasional gross errors.

Kurtosis is the name given to the 'pointedness' of a frequency distribution. A platykurtic frequency distribution is one with a flattened peak, while a leptokurtic frequency distribution is one with a pointed peak (Fig. 40.6). While descriptive terms can be used, based on visual observation of the shape and direction of skew, the degree of skewness and kurtosis can be quantified and statistical tests exist to test the 'significance' of observed values, but the calculations required are complex and best done with the aid of a computer.

41 Choosing and using statistical tests

This chapter outlines the philosophy of hypothesis-testing statistics, indicates the steps to be taken when choosing a test, and discusses features and assumptions of some important tests. For details of the mechanics of tests, consult appropriate texts (e.g. Miller and Miller, 2000). Most tests are now available in statistical packages for computers (see p. 315).

To carry out a statistical test:

1. Decide what it is you wish to test (create a null hypothesis and its alternative).
2. Determine whether your data fit a standard distribution pattern.
3. Select a test and apply it to your data.

Setting up a null hypothesis

Hypothesis-testing statistics are used to compare the properties of samples either with other samples or with some theory about them. For instance, you may be interested in whether two samples can be regarded as having different means, whether the concentration of a pesticide in a soil sample can be regarded as randomly distributed, or whether soil organic matter is linearly related to pesticide recovery.

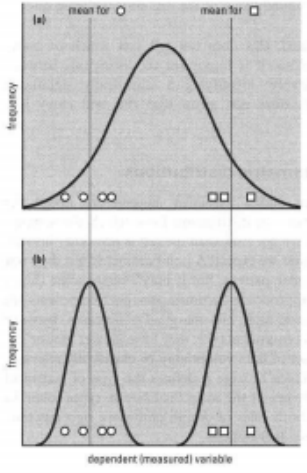

Fig. 41.1 Two explanations for the difference between two means. In case (a) the two samples happen by chance to have come from opposite ends of the same frequency distribution, i.e. there is no true difference between the samples. In case (b) the two samples come from different frequency distributions, i.e. there is a true difference between the samples. In both cases, the means of the two samples are the same.

KEY POINT **You can't use statistics to *prove* any hypothesis, but they can be used to assess *how likely* it is to be wrong.**

Statistical testing operates in what at first seems a rather perverse manner. Suppose you think a treatment has an effect. The theory you actually test is that it has no effect; the test tells you how improbable your data would be if this theory were true. This 'no effect' theory is the null hypothesis (NH). If your data are very improbable under the NH, then you may suppose it to be wrong, and this would support your original idea (the 'alternative hypothesis'). The concept can be illustrated by an example. Suppose two groups of subjects were treated in different ways, and you observed a difference in the mean value of the measured variable for the two groups. Can this be regarded as a 'true' difference? As Fig. 41.1 shows, it could have arisen in two ways:

1. Because of the way the subjects were allocated to treatments, i.e. all the subjects liable to have high values might, by chance, have been assigned to one group and those with low values to the other (Fig. 41.1a).
2. Because of a genuine effect of the treatments, i.e. each group came from a distinct frequency distribution (Fig. 41.1b).

A statistical test will indicate the probabilities of these options. The NH states that the two groups come from the same population (i.e. the treatment effects are negligible in the context of random variation). To test this, you calculate a test statistic from the data, and compare it with tabulated critical values giving the probability of obtaining the observed or a more extreme result by chance (see Boxes 41.1 and 41.2 below). This probability is sometimes called the significance of the test.

Note that you must take into account the degrees of freedom (d.f.) when looking up critical values of most test statistics. The d.f. is related to the size(s) of the samples studied; formulae for calculating it depend on the test being used. Chemists normally use two-tailed tests, i.e. we have no certainty

Definition

Modulus – the absolute value of a number, e.g. modulus −3.385 = 3.385.

beforehand that the treatment will have a positive or negative effect compared with the control (in a one-tailed test we expect one particular treatment to be bigger than the other). Be sure to use critical values for the correct type of test.

By convention, the critical probability for rejecting the NH is 5% (i.e. $P = 0.05$). This means we reject the NH if the observed result would have come up less than 1 time in 20 by chance. If the modulus of the test statistic is less than the tabulated critical value for $P = 0.05$, then we accept the NH and the result is said to be 'not significant' (NS for short). If the modulus of the test statistic is greater than the tabulated value for $P = 0.05$, then we reject the NH in favour of the alternative hypothesis that the treatments had different effects and the result is 'statistically significant'.

Two types of error are possible when making a conclusion on the basis of a statistical test. The first occurs if you reject the NH when it is true and the second if you accept the NH when it is false. To limit the chance of the first type of error, choose a lower probability, e.g. $P = 0.01$, but note that the critical value of the test statistic increases when you do this and results in the probability of the second error increasing. The conventional significance levels given in statistical tables (usually 0.05, 0.01, 0.001) are arbitrary. Increasing use of statistical computer programs is likely to lead to the actual probability of obtaining the calculated value of the test statistic being quoted (e.g. $P = 0.037$).

Choosing between parametric and non-parametric tests – always plot your data graphically when determining whether they are suitable for parametric tests as this may save a lot of unnecessary effort later.

Note that if the NH is rejected, this does not tell you which of many alternative hypotheses is true. Also, it is important to distinguish between statistical and practical significance: identifying a statistically significant difference between two samples does not mean that this will carry any chemical importance.

Comparing data with parametric distributions

A parametric test is one which makes particular assumptions about the mathematical nature of the population distribution from which the samples were taken. If these assumptions are not true, then the test is obviously invalid, even though it might give the answer we expect! A non-parametric test does not assume that the data fit a particular pattern, but it may assume some things about the distributions. Used in appropriate circumstances, parametric tests are better able to distinguish between true but marginal differences between samples than their non-parametric equivalents (i.e. they have greater 'power').

The distribution pattern of a set of data values may be chemically relevant, but it is also of practical importance because it defines the type of statistical tests that can be used. The properties of the main distribution types found in chemistry are given below with both rules of thumb and more rigorous tests for deciding whether data fit these distributions.

Binomial distributions

These apply to samples of any size from populations when data values occur independently in only two mutually exclusive classes (e.g. type A or type B). They describe the probability of finding the different possible combinations of the attribute for a specified sample size k (e.g. out of 10 samples, what is the chance of 8 being type A?). If p is the probability of the attribute being of type A and q the probability of it being type B, then the expected mean sample number of type A is kp and the standard deviation is $\sqrt{(kpq)}$. Expected frequencies can be calculated using mathematical expressions (see

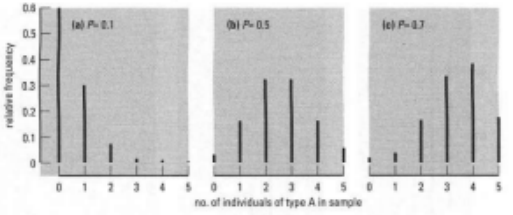

Fig. 41.2 Examples of binomial frequency distributions with different probabilities. The distributions show the expected frequency of obtaining n individuals of type A in a sample of 5. Here P is the probability of an individual being type A rather than type B.

Miller and Miller, 2000). Examples of the shapes of some binomial distributions are shown in Fig. 41.2. Note that they are symmetrical in shape for the special case $p = q = 0.5$ and the greater the disparity between p and q, the more skewed the distribution.

Chemical examples of data likely to be distributed in a binomial fashion occur when an observation or a set of trial results produce one of only two possible outcomes; for example, to determine the absence or presence of a particular pesticide in a soil sample. To establish whether a set of data is distributed in binomial fashion: calculate expected frequencies from probability values obtained from theory or observation, then test against observed frequencies using a χ^2-test or a G-test.

Tendency towards the normal distribution – under certain conditions, binomial and Poisson distributions can be treated as normally distributed:

- where samples from a binomial distribution are large (i.e. > 15) and p and q are close to 0.5;
- for Poisson distributions, if the number of counts recorded in each outcome is greater than about 15.

Poisson distributions

These apply to discrete characteristics which can assume low whole-number values, such as counts of events occurring in area, volume or time. The events should be 'rare' in that the mean number observed should be a small proportion of the total that could possibly be found. Also, finding one count should not influence the probability of finding another. The shape of Poisson distributions is described by only one parameter, the mean number of events observed, and has the special characteristic that the variance is equal to the mean. The shape has a pronounced positive skewness at low mean counts, but becomes more and more symmetrical as the mean number of counts increases (Fig. 41.3).

An example of data that we would expect to find distributed in a Poisson fashion is the number of radioactive disintegrations per unit time. One of the main uses for the Poisson distribution is to quantify errors in count data such as the number of minor accidents in the chemical laboratory over the course of an academic year. To decide whether data are Poisson distributed:

- Use the rule of thumb that if the coefficient of dispersion ≈ 1, the distribution is likely to be Poisson.
- Calculate 'expected' frequencies from the equation for the Poisson distribution and compare with actual values using a χ^2-test or a G-test.

Definition

Coefficient of dispersion $= s^2/\bar{Y}$. This is an alternative measure of dispersion to the coefficient of variation (p. 268)

It is sometimes of interest to show that data are *not* distributed in a Poisson fashion. If $s^2/\bar{Y} > 1$, the data are 'clumped' and occur together more than would be expected by chance; if $s^2/\bar{Y} < 1$, the data are 'repulsed' and occur together less frequently than would be expected by chance.

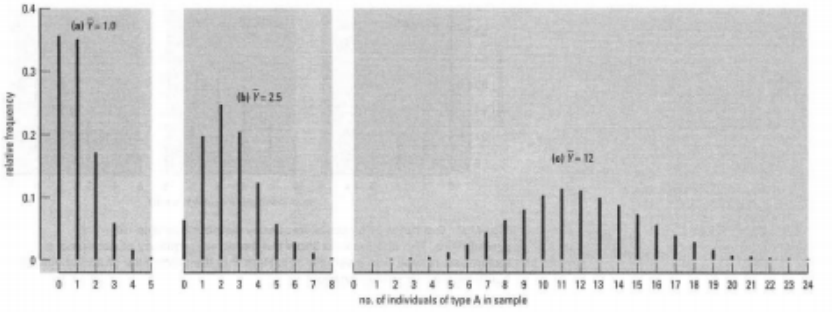

Fig. 41.3 Examples of Poisson frequency distributions differing in mean. The distributions are shown as line charts because the independent variable (events per sample) is discrete.

Normal distributions (Gaussian distributions)

These occur when random events act to produce variability in a continuous characteristic (quantitative variable). This situation occurs frequently in chemistry, so normal distributions are very useful and much used. The bell-like shape of normal distributions is specified by the population mean and standard deviation (Fig. 41.4): it is symmetrical and configured such that 68.27% of the data will lie within ± 1 standard deviation of the mean, 95.45% within ± 2 standard deviations of the mean, and 99.73% within ± 3 standard deviations of the mean.

Some chemical examples of data likely to be distributed in a normal fashion are pH of natural waters; melting point of a solid compound. To check whether data come from a normal distribution, you can:

- Use the rule of thumb that the distribution should be symmetrical and that nearly all the data should fall within $\pm 3s$ of the mean and about two-thirds within $\pm 1s$ of the mean.
- Plot the distribution on normal probability graph paper. If the distribution is normal, the data will tend to follow a straight line (see Fig. 41.5).

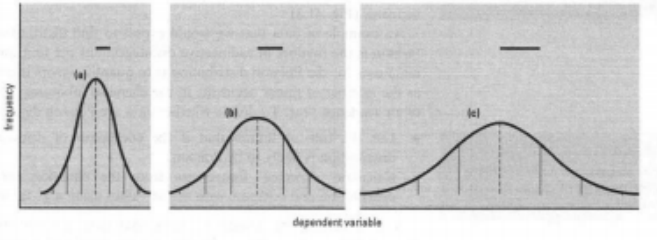

Fig. 41.4 Examples of normal frequency distributions differing in mean and standard deviation. The horizontal bars represent population standard deviations for the curves, increasing from (a) to (c). Vertical dashed lines are population means, while vertical solid lines show positions of values ± 1, 2 and 3 standard deviations from the means.

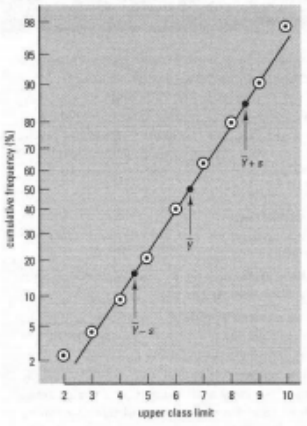

Fig. 41.5 Example of a normal probability plot. The plotted points are from a small data set where the mean $\bar{Y} = 6.93$ and the standard deviation $s = 1.895$. Note that values corresponding to 0% and 100% cumulative frequency cannot be used. The straight line is that predicted for a normal distribution with $\bar{Y} = 6.93$ and $s = 1.895$. This is plotted by calculating the expected positions of points for $\bar{Y} \pm s$. Since 68.3% of the distribution falls within these bounds, the relevant points on the cumulative frequency scale are $50 \pm 34.15\%$; thus this line was drawn using the points (4.495, 15.85) and (8.285, 84.15) as indicated on the plot.

Deviations from linearity reveal skewness and/or kurtosis (see p. 269), the significance of which can be tested statistically (see Miller and Miller, 2000).

- Use a suitable statistical computer program to generate predicted normal curves from the $\bar{Y}$ and s values of your sample(s). These can be compared visually with the actual distribution of data and can be used to give 'expected' values for a χ^2-test or a G-test.

The wide availability of tests based on the normal distribution and their relative simplicity means you may wish to transform your data to make them more like a normal distribution. Table 41.1 provides transformations that can be applied. The transformed data should be tested for normality as described above before proceeding – don't forget that you may need to check that transformed variances are homogeneous for certain tests (see below).

A very important theorem in statistics, the central limit theorem, states that as sample size increases, the distribution of a series of means from any frequency distribution will become normally distributed. This fact can be used to devise an experimental or sampling strategy that ensures that data are normally distributed, i.e. using means of samples as if they were primary data.

Choosing a suitable statistical test

Comparing location (e.g. means)

If you can assume that your data are normally distributed, the main test for comparing two means from independent samples is Student's t-test (see Boxes 41.1 and 41.2, and Table 41.2). This assumes that the variances of the data sets are homogeneous. Tests based on the t-distribution are also available for comparing paired data or for comparing a sample mean with a chosen value.

When comparing means of two or more samples, analysis of variance (ANOVA) is a very useful technique. This method also assumes data are normally distributed and that the variances of the samples are homogeneous. The samples must also be independent (e.g. not sub-samples). The nested types of ANOVA are useful for letting you know the relative importance of different sources of variability in your data. Two-way and multi-way ANOVAs are useful for studying interactions between treatments.

Table 41.1 Suggested transformations altering different types of frequency distribution to the normal type. To use, modify data by the formula shown; then examine effects with the tests described on p. 272–275.

Type of data; distribution suspected	Suggested transformation(s)
Proportions (including percentages); binomial	arcsine $\sqrt{x}$ (also called the angular transformation)
Scores; Poisson	$\sqrt{x}$ or $\sqrt{(x + 1/2)}$ if zero values present
Measurements; negatively skewed	x^2, x^3, x^4, etc. (in order of increasing strength)
Measurements; positively skewed	$1/\sqrt{x}$, $\sqrt{x}$, $\ln x$, $1/x$ (in order of increasing strength)

Box 41.1 How to carry out a *t*-test

The t-test was devised by a statistician who used the pen-name 'Student', so you may see it referred to as Student's t-test. It is used when you wish to decide whether two samples come from the same population or from different ones (Fig. 41.1). The samples might have been obtained from two different sources or by applying two different treatments to an originally homogeneous population.

The null hypothesis (NH) is that the two groups can be represented as samples from the same overlying population (Fig. 41.1a). If, as a result of the test, you accept this hypothesis, you can say that there is no significant difference between the groups.

The alternative hypothesis is that the two groups come from different populations (Fig. 41.1b). By rejecting the NH as a result of the test, you can accept the alternative hypothesis and say that there is a significant difference between the samples, or, where an experiment has been carried out, that the two treatments affected the samples differently.

How can you decide between these two hypotheses? On the basis of certain assumptions (see below), and some relatively simple calculations, you can work out the probability that the samples came from the same population. If this probability is very low, then you can reasonably reject the NH in favour of the alternative hypothesis, and if it is high, you will accept the NH.

To find out the probability that the observed difference between sample means arose by chance, you must first calculate a 't value' for the two samples in question. Some computer programs (e.g. Minitab®) provide this probability as part of the output, otherwise you can look up statistical tables (e.g. Table 41.2). These tables show 'critical values' – the borders between probability levels. If your value of t exceeds the critical value for probability P, you can reject the NH at this probability ('level of significance').

Note that:

- for a given difference in the means of the two samples, the value of t will get larger the smaller the scatter within each data set; and
- for a given scatter of the data, the value of t will get larger, the greater the difference between the means.

So, at what probability should you reject the NH? Normally, the threshold is arbitrarily set at 5% – in scientific literature you often see descriptions like 'the sample means were significantly different ($P < 0.05$)'. At this 'significance level' there is still up to a 5% chance of the t value arising by chance, so about 1 in 20 times, on average, the conclusion will be wrong. If P turns out to be lower, then this kind of error is much less likely.

Tabulated probability levels are generally given for 5%, 1% and 0.1% significance levels (see Table 41.2). Note that this table is designed for 'two-tailed' tests, i.e. where the treatment or sampling strategy could have resulted in either an increase *or* a decrease in the measured values. These are the most likely situations you will deal with in science.

Examine Table 41.2 and note the following:

- The larger the size of the samples (i.e. the greater the 'degrees of freedom'), the smaller t needs to be to exceed the critical value at a given significance level.
- The lower the probability, the greater t needs to be to exceed the critical value.

The mechanics of the test

A calculator that can work out means and standard deviations is helpful.

1. **Work out the sample means $\bar{Y}_1$ and $\bar{Y}_2$ and calculate the difference between them $(\bar{Y}_1 - \bar{Y}_2)$.**
2. **Work out the sample standard deviations s_1 and s_2.** (Note: if your calculator offers a choice, chose the '$n - 1$' option for calculating s – see p. 267.)
3. **Work out the sample standard errors $SE_1 = s_1/\sqrt{n_1}$ and $SE_2 = s_2/\sqrt{n_2}$; now square each, add the squares together, then take the square root of this** (n_1 and n_2 are the respective sample sizes, which may, or may not, be equal) $\sqrt{(SE_1)^2 + (SE_2)^2}$.
4. **Calculate t** from the formula:

$$t = \frac{\bar{Y}_1 - \bar{Y}_2}{\sqrt{(SE_1)^2 + (SE_2)^2}} \qquad [41.1]$$

   The value of t can be negative or positive, depending on the values of the means; this does not matter and you should compare the modulus (absolute value) of t with the values in tables.
5. **Work out the degrees of freedom** $= (n_1 - 1) + (n_2 - 1)$.
6. **Compare the t value with the appropriate critical value in a table**, e.g. Table 41.2.

Box 41.2 provides a worked example – use this to check that you understand the above procedures.

Assumptions that must be met before using the test

The most important assumptions are:

- The two samples are independent and randomly drawn (or if not, drawn in a way that does not create bias). The test assumes that the samples are quite large.
- The underlying distribution of each sample is normal. This can be tested with a special statistical test, but a rule of thumb is that a frequency distribution of the data should be (a) symmetrical about the mean and (b) nearly all of the data should be within 3 standard deviations of the mean and about two-thirds within 1 standard deviation of the mean (see p. 274).
- The two samples should have equal variances. This again can be tested (by an F-test), but may be gauged from inspection of the two standard deviations.

Box 41.2 Worked example of a *t*-test

Suppose the following data were obtained in an experiment (the units are not relevant):

Control: 6.6, 5.5, 6.8, 5.8, 6.1, 5.9
Treatment: 6.3, 7.2, 6.5, 7.1, 7.5, 7.3

Using the steps outlined in Box 41.1, the following values are obtained (denoting control with subscript 1, treatment with subscript 2):

1. $\bar{Y}_1 = 6.1167$; $\bar{Y}_2 = 6.9833$: difference between means $= \bar{Y}_1 - \bar{Y}_2 = -0.8666$
2. $s_1 = 0.49565$; $s_2 = 0.47504$
3. $SE_1 = 0.49565/2.44949 = 0.202348$
   $SE_2 = 0.47504/2.44949 = 0.193934$
4. $$t = \frac{-0.8666}{\sqrt{(0.202348^2 + 0.193934^2)}} = \frac{-0.8666}{0.280277} = -3.09$$
5. d.f. $= (5 + 5) = 10$
6. Looking at Table 41.2, we see that the modulus of this t value exceeds the tabulated value for $P = 0.05$ at 10 degrees of freedom ($= 2.23$). We therefore reject the NH, and conclude that the means are different at the 5% level of significance. If the modulus of t had been < 2.23, we would have accepted the NH. If the modulus of t had been > 3.17, we could have concluded that the means are different at the 1% level of significance.

Table 41.2 Critical values of Student's *t* statistic (for two-tailed tests). Reject the null hypothesis at probability P if your calculated t value exceeds the value shown for the appropriate degrees of freedom $= (n_1 - 1) + (n_2 - 1)$

Degrees of freedom	Critical values for $P = 0.05$	Critical values for $P = 0.01$	Critical values for $P = 0.001$
1	12.71	63.66	636.62
2	4.30	9.92	31.60
3	3.18	5.84	12.94
4	2.78	4.60	8.61
5	2.57	4.03	6.86
6	2.45	3.71	5.96
7	2.36	3.50	5.40
8	2.31	3.36	5.04
9	2.26	3.25	4.78
10	2.23	3.17	4.59
12	2.18	3.06	4.32
14	2.14	2.98	4.14
16	2.12	2.92	4.02
20	2.09	2.85	3.85
25	2.06	2.79	3.72
30	2.04	2.75	3.65
40	2.02	2.70	3.55
60	2.00	2.66	3.46
120	1.98	2.62	3.37
∞	1.96	2.58	3.29

Checking the assumptions of a test – always acquaint yourself with the assumptions of a test. If necessary, test them before using the test.

For data satisfying the ANOVA requirements, the least significant difference (LSD) is useful for making planned comparisons among several means. Any two means that differ by more than the LSD will be significantly different. The LSD is useful for showing on graphs.

The chief non-parametric tests for comparing locations are the Mann–Whitney *U*-test and the Kolmogorov–Smirnov test. The former assumes that the frequency distributions of the data sets are similar, whereas the latter makes no such assumption. In the Kolmogorov–Smirnov test, significant differences found with the test may be due to differences in location or shape of the distribution, or both.

Suitable non-parametric comparisons of location for paired quantitative data (sample size ≥ 6) include Wilcoxon's signed rank test, which assumes that the distributions have similar shape.

Non-parametric comparisons of location for three or more samples include the Kruskal–Wallis *H*-test. Here, the two data sets can be unequal in size, but again the underlying distributions are assumed to be similar.

Comparing dispersions (e.g. variances)

If you wish to compare the variances of two sets of data that are normally distributed, use the *F*-test. For comparing more than two samples, it may be sufficient to use the F_{max}-test, on the highest and lowest variances. The Scheffé–Box (log-ANOVA) test is recommended for testing the significance of differences between several variances. Non-parametric tests exist but are not widely available: you may need to transform the data and use a test based on the normal distribution.

Determining whether frequency observations fit theoretical expectation

The χ^2-test is useful for tests of 'goodness of fit', e.g. comparing expected and observed progeny frequencies in genetical experiments or comparing observed frequency distributions with some theoretical function. One limitation is that simple formulae for calculating χ^2 assume that no expected number is less than five. The *G*-test (*2I* test) is used in similar circumstances.

Comparing proportion data

When comparing proportions between two small groups (e.g. whether 3/10 is significantly different from 5/10), you can use probability tables such as those of Finney *et al.* (1963) or calculate probabilities from formulae; however, this can be tedious for large sample sizes. Certain proportions can be transformed so that their distribution becomes normal.

Placing confidence limits on an estimate of a population parameter

On many occasions, sample statistics are used to provide an estimate of the population parameters. It is extremely useful to indicate the reliability of such estimates. This can be done by putting a confidence limit on the sample statistic. The most common application is to place confidence limits on the mean of a sample from a normally distributed population. This is done by working out the limits as $\bar{Y} - (t_{P[n-1]} \times SE)$ and $\bar{Y} + (t_{P[n-1]} \times SE)$ where $t_{P[n-1]}$ is the tabulated critical value of Student's t statistic for a two-tailed test with $n - 1$ degrees of freedom and SE is the standard error of the mean (p. 268). A 95% confidence limit (i.e. $P = 0.05$) tells you that on average, 95 times out of 100, this limit will contain the population mean.

Confidence limits for statistics other than the mean – consult an advanced statistical text (e.g. Miller and Miller, 2000) if you wish to indicate the reliability of estimates of, for example, population variances.

Regression and correlation

These methods are used when testing relationships between samples of two variables. If one variable is assumed to be dependent on the other then regression techniques are used to find the line of best fit for your data. This does not tell you how well the data fit the line: for this, a correlation coefficient must be calculated. If there is no a priori reason to assume dependency between variables, correlation methods alone are appropriate.

If graphs or theory indicate a linear relationship between a dependent and an independent variable, linear regression can be used to estimate the

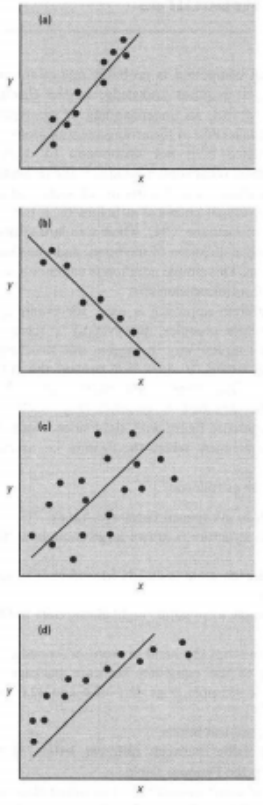

Fig. 41.6 Examples of correlation. The linear regression line is shown. In (a) and (b), the correlation between x and y is good: for (a) there is a positive correlation and the correlation coefficient would be close to 1; for (b) there is a negative correlation and the correlation coefficient would be close to -1. In (c) there is a weak positive correlation and r would be close to 0. In (d) the correlation coefficient may be quite large, but the choice of linear regression is clearly inappropriate.

equation that links them. If the relationship is not linear, a transformation may give a linear relationship. For example, this is sometimes used in analysis of chemical kinetics. However, 'linearizations' can lead to errors when carrying out regression analysis: take care to ensure (i) that the data are evenly distributed throughout the range of the independent variable and (ii) that the variances of the dependent variable are homogeneous. If these criteria cannot be met, weighting methods may reduce errors. In this situation, it may be better to use non-linear regression using a suitable computer program.

Model I linear regression is suitable for experiments where a dependent variable Y varies with an *error-free* independent variable X and the mean (expected) value of Y is given by $a + bX$. This might occur where you have carefully controlled the independent variable and it can therefore be assumed to have zero error (e.g. a calibration curve). Errors can be calculated for estimates of a and b and predicted values of Y. The Y values should be normally distributed and the variance of Y constant at all values of X.

Model II linear regression is suitable for experiments where a dependent variable Y varies with an independent variable X which has an error associated with it and the mean (expected) value of Y is given by $a + bX$. This might occur where the experimenter is measuring two variables and believes there to be a causal relationship between them; both variables will be subject to errors in this case. The exact method to use depends on whether your aim is to estimate the functional relationship or to estimate one variable from the other.

A correlation coefficient measures the strength of relationships but does not describe the relationship. These coefficients are expressed as a number between -1 and 1. A positive coefficient indicates a positive relationship while a negative coefficient indicates a negative relationship (Fig. 41.6). The nearer the coefficient is to -1 or 1, the stronger the relationship between the variables, i.e. the less scatter there would be about a line of best fit (note that this does not imply that one variable is dependent on the other!). A coefficient of 0 implies that there is no relationship between the variables. The importance of graphing data is shown by the case illustrated in Fig. 41.6d.

Pearson's product-moment correlation coefficient (r) is the most commonly used correlation coefficient. If both variables are normally distributed, then r can be used in statistical tests to test whether the degree of correlation is significant. If one or both variables are not normally distributed you can use Kendall's coefficient of rank correlation (τ) or Spearman's coefficient of rank correlation (r_S). They require that data are ranked separately and calculation can be complex if there are tied ranks. Spearman's coefficient is said to be better if there is uncertainty about the reliability of closely ranked data values.

42 Drawing chemical structures

Fig. 42.1 Methane.

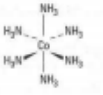

Fig. 42.2 Structure of hexaamminecobaltate.

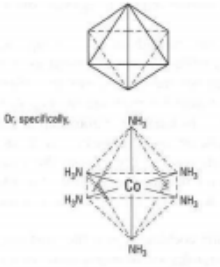

Fig. 42.3 An octahedron.

Drawing the structure of a chemical compound is probably one of the first basic requirements of any chemist. It requires knowledge of the chemical composition of the structure to be drawn, an understanding of the type of bonding, and frequently a mental visualization of the arrangement of atoms (or ions). Once this has been assimilated it is not uncommon to draw a representation of the structure on paper. What is often lacking is the realization that the molecule should be represented in three dimensions. To some extent it is possible to represent a three-dimensional chemical structure on a piece of paper. Fig. 42.1 shows the structure of methane, CH_4, where standard symbols e.g. the hatched line, are used to imply a direction of the bond, and one that is different to, for example, the solid line. This simple notation is commonly used to give a molecule the perception of three-dimensionality.

In co-ordination chemistry, a different approach is used; for example, in the structure for hexaamminecobaltate complex, $[Co(NH_3)_6]^{3+}$, there are several alternative approaches. The simplest way to express this structure is shown in Fig. 42.2. While this is useful, it does not convey the three-dimensional aspects of the molecule. Therefore, an alternative approach to expressing the visualization of this structure is possible (Fig. 42.3): the complex is an octahedron, i.e. a geometric figure with eight sides. Note that the octahedron has six points of attachment where the ligands, i.e. ammine (NH_3) groups, are attached.

The general points to remember are as follows:

- Always draw chemical structures in ink (pencil fades with time).
- Always ensure that the chemical structure is drawn large enough, so that no ambiguity is possible.
- If drawing by hand, ensure that each atom is clearly identified. This may require the use of coloured pens.
- Try and keep the structure as simple as possible, highlighting only the key features.
- Any text should appear in a clear script (by hand or word processed).
- When possible, it is advisable to use computer software packages to generate chemical structures. For example, Figs 42.1–42.4 and 42.6 were generated using ChemWindow®.
- Always indicate the number of chemical bonds.
- Make sure no confusion is possible between different letters of the alphabet representing elements in the Periodic Table.
- If necessary, show the number of electrons (pairs or individual electrons) clearly. Remember, a . (full stop) may be mistaken for a mark in the paper.
- It may be necessary to indicate the structural formula of a molecule, e.g. in isomerism.

Selected examples of drawing chemical structures

Lewis structures

When drawing Lewis structures it is important to show the position and number of electrons. For example, the Lewis structure for CO_3^{2-} is shown in Fig. 42.4a. Note the position of the double bond. Also, both carbon and

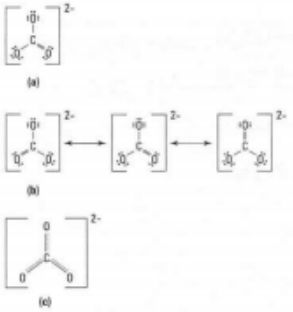

Fig. 42.4 Lewis diagrams for CO_3^{2-}.

oxygen obey the octet rule, i.e. the number of electrons around each atom adds up to eight (a single bond is composed of two electrons, a double bond of four electrons). Lewis structures sometimes give rise to canonical forms. The three possible canonical forms for CO_3^{2-} are shown in Fig. 42.4b. It is noted that as the position of the double bond moves the number of lone pairs of electrons changes. In reality, experimental evidence indicates that the C–O bond in carbonate is composed of neither single nor double bonds but is intermediate in bond length and strength. A more appropriate method of representing this structure is by delocalization of bonding electrons (Fig. 42.4c).

Ionic structures

In solid-state chemistry it is often necessary to draw a 'unit cell'. The most commonly found are the hexagonal close-packed (HCP) crystal structure and cubic close-packed (CCP) crystal structure. In the HCP structure atoms are arranged in an ABABA repeating pattern, while in the CCP structure the arrangement is an ABCABCA pattern. In both cases it is difficult to represent the structures without resorting to the drawing of circles. Fig. 42.5 shows the close-packing arrangement for both the HCP and CCP crystal structures. In the HCP structure the first and third layers of atoms are orientated in the same direction (directly above one another) while in the CCP structure, the first and third layers do not coincide, i.e. no atom in the third layer is directly above an atom in the first layer.

It is usually not recommended to attempt drawing ionic structures of greater complexity without resorting to a specialized computer-based drawing package.

Common chemical drawing packages:

ChemDraw (url: http://www.camsoft.com)
ChemWindow® (URL: http://www.softshell.com)
Isis/Draw (URL: http://www.mdli.com)

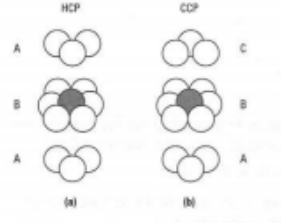

Fig. 42.5 Close-packing arrangement for (a) hexagonal close packing (ABABA etc.) and (b) cubic close packing (ABCABCA etc.).

Isomerism

The importance of drawing chemical structures can be further illustrated by considering isomerism. Isomers are substances that have the same molecular formula but different structural formulae. Different types of isomerism are found in chemistry, e.g. geometric, optical, etc. Fig. 42.6 shows the geometric isomers of dichlorocyclopropane, i.e. *cis*-dichlorocyclopropane and *trans*-dichlorocyclopropane.

KEY POINT **It is often important when drawing chemical structures to impart some structural identity.**

Atomic orbitals

You may need to draw a visualization of atomic orbitals, usually the s, p and d orbitals. This can be simplified by the use of Cartesian co-ordinates which allow a three-dimensional representation on paper. This is neither easy to replicate or often necessary. A simplified approach is, for example, to replace the spherical s orbital with a circle (Fig. 42.7). Similarly, the three p orbitals can be represented in two dimensions by the use of correct labelling of the axes (Fig. 42.7). Finally, the same approach can be replicated on the five d orbitals (Fig. 42.7). It is worthwhile remembering that the d_{xy}, d_{xz} and d_{yz} orbitals do not reside on the axes (x, y or z), but in the plane of their respective axes. In addition, the $d_{x^2-y^2}$ orbital occupies the x- and y-axes and the d_{z^2} orbital occupies the z-axis.

Electronic configuration

It is often difficult to recollect the order of filling of the electronic structures of atoms of different elements. Fig. 42.8 shows the usual order of filling of

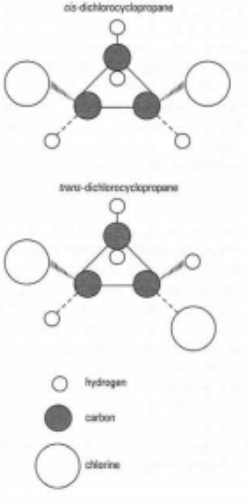

Fig. 42.6 Geometric isomers of dichlorocyclopropane.

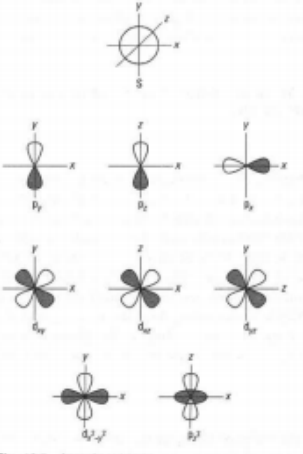

Fig. 42.7 Atomic orbitals.

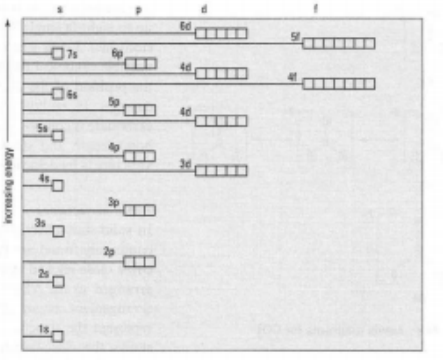

Fig. 42.8 Electronic configuration.

Fig. 42.9 Mnemonic for electronic configuration determination based on the Aufbau principle.

the orbitals of an atom according to the Aufbau principle (lowest energy first). However, a simple mnemonic exists to facilitate the correct order for each element in the Periodic Table (Fig. 42.9). In order to use the mnemonic all you need to remember is that:

- s orbitals can have up to $2\,e^-$
- p orbitals can have up to $6\,e^-$
- d orbitals can have up to $10\,e^-$
- f orbitals can have up to $14\,e^-$.

Then, it is simply a case of addition. In general terms, the order of electron filling is as follows (simply translating the mnemonic into an order):

1s 2s 2p 3s 3p 4s 3d 4p 5s 4d 5p 6s 4f 5d 6p 7s ...

For example, the atomic number of calcium is 20 (corresponding to 20 electrons). Therefore the electronic configuration is:

$1s^2\,2s^2\,2p^6\,3s^2\,3p^6\,4s^2$

Valence shell electron pair repulsion (VSEPR) theory

This is used to predict the shape of molecules. In order to be able to predict the geometry (or shape) of a molecule several simple steps are required. For example, consider the case of BrF_3. In this case we have indicated by

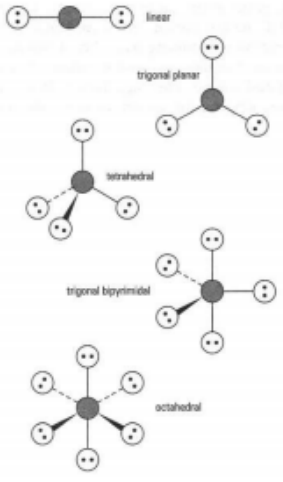

Fig. 42.10 Geometries of molecules.

underlining that the central atom is bromine. The first step is to determine the number of valence electrons for bromine; this is done by establishing the electron configuration for bromine, i.e. $1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 4s^2\ 3d^{10}\ 4p^5$. We can determine that the number of outer electrons is seven (from $4s^2$ and $4p^5$). We then determine how many atoms are attached to the central bromine atom – the answer is three. By simple addition we have $7 + 3 = 10$ electrons. We know that two electrons are required per bond; therefore we have enough electrons for five bonds. Then, it is simply the case to determine a geometry that allows for five bonds to be at the maximum distance from each other. Commonly, five different arrangements of atoms are found: linear, trigonal planar, tetrahedral, trigonal bipyramidal and octahedral. These arrangements are shown in Fig. 42.10. We can therefore see that the geometry of BrF_3 is trigonal bipyramidal. VSEPR also works for anions and cations using the same procedure. For a cation, a positively charged species, simply deduct one electron from the total; similarly for an anion, a negatively charged species, add one electron to the total. In all cases the total number of electrons obtained will be an even number.

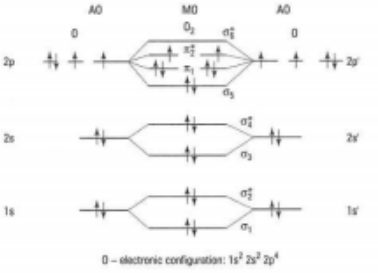

Fig. 42.11 Molecular orbital diagram for oxygen.

Molecular orbital diagrams

Molecular orbital diagrams (see Fig. 42.11) are required in the study of chemical bonding. For a diatomic molecule these consist of two atomic orbitals (AOs) and two molecular orbitals (MOs). In the case of the two MOs, one is the bonding MO and the other the anti-bonding MO. An asterisk (*) is used to represent an anti-bonding MO. Bonding MOs (of lower energy) are always occupied first. Two types of MO are shown, σ and π, using oxygen, O_2, as an example. The first task is to determine the electronic configuration for each oxygen atom, i.e. $1s^2\ 2s^2\ 2p^4$. An outline of the MO diagram is constructed such that one AO is located on the left-hand side of the page and the other on the right-hand side (the use of ′ indicates that a different AO is represented) with the MOs positioned in the centre (bonding MOs in the lowest position). You then have an MO diagram composed of three different energy levels corresponding to the 1s, 2s and 2p orbitals. Then, by simply adding the correct number of electrons into the two AOs first, total up the number of electrons. Electrons are then placed into the MO in the following sequence: lowest available position first; and then, individual electrons prior to pairing of electrons. The completed diagram is shown in Fig. 42.11 where all solid lines (in both the

AO and MO positions) represent places where up to two electrons can be paired up (dashed lines simply indicate association with a particular AO). Arrows (↑↓) indicate that the electrons are spinning (paired electrons have opposite spins). The numbering of bonding and anti-bonding MOs is merely for numerical sequencing and has no other significance. It is also worth noting that O_2 has unpaired electrons in the anti-bonding MO, i.e. π_2^*.

43 Chemometrics

Chemometrics has been defined as the chemical discipline that uses mathematical and statistical methods to design or select optimal measurement procedures and experiments and to provide maximum chemical information by analysing chemical data (Kowalski, 1978). It is a relatively new discipline that assists with (i) the planning of experiments, and (ii) the manipulation and interpretation of large data sets. Some aspects of chemometrics can be done using an appropriate speadsheet but the majority of applications require the use of dedicated software. The fundamental principles of most of the processes involved in chemometrics are those of statistics. You are therefore advised to become familiar with the material in Chapters 40 and 41 before proceeding.

When carrying out any experimental work, e.g. an undergraduate practical, you should always read the entire practical script before starting the experimentation. This is important as it allows you to plan each step of the process and to organize space and time to perform the experiment. This initial planning is further complicated in project work and research projects when, often, there is no laboratory script to follow. In these situations, you finally come down to planning the initial experiments after background research (e.g. reading the appropriate scientific literature on the subject area to be investigated), purchasing/obtaining the appropriate chemicals/reagents, etc. (see also Box 10.1). It is at this stage that chemometrics can be of some assistance. Assuming that you are able to identify the dependent variables in the experiment, then you can apply 'experimental design' which allows you to gain the maximum amount of knowledge about the system you are investigating from a limited number of experiments.

Once the experimental work has been completed you then need to consider how to interpret the results, i.e. how to maximize the chemical information inherent in the data. Initial attempts are often centred around plotting the data, to visualize trends and to allow conclusions to be drawn. The simplest form of data visualization is simply to tabulate the results (Chapter 38). As an example, if a class of students has determined the melting point of naphthalene, it is a relatively simple matter to tabulate the data (see Table 43.1). One possibility for the data is then to calculate the mean and standard deviation. Another approach would be to plot the data as a histogram, as in Fig. 43.1, so we are then able to make a visual interpretation of the quality of this univariate (one-variable) data.

However, what if we had more than one variable to consider? In other words, we have multivariate data. For example, what if we want to identify trends in the properties of a range of organic molecules? The variables we might want to consider could be: melting point, boiling point, M_r, solubility in a solvent and vapour pressure. We can, of course, tabulate the data, as before, but this does not allow us to consider any trends in the data. To do this we need to be able to plot the data. However, once we exceed three variables (which we need to be able to plot in three dimensions) it becomes impossible to produce a straightforward plot. It is in this context that chemometrics offers a solution, reducing the dimensionality to a smaller number of dimensions and hence the ability to display multivariate data. The most important technique in this context is called principal component analysis (PCA).

Table 43.1 Melting points for naphthalene

Student	m.pt. (°C)
A	79
B	81
C	80
D	77
E	80
F	80
G	79
H	76
I	81
J	80
K	79
L	78
M	82
N	81

Mean melting point of naphthalene is 79.5 °C.

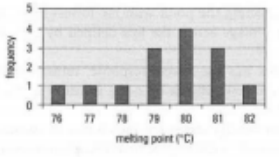

Fig. 43.1 Histogram: melting point of naphthalene.

Useful sources of information

Journals:

Annual Review of Physical Chemistry
Chemical Physics
Chemical Physics Letters
International Journal of Quantum Chemistry
Journal of Chemical Physics
Journal of Computational Chemistry
Journal of Molecular Structure/Theochem
Journal of Physical Chemistry
Reviews in Computational Chemistry
Theoretica Chimica Acta

Useful web sites:

Brookhaven databank (protein structures)
http:\\www.pdb.bnl.gov

Cambridge Crystallographic Data Centre
http:\\csdvx2.ccdc.cam.ac.uk\

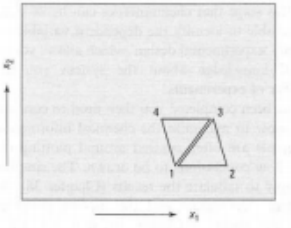

Fig. 43.2 Simplex optimization.

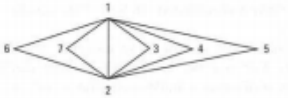

Fig. 43.3 Step-size simplex.

The following discussion highlights only the basic principles. For more detailed information you are advised to consult the literature and dedicated chemometric software packages. It should always be borne in mind, however, that the choice of which variables to optimize should be selected (i) by someone with prior knowledge of the system/instrument under investigation, or (ii) after performing preliminary experiments to determine which are the most important variables.

Experimental design

There are two main multivariate optimization strategies: those based on sequential designs and those based on simultaneous designs.

Sequential design

Sequential optimization is based on the one-at-a-time approach. The major limitation of this approach is that it assumes that no interaction effects occur between the variables. Unfortunately this is not always the case. A sequential design strategy involves carrying out a few experiments at a time and using the results of those experiments to determine the next experiment to be done. The best known of the sequential design approaches is called the simplex method. A simplex is essentially a geometric figure having a number of vertices equal to one more than the number of variables. For example, if we have two variables, the simplex is a triangle, three variables a tetrahedron, and so on.

Let us consider the case of two variables, x_1 and x_2. An algorithm describes the initial simplex to be performed (Fig. 43.2). By performing experiments 1–3, described by the initial simplex, and recording their responses, the next set of experiments can be described. If we obtain the lowest response for experiment 2, it can therefore be assumed that a higher response would be obtained in the opposite direction. By reflecting point 2, we can obtain point 4. By performing the experiment described by point 4 we obtain its response, thereby perpetuating the simplex.

Four rules can be described for a simplex design:

1. A new simplex is formed by rejecting the point with the lowest response and replacing it with its mirror image across the line defined by the two remaining points.
2. If the new point in the simplex has the lowest response, return to the preceding simplex and create the new simplex by using instead the point with the second-lowest response.
3. If a point is retained in three consecutive simplexes, then it can be assumed that an optimum has been reached. (Note: it may be that this optimum is not the true optimum, but that the simplex has been trapped at a false optimum. In this situation, it is necessary to start the simplex again, or use a modified simplex in which the step size is not fixed but variable, see Fig. 43.3.)
4. If a point is suggested by the simplex algorithm that is beyond the limit of the variables, i.e. it is beyond the safe working limits of an instrument, then the point is rejected and an artificially low response is assigned to it, and the simplex is continued with rules 1–3.

Simultaneous design

In a simultaneous approach the relationship between variables and results is studied as follows: carry out an appropriate design, apply a mathematical model to the design, and then apply a response surface method to the data.

Appropriate designs might be based on factorial designs (full or fractional) or a central composite design. Response surface methods frequently rely on visualization of the data for interpretation.

Factorial design

In general terms, consider the case of two variables at two levels, e.g. a high value and a low value. This is termed a two-level design or a (full) 2^k factorial design, where k is the number of variables. Therefore we have $2^2 = 4$ experiments to be done. Often the values of the variables are coded; this is done for convenience purposes only. In this example, high and low values will be coded as (+) and (−). Alternatively, it might be the case that the number of variables is three. In this situation we would have a 2^3 factorial design, requiring eight experiments. An example of a two-level factorial design is shown in Box 43.1.

Table 43.2 Two-level factorial design

Experiment	Volume of solvent	salt	pH	Result
1	−1	−1	−1	Y_1
2*	+1	−1	−1	Y_2
3	+1	+1	−1	Y_3
4*	−1	+1	−1	Y_4
5*	−1	−1	+1	Y_5
6	+1	−1	+1	Y_6
7	+1	+1	+1	Y_7
8*	−1	+1	+1	Y_8

The limitation of the two-level factorial design approach is that no estimation of curvature can be determined. In order to take this into account the use of designs with at least three levels is required. Three-level designs are therefore often known as response surface designs. Probably the most important design in this context is the central composite design (CCD). Central composite designs consist of a full (or fractional) factorial design onto which is superimposed a star design. The number of experiments to be done (R) can be worked out as follows:

$$R = 2^k + 2k + n_0 \qquad [43.1]$$

Box 43.1 Example of a two-level factorial design

The recovery of phenols by liquid–liquid extraction, from 1 litre of river water, and subsequent analysis by high-performance liquid chromatography is to be optimized. It has been determined that the following are critical to achieving an optimum extraction: the volume of extraction solvent, the mass of salt added and the pH of the water sample. Therefore, a 2^3 design is required. The experimental levels are: volume of extraction solvent (5 and 50 mL); mass of salt added (0.0 and 1.0 g); and pH (4 and 7). The coded values for the experiment are shown in Table 43.2. The +1 values represent the higher value, e.g. pH 7, while −1 represents the lower value, e.g. pH 4. The number of experiments can be reduced if a fractional factorial design is used. For example, in this situation the fractional factorial design would become $2^{3-1} = 4$ experiments. In this situation the experiments labelled with an asterisk in Table 43.2 would be done.

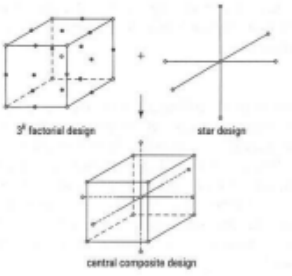

Fig. 43.4 Central composite design.

where k is the number of variables, and n_0 is the number of experiments in the centre of the design.

For a design with three variables we would require $[2^3 + (2 \times 3) + 1] = 15$ experiments. In order to obtain repeatability information it is necessary to run an experiment several times. This is done by performing the centre point experiment twice. The total number of experiments would therefore be 16. The list of experiments is shown in Table 43.3 while Fig. 43.4 shows a diagrammatic representation of the CCD. The CCD is composed of a 3^k factorial design superimposed with a star design ($+\alpha$, $-\alpha$). In order to minimize systematic eror (bias) it is necessary randomize the experimental run order. This is shown in Table 43.4.

Response surface methodology

Response surface methodology allows the relationship between the responses and variables to be quantified, using a mathematical model, and to be visualized. Thus the equation for a straight-line graph can be written as:

$$y = mx + c \qquad [43.2]$$

Table 43.3 Central composite design for three variables

Experiment	Variable 1	Variable 2	Variable 3	Result
Factorial design, 2^3				
1	−1	−1	−1	Y_1
2	+1	−1	−1	Y_2
3	+1	+1	−1	Y_3
4	−1	+1	−1	Y_4
5	−1	−1	+1	Y_5
6	+1	−1	+1	Y_6
7	+1	+1	+1	Y_7
8	−1	+1	+1	Y_8
Star design, 2*k*				
9	$-\alpha$	0	0	Y_9
10	$+\alpha$	0	0	Y_{10}
11	0	$-\alpha$	0	Y_{11}
12	0	$+\alpha$	0	Y_{12}
13	0	0	$-\alpha$	Y_{13}
14	0	0	$+\alpha$	Y_{14}
Centre points				
15	0	0	0	Y_{15}
16	0	0	0	Y_{16}

where m is a constant and c is the intercept. This describes the relationship between a single variable (x) and its response (y). Using the previous example, with three variables (x_1, x_2 and x_3) it is possible to extend this mathematical model.

First of all we can consider how each of the variables influences the response (y) in a linear manner. However, the relationship between y and x_1, x_2 and x_3 may not be linear, so it is necessary to consider the possibility of curvature. This is done in terms of a quadratic variable, i.e. a squared dependence (x_1^2, x_2^2 and x_3^2). Finally, it is also important to consider the effects of possible interactions between the variables, $x_1 \rightarrow x_2 \rightarrow x_3$, i.e. x_1x_2, x_1x_3 and x_2x_3. The overall general equation can therefore be written as:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_1^2 + b_5x_2^2 + b_6x_3^2 + b_7x_1x_2 + b_8x_1x_3 + b_9x_2x_3 \qquad [43.3]$$

Table 43.4 A typical randomized CCD

Original experiment (from Table 43.3)	(New) Experiment run order	Variable 1	Variable 2	Variable 3	Result
8	1	−1	+1	+1	Y_1
3	2	+1	+1	−1	Y_2
14	3	0	0	+α	Y_3
6	4	+1	−1	+1	Y_4
12	5	0	+α	0	Y_5
13	6	0	0	−α	Y_6
4	7	−1	+1	−1	Y_7
1	8	−1	−1	−1	Y_8
7	9	1	1	1	Y_9
15	10	0	0	0	Y_{10}
10	11	+α	0	0	Y_{11}
16	12	0	0	0	Y_{12}
9	13	−α	0	0	Y_{13}
2	14	+1	−1	−1	Y_{14}
5	15	−1	−1	+1	Y_{15}
11	16	0	−α	0	Y_{16}

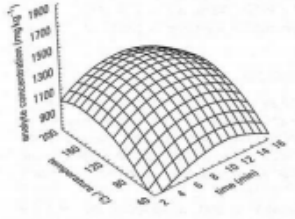

Fig. 43.5 Example of a response surface.

where b_0 is the intercept parameter and $b_1 - b_9$ are the regression coefficients for linear, quadratic and interaction effects.

This equation can be analysed using multiple linear regression and tested for statistical significance at, for example, the 95% confidence interval (see p. 278). In addition, the response can be explored by plotting a three-dimensional graph. Unfortunately, in the above example, three variables are present. This immediately constrains what it is possible to plot on the graph (one of the axes must be the response). One way to select the two variables to plot is by considering their statistical significance and then selecting two variables which are significant at the 95% confidence interval. An alternative approach might be simply to plot the two variables you might wish to discuss in your experimental report. A typical response surface is shown in Fig. 43.5. It can be seen that the 'time' variable has a maximum at 8–12 min while the 'temperature' variable has a maximum at 160–180 °C. Further experiments might be carried out at these two maxima to determine the repeatability of the approach. However, it is necessary to plot all variables consecutively to identify all maxima.

In general, it is important to consider the following issues when carrying out an experimental design:

- Carry out repeat measurements for a particular combination of variables, to determine the repeatability of the approach.
- To remove systematic error (bias), you should randomize the order in which experiments are done (p. 66).
- It is important to eliminate intervariable effects (confounding), i.e. the situation where one variable is inter-related to another.
- Often, the large number of experiments to be carried out makes it impossible to run all of them on the same day. If this happens run your experiments in discrete groups or 'blocks'.

Principal component analysis

The use of modern automated instrumentation allows the acquisition of large amounts of chemical data. As well as simply tabulating the data, other forms

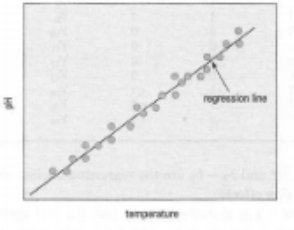

Fig. 43.6 Determination of principal component 1.

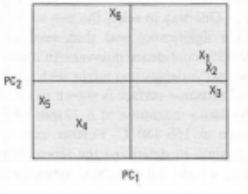

Fig. 43.7 Principal component analysis.

of 'analysis' are required to interrogate the chemical information contained within the data. One such approach, enabling the simplification of large data sets by reducing the number of independent variables, is principal component analysis (PCA). The basis of this approach is:

- To reduce the number of original independent variables into new axes, so-called 'principal components', PCs, each of which can be estimated unambiguously. The data contained in these new PCs, and which are expressed as 'scores', are uncorrelated with each other.
- To express, in a few PCs, the amount of variation in the data.
- To have each new PC express a decreasing amount of variation.

An example of the application of PCA is shown in Box 43.2.

Box 43.2 Example of principal component analysis

Consider a chemical reaction where reactants, X and Y, produce product Z. The yield of Z is dependent upon the temperature of the reaction and its pH. And suppose that the reaction has been carried out by a class of students, providing a large amount of data. By plotting temperature against pH (Fig. 43.6), we can identify a single new variable, PC_1, which obviously contains aspects of pH and temperature, i.e.

$$PC_1 = a(\text{temperature}) + b(\text{pH}) \qquad [43.4]$$

PC_1 can then be used to replace the original two variables. In addition, the direction of PC_1 indicates where the greatest variation in the data lies. The other information that can be obtained is the scatter of the data on either side of the regression line. This is due to random variation rather than a trend. In this example therefore it is not possible to extract any further PCs.

In eqn [43.4], the coefficients a and b indicate the relative importance of the two variables to PC_1, and are called factor loadings. In general, therefore, the plotting of factor loadings between any two PCs can provide useful information as to the relationship of variables to the PCs. Fig. 43.7 shows such a plot. It is seen that variables 1–3 contribute strongly (positively) and variable 5 (negatively) to PC_1. Variable 6 contributes strongly to PC_2, whereas variable 4 contributes significantly to both PCs.

44 Computational chemistry

A chemist is normally visualized as someone in a white laboratory coat performing an experiment. However, some chemists never actually go into a 'wet chemical' laboratory but utilize computers to perform experiments.

Why do chemistry on computers?

- Safety: invariably all laboratory experiments carry some risk, associated with the chemicals or apparatus to be used. It is usual to perform a COSHH assessment (p. 7) prior to experimental work. Computational chemistry allows the user to carry out work on 'dangerous' chemicals with no risk!
- Cost: apart from the financial outlay on a computer and associated peripherals together with the appropriate software, no further costs are involved, unlike the experimental laboratory where most chemicals require disposal after use.
- Understanding: computational chemistry has the ability to provide a basis for understanding chemical principles.

Computational chemistry uses commercially available software to enhance chemical knowledge.

This branch of chemistry, sometimes referred to as theoretical chemistry, molecular modelling or computational chemistry, used to be restricted to a few researchers with access to expensive computers. This has changed in recent years with the availability of low-cost, high-power computers, coupled with the availability of software packages that require no knowledge of computer programming, making computational chemistry accessible to undergraduate students. The new user of computational chemistry will quickly discover the ease with which it is possible to observe complex molecules on the screen using the software's computer graphics (Fig. 44.1). However, computational chemistry is much more than pretty pictures. It allows the chemist to perform theoretical experiments in three distinct areas:

1. single molecule calculations
2. molecular interactions
3. reactions of molecules.

Definitions

An **ab initio method** is a quantum-mechanical approach which attempts to calculate, from first principles, solutions to the Schrödinger wave equation.

Molecular mechanics describes a system in which the energy depends only on the nuclei present.

Quantum mechanics describes a molecular system in which both electrons and nuclei are involved.

A **semi-empirical method** is a type of quantum-mechanical theory that incorporates information derived from experimental data.

In all cases, the basis of the calculation is the determination of the energy of the system. Two approaches are used: quantum mechanics and molecular mechanics. Molecular mechanics is best suited to large molecules, e.g. proteins, whereas quantum mechanics, while offering a more fundamental approach, is restricted to smaller molecules.

KEY POINT **Just as in spectroscopy or chromatography, where not every spectrum or peak is resolved, so in computational chemistry do not assume every computed number is exact. However, computational chemistry can allow a qualitative or approximate insight into chemical processes provided the user understands the basis behind each approach and can interpret the results.**

Quantum mechanics

A quantum-mechanical calculation commences with the Schrödinger wave equation:

$$H\Psi = \varepsilon\Psi \qquad [44.1]$$

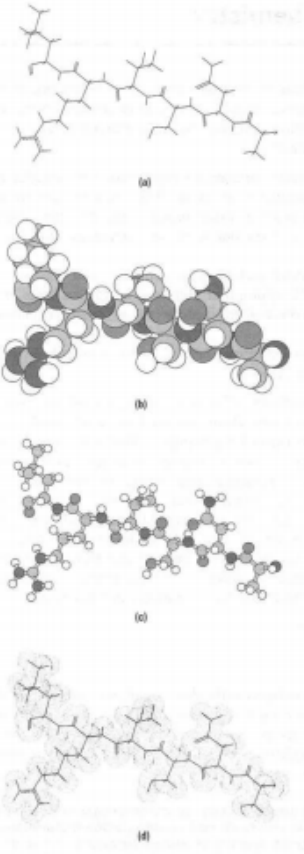

Figure 44.1 Computer generated images of six amino acids using different representations (a) stick, (b) balls, (c) balls and cylinders and (d) sticks and dots.

where H is called the Hamiltonian operator (it describes the kinetic energies of the nuclei and electrons and the electrostatic interactions felt between individual particles), ε is the energy of the system, and Ψ is called a wavefunction – Ψ^2 describes the probability of finding an electron at a particular set of co-ordinates.

The equation is used to describe the behaviour of an atom or molecule in terms of its wave-like (or quantum) nature. By trying to solve the equation the energy levels of the system are calculated. However, the complex nature of multielectron/nuclei systems is simplified using the Born–Oppenheimer approximation. Unfortunately it is not possible to obtain an exact solution of the Schrödinger wave equation except for the simplest case, i.e. hydrogen. Theoretical chemists have therefore established approaches to find approximate solutions to the wave equation. One such approach uses the Hartree–Fock self-consistent field method, although other approaches are possible. Two important classes of calculation are based on *ab initio* or semi-empirical methods. *Ab initio* literally means 'from the beginning'. The term is used in computational chemistry to describe computations which are not based upon any experimental data, but based purely on theoretical principles. This is not to say that this approach has no scientific basis – indeed the approach uses mathematical approximations to simplify, for example, a differential equation. In contrast, semi-empirical methods utilize some experimental data to simplify the calculations. As a consequence semi-empirical methods are more rapid than *ab initio*.

Molecular mechanics

Most molecular modelling packages allow the use of empirical methods which only consider the nuclei. These are called molecular mechanical methods and are faster than the quantum-mechanical methods. They are based on classical mechanics and therefore allow treatment of larger molecules. However, as electrons are not included in the calculation, this approach does not provide information on bond breaking or formation, or any details of orbitals involved in any interactions.

The computational laboratory

Like all laboratory classes it is important to go prepared so that you will get the most out of your time in the laboratory. This might include background reading, making an outline of the experimental procedure, a sketch (or photocopy) of the chemical structure of all molecules to be worked on, and a plan of how you will draw each molecule – obviously the more complex molecules may require more thought than a simple molecule. Once there, it is important to:

- Get a comfortable chair – you may be sitting in it for quite a while. Make yourself feel at ease and relax.
- Plan your work – a considerable amount of time in the laboratory will be spent constructing models, setting up calculations and evaluating results. It is therefore important to maximize your time on the computer by planning in advance.
- Follow all instructions carefully – remember that a computer carries out your instructions.
- Examine all results carefully – do not accept everything the computer prints out/displays. Question the results yourself – do they make sense? If not recheck your initial data entry.

- Save all your results for rechecking by yourself at a later date or for assessment by your tutor.

Computer software

A typical software package used to perform computational chemistry should be able to:

- Build and display molecules.
- Optimize the structure of molecules.
- Investigate the reactivity of molecules.
- Generate and view orbitals and electronic plots.
- Evaluate chemical pathways and mechanisms.
- Study the dynamic behaviour of molecules.

It is inappropriate to describe any particular software system. Nevertheless, all software is usually accompanied by a user manual or a 'help' file to make the use of commercial software packages user-friendly.

Applications

Computational chemistry is being applied to a wide variety of areas, in particular its ability to predict chemical properties, such as:

- molecular structure
- reaction enthalpies
- dipole moments and infrared intensities
- vibrational frequencies
- reaction free energies
- relative acid constants
- reaction rates.

In addition, computational chemistry is widely applied in drug design in the pharmaceutical industry, where it can allow the modeller to investigate whether a molecule will bind tightly to an enzyme or receptor. In addition, it is possible to assess whether a molecule will fit into an active site of an enzyme, how well the molecule will bind, and, ultimately, to assess whether it is possible to design the 'best' molecule.

Resources for analysis and presentation of data

Books on statistics and chemometrics

Main supplementary text:

Miller, J.N. and Miller, J.C. (2000) *Statistics and Chemometrics for Analytical Chemistry*, 4th Edn, Prentice Hall, Harlow, Essex.

Other useful sources (chronological order):

Adams, M.J. (1995) *Chemometrics in Analytical Chemistry*, Royal Society of Chemistry, Cambridge.

De Levie, R. (2001) *How to Use Excel in Analytical Chemistry and in General Scientific Data Analysis*, Cambridge University Press.

Doggett, G. and Sutcliffe, B.T. (1995) *Mathematics for Chemistry*, Longman, Harlow, Essex.

Farrant, T.J. (1997) *Practical Statistics for the Analytical Chemist*, Royal Society of Chemistry, Cambridge.

Gardiner, W.P. (1997) *Statistical Analysis Methods for Chemists. A software-based approach*, Royal Society of Chemistry, Cambridge.

Gormally, J. (2000) *Essential Mathematics for Chemists*, Prentice Hall, Harlow, Essex.

Massart, D.L., Vandeginste, B.G.M., Buydens, L.M.C., de Jong, S., Lewi, P.J. and Smeyers-Verbeke, J. (1997) *Handbook of Chemometrics and Qualimetrics: Part A*, Elsevier Science B.V., Amsterdam.

Meier, P.C. (2000) *Statistical Methods in Analytical Chemistry*, 2nd Edn, John Wiley and Sons Ltd, Chichester.

Books on computational chemistry

Main supplementary text:

Goodman, J. (1999) *Chemical Applications of Molecular Modelling*, Royal Society of Chemistry, Cambridge.

Other useful sources (chronological order):

Allen, M.P. and Tildesley, D.J. (1991) *Computer Simulation of Liquids*, Clarendon, Oxford.

Brooks, C.L., Karplus, M. and Pettitt, B.M. (1991) *Proteins: A theoretical Perspective of Dynamics, Structure and Thermodynamics*, Wiley-Interscience, New York.

Clark, T. (1985) *A Handbook of Computational Chemistry*, Wiley-Interscience, New York.

Comba, P. and Hambley, T.W. (2000) *Molecular Modelling of Inorganic Compounds*, John Wiley and Sons Ltd, Chichester.

Foresman, J.B. and Frisch, A.E. (1993) *Exploring Chemistry with Electronic Structure Methods*, Gaussian Inc., Pittsburgh.

Grant, G.H. and Richards, W.G. (1995) *Computational Chemistry*, Oxford University Press, Oxford.

Haile, J.M. (1992) *Molecular Dynamics Simulation: Elementary Methods*, John Wiley and Sons, New York.

Hehre, W.J., Radom, L., Schleyer, P.V.R. and Pople, J.A. *Ab Initio Molecular Orbital Theory*, Wiley-interscience, New York.

Hehre, W.J., Shusterman, A.J. and Huang, W.W. (1996) *A Laboratory Book of Computational Organic Chemistry*, Wavefunction Inc., Irvine.

Hehre, W.J., Shusterman, A.J. and Nelson, J.E. (1998) *Molecular Modelling, Workbook for Organic Chemistry*, Wiley, New York.

Jensen, F. (1999) *Introduction to Computational Chemistry*, Wiley, London.

Jensen, P. and Bunker, P. (2000) *Computational Molecular Spectroscopy*, John Wiley and Sons Ltd, Chichester.

Leach, A.R. (1996) *Molecular Modelling, Principles and Applications*, Longman, Harlow.

McCammon, J.A. and Harvey, S.C. (1987) *Dynamics of Proteins and Nucleic Acids*, Cambridge University Press, New York.

Schlecht, M.F. (1998) *Molecular Modelling on the PC*, Wiley-VCH, New York.

Szabo, A. and Ostlund, N.S. (1989) *Modern Quantum Chemistry. Introduction to Advanced Electronic Structure Theory*, McGraw-Hill Publishing Co., New York.

Software for drawing chemical structures

ChemDraw, Adept Scientific plc, Letchworth, Herts, UK.

ChemSketch, Advanced Chemical Development, Inc., Toronto, Canada [www.acdlabs.com].

Software for statistics and chemometrics

MultiSimplex, Karlskrona, Sweden (http://www.multisimplex.com).

Statistica, 6.0, Statsoft (http://www.statsoft.com).

Statistics for the Analytical Chemist Softbook, Royal Society of Chemistry, Cambridge.

Information technology and library resources

45 The Internet and World Wide Web

Definitions

Browser – a program to display web pages and other Internet resources.

FAQ – Frequently Asked Question; sometimes used as a file extension (.faq) for downloadable files.

FTP – File Transfer Protocol; means of downloading files.

URL – Uniform Resource Locator: the 'address' for WWW resources.

Making the most of information and communication technology ('ICT') requires skills related to the use of computers for finding, retrieving, recording, analysing and communicating information, especially via the developing global Internet environment. This includes using programs for:

- searching databases and Internet resources, e.g. using Web 'browsers' such as Netscape® and Internet Explorer®;
- retrieving network resources, e.g. applying FTP to obtain copies of files;
- storing, modifying and analysing information, e.g. using databases, spreadsheets and statistical packages (see Chapters 47 and 48);
- communicating information, e.g. by e-mail, word processors, desktop publishing packages and programs for making presentations.

The Internet as a global resource

The key to the rapid development of the Internet was the evolution and expansion of networks – collections of computers which can communicate with each other. They operate at various scales such as local area networks (LANs) and wide area networks (WANs) and these can be connected to the Internet, which is a complex network of networks (Fig. 45.1). The Internet is loosely organized; no one group runs it or owns it. Instead, many private organizations, universities and government departments pay for and run discrete parts of it. Private organizations include commercial online service providers such as America Online® and CompuServe®.

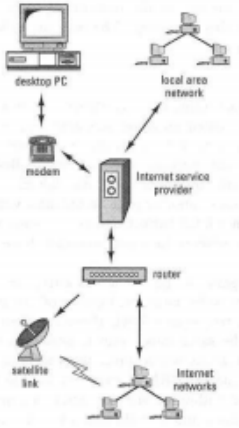

Fig. 45.1 Diagram of a network system.

You can gain access to the Internet either through a LAN at your place of work or from home via a modem connected to a dial-in service provider over the telephone line. You do not need to understand the technology of the network to use it – most of it is invisible to the user. However, if you do wish to understand more, sources such as Gralla (1996) and Winship and McNab (2000) are recommended. What you do need to know are the options available to you in terms of using its facilities and their relative merits or disadvantages. You also need to understand a little about the nature of Internet addresses.

KEY POINT **Most material on the Internet has not been subject to peer review, vetting or editing. Information obtained from the WWW or posted on newsgroups may be inaccurate, biased or spoof; do not assume that everything you read is true or even legal.**

Communicating on the Internet: e-mail and newsgroups

Using e-mail, you are able to send messages to anyone who is connected to the Internet, directly or indirectly. You can attach text files, data files, pictures, video clips, sounds and executable files to your messages. The messages themselves are usually only very simple in format but sufficient for most purposes: formatted material can be attached as a file if necessary. The uses that can be made of the system vary from personal and business related to the submission of work to a tutor in an educational system. Note, however, that the system is not secure (confidential) and the transmission of

Junk and chain mail on the Internet – do not get involved in distributing junk or chain mail (the electronic equivalent of chain letters). This activity is likely to be against your institute's rules for the use of its computer facilities because it can foul up the operation of networks.

Examples Common domains and sub-domains include:

.com	commercial (USA mainly)
.edu	education (USA mainly)
.gov	government (USA only)
.mil	military (USA only)
.net	network companies
.org	organization
.uk	United Kingdom

sensitive information should be done with caution. Another downside of e-mail is the junk mail that you may receive once your e-mail address is distributed, e.g. in newsletters.

Specific address information is required to exchange information and mail between computers. Although the computer actually uses a complex series of numbers for this purpose (the 'I.P.' address), the Domain Name System/Service (DNS) was developed to make this easier for users. Each computer on the Internet is given a domain name (= Internet address) which is a hierarchy of lists and addresses. Thus, 'unn.ac.uk' is the DNS-registered name of the University of Northumbria at Newcastle: the top (root) level domain is 'uk', identifying its country as the United Kingdom, and the next is 'ac', identifying the academic community sub-domain. The final sub-domain is 'unn' identifying the specific academic institute. For the purposes of e-mail, the names of individuals at that site may be added before the domain name and the @ sign is used to separate them. Thus, the e-mail address of the first author of this book is 'John.Dean@unn.ac.uk'.

Internet tools

There are various facilities available for use on the Internet depending on your own system and method of accessing the system. The best way to learn how to use them is simply to try them out.

Locating information on the WWW – useful searching systems are located at the following URLs (some may be directly accessible from your browser):

http://www.altavista.com
http://www.goto.com/
http://www.lycos.com/
http://www.webcrawler.com/
http://www.yahoo.com
http://www.google.com
http://www.askjeeves.com

The World Wide Web (WWW)

The 'web' is the most popular Internet application. It allows easy links to information and files which may be located on computers anywhere in the world. The WWW allows access to millions of 'home pages' or 'websites', the initial point of reference with companies, institutes and individuals. Besides their own text and images, these contain 'hypertext links', highlighted words or phrases that you click on to take you to another page on the same website or to a completely different site with related subject material. Certain sites specialize in such links, acting like indexes to other websites: these are particularly useful.

When using a web browser program to get to a particular page of information on the web all you require is the name and location of that page. The page location is commonly referred to as a URL (Uniform Resource Locator). The URL always takes the same basic format, beginning with 'http://' and followed by the various terms which direct the system to the resource pages. If you don't have a specific URL in mind but wish to find appropriate sites, use a 'search engine' within the browser: enter appropriate and limited key words on which to search and note the site(s) which may be of interest. There is so much available on the web, from company products through library resources to detailed information on specialist chemical and environmental topics, that much time can be spent searching and reading information: try to stay focused!

FTP (File Transfer Protocol) and file transmission

FTP is a method of transferring files across the Internet. In may cases the files are made available for 'anonymous' FTP access, i.e. you do not need previously arranged passwords. Log in as 'anonymous' and give your e-mail address as the password. Use your web browser to locate the file you want and then use its FTP software to transfer it to your computer.

KEY POINT **The transfer of files can result in the transfer of associated viruses. Always check your files for viruses before running them.**

When using ICT, including the Internet, always remember the basic rules of using computers and networks (Box 45.1).

Box 45.1 Important guidelines for using computers and networks

Hardware
- Don't drink or smoke around the computer.
- Try not to turn the computer off more than is necessary.
- Never turn off the electricity supply to the machine while in use.
- Rest your eyes at frequent intervals if working for extended periods at a computer monitor.
- Never try to reformat the hard disk except in special circumstances.

Floppy disks
- Protect floppy disks when not in use by keeping them in holders or boxes.
- Never touch a floppy disk's recording surface.
- Keep disks away from dampness, excess heat or cold.
- Keep disks well away from magnets; remember that these are present in loudspeakers, TVs, etc.
- Don't use disks from others unless first checked for viruses.
- Don't insert or remove a disk from the drive when it is operating (drive light on).
- Try not to leave a disk in the drive when you switch the computer off.

File management
- Always use virus-checking programs on imported files before running them.
- Make backups of all important files and at frequent intervals (say every half hour) during the production of your own work, e.g. when using a word processor or spreadsheet.
- Periodically clear out and reorganize your file storage areas.

Network rules
- Be polite when sending messages.
- Never attempt to 'hack' into other people's files.
- Don't play games without approval – they can hinder the operation of the system.
- Periodically reorganize your e-mail folder(s). These rapidly become filled with acknowledgements and redundant messages which reduce server efficiency.
- Remember to log out of the network when finished: others can access your files if you forget to log out.

The golden rule: always make backup copies of important disks/files and store them well away from your working copies. Be sure that the same accident cannot happen to both copies.

46 Internet resources for chemistry

A common way to find information on the Internet is by browsing ('surfing') the World Wide web (WWW). However, as this can be time consuming and wasteful, browsing should be focused on relevant sites. Many of the most useful websites are those providing detailed lists and hypertext links to other locations. A useful place to find out what the Internet offers the chemist can be found at the URL http://www.chemdex.org.

KEY POINT **Remember that the Internet should not be viewed as a substitute for your university library and other local resources, but should complement, rather than replace, more traditional printed texts and CD-ROM material.**

Revisiting websites – 'bookmark' sites of interest, so that you can return to them at a later date. Make a copy of your bookmark file occasionally, to avoid loss of relevant information.

Using Internet addresses – note that the locations given in the chapter may change as sites are updated: you can make a key-word search using a searching system or 'search engine' to find a particular website if necessary.

General information

Some of the principal resources you can utilize via the WWW are:

- Libraries, publishers and companies. These organizations recognize the significance of the Internet as a means of communication; for example, the Addison Wesley Longman higher education website at http://www.awl-he.com/ allows information on specific catalogues and books to be requested online. A large number of scientific journals are also available in electronic format *via* ingenta.com (http://www.ingenta.com). Ingenta journals offer a single point of access to over 2500 academic and professional journals from over 30 publishers. Access to browse and search the database of articles is free, as is the ability to display tables of contents, bibliographic information and abstracts. Full-text articles require a fee for access – check whether your institution subscribes. A broad range of scientific journals published by Academic Press is available *via* the site http://www.apnet.com/, and students at UK colleges and universities should have full access without additional subscription (via http://www.janet.idealibrary.com/). You can keep up to date using *New Scientist* pages (at http://www.newscientist.com/), *Scientific American* (at http://sciam.com/) or *Nature* (at http:/www.nature.com/). A few of the newer titles are available only in electronic format, e.g. the e-journal CrystEngComm can be found at http://www.rsc.org/is/journals/current/crystengcomm/ceepub.htm. Electronic publishing is likely to develop further in future years.
- Institutions. Many research organizations, societies and educational institutions around the world are now online, with their own web pages. There is a detailed list of scholarly chemical societies at http://www.chemdex.org/chemdex/learned-society.html. You can use the websites of these organizations to obtain specific information. They frequently provide hypertext links to other relevant sites for particular groups or topics: for example, the Royal Society of Chemistry web page (http://www.rsc.org) has links to various sites of interest to chemists. Use the WWW to obtain details of the activities of research organizations (e.g. GlaxoSmithKline, at http://www.glaxosmithkline.co.uk) or individual laboratories and researchers at specific universities. Some other relevant websites are given in Table 46.1.

Table 46.1 Selected examples of useful websites

American Chemical Society http://www.acs.org
The Royal Society of Chemistry http://www.rsc.org
Society of Chemical Industry http://sci.mond.org
International Union of Pure and Applied Chemistry (IUPAC) http://iupac.chemsoc.org
Laboratory of the Government Chemist http://www.lgc.co.uk
Daresbury Laboratory http://www.dl.ac.uk
Brookhaven National Laboratory http://www.chemistry.bnl.gov
United States Environmental Protection Agency http://www.epa.gov
National Institute of Standards and Technology (NIST) Laboratory http://www.cstl.nist.gov
National Institute of Standards and Technology (NIST) WebBook http://webbook.nist.gov
University of Sheffield http://www.chemdex.org
Specialized Information Services – Chemical information page on drugs, pesticides, environmental pollutants and other potential toxins http://chem.sis.nlm.nih.gov
Reactive Reports – A chemistry web magazine http://www.acdlabs.com/webzine
WebElements Periodic Table http://www.webelements.com

Table 46.2 Examples of chemical databases

Beilstein http://www.beilstein.com
Scientific and Technical Information Network http://www.fiz-karlsruhe.de/onlin_db.html
ChemExper Chemical Directory http://www.chemexper.be/main.shtml
Cambridge Crystallographic Data Centre http://www.ccdc.cam.ac.uk
The Chemical Database Service http://cds3.dl.ac.uk/cds/cds.html
National Institute of Standards and Technology (NIST) WebBook http://webbook.nist.gov
The Wired Chemist http://wulfenite.fandm.edu

- Data and pictures. Archives of text material, photographs and video clips can be accessed and easily downloaded. However, downloading graphical images may take quite a while, especially for remote links or at busy times, leading to high costs.
- Newsgroups (Usenet). News articles on a wide range of topics are 'posted' at appropriate sites, where they are placed into subject groups (newsgroups). Any user can contribute to the discussion by posting his/her own message, to be read by other users via appropriate news software. While the number of newsgroups is very large, some of them may include relevant information. Further information on topics of chemistry interest is available at http://www.chemdex.org/index.html. Student access to newsgroups may be limited at some universities and colleges.

KEY POINT **Remember that the information from Internet newsgroups and similar websites may be unedited and may represent the personal opinion of the author of the article.**

- Mailing lists. Messages sent to the list address are distributed automatically to all members of the mailing list, *via* their personal e-mailbox, keeping them up to date on the particular topic of the mailing list. To receive such messages, you will need to join the mailing list. Relevant mailing lists for chemistry can be found at http://www.chemdex.org/chemdex/listserv.html. Take care not to join too many lists, as you will receive a large number of messages, and many are likely to be of only marginal interest. A number of mailing lists also have archived files, offering a more selective means of locating relevant material.
- Databases. Sites such as Bath Information and Data Services (BIDS) and web of Science (WOS) provide access to abstracts of recent publications: use these to find relevant literature for specific topics. Access is via the websites at http://www.bids.ac.uk/ or http://www.webofscience.com; you will need a username and password – check with your department or library. In the case of BIDS, it provides access to databases covering subjects from science, engineering and medicine to economics, politics, education and the arts. Specific databases offered include: ISI citation indexes; EMBASE (international biomedical information); INSPEC (physics, electronic engineering and computing); international bibliography of the social sciences (IBSS); The Royal Society of Chemistry databases; and education databases. See also Table 46.2.

The BIDS Royal Society of Chemistry service

The BIDS RSC service provides access to four bibliographic databases and a further two databases (Chemical Safety Data Sheets and the UK Nutrient Databank via the WWW at http://www.bids.ac.uk/websearch.html) are supported by The Royal Society of Chemistry (URL: http://www.bids.ac.uk/Tfedocs/rsc.html). The databases are:

- Analytical Abstracts
- Chemical Business NewsBase
- Chemical Safety NewsBase
- Mass Spectrometry Bulletin
- Chemical Safety Data Sheets
- UK Nutrient Databank.

The BIDS RSC bibliographic databases can be searched by using:

- Words or phrases in articles, key words or abstracts
- Author names and/or addresses
- Journal names
- CAS registry numbers
- Chemical/product names
- Specialist index terms.

Information (e.g. abstracts) can be displayed, downloaded or e-mailed to your personal address. In additional, electronic full-text articles may also be available via ingenta journals (http://www.ingenta.com). This is normally offered in PDF via a viewer, e.g. Adobe Acrobat Reader.

In contrast, the Chemical Safety Data Sheet database is searched using chemical name or synonyms, while the UK Nutrient databank is searched by food name.

Analytical Abstracts (http://www.rsc.org/is/database/aahome.htm)
This is database specifically aimed at analytical chemistry. In particular, the following areas are covered: general analytical chemistry; chromatography and electrophoresis; spectrometry and radiochemical methods; inorganic, organic and organometallic analysis; applied and industrial analysis; clinical and biochemical analysis; pharmaceutical analysis, including drugs in biological fluids; environmental analysis; agricultural analysis; and, food analysis. The database is updated weekly and covers the period from 1980 onwards. The database is sourced from over 269 journals. However, a more limited number of journals are available in full-text format. A typical sample record is shown in Table 46.3.

Chemical Business NewsBase (http://www.rsc.org/is/database/cbnbhome.htm)
This provides information on the business environment of the chemical industry. It provides company, product and market information for the chemical industry and its end-use sectors worldwide. It also covers environmental issues and regulatory developments. More than 580 000 items are contained within the database since its initiation in 1985. The database is updated daily, from more than 700 sources. As well as information from journals and newspapers, information is also sourced from company reports, newsletters and press releases.

Chemical Safety NewsBase (http://www.rsc.org/is/database/csnbhome.htm)
This provides information on the health and safety effects of chemicals used in industry, and all health and safety aspects relevant to the laboratory and office environment. The database contains over 50 000 items. Each reference includes the document title, full bibliographic details and a detailed abstract. The database is updated each month with coverage of scientific journals, new books, standards, data sheets, audiovisual aids and technical reports.

Mass Spectrometry Bulletin (http://www.rsc.org/is/database/msbhome.htm)
This is a current awareness bulletin providing information on mass spectrometry and related ion processes. The bulletin is sub-divided as follows: instrument design and techniques; isotopic analysis, precision mass measurement, isotope separation, age determination, etc.; chemical analysis; organic chemistry; atomic and molecular processes; surface phenomena and solid-state studies; and thermodynamics and reaction kinetics. The database

Table 46.3 Example record from Analytical Abstracts

AA Accession No.: 60-07-H-00251 DOC. TYPE: Journal

Kinetic determination of ultratrace amounts of ascorbic acid with spectrofluorimetric detection.

AUTHOR: Feng, S. L.; Chen, X. L.; Fan, J.; Zhang, G.; Jiang, J. H.; Wei, X. J.
CORPORATE SOURCE: Dept. CHem., Henan Normal Univ., Xinxiang, Henan, 453002, China
JOURNAL: Anal. Lett., (Analytical Letters), Volume: 31, Issue: 3, Page(s): 463–474
CODEN: ANALBP ISSN: 0003-2719
PUBLICATION DATE: Feb 1998 (980200)
LANGUAGE: English

ABSTRACT: To the test solution, 1.2 mL of phosphate buffer of pH 2.6, 0.3 mL of 0.1 mM-rhodamine 6G, 1.2 mL of 10 mu.g/mL vanadium(V) solution and 1.5 mL of 0.1M-potassium bromate were added. The mixture was made up to 25 mL with H2O and allowed to stand for 4 min. The reaction was quenched by addition of 1 mL of 1m-sodium acetate and the fluorescence intensity was measured at 548.4 nm (excitation at 348.4 nm). Calibration graphs were linear for the range 1.6 to 28 ng/mL. Detection limit was 0.62 ng/mL. None of the common ions and organic compounds investigated interfered. The method was used in the analysis of pharmaceutical preparations, fruit juices and vegetables. Results were in good agreement with those obtained by the iodimetric method.

ANALYTE: ascorbic acid (50-81-7) –detmn. of, in fruit juices, pharmaceuticals and vegetables, by fluorimetry
MATRIX: pharmaceutical preparations –detmn. of ascorbic acid in, by fluorimetry
fruit juices –detmn. of ascorbic acid in, by fluorimetry
vegetables –detmn. of ascorbic acid in, by fluorimetry
SECTION: H-87000 (Environmental, Agriculture and Food)
SECTION CROSS REFERENCE: G001 (Pharmaceutical Analysis)

is updated monthly. More than 900 sources are accessed to provide up-to-date information.

Chemical Safety Data Sheets (http://www.bids.ac.uk/TFedocs/rsc_safetydata.html)
These provide safety information for over 550 common chemicals. The database is arranged alphabetically to enable easy access.

UK Nutrition Databank (http://www.rsc.org/is/database/nutshome.htm)
This provides nutrient composition data on foods consumed in the UK. The database provides data for 1188 foods covering all food groups, providing values per 100 g (or 100 mL) for over 40 nutrients. In addition, a food index, listing alternative and taxonomic names and recipe information, is also provided. The particular food groups covered include: cereals and cereal products; milk products and eggs; vegetables, herbs and spices; fruit and nuts; vegetable dishes; fish and fish products; miscellaneous foods; meat, poultry and game; meat products and dishes; and fatty acids.

The Royal Society of Chemistry: Bibliographic Databases and Reference Databanks

In addition to the BIDS RSC service, The Royal Society of Chemistry (http://www.rsc.org/is/database/sec_serv.htm) also offers a range of bibliographic databases and reference databanks. These are (excluding the ones available via the BIDS RSC service) described below.

Analytical chemistry
- Analytical Abstracts
- Chromatography Abstracts
- Mass Spectrometry Bulletin
- Window on Chemometrics.

Chemical business
- Chemicals, Formulated Products and their Company Sources
- Focus on Catalysts
- Focus on Pigments
- Focus on Polyvinyl Chloride
- Focus on Powder Coatings
- Focus on Surfactants.

Health, safety and toxicology
- Chemical Hazards in Industry
- The Dictionary of Substances and their Effects
- Environmental Chemistry, Health and Safety
- Hazards in the Office
- Laboratory Hazards Bulletin.

As an example, Chromatography Abstracts (http://www.rsc.org/is/database/chroabs.htm) provides an update of developments in separation science. It is arranged into the following sections: general and miscellaneous techniques; gas chromatography; liquid chromatography; electrophoresis; and thin-layer chromatography. An example of a typical record is shown in Table 46.4.

Table 46.4 Example record from Chromatography Abstracts

1854 Bovine whey fractionation based on cation-exchange chromatography.

Hahn, R.; Shulz, P. M.; Schaupp, C.; Jungbauer, A.J.
Chromatogr., A, 6 Feb 1998, **795(2)**, 277–287

Four cation-exchange resins, namely (i) S-HyperD-F, (ii) S-Sepharose FF, (iii) Fractogel EMD SO3-650(S) and (iv) Macro-Prep High S Support, were compared for the separation of proteins such as IgG, lactoferrin and lactoperoxidase from bovine whey. The chromatographic method was based on flushing .alpha.-lactalbumin through the column and then eluting the proteins by a sequential step gradient with 0.1, 0.2, 0.3, 0.4 and 1M-NaCl. The collected fractions were analysed by GPC and SDS-polyacrylamide gel electrophoresis. The lactoperoxidase activity was measured by the oxidation of o-phenylenediamine. Resin iii exhibited the highest binding capacity for IgG of 3.7 mg/mL gel at a flow rate of 100 cm/h but the lowest protein resolution. Resins i and ii exhibited slightly poorer dynamic capacities for IgG but better protein resolution. For i, ii and iii, the binding capacities were not dependent on flow rate for 100–600 cm/h and the binding capacities were not improved by removing low molecular weight compounds from the feed solution. Resins i, ii and iii were considered suitable for the large scale purification of bovine whey proteins.

47 Using spreadsheets

Definitions

Spreadsheet – a display of a grid of cells into which numbers, text or formulae can be typed to form a worksheet. Each cell is uniquely identifiable by its column and row number combination (i.e. its two-dimensional co-ordinates) and can contain a formula which makes it possible for an entry to one cell to alter the contents of one or more other cells.

Template – a pre-designed spreadsheet without data but including all formulae necessary for (repeated) data analysis.

Macro – a sequence of user-defined instructions carried out by the spreadsheet, allowing complex repeated tasks to be 'automated'.

KEY POINT **The spreadsheet is one of the most powerful and wide ranging of all microcomputer applications. It is the electronic equivalent of a huge sheet of paper with calculating powers and provides a dynamic method of storing and manipulating data sets.**

Statistical calculations and graphical presentations are available in many versions and most have scientific functions. Spreadsheets can be used to:

- manipulate raw data by removing the drudgery of repeated calculations, allowing easy transformation of data and calculation of statistics;
- graph out your data rapidly to get an instant evaluation of results – printout can be used in practical and project reports;
- carry out limited statistical analysis by built-in procedures or by allowing construction of formulae for specific tasks;
- model 'what if' situations where the consequences of changes in data can be seen and evaluated;
- store data sets with or without statistical and graphical analysis.

The spreadsheet (Fig. 47.1) is divided into rows (identified by numbers) and columns (identified by alphabetic characters). Each individual combination of column and row forms a cell which can contain a data item, a formula, or a piece of text called a label. Formulae can include scientific and/or statistical functions and/or a reference to other cells or groups of cells (often called a range). Complex systems of data input and analysis can be constructed (models). The analysis, in part or complete, can be printed out. New data can be added at any time and the sheet recalculated. You can construct templates, pre-designed spreadsheets containing the formulae required for repeated data analyses.

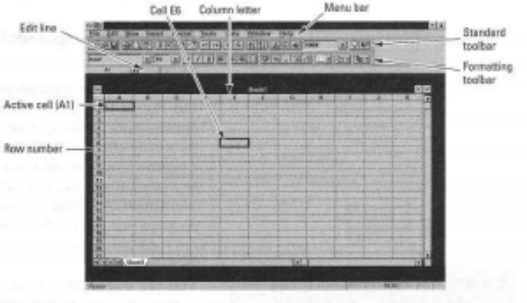

Fig. 47.1 A screen dump of a typical spreadsheet, showing cells, rows and columns; toolbars etc. Screen shot reprinted by permission from Microsoft Corporation.

Templates – these should contain:
- a data input section,
- data transformation and/or calculation sections,
- a results section, which can include graphics,
- text in the form of headings and annotations,
- a summary section.

Constructing a spreadsheet – start with a simple model and extend it gradually, checking for correct operation as you go.

The power a spreadsheet offers is directly related to your ability to create models that are accurate and templates that are easy to use. The sequence of operations required is:

1. Determine what information/statistics you want to produce.
2. Identify the variables you will need to use, both for original data that will be entered and for any intermediate calculations that might be required.
3. Set up areas of the spreadsheet for data entry, calculation of intermediate values (statistical values such as sums of squares etc.), calculation of final parameters/statistics and, if necessary, a summary area.
4. Establish the format of the numeric data if it is different from the default values. This can be done globally (affecting the entire spreadsheet) or locally (affecting only a specified part of the spreadsheet).
5. Establish the column widths required for the various activities.
6. Enter labels: use extensively for annotation.
7. Enter a test set of values to use during formula entry: use a fully worked example to check that formulae are working correctly.
8. Enter the formulae required to make all the calculations, both intermediate and final. Check that results are correct using the test data.

The spreadsheet is then ready for use. Delete all the test data values and you have created your template. Save the template to a disk and it is then available for repeated operations.

Data entry

Spreadsheets have built-in commands which allow you to control the layout of data in the cells. These include number format, the number of decimal places to be shown (the spreadsheet always calculates using eight or more places), the cell width and the location of the entry within the cell (left, right or centre). An auto-entry facility assists greatly in entering large amounts of data by moving the entry cursor either vertically or horizontally as data are entered. Recalculation default is usually automatic so that when a new data value is entered the entire sheet is recalculated immediately. This can dramatically slow down data entry so select manual recalculation mode before entering new data sets if the spreadsheet is large with many calculations.

The parts of a spreadsheet

Labels

These identify the contents of rows and columns. They are text characters, and cannot be used in calculations. Separate them from the data cells by drawing lines, if this feature is available. Programs make assumptions about the nature of the entry being made: most assume that if the first character is a number, then the entry is a number or formula. If it is a letter, then it will be a label. If you want to start a label with a number, you must override this assumption by typing a designated character before the number to tell the program that this is a label; check your program manual for details.

Using hidden (or zero-width) columns – these are useful for storing intermediate calculations which you do not wish to be displayed on the screen or printout.

Numbers

You can also enter numbers (values) in cells for use in calculations. Many programs let you enter numbers in more than one way and you must decide which method you prefer. The way you enter the number does not affect the way it is displayed on the screen as this is controlled by the cell format at the

point of entry. There are usually special ways to enter data for percentages, currency and scientific notation for very large and small numbers.

Formulae

These are the 'power tools' of the spreadsheet because they do the calculations. A cell can be referred to by its alphanumeric code, e.g. A5 (column A, row 5) and the value contained in that cell manipulated within a formula, e.g. (A5 + 10) or (A5 + B22) in another cell. Formulae can include a diverse array of pre-programmed functions which can refer to a cell, so that if the value of that cell is changed, so is the result of the formula calculation. They may also include limited branching options through the use of logical operators.

Definition

Function – a pre-programmed code for the transformation of values (mathematical or statistical functions) or selection of text characters (string functions).

Functions

A variety of functions is usually offered, but only mathematical and statistical functions will be considered here.

Example = sin(A5) is an example of a function in Microsoft® Excel®. If you write this in a cell, the spreadsheet will calculate the sine of the number in cell A5 (assuming it to be an angle in radians) and write it in the cell. Different programs may use a slightly different syntax.

Mathematical functions

Spreadsheets have program-specific sets of predetermined functions but they almost all include trigonometrical functions, angle functions, logarithms (p. 262) and random number functions. Functions are invaluable for transforming sets of data rapidly and can be used in formulae required for more complex analyses. Spreadsheets work with an order of preference of the operators in much the same way as a standard calculator and this must always be taken into account when operators are used in formulae. They also require a very precise syntax – the program should warn you if you break this!

Empty cells – note that these may be given the value 0 by the spreadsheet for certain functions. This may cause errors, e.g. by rendering a minimum value inappropriate. Also, an error return may result for certain functions if the cell content is zero.

Statistical functions

Modern spreadsheets incorporate many sophisticated statistical functions, and if these are not appropriate, the spreadsheet can be used to facilitate the calculations required for most of the statistical tests found in textbooks. The descriptive statistics normally available include:

- sums of all data present in a column, row or block;
- minima and maxima of a defined range of cells;
- counts of cells – a useful operation if you have an unknown or variable number of data values;
- averages and other statistics describing location;
- standard deviations and other statistics describing dispersion.

A useful function where you have large numbers of data allows you to create frequency distributions using pre-defined class intervals.

Statistical calculations – make sure you understand whether any functions you employ are for populations or samples (see p. 263).

The hypothesis-testing statistical functions may be reasonably powerful (e.g. *t*-test, ANOVA, regressions) and they often return the *probability* P of obtaining the test statistic (where $0 < P < 1$), so there may be no need to refer to statistical tables. Again, check on the effects of including empty cells.

Copying

All programs provide a means of copying (replicating) formulae or cell contents when required and this is a very useful feature. When copying, references to cells may be either relative, changing with the row/column as they are copied, or absolute, remaining a fixed cell reference and not changing as the formulae are copied. This distinction between cell references

Example In Microsoft® Excel®, copying is normally *relative*, and if you wish a cell reference to be *absolute* when copied, this is done by putting a dollar ($) sign before and after the column reference letter, e.g. C56.

is very important and must be understood; it provides one of the most common forms of error when copying formulae. Be sure to understand how your spreadsheet performs these operations.

Naming blocks

When a group of cells (a block) is carrying out a particular function, it is often easier to give the block a name which can then be used in all formulae referring to that block. This powerful feature also allows the spreadsheet to be more readable.

Graphics display

Most spreadsheets now offer a wide range of graphics facilities which are easy to use and this represents an ideal way to examine your data sets rapidly and comprehensively. The quality of the final graphics output (to a printer) is variable but is usually sufficient for initial investigation of your data. Many of the options are business graphics styles but there are usually histogram, bar chart, X–Y plotting, line and area graphics options available. Note that some spreadsheet graphics may not come up to the standards expected for the formal presentation of scientific data (p. 343).

Printing spreadsheets

This is usually a straightforward menu-controlled procedure, made difficult only by the fact that your spreadsheet may be too big to fit on one piece of paper. Try to develop an area of the sheet which contains only the data that you will be printing, i.e. perhaps a summary area. Remember that columns can usually be hidden for printing purposes and you can control whether the printout is in portrait or landscape mode, and for continuous paper or single sheets (depending on printer capabilities). Use a screen preview option, if available, to check your layout before printing. Most spreadsheets are now WYSIWYG (What You See Is What You Get) so that the appearance on the screen is a realistic impression of the printout. A 'print to fit' option is also available in some programs, making the output fit the page dimensions.

Using string functions – these allow you to manipulate text within your spreadsheet and include functions such as 'search and replace' and alphabetical or numerical 'sort'.

Use as a database

Many spreadsheets can be used as databases, using rows and columns to represent the fields and records (see Chapter 48). For many applications in chemistry, the spreadsheet form of database is perfectly adequate and should be seriously considered before using a full-feature database program.

48 Word processors, databases and other packages

Word processors

The word processor has facilitated writing because of the ease of revising text. Word processing is a transferable skill valuable beyond the immediate requirements of your chemistry course. Using a word processor should improve your writing skills and speed because you can create, check and change your text on the screen before printing it as 'hard copy' on paper. Once entered and saved, multiple uses can be made of a piece of text with little effort.

When using a word processor you can:

- refine material many times before submission;
- insert material easily, allowing writing to take place in any sequence;
- use a spell-checker to check your text;
- use a thesaurus when composing your text;
- produce high-quality final copies;
- reuse part or all of the text in other documents.

The potential disadvantages of using a word processor include:

- lack of ready access to a computer, software and/or a printer;
- time taken to learn the operational details of the program;
- the temptation to make 'trivial' revisions;
- loss of files due to computer breakdown or disk loss or failure.

The computerized office – many word processors are now sold as part of an integrated suite, e.g. PerfectOffice® and Microsoft® Office, with the advantage that they share a common interface in the different components (word processor, spreadsheet, database, etc.) and allow ready exchange of information (e.g. text, graphics) between component programs.

Word processors come as 'packages' comprising the program and a manual, often with a tutorial program. Examples are Microsoft® Word and WordPerfect®. Most word processors have similar general features but differ in operational detail; it is best to pick one and stick to it as far as possible so that you become familiar with it. Learning to use the package is like learning to drive a car – you need only to know how to drive the computer and its program, not to understand how the engine (program) and transmission (data transfer) work, although a little background knowledge is often helpful and will allow you to get the most from the program.

Using textbooks, manuals and tutorials – the manuals that come with some programs may not be very user-friendly and it is often worth investing in one of the textbooks that are available for most word processing programs. Alternatively, use an online 'help' tutorial, available with the more sophisticated packages.

In most word processors, the appearance of the screen realistically represents what the printout on paper will look like (WYSIWYG). Word processing files actually contain large amounts of code relating to text format etc., but these clutter the screen if visible, as in non-WYSIWYG programs. Some word processors are menu driven, others require keyboard entry of codes: menus are easier to start with and the more sophisticated programs allow you to choose between these options.

Because of variation in operational details, only general and strategic information is provided in this chapter: you must learn the details of your word processor through use of the appropriate manual and 'help' facilities.

Before starting you will need:

- the program (ideally on a hard disk);
- a floppy disk for storage, retrieval and backup of your own files when created;
- the appropriate manual or textbook giving operational details;

- a draft page layout design: in particular you should have decided on page size, page margins, typeface (font) and size, type of text justification, and format of page numbering;
- an outline of the text content;
- access to a suitable printer: this need not be attached to the computer you are using since your file can be taken to an office where such a printer is available, providing that it has the same word processing program.

Using a word processor – take full advantage of the differences between word processing and 'normal' writing (which necessarily follows a linear sequence and requires more planning):

- Simply jot down your initial ideas for a plan, preferably to paragraph topic level. The order can be altered easily and if a paragraph grows too much it can easily be split.
- Start writing wherever you wish and fill in the rest later.
- Just put down your ideas as you think, confident in the knowledge that it is the concepts that are important to note; their order and the way you express them can be adjusted later.
- Don't worry about spelling and use of synonyms – these can (and should) be checked during a special revision run through your text, using the spelling checker first to correct obvious mistakes, then the thesaurus to change words for style or to find the exact word.
- Don't forget that a draft printout may be required to check (i) for pace and spacing – difficult to correct for on a screen; and (ii) to ensure that words checked for spelling fit the required sense.

Laying out (formatting) your document

Although you can format your text at any time, it is good practice to enter the basic commands at the start of your document: entering them later can lead to considerable problems due to reorganization of the text layout. If you use a particular set of layout criteria regularly, e.g. an A4 page with space for a letterhead, make a template containing the appropriate codes that can be called up whenever you start a new document. Note that various printers may respond differently to particular codes, resulting in a different spacing and layout.

Typing the text

Think of the screen as a piece of typing paper. The cursor marks the position where your text/data will be entered and can be moved around the screen by use of the cursor-control keys. When you type, don't worry about running out of space on the line because the text will wrap around to the next line automatically. Do not use a carriage return (usually the ENTER or ↵ key) unless you wish to force a new line, e.g. when a new paragraph is wanted. If you make a mistake when typing, correction is easy. You can usually delete characters or words or lines and the space is closed automatically. You can also insert new text in the middle of a line or word. You can insert special codes to carry out a variety of tasks, including changing text appearance, such as underlining, **bold** and *italics*. Paragraph indentations can be automated using TAB or ⇥ as on a typewriter but you can also indent or bullet whole blocks of text using special menu options. The function keys are usually pre-programmed to assist in many of these operations.

Deleting and restoring text – because deletion can sometimes be made in error, there is usually an 'undelete' or 'restore' feature which allows the last deletion to be recovered.

Editing features

Word processors usually have an array of features designed to make editing documents easy. In addition to the simple editing procedures described above, the program usually allows blocks of text to be moved ('cut and paste'), copied or deleted.

An extremely valuable editing facility is the search procedure: this can rapidly scan through a document looking for a specified word, phrase or punctuation. This is particularly valuable when combined with a replace facility so that, for example, you could replace the word 'test' with 'trial' throughout your document simply and rapidly.

Most WYSIWYG word processors have a command (e.g. Show/Hide in Microsoft® Word) which reveals the non-printing characters, including paragraph and space markers. This can be useful when editing text, as spacing may alter in apparently mysterious ways if the precise position of the cursor *vis-à-vis* these markers is not taken into account.

Fonts and line spacing

Most word processors offer a variety of fonts depending upon the printer being used. Fonts come in a wide variety of types and sizes, but they are defined in particular ways as follows:

Presenting your documents – it is good practice not to mix typefaces too much in a formal document; also the font size should not differ greatly for different headings, sub-headings and the text.

- Typeface: the term for a family of characters of a particular design, each of which is given a particular name. The most commonly used for normal text is Times Roman, as used here for the main text, but many others are widely available, particularly for the better quality printers. They fall into three broad groups: serif fonts with curves and flourishes at the ends of the characters (e.g. Times Roman); sans serif fonts without such flourishes, providing a clean, modern appearance (e.g. Helvetica, also known as Swiss); and decorative fonts used for special purposes only, such as the production of newsletters and notices (e.g. *Freestyle Script*).
- Size: measured in points. A point is the smallest typographical unit of measurement, there being 72 points to the inch (about 28 points per cm). The standard sizes for text are 10, 11 and 12 point, but typefaces are often available up to 72 point or more.
- Appearance: many typefaces are available in a variety of styles and weights. Many of these are not designed for use in scientific literature but for desktop publishing.

Preparing draft documents – use double spacing to allow room for your editing comments on the printed page.

- Spacing: can be either fixed, where every character is the same width, or proportional, where the width of every character, including spaces, is varied. Typewriter fonts such as Elite and Prestige use fixed spacing and are useful for filling in forms or tables, but proportional fonts make the overall appearance of text more pleasing and readable.
- Pitch: specifies the number of characters per horizontal inch of text. Typewriter fonts are usually 10 or 12 pitch, but proportional fonts are never given a pitch value since it is inherently variable.
- Justification is the term describing the way in which text is aligned vertically. Left justification is normal, but for formal documents, both left and right justification may be used (as here).

Preparing final documents – for most work, use a 12 point proportional serif typeface with spacing dependent upon the specifications for the work.

You should also consider the vertical spacing of lines in your document. Drafts and manuscripts are frequently double spaced to allow room for editing. If your document has unusual font sizes, this may well affect line spacing, although most word processors will cope with this automatically.

Table construction

Tables can be produced by a variety of methods:

- Using the tab key ⇥ as on a typewriter: this moves the cursor to predetermined positions on the page, equivalent to the start of each tabular column. You can define the positions of these tabs as required at the start of each table.
- Using special table-constructing procedures. Here the table construction is largely done for you and it is much easier than using tabs, providing you enter the correct information when you set up the table.
- Using a spreadsheet to construct the table and then copying it to the word processor. This procedure requires considerably more manipulation than using the word processor directly and is best reserved for special circumstances, such as the presentation of a very large or complex table of data, especially if the data are already stored as a spreadsheet.

Graphics and special characters

Many word processors can incorporate graphics from other programs into the text of a document. Files must be compatible (see your manual) but if this is so, it is a relatively straightforward procedure. For highly professional documents this is a valuable facility, but for most undergraduate work it is probably better to produce and use graphics as a separate operation, e.g. employing a spreadsheet.

You can draw lines and other graphical features directly within most word processors and special characters may be available dependent upon your printer's capabilities. It is a good idea to print out a full set of characters from your printer so that you know what it is capable of. These may include symbols and Greek characters, often useful in chemistry.

Tools

Many word processors also offer you special tools, the most important of which are:

- Macros: special sets of files you can create when you have a frequently repeated set of keystrokes to make. You can record these keystrokes as a 'macro' so that it can provide a shortcut for repeated operations.
- Thesaurus: used to look up alternative words of similar or opposite meaning while composing text at the keyboard.
- Spell-check: a very useful facility which will check your spellings against a dictionary provided by the program. This dictionary is often expandable to include specialist words which you use in your work. The danger lies in becoming too dependent upon this facility as they all have limitations: in particular, they will not pick up incorrect words which happen to be correct in a different context (i.e. 'was' typed as 'saw' or 'meter' rather than 'metre'). Beware of American spellings in programs from the USA, e.g. 'color' instead of 'colour'. The rule, therefore, is to use the spell-check first and then carefully read the text for errors which have slipped through.

Using a spell-check facility – do not rely on this to spot all errors.

- Word count: useful when you are writing to a prescribed limit.

Printing from your program

Word processors require you to specify precisely the type of printer and/or other style details you wish to use. Most printers also offer choices as to text and graphics quality, so choose draft (low) quality for all but your final copy since this will save both time and materials.

Use a print preview option to show the page layout if it is available. Assuming that you have entered appropriate layout and font commands, printing is a straightforward operation carried out by the word processor at your command. Problems usually arise because of some incompatibility between the criteria you have entered and the printer's own capabilities. Make sure that you know what your printer offers before starting to type: although parameters are modifiable at any time, changing the page size, margin size, font size, etc., all cause your text to be rearranged, and this can be frustrating if you have spent hours carefully laying out the pages!

Using the print preview mode – this can reveal errors of several types, e.g. spacing between pages, that can prevent you wasting paper and printer ink unnecessarily.

KEY POINT **It is vital to save your work frequently to a hard or floppy disk (or both). This should be done every 10 min or so. If you do not save regularly, you may lose hours or days of work. Many programs can be set to 'autosave' every few minutes.**

Databases

A database is an electronic filing system whose structure is similar to a manual record card collection. Its collection of records is termed a file. The individual items of information on each record are termed fields. Once the database is constructed, search criteria can be used to view files through various filters according to your requirements. The computerized catalogues in your library are just such a system; you enter the filter requirements in the form of author or subject keywords.

You can use a database to catalogue, search, sort and relate collections of information. The benefits of a computerized database over a manual card-file system are:

- The information content is easily amended/updated.
- Printout of relevant items can be obtained.
- It is quick and easy to organize through sorting and searching/selection criteria, to produce sub-groups of relevant records.
- Record displays can easily be redesigned, allowing flexible methods of presenting records according to interest.
- Relational databases can be combined, giving the whole system immense flexibility. The older 'flat-file' databases store information in files which can be searched and sorted, but cannot be linked to other databases.

Choosing between a database and a spreadsheet – use a database only after careful consideration. Can the task be done better within a spreadsheet? A database program can be complex to set up and usually needs to be updated regularly.

Relatively simple database files can be constructed within the more advanced spreadsheets using the columns and rows as fields and records respectively. These are capable of limited sorting and searching operations and are probably sufficient for the types of databases you are likely to require as an undergraduate. You may also make use of a bibliography database especially constructed for that purpose.

Statistical analysis packages

Statistical packages vary from small programs designed to carry out very specific statistical tasks to large sophisticated packages (Statgraphics®, Minitab®, Statistica® etc.) intended to provide statistical assistance, from experimental design to the analysis of results. Consider the following features when selecting a package:

- The data entry and editing section should be user-friendly, with options for transforming data.
- Options should include descriptive statistics and exploratory data analysis techniques.
- Hypothesis testing techniques should include ANOVA, regression analysis, multivariate techniques and parametric and non-parametric statistics.
- Output facilities should be suitable for graphical and tabular formats.

Some programs have complex data entry systems, limiting ease of use. The data entry and storage system should be based upon a spreadsheet system, so that subsequent editing and transformation operations are straightforward.

KEY POINT **Make sure that you understand the statistical basis for your test and the computational techniques involved *before* using a particular program.**

Graphics/presentation packages

ChemWindow® and ChemDraw® allow you to draw chemical structures easily. They contain, for example, benzene rings which can be easily selected and moved around on the computer screen.

Many of these packages are specifically designed for business graphics rather than science. They do, however, have considerable value in the preparation of materials for posters and talks where visual quality is an important factor. There are several packages available for microcomputers such as Freelance Graphics®, Harvard Graphics® and Microsoft® PowerPoint®, which provide templates for the preparation of overhead transparencies, slide transparencies and paper copy, both black and white and in colour. They usually incorporate a 'freehand' drawing option, allowing you to make your own designs.

Although the facilities offered are often attractive, the learning time required for some of the more complex operations is considerable and they should be considered only for specific purposes: routine graphical presentation of data sets is best done from within a spreadsheet or statistical package. There may be a service provided by your institution for the preparation of such material and this should be seriously considered before trying to learn to use these programs.

The most important points regarding the use of graphics packages are:

- Graphics quality: the built-in graphics are sometimes of only moderate quality. Use of annotation facilities can improve graphics considerably. Do not use inappropriate graphics for scientific presentation.
- The production of colour graphics: this requires a good-quality colour printer/plotter.
- Importing graphics files: graphs produced by spreadsheets or other statistical programs can usually be imported into graphics programs – this is useful for adding legends, annotations, etc., when the facilities offered by the original programs are inadequate. Check that the format of files produced by your statistics/spreadsheet program can be recognized by your graphics program. The different types of file are distinguished by the three-character filename extension.

KEY POINT **Computer graphics are not always satisfactory for scientific presentation. While they may be useful for exploratory procedures, they may need to be redrawn by hand for your final report. It may be helpful to use a computer-generated graph as a template for the final version.**

49 Finding and citing published information

The ability to find scientific information is a skill required for many exercises in your degree programme. You will need to research facts and published findings as part of writing essays, literature reviews and project introductions, and when amplifying your lecture notes and revising for exams. You must also learn how to follow scientific convention in citing source material as the authority for each statement you make.

Sources of information

For essays and revision

You are unlikely to delve into the primary literature for these purposes – books and reviews are much more readable! If a lecturer or tutor specifies a particular book, then it should not be difficult to find out where it is shelved in your library, as most libraries now have a computerized index system and their staff will be happy to assist with any queries. If you want to find out which books your library holds on a specified topic, use the system's subject index. You will also be able to search by author or by key words.

There are two main systems used by libraries to classify books: the Dewey Decimal system and the Library of Congress system. Libraries differ in the way they employ these systems, especially by adding further numbers and letters after the standard classification marks to signify, say, shelving position or edition number. Enquire at your library for a full explanation of local usage.

Browsing in a library – this *may* turn up interesting material, but remember the books on the shelves are those *not* currently out on loan. Almost by definition, the latter may be more up to date and useful. To find out a library's full holding of books in any subject area, you need to search its catalogue (normally available as a computerized database).

Example The book *Inorganic Chemistry*, 3rd edn, by D.F. Shriver and P.W. Atkins (1999; Oxford University Press) is likely to be classified as follows:

Dewey decimal system: 546

where	500	refers to natural sciences and mathematics
	540	refers to chemistry
	546	refers to inorganic chemistry

Library of Congress system: QD146–197

where	Q	refers to science
	QD	refers to chemistry
	QD146–197	refers to inorganic chemistry

Example of book classification

	Dewey	Library of Congress
Chemistry	540	QD1–999
Analytical chemistry	543	QD71–145
Chromatography	543.544	QD117.C5; QD79.C4
Crystallography	548	QD901–999
Electrochemistry	541.13	QD551–671; QD261; TP250–261
Inorganic chemistry	546	QD146–197
Organic chemistry	547	QD241–449
Physical chemistry	541.1	QD450–731
Radiochemistry	541.28	QD601–655
Spectroscopy	543.42	QD95–96; QC450–467; QD272.S6
Surface chemistry	541.18	QD506–508
Synthesis (organic)	547.07	QD262

Computer databases – several databases are now produced on CD-ROM for open use in libraries (e.g. Medline, Applied Science and Technology Index). Especially useful to chemists are *Chemical Abstracts* and *Analytical Abstracts*. Some databases can be accessed via the Internet, such as BIDS (Bath Information and Data Service), a UK service providing access to information from over 7000 periodicals (username and password required via your library). Each of these databases usually has its own easy-to-follow menu instructions. It is worthwhile to consider key words for your search beforehand to focus your search and save time.

The World Wide Web is an expanding resource for gathering both general and specific information (see Chapter 45). Sites fall into analogous categories to those in the printed literature: there are sites with original information, sites that review information and bibliographic sites. One considerable problem is that websites may be frequently updated, so information present when you first looked may be altered or even absent when the site is next consulted. Further, very little of the information on the WWW has been monitored or refereed. Another disadvantage is that the site information may not state the origin of the material, who wrote it or when it was written.

Definitions

Journal/periodical/serial – any publication issued at regular intervals. In chemistry, usually containing papers describing original research findings and reviews of literature.
The primary literature – this comprises original research papers, published in specialist scientific periodicals. Certain prestigious general journals (e.g. *Nature*, *Science*) contain important new advances from a wide subject area.
Monograph – a specialized book covering a single topic.
Review – an article in which recent advances in a specific area are outlined and discussed.
Proceedings – volume compiling written versions of papers read at a scientific meeting on a specific topic.
Abstracts – shortened versions of papers, often those read at scientific meetings. These may later appear in the literature as full papers.
Bibliography – a summary of the published work in a defined subject area.

For literature surveys and project work

You will probably need to consult the primary literature. If you are starting a new research project or writing a report from scratch, you can build up a core of relevant papers by using the following methods:

- Asking around: supervisors or their postgraduate students will almost certainly be able to supply you with a reference or two that will start you off.
- Searching a computer database: these cover very wide areas and are a convenient way to start a reference collection, although a charge is often made for access and sending out a listing of the papers selected (your library may or may not pass this on to you).
- Consulting the bibliography of other papers in your collection – an important way of finding the key papers in your field. In effect, you are taking advantage of the fact that another researcher has already done all the hard work!
- Referring to 'current awareness' journals or computer databases: these are useful for keeping you up to date with current research; they usually provide a monthly listing of article details (title, authors, source, author address) arranged by subject and cross-referenced by subject and author. Current awareness journals cover a wider range of primary journals than could ever be available in any one library. Examples include:
  (a) *Current Contents*, published by the Institute of Scientific Information, Philadelphia, USA, which reproduces the contents pages of journals of a particular subject area and presents an analysis by author and subject.
  (b) *Current Advances*, published by Elsevier Science, Oxford, UK, which sub-divides papers by subject within research areas and cross-references by subject and author.
  (c) *Chemical Abstracts and Analytical Abstracts*, in which each paper's abstract is also reproduced. Papers may be cross-referenced according to various taxa, which is useful in allowing you to find out what work has been done on a particular organism.
- Using the *Science Citation Index* (SCI): this is a very valuable source of new references, because it lets you see who has cited a given paper; in effect, SCI allows you to move forward through the literature from an existing reference. SCI is published regularly during the year and issues are collated annually. Some libraries have copies on CD-ROM: this allows rapid access and output of selected information.

For specialized information

You may need to consult reference works such as encyclopaedias, maps and books providing specialized information. Much of this is now available on CD-ROM (consult your library's information service). Two books worth noting are:

1. The *Handbook of Chemistry and Physics* (Lide, 2000): the Chemical Rubber Company's publication (affectionately known as the 'Rubber Bible') giving all manner of physical constants, radioisotope half-lives, etc.
2. *The Merck Index* (Budavari, 1999), which gives useful information about organic chemicals, e.g. solubility, whether poisonous, etc.

Copyright law – in Europe, copyright regulations were harmonized in 1993 (Directive 93/98/EEC) to allow literary copyright for 70 years after the death of an author and typographical copyright for 25 years after publication. This was implemented in the UK in 1996, where, in addition, the Copyright, Designs and Patents Act (1988) allows the Copyright Licensing Agency to license institutions so that lecturers, students and researchers may take copies for teaching and personal research purposes – no more than a single article per journal issue, one chapter of a book, or extracts to a total of 10% of a book.

Storing research papers – these can easily be kept in alphabetical order within filing boxes or drawers, but if your collection is likely to grow large, it will need to be refiled as it outgrows the storage space. An alternative is to keep an alphabetical card index system (useful when typing out lists of references) and file the papers by 'accession number' as they accumulate. New filing space is only required at one 'end' and you can use the accession numbers to form the basis of a simple cross-referencing system.

Fig. 49.1 A typical reference card. Make sure the index card carries all the bibliographic information of potential relevance.

Obtaining and organizing research papers

Obtaining a copy

It is usually more convenient to have personal copies of key research articles for direct consultation when working in a laboratory or writing. The simplest way of obtaining these is to photocopy the originals. For academic purposes, this is normally acceptable within copyright law. If your library does not take the journal, it may be possible for the library to borrow it from a nearby institute or obtain a copy via a national borrowing centre (an 'inter-library loan'). If the latter, you will have to fill in a form giving full bibliographic details of the article and where it was cited, as well as signing a copyright clearance statement concerning your use of the copy.

Recent advances in electronic publishing mean that full-text copies of research articles can be downloaded either from a publisher or via BIDS. In the case of the former you will need to establish whether your institute has an electronic subscription to the publication of interest. Viewing of an electronic research paper is done via a particular software programme, e.g. the reader Adobe Acrobat®. It is often possible to download a free copy of the reader to your computer the first time you try this.

Your department might be able to supply 'reprint request' postcards to be sent to the designated author of a paper. This is an unreliable method of obtaining a copy because it may take some time (allow at least 1–3 months!) and some requests will not receive a reply. Taking into account the waste involved in postage and printing, it is probably best simply to photocopy or send for a copy via inter-library loan.

Organizing papers

Although the numbers of papers you accumulate may be small to start with, it is worth putting some thought into their storage and indexing before your collection becomes disorganized and unmanageable. Few things are more frustrating than not being able to lay your hands on a vital piece of information, and this can seriously disrupt your flow when writing or revising.

Card index systems

Index cards (Fig. 49.1) are a useful adjunct to any filing system. Firstly, you may not have a copy of the paper to file yet may still wish the reference information to be recorded somewhere for later use. Secondly, a selected pile of cards can be used when typing out different bibliographies. Thirdly, the cards can help when organizing a review (see p. 339). Fourthly, the card can be used to record key points and comments on the paper. The priority rule for storage in card boxes is again first-author name, subsequent author name(s), date. Computerized card index systems simplify cross-referencing and can provide computer files for direct insertion into word processed documents; however, they are very time consuming to set up and maintain, so you should only consider using one if the time invested will prove worthwhile.

Making citations in text

There are two main ways of citing articles and creating a bibliography (also referred to as 'References' or 'Literature Cited').

The Harvard system

For each citation, the author name(s) and the date of publication are given at the relevant point in the text. The bibliography is organized alphabetically

and by date of publication for papers with the same authors. Formats normally adopted are, for example, 'Smith and Jones (1983) stated that ...' or 'it has been shown that ... (Smith and Jones, 1983)'. Lists of references within parentheses are separated by semi-colons, e.g. '(Smith and Jones, 1983; Jones and Smith, 1985)', normally in order of date of publication. To avoid repetition within the same paragraph, a formula such as 'the investigations of Smith and Jones indicated that' could be used following an initial citation of the paper. Where there are more than two authors it is usual to write 'et al.' (or *et al.* if an italic font is available); this stands for the Latin *et alia* meaning 'and others'. If citing more than one paper with the same authors, put, for example, 'Smith and Jones (1987; 1990)' and if papers by a given set of authors appeared in the same year, letter them (e.g. Smith and Jones, 1989a; 1989b).

The numerical or Vancouver system

Papers are cited via a superscript or bracketed reference number inserted at the appropriate point. Normal format would be, for example 'computational chemistry[4,5] has shown that ...' or 'Jones [55,82] has claimed that ...'. Repeated citations use the number from the first citation. In the true numerical method (e.g. as in *Nature*, *Science*), numbers are allocated by order of citation in the text. This is by far the most common approach in chemistry journals. Note that adding or removing references is tedious, so the numbering should be done only when the text is finalized.

The 'Katritzky' system

A third system has been popularized in publications involving Professor Roy Katritzky (University of Florida, USA) and uses the best features of both the Harvard and Vancouver systems. For each reference in the text a code is written comprising the year of publication, the journal and the page of the journal. In the reference section the coded references are cited, together with the full reference – authors, journal, year, volume and page – *in year order* and a list of journal codes in alphabetical order can be provided. For example:

in the text

... Robinson and Watt (34JCS1536) found ...

in the reference section

34JCS1536 R. Robinson and S.J. Watt, *J. Chem. Soc.*, 1934, 1536.

The advantage of this system is that it is easy to insert missed references into the text, a great disadvantage of the otherwise neat Vancouver system. For details consult the reference section in *Comprehensive Heterocyclic Chemistry*, ed. A.R. Katritzky and C.W. Rees, Pergamon, Oxford, 1984.

Examples

Paper in journal:
Smith, A. B., Jones, C.D. and Professor, A. Journal of New Results, **11** (2000) 19.

Book:
Smith, A. B. (1998). Summary of My Life's Work. Megadosh Publishing Corp., Bigcity.

Chapter in edited book:
Jones, C. D. and Smith, A. B. (1998). Earth-shattering research from our laboratory. In: Research Compendium 1998 (ed. A. Professor), pp. 123–456. Bigbucks Press, Booktown.

Thesis:
Smith, A. B. (1995). Investigations on my favourite topic. PhD thesis, University of Life, Fulchester.

Note that underlining used here specifies italics in print; use an italic font if working with a word processor.

How to list your citations in a bibliography

Whichever citation method is used in the text, comprehensive details are required for the bibliography so that the reader has enough information to find the reference easily. Citations should be listed in alphabetical order with the priority: first author, subsequent author(s), date. Unfortunately, in terms of punctuation and layout, there are almost as many ways of citing papers as there are journals!

KEY POINT **Your department may specify an exact format for project work; if not, decide on a style and be consistent – if you do not pay attention to the details of citation you may lose marks.**

Take special care with the following aspects:

- Authors and editors: give details of *all* authors and editors in your bibliography, even if given as *et al.* in the text.
- Abbreviations for journals: while there are standard abbreviations for the titles of journals (consult library staff), it is a good idea to give the whole title, if possible.
- Books: the edition should always be specified as contents may change between editions. Add, for example, '(5th edition)' after the title of the book. You may be asked to give the International Standard Book Number (ISBN), a unique reference number for each book published.
- Unsigned articles, e.g. unattributed newspaper articles and instruction manuals: refer to the author(s) in text and bibliography as 'Anon.'.
- Unread articles: you may be forced to refer to a paper via another without having seen it. If possible, refer to another authority who has cited the paper, e.g. '... Jones (1980), cited in Smith (1990), claimed that ...'. Alternatively, you could denote such references in the bibliography by an asterisk and add a short note to explain at the start of the reference list.
- Personal communications: information received in a letter, seminar or conversation can be referred to in the text as, for example, '... (Smith, pers. comm.)'. These citations are not generally listed in the bibliography of papers, though in a thesis you could give a list of personal communicants and their addresses.

Citing websites – there is no widely accepted format at present. We suggest providing author name(s) and date in the text if using the Harvard system, while in the bibliography giving the above, plus site title and full URL reference (e.g. Hacker, A. (1998) University of Cybertown homepage on aardvarks. http://www.myserver.ac.uk/homepage).

Communicating information

50 General aspects of scientific writing

Written communication is an essential component of all sciences. Most courses include writing exercises in which you will learn to describe ideas and results accurately, succinctly and in an appropriate style and format. The following are features common to all forms of scientific writing.

Organizing time

Making a timetable at the outset helps ensure that you give each stage adequate attention and complete the work on time. To create and use a timetable:

1. Break down the task into stages.
2. Decide on the proportion of the total time each stage should take.
3. Set realistic deadlines for completing each stage, allowing some time for slippage.
4. Refer to your timetable frequently as you work: if you fail to meet one of your deadlines, make a serious effort to catch up as soon as possible.

KEY POINT **The appropriate allocation of your time to reading, planning, writing and revising will differ according to the task in hand (see Chapters 51 and 53).**

Organizing information and ideas

Before you write, you need to gather and/or think about relevant material (Chapter 49). You must then decide:

- what needs to be included and what doesn't;
- in what order it should appear.

Start by jotting down headings for everything of potential relevance to the topic (this is sometimes called 'brainstorming'). A spider diagram (Fig. 50.1) will help you organize these ideas. The next stage is to create an outline of your text (Fig. 50.2). Outlines are valuable because they:

Creating an outline – an informal outline can be made simply by indicating the order of sections on a spider diagram (as in Fig. 50.1).

- force you to think about and plan the structure;
- provide a checklist so nothing is missed out;
- ensure the material is balanced in content and length;
- help you organize figures and tables by showing where they will be used.

In an essay or review, the structure of your writing should help the reader to assimilate and understand your main points. Sub-divisions of the topic could simply be related to the nature of the subject matter (e.g. levels of organization of a protein) and should proceed logically (e.g. primary structures, then secondary, etc.).

A chronological approach is good for evaluation of past work (e.g. the development of the concept of aromaticity), whereas a step-by-step comparison might be best for certain exam questions (e.g. 'Discuss the differences between solid phase and Soxhlet extraction in the analysis of pesticides'). There is little choice about structure for practical and project reports (see Chapter 52).

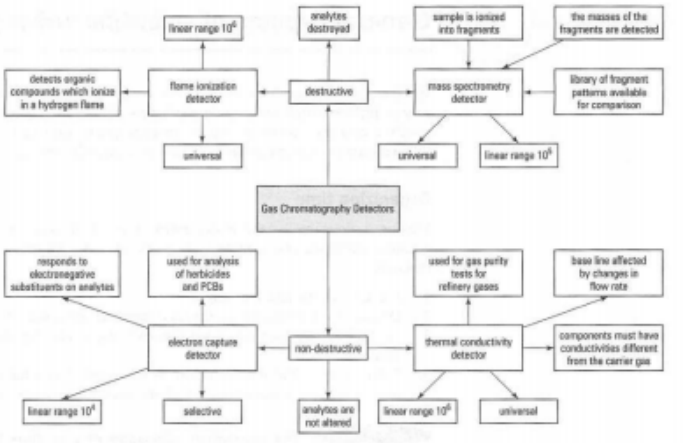

Fig. 50.1 Spider diagram showing how you might 'brainstorm' an essay with the title 'Detectors for Gas Chromatography'. Write out the essay title in full to form the spider's body, and as you think of possible content, place headings around this to form its legs. Decide which headings are relevant and which are not and use arrows to note connections between subjects, if required. This may influence your choice of order and may help to make your writing flow because the links between paragraphs will be natural. You can make an informal outline directly on a spider diagram by adding numbers indicating a sequence of paragraphs (as shown). This method is best when you must work quickly, as with an essay written under exam conditions.

Fig. 50.2 Formal outlines. These are useful for a long piece of work where you or the reader might otherwise lose track of the structure. The headings for sections and paragraphs are simply written in sequence with the type of lettering and level of indentation indicating their hierarchy. Two different forms of formal outline are shown, a minimal form (a) and a numbered form (b). Note that the headings used in an outline are often repeated within the essay to emphasize its structure. The content of an outline will depend on the time you have available and the nature of the work, but the most detailed hierarchy you should reasonably include is the subject of each paragraph.

Gas chromatography detectors

Introduction
Destructive detectors
  Flame ionization
  Mass spectrometry
Non-destructive detectors
  Thermal conductivity
  Electron capture
Conclusions

(a)

Gas chromatography detectors

1. Introduction
2. Destructive detectors
   2.1 Flame ionization
   2.2 Mass spectrometry
3. Non-destructive detectors
   3.1 Thermal conductivity
   3.2 Electron capture
4. Conclusions

(b)

Writing

Adopting a scientific style

Your main aim in developing a scientific style should be to get your message across directly and unambiguously. While you can try to achieve this through a set of 'rules' (see Box 50.1), you may find other requirements driving your writing in a contradictory direction. For instance, the need to be accurate and complete may result in text littered with technical terms, and the flow may be continually interrupted by references to the literature. The need to be succinct

Box 50.1 How to achieve a clear, readable style

Words and phrases

- Choose short clear words and phrases rather than long ones, e.g. use 'build' rather than 'fabricate' 'now' rather than 'at the present time'. At certain times, technical terms must be used for precision, but don't use jargon if you don't have to.
- Don't worry too much about repeating words, especially when to introduce an alternative might subtly alter your meaning.
- Where appropriate, use the first person to describe your actions ('We decided to...'; 'I conclude that...'), but not if this is specifically discouraged by your supervisor.
- Favour active forms of speech ('the solution was placed in a beaker') rather than the passive voice ('the beaker was filled with the solution').
- Use tenses consistently. Past tense is always used for the experimental section ('samples were taken from...') and for reviewing past work ('Smith (1990) concluded that...'). The present tense is used when describing data ('Fig. 1 shows...'), for generalizations ('Most authorities agree that...') and conclusions ('I conclude that...').
- Use statements in parentheses sparingly – they disrupt the reader's attention to your central theme (see above section for examples).
- Avoid clichés and colloquialisms – they are usually inappropriate in a scientific context.

Sentences

- Don't make them overlong or complicated.
- Introduce variety in structure and length.
- Make sure you understand how and when to use punctuation.
- If unhappy with the structure of a sentence, try chopping it into a series of shorter sentences.

Paragraphs

- Keep short and restrict them to a distinct theme.
- Use repeated key words (same subject or verb) or appropriate linking phrases (e.g. 'On the other hand...') to connect sentences and emphasize the flow of text.
- The first sentence should introduce the topic of a paragraph and the following sentences explain, illustrate or give examples.

Note: If you're not sure what is meant by any of the terms used here, consult a guide on writing (see Box 50.2).

also affects style and readability through the use of, for example, stacked noun–adjectives (e.g. 'ring-opening metathesis polymerization') and acronyms (e.g. 'Romp'). Finally, style is very much a matter of taste and each tutor, examiner, supervisor or editor will have pet loves and hates which you may have to accommodate.

Developing technique

Writing is a skill that can be improved, but not instantly. You should analyse your deficiencies with the help of feedback from your tutors, be prepared to change work habits (e.g. start planning your work more carefully), and be willing to learn from some of the excellent texts that are available on scientific writing.

KEY POINT **You need to take a long-term view if you wish to improve your writing skills. An essential preliminary is to invest in and *make full use of* a personal reference library (see Box 50.2).**

Getting started

A common problem is 'writer's block' – inactivity or stalling brought on by a variety of causes. If blocked, ask yourself these questions:

- Are you comfortable with your surroundings? Make sure you are seated comfortably at a reasonably clear desk and have minimized the possibility of interruptions and distractions.

Box 50.2 Improve your writing ability by consulting a personal reference library

Using dictionaries

We all know that a dictionary helps with spelling and definitions, but how many of us use one effectively? You should:

- Keep a dictionary beside you when writing and always use it if in any doubt about spelling or definitions.
- Use it to prepare a list of words which you have difficulty in spelling: apart from speeding up the checking process, the act of writing out the words helps commit them to memory.
- Use it to write out a personal glossary of terms. This can help you memorize definitions. From time to time, test yourself.

Not all dictionaries are the same! Ask your tutor or supervisor whether he/she has a preference and why. Try out the *Oxford Advanced Learner's Dictionary*, which is particularly useful because it gives examples of use of all words and helps with grammar, e.g. by indicating which prepositions to use with verbs.

Using a thesaurus

A thesaurus contains lists of words of similar meaning grouped thematically; words of opposite meaning always appear nearby.

- Use a thesaurus to find a more precise and appropriate word to fit your meaning, but check definitions of unfamiliar words with a dictionary.
- Use it to find a word or phrase 'on the tip of your tongue' by looking up a word of similar meaning.
- Use it to increase your vocabulary.

Roget's Thesaurus is the standard. Collins publish a combined dictionary and thesaurus.

Using guides for written English

These provide help with the use of words.

- Use a guide to solve grammatical problems such as when to use 'shall' or 'will', 'which' or 'that', 'effect' or 'affect', etc.
- Use it for help with the paragraph concept and the correct use of punctuation.
- Use it to learn how to structure writing for different tasks.

Recommended guides include the following:

Kane, T.S. (1983) *The Oxford Guide to Writing*. Oxford University Press, New York. This is excellent for the basics of English – it covers grammar, usage and the construction of sentences and paragraphs.

Partridge, E. (1953) *You Have a Point There*. Routledge and Kegan Paul, London. This covers punctuation in a very readable manner.

Tichy, H.J. (1988) *Effective Writing for Engineers, Managers and Scientists*. John Wiley and Sons, New York. This is strong on scientific style and clarity in writing.

- Are you trying to write too soon? Have you clarified your thoughts on the subject? Have you done enough preliminary reading? Talking to a friend about your topic might bring out ideas or reveal deficiencies in your knowledge.
- Are you happy with the underlying structure of your work? If you haven't made an outline, try this. If you are unhappy because you can't think of a particular detail at the planning stage, just start writing – it is more likely to come to you while you are thinking of something else.
- Are you trying to be too clever? Your first sentence doesn't have to be earth-shattering in content or particularly smart in style. A short statement of fact or a definition is fine. If there will be time for revision, get your ideas down on paper and revise grammar, content and order later.
- Do you really need to start writing at the beginning? Try writing the opening remarks after a more straightforward part. With reports of experimental work, the materials and methods section may be the easiest to start at.
- Are you too tired to work? Don't try to 'sweat it out' by writing for long periods at a stretch: stop frequently for a rest.

Revising your text

Wholesale revision of your first draft is strongly advised for all writing apart from in exams. If a word processor is available, this can be a simple process. Where possible, schedule your writing so you can leave the first draft to 'settle' for at least a couple of days. When you return to it fresh, you will see more easily where improvements can be made. Try the following structured revision process, each stage being covered in a separate scan of your text:

1. Examine content. Have you included everything you need to? Is all the material relevant?
2. Check the grammar and spelling. Can you spot any 'howlers'?
3. Focus on clarity. Is the text clear and unambiguous? Does each sentence really say what you want it to say?
4. Try to achieve brevity. What could be missed out without spoiling the essence of your work? It might help to imagine an editor has set you the target of reducing the text by 15%.
5. Improve style. Could the text read better? Consider the sentence and paragraph structure and the way your text develops to its conclusion.

Revising your text – to improve clarity and shorten your text, 'distil' each sentence by taking away unnecessary words and 'condense' words or phrases by choosing a shorter alternative.

51 Writing essays

The function of an essay is to show how much you understand about a topic and how well you can organize and express your knowledge.

Organizing your time

Most essays have a relatively straightforward structure and it is best to divide your time into three main parts (Fig. 51.1). For exam strategies, see Chapter 56.

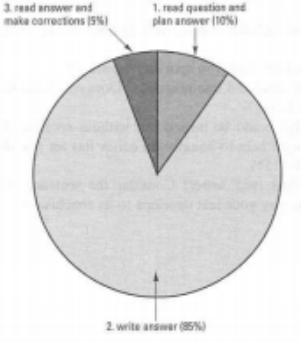

Fig. 51.1 Pie chart showing a typical division of time for an essay.

Making a plan for your essay

Dissect the meaning of the essay question or title

Read the title very carefully and think about the topic before starting to write. Consider the definitions of each of the important nouns (this can help in approaching the introductory section). Also think about the meaning of the verb(s) used and try to follow each instruction precisely (see Table 51.1). Don't get side-tracked because you know something about one word or phrase in the title: consider the whole title and all its ramifications. If there are two or more parts to the question, make sure you give adequate attention to each part.

Table 51.1 Instructions often used in essay questions and their meanings. When more than one instruction is given (e.g. compare and contrast; describe and explain), make sure you carry out *both* or your may lose a large proportion of the available marks

Account for:	give the reasons for
Analyse:	examine in depth and describe the main characteristics of
Assess:	weigh up the elements of and arrive at a conclusion about
Comment:	give an opinion on and provide evidence for your views
Compare:	bring out the similarities between
Contrast:	bring out dissimilarities between
Criticize:	judge the worth of (give both positive and negative aspects)
Define:	explain the exact meaning of
Describe:	use words and diagrams to illustrate
Discuss:	provide evidence or opinions about, arriving at a balanced conclusion
Enumerate:	list in outline form
Evaluate:	weigh up or appraise; find a numerical value for
Explain:	make the meaning of something clear
Illustrate:	use diagrams or examples to make clear
Interpret:	express in simple terms, providing a judgement
Justify:	show that an idea or statement is correct
List:	provide an itemized series of statements about
Outline:	describe the essential parts only, stressing the classification
Prove:	establish the truth of
Relate:	show the connection between
Review:	examine critically, perhaps concentrating on the stages in the development of an idea or method
State:	express clearly
Summarize:	without illustrations, provide a brief account of
Trace:	describe a sequence of events from a defined point of origin

Consider possible content and examples

The spider diagram technique (p. 326) is a speedy way of doing this. If you have time to read several sources, consider their content in relation to the essay title. Can you spot different approaches to the same subject? Which do you prefer as a means of treating the topic in relation to your title? Which examples are most relevant to your case, and why?

Construct an outline

Every essay should have a structure related to its title. Most marks for essays are lost because the written material is badly organized or is irrelevant. An essay plan, by definition, creates order and, if thought about carefully, can ensure relevance. Your plan should be written down (but scored through later if written in an exam book). Think about an essay's content in three parts:

Essay content – it is rarely enough simply to lay down facts for the reader – you must analyse them and comment on their significance.

1. The introductory section, in which you should include definitions and some background information on the context of the topic being considered. You should also tell your reader how you plan to approach the subject.
2. The middle of the essay, where you develop your answer and provide relevant examples. Decide whether a broad analytical approach is appropriate or whether the essay should contain more factual detail.
3. The conclusion, which you can make quite short. You should use this part to summarize and draw together the components of the essay, without merely repeating previous phrases. You might mention such things as: the broader significance of the topic; its future; its relevance to other important areas of chemistry. Always try to mention both sides of any debate you have touched on, but beware of 'sitting on the fence'.

KEY POINT **Use paragraphs to make the essay's structure obvious. Emphasize them with headings and sub-headings unless the material beneath the headings would be too short or trivial.**

Now start writing!

Using diagrams – give a title and legend for each diagram so that it makes sense in isolation and point out in the text when the reader should consult it (e.g. 'as shown in Fig. 1 . . .' or 'as can be seen in the accompanying diagram, . . .').

- Never lose track of the importance of content and its relevance. Repeatedly ask yourself: 'Am I really answering this question?' Never waffle just to increase the length of an essay. Quality rather than quantity is important.
- Illustrate your answer appropriately. Use examples to make your points clear, but remember that too many similar examples can stifle the flow of an essay. Use diagrams where a written description would be difficult or take too long. Use tables to condense information.
- Take care with your handwriting. You can't get marks if your writing is illegible! Try to cultivate an open form of handwriting, making the individual letters large and distinct. If there is time, make out a rough draft from which a tidy version can be copied.

Reviewing your answer

Learning from lecturers' and tutors' comments – ask for further explanations if you don't understand a comment or why an essay was less successful than you thought it should have been.

Don't stop yet!

- Reread the question to check that you have answered all points.
- Reread your essay to check for errors in punctuation, spelling and content. Make any corrections obvious. Don't panic if you suddenly realize you've missed a large chunk out as the reader can be redirected to a supplementary paragraph if necessary.

52 Reporting practical and project work

Practical reports, project reports, theses and scientific papers differ greatly in depth, scope and size, but they all have the same basic structure (Box 52.1). Some variation is permitted, however (see Box 52.1), and you should always follow the advice or rules provided by your department.

Additional parts may be specified: for theses, a title page is often required and a List of Figures and Tables as part of the Contents. When work is submitted for certain degrees, you may need to include certain declarations and statements made by the student and supervisor. In scientific papers, a list of Key Words is often added following the Abstract: this information may be combined with words in the title for computer cross-referencing systems.

KEY POINT **Department or faculty regulations may specify an exact format for producing your report or thesis. Obtain a copy of these rules at an early stage and follow them closely.**

Options for discussing data – the main optional variants of the general structure include combining Results and Discussion into a single section and adding a separate conclusions section.

- The main advantage of a joint results and discussion section is that you can link together different experiments, perhaps explaining why a particular result led to a new hypothesis and the next experiment.
- The main advantage of having a separate conclusions section is to draw together and emphasize the chief points arising from your work, when these may have been 'buried' in an extensive discussion section.

Practical and project reports

These are exercises designed to make you think more deeply about your experiments and to practise and test the skills necessary for writing up research work. Special features are:

- Introductory material is generally short and unless otherwise specified should outline the aims of the experiment(s) with a minimum of background material.
- Experimental instructions may be provided by your supervisor for practical reports. With project work, your lab notebook (see p. 67) should provide the basis for writing this section.
- Great attention in assessment will be paid to presentation and analysis of data. Take special care over graphs (see p. 251). Make sure your conclusions are justified by the evidence.

Theses

Theses are submitted as part of the examination for a degree following an extended period of research. They act to place on record full details about your experimental work and will normally only be read by those with a direct interest in it – your examiners or colleagues. Note the following:

- You are allowed scope to expand on your findings and to include detail that might otherwise be omitted in a scientific paper.
- You may have problems with the volume of information that has to be organized. One method of coping with this is to divide your thesis into chapters, each having the standard format (as in Box 52.1). A general introduction can be given at the start and a general discussion at the end. Discuss this option with your supervisor as it is not universally favoured.
- There may be an oral exam ('viva') associated with the submission of the thesis. The primary aim of the examiners will be to ensure that you understand what you did and why you did it.

Box 52.1 The structure of reports of experimental work

Undergraduate practical and project reports are generally modelled on this structure or a close variant of it, because this is the structure used for nearly all research papers and theses. The more common variations include Results and Discussion combined into a single section for convenience and Conclusions appearing separately as a series of points arising from the work. In scientific papers, a list of Key Words (for computer cross-referencing systems) may be included following the Abstract. Regarding variations in positioning, Acknowledgements may appear after the Contents, rather than near the end. Department or faculty regulations for producing theses and reports may specify a precise format; they often require a title page to be inserted at the start and a List of Figures and Tables as part of the Contents. These regulations may also specify declarations and statements to be made by the student and supervisor.

Part (in order)	Contents/purpose	Checklist for reviewing content
Title	Explains what the project was about	Does it explain what the text is about succinctly?
Authors plus their institutions	Explains who did the work and where; also where they can be contacted now	Are all the details correct?
Abstract/Summary	Synopsis of methods, results and conclusion of work described. Allows the reader to grasp quickly the essence of the work	Does it explain why the work was done? Does it outline the whole of your work and your findings?
List of Contents	Shows the organization of the text (not required for short papers)	Are all the sections covered? Are the page numbers correct?
Abbreviations	Lists all the abbreviations used (but not those of SI, chemical elements, or standard chemical terms)	Have they all been explained? Are they all in the accepted form? Are they in alphabetical order?
Introduction	Orientates the reader, explains why the work has been done and its context in the literature, why the methods used were chosen. Indicates the central hypothesis behind the experiments	Does it provide enough background information and cite all the relevant references? Is it of the correct depth for the readership? Have all the technical terms been defined? Have you explained why you investigated the problem? Have you explained your methodological approach to the problem?
Experimental	Explains how the work was done. Should contain sufficient detail to allow another competent worker to repeat the work	Is each experiment covered and have you avoided unnecessary duplication? Is there sufficient detail to allow repetition of the work? Are the correct names, sources and grades given for all chemicals?
Results	Displays and describes the data obtained. Should be presented in a form which is easily assimilated (graphs rather than tables, small tables rather than large ones)	Is the sequence of experiments logical? Are the parts adequately linked? Are the data presented in the clearest possible way? Have SI units been used properly throughout? Has adequate statistical analysis been carried out? Is all the material relevant? Are the figures and tables all numbered in the order of their appearance? Are their titles appropriate? Do the figure and table legends provide all the information necessary to interpret the data without reference to the text? Have you presented the same data more than once?
Discussion/ Conclusions	Discusses the results: their meaning, their importance; compares the results with those of others; suggests what to do next	Have you explained the significance of the results? Have you compared your data with other published work? Are your conclusions justified by the data presented?
Acknowledgements	Gives credit to those who helped carry out the work	Have you listed everyone that helped, including any grant-awarding bodies?
Literature Cited (Bibliography)	Lists all references cited in appropriate format: provides enough information to allow the reader to find the reference in a library	Do all the references in the text appear on the list? Do all the listed references appear in the text? Do the years of publications and authors match? Are the journal details complete and in the correct format? Is the list in alphabetical order, or correct numerical order?

Steps in the production of a practical report or thesis

Choose the experiments you wish to describe and decide how best to present them

Try to start this process before your lab work ends, because at the stage of reviewing your experiments, a gap may become apparent (e.g. a missing control) and you might still have time to rectify the deficiency. Irrelevant material should be ruthlessly eliminated, at the same time bearing in mind that negative results can be extremely important. Use as many different forms of data presentation as are appropriate, but avoid presenting the same data in more than one form. Graphs are generally easier for the reader to assimilate, while tables can be used to condense a lot of data into a small space. Relegate large tables of data to an appendix and summarize the important points. Make sure that the experiments you describe are representative: always state the number of times they were repeated and how consistent your findings were.

Repeating your experiments – remember, if you do an experiment twice, you have repeated it only once!

Make up plans or outlines for the component parts

The overall plan is well defined (see Box 52.1), but individual parts will need to be organized as with any other form of writing (see Chapter 50).

Write!

The experimental section is often the easiest to write once you have decided what to report. There may be minor variations in style, but the one shown in Box 52.2 will be suitable in the majority of cases. The results section is the next easiest as it should only involve description. At this stage your should be jotting down ideas for the Discussion – this may be the hardest part to compose, as you need an overview of both your own work and the relevant literature. It is also liable to become wordy, so try hard to make it succinct. The Introduction should not be too difficult if you have understood fully the aims of the experiments. Write the Abstract and complete the list of references at the end. To assist with the latter, it is a good idea to use or pull out their cards from your index system.

Presenting your results – remember that the order of results presented in a report need not correspond with the order in which you carried out the experiments: you are expected to rearrange them to provide a logical sequence of findings.

Revise the text

Once your first draft is complete, try to answer all the questions given in Box 52.1. Show your work to your supervisors and learn from their comments. Let a friend or colleague who is unfamiliar with your subject read your text; he/she may be able to pinpoint obscure wording and show where information or explanation is missing. If writing a thesis, double-check that you are adhering to your institution's thesis regulations.

Prepare the final version

Markers appreciate neatly produced work but a well-presented document will not disguise poor science! If using a word processor, print the final version with the best printer available. Make sure figures are clear and in the correct size and format.

Remember, there is no excuse for sloppy presentation if you use a word processor.

Submit your work

Your department will specify when to submit a thesis or project report, so plan your work carefully to meet this deadline or you may lose marks. Tell your supervisor early of any circumstances that may cause delay.

Box 52.2 Writing experimental procedures

The main purpose of the methods described in the experimental section of a laboratory report, project report, thesis or paper is to communicate sufficient information to allow an experienced chemist to repeat your experiments. One of the goals of writing laboratory reports is to provide you with practice in writing in the generally accepted style required for more professional publications. The following points should be noted:

- Always write in the third person, past tense.
- Do not copy word for word the instructions given in the experiment protocol. You are in a learning situation in the laboratory and the protocol may contain information, which may be new to you, but is general knowledge to experienced chemists.
- Condensing the experimental protocol for your report will help you to see the important steps in the experiment.
- All sentences begin with a capital letter not a number or bracket.

The following examples illustrate the differences between the instructions of the experiment protocol and the accepted style required for the experimental section.

Example 1 A preparative experiment: the synthesis of methyl 2,4-dimethylbenzoate

Experiment protocol

'In a round-bottom flask (100 mL) place 2,4-dimethylbenzoic acid (3.0 g, 0.02 mol), anhydrous potassium carbonate (3.3 g, 0.024 mol) and a small magnetic flea. In the fume cupboard and wearing protective gloves, carefully weigh out dimethylsulphate (2.8 g, 0.022 mol) into a glass sample tube (10 mL) using a Pasteur pipette to transfer the liquid. Using the Pasteur pipette, transfer the dimethylsulphate to the reaction flask and use anhydrous propanone (10 mL) to rinse the sample tube. Add the rinsings to the reaction flask and add more dry propanone (10 mL). Fit the flask with a reflux condenser and boil the mixture under reflux for 3 hours using an oil bath on a stirrer hot plate in the fume cupboard. Allow the reaction mixture to cool to room temperature and pour into water (100 mL) washing the reaction flask with a few millilitres of water. Extract the aqueous solution three times with dichloromethane (20 mL), dry the dichloromethane ($MgSO_4$), filter off the drying agent, remove the solvent on the rotary evaporator and record the weight of the crude product. Recrystallize and charcoal the crude ester from ethanol and dry in a vacuum desiccator. Record the weight and melting point of the purified ester and obtain and interpret infrared and ^{1}H-NMR spectra. Check the purity of your product usingTLC (SiO_2 plates and CH_2Cl_2 as eluent) and calculate the percentage yield of the product.'

Experimental

A mixture of 2,4-dimethylbenzoic acid (3.0 g, 0.02 mol), dimethylsulphate (2.8 g, 0.022 mol) and anhydrous potassium carbonate (3.3 g, 0.024 mol) in dry propanone (20 mL) was boiled under reflux (3h), cooled, poured into water (100 mL) and extracted with dichloromethane (3 × 20 mL). Removal of the dried ($MgSO_4$) organic solvent gave a white solid (2.3 g), which was recrystallized from ethanol, using charcoal to improve the colour, to yield white needles of methyl 2,4-dimethylbenzoate (2.28 g). The infrared (Nujol) and ^{1}H-NMR ($CDCl_3$) spectra were recorded and TLC (SiO_2/CH_2Cl_2) showed the product to be pure. The melting point was determined and the % yield calculated.

Note 1. Experimental gives reagents, solvents, quantities, times, yields, etc., and sufficient detail for the experiment to be repeated by a proficient chemist. Details of standard techniques such as weighing liquids, setting up apparatus, recrystallization, drying the product, etc., are 'understood' by the experimentalist from experience and training.

Note 2. The weights and volumes used are quoted to one decimal place, since this is the level of accuracy required for preparative experiments which results from the techniques used, distillation, recrystallization, extraction, etc., and the non-quantitative conversion of the reactants to products may be more significant than errors in weighing.

Note 3. All analytical data such as IR and NMR spectra, TLC, melting point and yield calculations are entered in the results section.

Note 4. The % yield for the product represents a comparison with the theoretical yield of the reaction and the practical yield. Calculation of the theoretical yield based on:

(a) the molar quantities of the reactants used;
(b) the number of moles of each reactant required to make 1 mole of product;
(c) the assumption that reactions go 100%;
(d) the 'limiting quantity' of one of the reactants.

To calculate the theoretical yield of a reaction, it is essential that you recognize the reactant, which is the limiting quantity.

If a reaction requires 1 mole of reactant A and 1 mole of reactant B to give 1 mole of product AB, i.e.:

$$A + B \rightarrow AB$$

if we use A (1 mol) and B (1.5 mol), then only 1 mol of AB can be formed and there is 0.5 mol of B in excess

Box 52.2 (continued)

and unreacted. Therefore the limiting quantity is the amount of reactant A (1 mol). The excess of reactant B may be necessary to ensure complete conversion of A into AB, i.e. 100% reaction. In the example above, the limiting quantity is the amount of 2,4-dimethylbenzoic acid (0.02 mol) and only 0.02 mol of the methyl ester can be formed (164 × 0.02 = 3.28 g). Therefore:

$$\%\ \text{yield} = \frac{\text{reaction yield}}{\text{theory yield}} \times 100 = \frac{2.28}{3.28} \times 100 = 70\%$$

Example 2 A quantitative experiment: standardization of sodium thiosulphate solution

Experiment protocol

'Prepare a standard solution of potassium iodate by dissolving potassium iodate (about 1.34 g, accurately weighed) in distilled water (100 mL), quantitatively transferring the solution to a volumetric flask (250.00 mL) making up to the mark using distilled water and mixing well. Pipette an aliquot (25.00 mL) of the solution into a conical flask (250 mL) and add sulphuric acid (50 mL, 1 M). Weigh out potassium iodide (1 g), add it to the conical flask and swirl until it has all dissolved. Titrate the liberated iodine (brown solution) with the sodium thiosulphate solution until a pale straw colour is reached. Add iodine indicator (two drops) or freshly prepared starch solution (two drops) and continue the titration until the colour changes from blue–black to colourless. If the solution does not turn blue–black when you add the iodine indicator or starch, you have overshot the end-point. Repeat the experiment until consistent results are obtained. Calculate the molarity of the sodium thiosulphate solution.'

Experimental

Potassium iodate (1.3402 g) was dissolved in distilled water (100 mL), transferred to a volumetric flask (250.00 mL) and made up to the mark using distilled water. An aliquot (25.00 mL) was transferred to a conical flask (250 mL), sulphuric acid (50 mL, 1 M) and potassium iodide (1 g) were added and the mixture swirled to effect solution. The iodine liberated was titrated with the sodium thiosulphate solution to a pale straw colour. Iodine indicator (two drops) was then added and the titration continued to a blue–black to colourless end-point. The experiment was repeated until consistent results were obtained and the concentration of the sodium thiosulphate solution calculated.

Note 1. The differences in accuracy, indicating the equipment you must use, are shown by the decimal point quoted in the quantities.

Note 2. Titrations should be repeated to give consecutive results to within one or two drops of titrant. Do not average widely differing results.

Note 3. Balance readings, burette readings and calculations should be included in the results section.

Example 3 Reaction kinetics: determination of rate constant and energy of activation using titrimetry

Experiment protocol

'The following solutions are provided:

A. Potassium persulphate (0.04 M)
B. Sodium thiosulphate (0.01 M)
C. Potassium iodide (0.4 M).

i. Place solution A (100 mL) and solution C (100 mL) into separate conical flasks and suspend them in a thermostat bath at 25 °C.
ii. Mix solutions A and C (50 mL of each) in a stoppered flask and immerse in hot water until the end of the experiment.
iii. After about 15 minutes from step i, mix the solutions A and C and start the clock. This mixture must be kept in the thermostat bath at 25 °C, throughout the experiment.
iv. Just before 2 minutes have elapsed, take a sample (10.00 mL) from the flask by pipette and add it to distilled water (200 mL) in a separate flask. This quenches (stops) the reaction.
v. Take further samples at 4, 6, 9, 13, 18, 24, 32, 44 and 60 minutes and quench in the same way.
vi. Titrate each quenched solution with sodium thiosulphate, including a sample of the 'hot-water' sample. Use a small amount of freshly prepared starch indicator solution and titrate from blue to colourless. The titres for the samples are the values (T) and that for the 'hot-water' sample is T_∞.
vii. Now repeat the whole experiment using a thermostat bath set at 35–40 °C. Make an accurate record of the temperature. You do not need to repeat the 'hot-water' sample since this will serve for both experiments. Since this higher tempera-ture reaction is faster, there is no need to take samples at 44 and 60 minutes.
viii. For each temperature plot a graph of $-\ln(T_\infty - T)$ versus time (s).'

Experimental

Solutions (100 mL) of potassium persulphate (0.04 M) and potassium iodide (0.4 M) were equilibrated (0.25 h) in a constant temperature bath (25 °C). The two solutions were mixed in a conical flask (250 mL) in the constant temperature bath and the clock started. After 2 minutes, an aliquot (10.00 mL) of the mixture was quenched with distilled water (200 mL) and the sampling process repeated after 4, 6, 9, 13, 18, 24, 32, 44 and 60 minutes. Each sample was titrated with

Box 52.2 (continued)

sodium thiosulphate solution (0.01 M), using iodine indicator, to a colourless end-point and the values recorded (7). The experiment was then repeated at a known temperature between 35°C and 40°C but the samples at 44 and 60 minutes were not taken. Solutions (50 mL) of the potassium persulphate and potassium iodide were mixed and kept in a water bath (60–70°C) for 2 hours. An aliquot (10.00 mL) of this solution was quenched in distilled water (200 mL) and titrated (T_∞) with the sodium thiosulphate solution. A graph of $-\ln(t_\infty - T)$ versus time (s) was plotted.

Note 1. Only experimental detail is given. Calculations, results and theory are in the appropriate sections.

Box 52.3 Steps in producing a scientific paper

Scientific papers are the life-blood of any science and it is a major landmark in your scientific career to publish your first paper. The major steps in doing this should include the following.

Assessing potential content
The work must be of an appropriate standard to be published and should be 'new, true and meaningful'. Therefore, before starting, the authors need to review their work critically under these headings. The material included in a scientific paper will generally be a subset of the total work done during a project, so it must be carefully selected for relevance to a clear central hypothesis – if the authors won't prune, the referees and editors of the journal certainly will!

Choosing a journal
There are thousands of journals covering chemistry and each covers a specific area (which may change through time). The main factors in deciding on an appropriate journal are the range of subjects it covers, the quality of its content and the number and geographical distribution of its readers. The choice of journal always dictates the format of a paper since authors must follow to the letter the journal's 'Instructions to Authors'.

Deciding on authorship
In multiauthor papers, a contentious issue is often who should appear as an author and in what order they should be cited. Where authors make an equal contribution, an alphabetical order of names may be used. Otherwise, each author should have made a substantial contribution to the paper and should be prepared to defend it in public. Ideally, the order of appearance will reflect the amount of work done rather than seniority. This may not happen in practice!

Writing
The paper's format will be similar to that shown in Box 52.1 and the process of writing will include outlining, reviewing, etc., as discussed elsewhere in this chapter. Figures must be finished to an appropriate standard and this may involve preparing photographs of them.

Submitting
When completed, copies of the paper are submitted to the editor of the chosen journal with a simple covering letter. A delay of 1 to 2 months usually follows while the manuscript is sent to one or more anonymous referees who will be asked by the editor to check that the paper is novel, scientifically correct and that its length is fully justified.

Responding to referees' comments
The editor will send on the referees' comments and the authors then have a chance to respond. The editor will decide on the basis of the comments and replies to them whether the paper should be published. Sometimes quite heated correspondence can result if the authors and referees disagree!

Checking proofs and waiting for publication
If a paper is accepted, it will be sent off to the typesetters. The next the authors see of it is the proofs (first printed version in style of journal), which have to be corrected carefully for errors and returned. Eventually, the paper will appear in print, but a delay of 6 months following acceptance is not unusual. Most journals offer the authors reprints, which can be sent to other researchers in the field or to those who send in reprint request cards.

Producing a scientific paper

Scientific papers are the means by which research findings are communicated to others. They are published in journals with a wide circulation among academics and are 'peer reviewed' by one or more referees before being accepted. Each journal covers a well-defined subject area and publishes details of the format they expect. It would be very unusual for an undergraduate to submit a paper on his/her own – this would normally be done in collaboration with your project supervisor and only then if your research has satisfied appropriate criteria. However, it is important to understand the process whereby a paper comes into being (Box 52.3), as this can help you when interpreting the primary literature.

53 Writing literature surveys and reviews

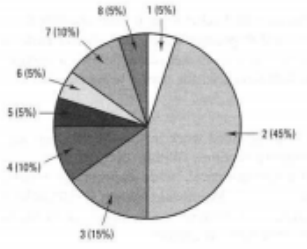

Fig. 53.1 Pie chart showing how you might allocate time for a literature survey:
1. select a topic;
2. scan the literature;
3. plan the review;
4. write first draft;
5. leave to settle;
6. structure-review the text;
7. write final draft;
8. produce top copy.

The literature survey or review is a specialized form of essay which summarizes and reviews the evidence and concepts concerning a particular area of research.

KEY POINT **A literature review should *not* be a recitation of facts. The best reviews are those which analyse information rather than simply describe it.**

Making up a timetable

Fig. 53.1 illustrates how you might divide up your time for writing a literature survey. There are many sub-divisions in this chart because of the size of the task: in general, for lengthy tasks, it is best to divide up the work into manageable chunks. Note also that proportionately less time is allocated to writing itself than with an essay. In a literature survey, make sure that you spend adequate time on research and revision.

Selecting a topic

You may have no choice in the topic to be covered, but if you do, carry out your selection as a three-stage process:

1. Identify a broad subject area that interests you.
2. Find and read relevant literature in that area. Try to gain a broad impression of the field from books and general review articles. Discuss your ideas with your supervisor.
3. Select a relevant and concise title. The wording should be considered very carefully as it will define the content expected by the reader. A narrow subject area will cut down on the amount of literature you will be expected to review, but will also restrict the scope of the conclusions you can make (and vice versa for a wide subject area).

Scanning the literature and organizing your references

You will need to carry out a thorough investigation of the literature before you start to write. The key problems are as follows:

- Getting an initial toe-hold in the literature. Seek help from your supervisor, who may be willing to supply a few key papers to get you started. Hints on expanding your collection of references are given on p. 317.
- Assessing the relevance and value of each article. This is the essence of writing a review, but it is difficult unless you already have a good understanding of the field (Catch 22!). Try reading earlier reviews in your area.
- Clarifying your thoughts. Sometimes you can't see the wood for the trees! Sub-dividing the main topic and assigning your references to these smaller subject areas may help you gain a better overview of the literature.

Using index cards (see p. 319) – these can help you organize large numbers of references. Write key points on each card – this helps when considering where the reference fits into the literature. Arrange the cards in subject piles, eliminating irrelevant ones. Order the cards in the sequence you wish to write in.

Deciding on structure and content

The general structure and content of a literature survey are described below.

Introduction

The introduction should give the general background to the research area, concentrating on its development and importance. You should also make a statement about the scope of your survey; as well as defining the subject matter to be discussed, you may wish to restrict the period being considered.

Main body of text

The review itself should discuss the published work in the selected field and may be sub-divided into appropriate sections. Within each portion of a review, the approach is usually chronological, with appropriate linking phrases (e.g. 'Following on from this, ...'; 'Meanwhile, Bloggs (1980) tackled the problem from a different angle ...'). However, a good review is much more than a chronological list of work done. It should:

- allow the reader to obtain an overall view of the current state of the research area, identifying the key areas where knowledge is advancing;
- show how techniques are developing and discuss the benefits and disadvantages of using particular chemicals or experimental systems;
- assess the relative worth of different types of evidence – this is the most important aspect: do not be intimidated from taking a critical approach as the conclusions you may read in the primary literature aren't always correct;
- indicate where there is conflict in findings or theories, suggesting if possible which side has the stronger case;
- indicate gaps in current knowledge.

Balancing opposing views – even if you favour one side of a disagreement in the literature, your review should provide a fair description of all the published views of the topic. Having done this, if you do wish to state a preference, give reasons for your opinion.

Conclusions

The conclusions should draw together the threads of the preceding parts and point the way forward, perhaps listing areas of ignorance or where the application of new techniques may lead to advances.

References etc.

The references or literature cited section should provide full details of all papers referred to in the text (see p. 320). The regulations for your department may also specify a format and position for the title page, list of contents, acknowledgements, etc.

Making citations – a review of literature poses stylistic problems because of the need to cite large numbers of papers; in *Chemical Society Reviews* this is overcome by using numbered references (see p. 320).

Style of literature surveys

The Chemical Society Review series (available in most university libraries) provides good examples of appropriate style for reviews of the chemical sciences.

54 Organizing a poster display

A scientific poster is a visual display of the results of an investigation, usually mounted on a rectangular board. Posters are used at scientific meetings, to communicate research findings, and in undergraduate courses, to display project results or assignment work.

In a written report you can include a reasonable amount of specific detail and the reader can go back and reread difficult passages. However, if a poster is long winded or contains too much detail, your reader is likely to lose interest.

KEY POINT **A poster session is like a competition – you are competing for the attention of people in a room. Because you need to attract and hold the attention of your audience, make your poster as interesting as possible. Think of it as an advertisement for your work and you will not go far wrong.**

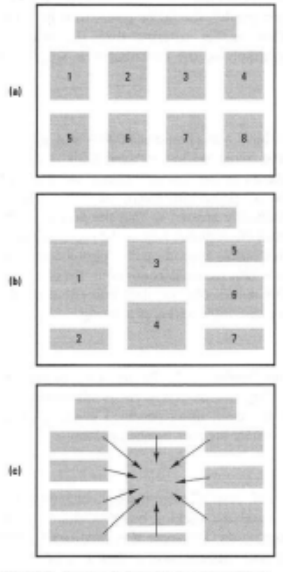

Fig. 54.1 Poster design. (a) An uninspiring design: sub-units of equal area, reading left to right, are not recommended. (b) This design is more interesting and the text will be easier to read (column format). (c) An alternative approach, with a central focus and arrows/tapes to guide the reader.

Preliminaries

Before considering the content of your poster, you should find out:

- the linear dimensions of your poster area, typically up to 1.5 m wide by 1.0 m high;
- the composition of the poster board and the method of attachment, whether drawing pins, Velcro® tape, or some other form of adhesive; and whether these will be provided (in any case, it's safer to bring your own);
- the time(s) when the poster should be set up and when you should attend;
- the room where the poster session will be held.

Design

Plan your poster with your audience in mind, as this will dictate the appropriate level for your presentation. Aim to make your poster as accessible as possible to a broad audience. Since a poster is a visual display, you must pay particular attention to the presentation of information: work that may have taken hours to prepare can be ruined in a few minutes by the ill-considered arrangement of items (Fig. 54.1). Begin by making a draft sketch of the major elements of your poster. It is worth discussing your intended design with someone else, as constructive advice at the draft stage will save a lot of time and effort when you prepare the final version (or consult Simmonds and Reynolds, 1994).

Layout

Usually the best approach is to divide the poster into several smaller areas, perhaps six or eight in all, and prepare each as a separate item on a piece of thick card. Some people prefer to produce a single large poster on one sheet of paper or card and store it inside a protective cardboard tube. However, a single large poster will bend and crease, making it difficult to flatten out. In addition, photographs and text attached to the backing sheet often work loose.

Sub-dividing your poster means that each smaller area can be prepared on a separate piece of paper or card, of A4 size or slightly larger, making

transport and storage easier. It also breaks the reading matter up into smaller pieces, looking less formidable to a potential reader. By using pieces of card of different colours you can provide emphasis for key aspects, or link text with figures or photographs.

You will need to guide your reader through the poster. It is often appropriate to use either a numbering system, with large, clear numbers at the top of each piece of card, or a system of arrows (or thin tapes), to show the relationship of sections within the poster (see Fig. 54.1). Make sure that the relationship is clear and that the arrows or tapes do not cross.

Title

Your chosen title should be concise (no more than eight words), specific and interesting, to encourage people to read the poster. Make the title large and bold – it should run across the top of your poster, in letters at least 4 cm high, so that it can be read from the other side of the room. Coloured spirit-based marker and block capitals drawn with a ruler work well, as long as your writing is readable and neat (the colour can be used to add emphasis). Alternatively, you can use Letraset®, or similar lettering. Details of authors, together with their addresses (if appropriate), should be given, usually in the top right-hand corner in somewhat smaller lettering than the title. At conferences, a passport-sized photograph of the contributor is sometimes useful for identification.

Making up your poster – text and graphics printed on good-quality paper can be glued directly onto a contrasting mounting card: use photographic spray mountant or Pritt® rather than liquid glue. Trim carefully using a guillotine to give equal margins, parallel with the paper. Photographs should be placed in a window mount to avoid the tendency for their corners to curl. Another approach is to trim pages or photographs to their correct size, then encapsulate in plastic film: this gives a highly professional finish and is less weighty to transport.

Text

Keep text to a minimum – aim to have a maximum of 500 words in your poster. Write in short sentences and avoid verbosity. Keep your poster as visual as possible and make effective use of the spaces between the blocks of text. Your final text should be double spaced and should have a minimum capital letter height of 5–10 mm, preferably greater, so that the poster can be read at a distance of 1 m. One method of obtaining text of the required size is to photo-enlarge standard typescript (using a good-quality photocopier), or use a high-quality (laser) printer. It is best to avoid continuous use of text in capitals, since it slows reading and makes the text less interesting to the reader. Also avoid italic, 'balloon' or decorative styles of lettering.

Sub-titles and headings

These should have a capital letter height of 12–20 mm, and should be restricted to two or three words. They can be produced by photo-enlargement, by stencilling, Letraset® or by hand, using pencilled guidelines (but make sure that no pencil marks are visible on your finished poster).

Colour

Consider the overall visual effect of your chosen display, including the relationship between text, diagrams and the backing board. Colour can be used to highlight key aspects of your poster. However, it is very easy to ruin a poster by the inappropriate choice and application of colour. Careful use of two, or at most three, complementary colours will be easier on the eye and may aid comprehension. Colour can be used to link the text with the visual images (e.g. by picking out a colour in a photograph and using the same colour on the mounting board for the accompanying text). Use coloured inks or water-based paints to provide colour in diagrams and figures, as felt pens rarely give satisfactory results.

Content

The typical format is that of a scientific report (see Box 52.1), i.e. with the same headings, but with a considerably reduced content. Never be tempted to spend the minimum amount of time converting a piece of scientific writing into poster format. At scientific meetings, the least interesting posters are those where the author simply displays pages from a written communication (e.g. a journal article) on the poster board! Keep references within the text to a minimum – interested parties can always ask you for further information.

Introduction

This should give the reader background information on the broad field of study and the aims of your own work. It is vital that this section is as interesting as possible, to capture the interest of your audience. It is often worth listing your objectives as a series of numbered points.

Designing the materials and methods section – photographs or diagrams of apparatus can help to break up the text of the experimental section and provide visual interest. It is sometimes worth preparing this section in a smaller typeface.

Experimental

Keep this short, and describe only the principal techniques used. You might mention any special techniques, or problems of general interest.

Results

Don't present your raw data: use data reduction wherever possible, i.e. figures and simple statistical comparisons. Graphs, diagrams, histograms and pie charts give clear visual images of trends and relationships and should be used in place of tabulated data (see p. 251). Final copies of all figures should be produced so that the numbers can be read from a distance of 1 m. Each should have a concise title and legend, so that it is self-contained: if appropriate, a series of numbered points can be used to link a diagram with the accompanying text. Where symbols are used, provide a key on each graph (symbol size should be at least 5 mm). Avoid using graphs straight from a written version, e.g. a project report, textbook or a paper, without considering whether they need modification to meet your requirements.

Keeping graphs and diagrams simple – avoid composite graphs with different scales for the same axis, or with several trend lines (use a maximum of three trend lines per graph).

Conclusions

This is where many readers will begin, and they may go no further unless you make this section sufficiently interesting. This section needs to be the strongest part of your poster. Refer to your figures here to draw the reader into the main part of your poster. A slightly larger or bolder typeface may add emphasis, though too many different typefaces can look messy.

Listing your conclusions – a series of numbered points is a useful approach, if your findings fit this pattern.

The poster session

If you stand at the side of your poster throughout the session, you are likely to discourage some readers, who may not wish to become involved in a detailed conversation about the poster. Stand nearby. Find something to do – talk to someone else, or browse among the other posters, but remain aware of people reading your poster and be ready to answer any queries they may raise. Do not be too discouraged if you aren't asked lots of questions: remember, the poster is meant to be a self-contained, visual story, without need for further explanation.

A poster display will never feel like an oral presentation, where the nervousness beforehand is replaced by a combination of satisfaction and relief as you unwind after the event. However, it can be a very satisfying means of communication, particularly if you follow these guidelines.

Consider providing a handout – this is a useful way to summarize the main points of your poster, so that your readers have a permanent record of the information you have presented.

55 Giving an oral presentation

Most students feel very nervous about giving talks. This is natural, since very few people are sufficiently confident and outgoing that they look forward to speaking in public. Additionally, the technical nature of your subject matter may give you cause for concern, especially if you feel that some members of the audience have a greater knowledge than you have. However, this is a fundamental method of scientific communication and it therefore forms an important component of many courses.

The comments in this chapter apply equally to informal talks, e.g. those based on assignments and project work, and to more formal conference presentations. It is hoped that the advice and guidance given below will encourage you to make the most of your opportunities for public speaking, but there is no substitute for practice. Do not expect to find all of the answers from this, or any other, book. Rehearse, and learn from your own experience.

KEY POINT **The three 'Rs' of successful public speaking are: Reflect – give sufficient thought to all aspects of your presentation, particularly at the planning stage. Rehearse – to improve your delivery. Rewrite – modify the content and style of your material in response to your own ideas and to the comments of others.**

Learning from experience – use your own experience of good and bad lecturers to shape your performance. Some of the more common errors include:

- speaking too quickly
- reading to notes and ignoring the audience
- unexpressive, impersonal or indistinct speech
- distracting mannerisms
- poorly structured material with little emphasis on key information
- factual information too complex and detailed
- too few visual aids.

Preparation

Preliminary information

Begin by marshalling all of the details you need to plan your presentation, including:

- the duration of the talk;
- whether time for questions is included;
- the size and location of the room;
- the projection/lighting facilities provided, and whether pointers or similar aids are available.

It is especially important to find out whether the room has the necessary equipment for slide projection (slide projector and screen, black-out curtains or blinds, appropriate lighting) or overhead projection before you prepare your audiovisual aids. If you concentrate only on the spoken part of your presentation at this stage, you are inviting trouble later on. Have a look around the room and try out the equipment at the earliest opportunity, so that you are able to use the lights, projector, etc., with confidence.

Audiovisual aids

Find out whether your department has facilities for preparing overhead transparencies and slides, whether these facilities are available for your use and the cost of materials. Adopt the following guidelines:

- Keep text to a minimum: present only the key points, with up to 20 words per slide/transparency.
- Make sure the text is readable: try out your material beforehand.
- Use several simpler figures rather than a single complex graph.
- Avoid too much colour on overhead transparencies: blue and black are easier to read than red or green.
- Don't mix slides and transparencies as this is often distracting.

- Use spirit-based pens for transparencies; use alcohol for corrections.
- Transparencies can be produced from typewritten or printed text using a photocopier, often giving a better product than pens. Note that you must use special heat-resistant acetate sheets for photocopying.

Audience

You should consider your audience at the earliest stage, since they will determine the appropriate level for your presentation. If you are talking to fellow students you may be able to assume a common level of background knowledge. In contrast, a research lecture given to your department, or a paper at a meeting of a scientific society, will be presented to an audience from a broader range of backgrounds. An oral presentation is not the place for a complex discussion of specialized information: build up your talk from a low level. The speed at which this can be done will vary according to your audience. As long as you are not boring or patronizing, you can cover basic information without losing the attention of the more knowledgeable members in your audience. The general rule should be: 'do not overestimate the background knowledge of your audience'. This sometimes happens in student presentations, where fears about the presence of 'experts' can encourage the speaker to include too much detail, overloading the audience with facts.

Content

While the specific details in your talk will be for you to decide, most oral presentations share some common features of structure, as described below.

Introductory remarks

It is vital to capture the interest of your audience at the outset. Consequently, you must make sure your opening comments are strong, otherwise your audience will lose interest before you reach the main message. Remember it takes a sentence or two for an audience to establish a relationship with a new speaker. Your opening sentence should be some form of preamble and should not contain any key information. For a formal lecture, you might begin with 'Mr Chairman, ladies and gentlemen, my talk today is about …' then restate the title and acknowledge other contributors etc. You might show a transparency or slide with the title printed on it, or an introductory photograph, if appropriate. This should provide the necessary settling-in period.

After these preliminaries, you should introduce your topic. Begin your story on a strong note – this is no place for timid or apologetic phrases. You should:

- explain the structure of your talk;
- set out the aims and objectives of your work;
- explain your approach to the topic.

Opening remarks are unlikely to occupy more than 10% of the talk. However, because of their significance, you might reasonably spend up to 25% of your preparation time on them. Make sure you have practised this section, so that you can deliver the material in a flowing style, with less chance of mistakes.

The main message

This section should include the bulk of your experimental results or literature findings, depending on the type of presentation. Keep details of methods to the minimum needed to explain your data. This is *not* the place for a detailed description of equipment and experimental protocol (unless it is a talk about methodology!). Results should be presented in an easily digested format.

Allowing time for slides – as a rough guide you should allow at least 2 minutes per illustration, although some diagrams may need longer, depending on content.

Do not expect your audience to cope with large amounts of data; use a maximum of six numbers per slide. Present summary statistics rather than individual results. Show the final results of any analyses in terms of the statistics calculated, and their significance (p. 271), rather than dwelling on details of the procedures used. Remember that graphs and diagrams are usually better than tables of raw data, since the audience will be able to see the trends and relationships in your data (p. 251). However, figures should not be crowded with unnecessary detail. Every diagram should have a concise title and the symbols and trend lines should be clearly labelled, with an explanatory key where necessary. When presenting graphical data always 'introduce' each graph by stating the units for each axis and describing the relationship for each trend line or data set. Summary slides can be used at regular intervals, to maintain the flow of the presentation and to emphasize the key points.

Take the audience through your story step by step at a reasonable pace. Try not to rush the delivery of your main message owing to nervousness. Avoid complex, convoluted story-lines – one of the most distracting things you can do is to fumble backwards through slides or overhead transparencies. If you need to use the same diagram or graph more than once then you should make two (or more) copies. In a presentation of experimental results, you should discuss each point as it is raised, in contrast to written text, where the results and discussion may be in separate sections. The main message typically occupies approximately 80% of the time allocated to an oral presentation.

Final remarks – make sure you give the audience sufficient time to assimilate your final slide: some of them may wish to write down the key points. Alternatively, you might provide a handout, with a brief outline of the aims of your study and the major conclusions.

Concluding remarks

Having captured the interest of your audience in the introduction and given them the details of your story in the middle section, you must now bring your talk to a conclusion. At all costs, do not end weakly, e.g. by running out of steam on the last slide. Provide your audience with a clear 'take-home message', by returning to the key points in your presentation. It is often appropriate to prepare a slide or overhead transparency listing your main conclusions as a numbered series.

Signal the end of your talk by saying 'finally …', 'in conclusion …', or a similar comment and then finish speaking after that sentence. Your audience will lose interest if you extend your closing remarks beyond this point. You may add a simple end phrase (e.g. 'thank you') as you put your notes into your folder, but do not say 'that's all folks!', or make any similar offhand remark. Finish as strongly and as clearly as you started.

Hints on presentation

Notes

Many accomplished speakers use abbreviated notes for guidance, rather than reading from a prepared script. When writing your talk:

- Prepare a first draft as a full script: write in spoken English, keeping the text simple and avoiding an impersonal style. Aim to *talk* to your audience, not read to them.
- Use note cards with key phrases and words: it is best to avoid using a full script at the final presentation. As you rehearse and your confidence improves, a set of cards may be a more appropriate format for your notes.
- Consider the structure of your talk: keep it as simple as possible and announce each sub-division, so your audience is aware of the structure.

Using slides – check that the lecture theatre has a lectern light, otherwise you may have problems reading your notes when the lights are dimmed.

- Mark the position of slides/key points, etc.: each note card should contain details of structure, as well as content.
- Memorize your introductory/closing remarks: you may prefer to rely on a full written version for these sections, in case your memory fails.
- Use notes: write on only one side of the card/paper, in handwriting large enough to be read easily during the presentation. Each card or sheet must be clearly numbered, so that you do not lose your place.
- Rehearse your presentation: ask a friend to listen and to comment constructively on parts that were difficult to follow.
- Use 'split times' to pace yourself: following rehearsal, note the time at which you should arrive at key points of your talk. These timing marks will help you keep to time during the 'real thing'.

Image

Ensure that the image you project is appropriate for the occasion:

- Consider what to wear: aim to be respectable without 'dressing up', otherwise your message may be diminished.
- Develop a good posture: it will help your voice projection if you stand upright, rather than slouching or leaning over the lectern.
- Project your voice: speak towards the back of the room.
- Make eye contact: look at members of the audience in all parts of the room. Avoid talking to your notes, or to only one section of the audience.
- Deliver your material with expression: arm movements and subdued body language will help maintain the interest of your audience. However, you should avoid extreme gestures (it may work for some TV personalities, but it isn't recommended for the beginner!).
- Manage your time: avoid looking at your watch as it gives a negative signal to the audience. Use a wall clock, if one is provided, or take off your watch and put it beside your notes, so you can glance at it without distracting your audience.
- Try to identify and control any distracting repetitive mannerisms, e.g. repeated empty phrases, fidgeting with pens, keys, etc., as this will distract your audience. Practising in front of a mirror may help.
- Practise your delivery: use the comments of your friends to improve your performance.

Questions

Many speakers are worried by the prospect of questions after their oral presentation. Once again, the best approach is to prepare beforehand:

- Consider what questions you may be asked: prepare brief answers.
- Do not be afraid to say 'I don't know': your audience will appreciate honesty, rather than vacillation, if you don't have an answer for a particular question.
- Avoid arguing with a questioner: suggest a discussion afterwards rather than becoming involved in a debate about specific details.
- If no questions are forthcoming you may pose a question yourself, and then ask for opinions from the audience: if you use this approach you should be prepared to comment briefly if your audience has no suggestions. This will prevent the presentation from ending in an em-barrassing silence.

Examples Commonly used abbreviations include:

$\exists$	there are, there exist(s)
$\therefore$	therefore
$\because$	because
$\propto$	is proportional to
$\rightarrow$	leads to, into
$\leftarrow$	comes from, from
$\rightarrow\!\!\!\!\rightarrow$	involves several processes in a sequence
1°, 2°	primary, secondary (etc.)
$\approx$, $\cong$	approximately, roughly equal to
$=$, $\neq$	equals, not equal to
$\equiv$, $\not\equiv$	equivalent, not equivalent to
$<$, $>$	smaller than, bigger than
$\gg$	much bigger than
[X]	concentration of X
$\sum$	sum
f	function
#	number
∞	infinity, infinite

You should also make up your own abbreviations relevant to the context, e.g. if a lecturer is talking about resonance-stabilized carbocations, you could write 'RSC^{+}' instead.

56 Examinations

You are unlikely to have reached this stage in your education without being exposed to the examination process. However, the following comments should help you to identify and improve on the skills required for exam success.

Information gathering

KEY POINT **To do well in an examination, you need to put in effective work long before you go into the examination hall and even before you start to revise. You need to base your revision on accurate, tidy notes with an appropriate amount of subject detail and depth.**

Taking notes from lectures

Taking good lecture notes is essential if you are to make sense of them later. Start by noting the date, course, topic and lecturer. Number every page in case they get mixed up later. The most popular way of taking notes is to write in a linear sequence down the page; however, the alternative 'pattern' method (Fig. 56.1) has its advocates: experiment to see which you prefer.

Whatever method you use to take notes, you shouldn't try to take down all the lecturer's words, except when an important definition or example is being given, or when the lecturer has made it clear that he/she is dictating. Listen first, then write. Your goal should be to abstract the structure and reasoning behind the lecturer's approach. Use headings and leave plenty of space, but don't worry too much about being tidy at this stage – it is more important that you get down the appropriate information in a form that you at least can read. Use abbreviations to save time. Make sure you note down references to texts and take special care to ensure accuracy of definitions and numerical examples. If the lecturer repeats or otherwise emphasizes a point, make a margin note of this – it could come in useful when revising. If there is something you don't understand, ask at the end of the lecture, and make an appointment to discuss the matter if there isn't time to deal with it then. Tutorials may provide an additional forum for discussing course topics.

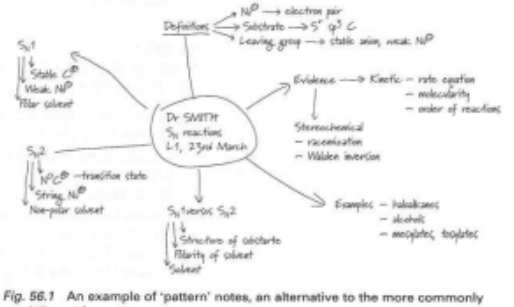

Fig. 56.1 An example of 'pattern' notes, an alternative to the more commonly used 'linear' format.

'Making up' your notes

As soon as possible after the lecture, work through your notes, tidying them up and adding detail where necessary. Add emphasis to any headings you have made, so that the structure is clearer. Compare your notes with material in a textbook and correct any inconsistencies. Make notes from, or photocopy, any useful material you see in textbooks, ready for revision.

Skimming texts

This is a valuable means of gaining the maximum amount of information in the minimum amount of time, by reading as little of a text as is required. It can be used to decide what parts to read in detail and to make notes of, perhaps when you have already read the text in detail at some point in the past.

Essentially, the technique requires you to look at the structure of the text, rather than the detail. In a sense, you are trying to see the writer's original plan and the purpose behind each part of the text. Look through the whole of the piece first, to gain an overview of its scope and structure. Headings provide an obvious clue to structure, if present. Next, look for the 'topic sentence' in each paragraph, which is often the first. You might then decide that the paragraph contains a definition that is important to note, or it may contain examples, so may not be worth reading for your purpose.

Seeing sequences – writers often number their points (firstly, secondly, thirdly, etc.) and looking for these words in the text can help you skim it quickly.

Preparing for an exam

Begin by finding out as much as you can about the exam, including:

- its format and duration;
- the date and location;
- the types of question;
- whether any questions/sections are compulsory;
- whether the questions are internally or externally set or assessed;
- whether calculators are required.

Your course tutor is likely to give you details of exam structure and timing well beforehand, so that you can plan your revision: the course handbook and past papers (if available) can provide further useful details. Check with your tutor that the nature of the exam has not changed before you consult past papers.

Organizing and using lecture notes, assignments and practical reports

Given their importance as a source of material for revision, you should have sorted out any deficiencies or omissions in lecture notes/practical reports at an early stage. For example, you may have missed a lecture or practical due to illness etc., but the exam is likely to assume attendance throughout the year. Make sure you attend classes whenever possible and keep your notes up to date.

Your practical reports and any assignment work will contain specific comments from the teaching staff, indicating where marks were lost, corrections, mistakes, inadequacies, etc. It is always worth reading these comments as soon as your work is returned, to improve the standard of your subsequent reports. If you are unsure about why you lost marks in an assignment, or about some particular aspects of a topic, ask the appropriate member of staff for further explanation. Most lecturers are quite happy to discuss such details with students on a one-to-one basis and this information may provide you with 'clues' to the expectations of individual lecturers that

may be useful in exams set by the same members of staff. However, you should *never* 'fish' for specific information on possible exam questions, as this is likely to be counter-productive.

Revision

Begin your revision early, to avoid last-minute panic. Start in earnest about 6 weeks beforehand:

- Prepare a revision timetable – an 'action plan' that gives details of specific topics to be covered. Find out at an early stage when (and where) your exams are to be held, and plan your revision around this. Try to keep to your timetable. Time management during this period is as important as keeping to time during the exam itself.
- Remember, your concentration span is limited to 15–20 min: make sure you have two or three short (5 min) breaks during each hour of revision.
- Make your revision as active and interesting as possible: the least productive approach is simply to read and reread your notes.
- Include recreation within your schedule: there is little point in tiring yourself with too much revision, as this is unlikely to be profitable.
- Ease back on the revision near the exam: plan your revision, to avoid last-minute cramming and overload fatigue.

Active revision

The following techniques may prove useful in devising an active revision strategy:

- Prepare revision sheets with details for a particular topic on a single sheet of paper, arranged as a numbered checklist. Wall posters are another useful revision aid.
- Memorize definitions and key phrases: definitions can be a useful starting point for many exam answers.
- Use mnemonics and acronyms to commit specific factual information to memory. The dafter they are, the better they work!
- Prepare answers to past or hypothetical questions, e.g. write essays or work through calculations and problems, within appropriate time limits. However, you should not rely on 'question spotting': this is a risky practice!
- Use spider diagrams as a means of testing your powers of recall on a particular topic (p. 326).
- Try recitation as an alternative to written recall.
- Draw diagrams from memory: make sure you can label them fully.
- Form a revision group to share ideas and discuss topics with other students.
- Use a variety of different approaches to avoid boredom during revision (e.g. record information on audio tape, use cartoons, or *any* other method, as long as it's not just reading notes!).

Preparing for an exam – make a checklist of the items you'll need (e.g. pens, pencils, sharpener and eraser, ruler, calculator, paper tissues, watch).

The evening before your exam should be spent in consolidating your material, and checking through summary lists and plans. Avoid introducing new material at this late stage: your aim should be to boost your confidence, putting yourself in the right frame of mind for the exam itself.

Final preparations – try to get a good night's sleep before an exam. Last-minute cramming will be counter-productive if you are too tired during the exam.

The examination

On the day of the exam, give yourself sufficient time to arrive at the correct room, without the risk of being late (e.g. what if your bus breaks down?).

Using the exam paper – unless this is specifically forbidden, you *should* write on the question paper to plan your strategy, keep to time and organize your answers.

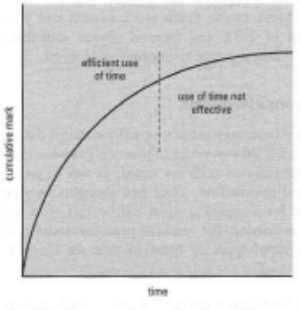

Fig. 56.2 Exam marks as a function of time. The marks awarded in a single answer will follow the law of diminishing returns – it will be far more difficult to achieve the final 25% of the available marks than the initial 25%. Do not spend too long on any one question.

The exam paper

Begin by reading the instructions at the top of the exam paper carefully, so that you do not make any errors based on lack of understanding. Make sure that you know:

- how many questions are set;
- how many must be answered;
- whether the paper is divided into sections;
- whether any parts are compulsory;
- what each question/section is worth, as a proportion of the total mark;
- whether different questions should be answered in different books.

If you are unsure about anything, ask! – the easiest way to lose marks in an exam is to answer the wrong number of questions, or to answer a different question from the one set by the examiner. Underline the key phrases in the instructions, to reinforce their message.

Next, read through the set of questions. If there is a choice, decide on those questions to be answered and decide on the order in which you will tackle them. Prepare a timetable which takes into account the amount of time required to complete each question and which reflects the allocation of marks – there is little point in spending one-quarter of the exam period on a question worth only 5% of the total marks! Use the exam paper to mark the sequence in which the questions will be answered and write the finishing times alongside: refer to this timetable during the exam to keep yourself on course.

Do not be tempted to spend too long on any one question: the return in terms of marks will not justify the loss of time from other questions (see Fig. 56.2). Take the first 10 min or so to read the paper and plan your strategy, before you begin writing. Do not be put off by those who begin immediately; it is almost certain they are producing unplanned work of a poor standard.

Providing answers

Before you tackle a particular question, you must be sure of what is required in your answer. Ask yourself 'What is the examiner looking for in this particular question?' and then set about providing a *relevant* answer. Consider each individual word in the question and highlight, underline or circle the key words. Make sure you know the meaning of the terms given in Table 51.1 (p. 330) so that you can provide the appropriate information, where necessary. Refer back to the question as you write, to confirm that you are keeping to the subject matter. Box 56.1 gives advice on writing essays under exam conditions.

It is usually a good idea to begin with the question that you are most confident about. This will reassure you before tackling more difficult parts of the paper. If you run out of time, write in note form. Examiners are usually understanding, as long as the main components of the question have been addressed and the intended structure of the answer is clear.

The final stage

At the end of the exam, you should allow at least 10 min to read through your script, to check for:

- grammatical and spelling errors;
- mathematical errors.

Box 56.1 Writing under exam conditions

Never go into an exam without a strategy for managing the available time.

- **Allocate some time (say 5% of the total) to consider which questions to answer and in which order.**
- **Share the rest of the time among the questions.** Aim to optimize the marks obtained. A potentially good answer should be allocated *slightly* more time than one you don't feel so happy about. However, don't concentrate on any one answer (see Fig. 56.2).
- **For each question divide the time into planning, writing and revision phases** (see p. 308).

Employ time-saving techniques as much as possible.

- **Use spider diagrams** (p. 326) to organize and plan your answer.
- **Use diagrams and tables** to save time in making difficult and lengthy explanations.
- **Use abbreviations** to save time repeating text but *always* explain them at the first point of use.
- **Consider speed of writing and neatness** especially when selecting the type of pen to use – ball-point pens are fastest, but they can smudge. You can only gain marks if the examiner can read your script!
- **Keep your answer simple and to the point**, with clear explanations of your reasoning.

Make sure your answer is relevant.

- **Don't** include irrelevant facts just because you memorized them during revision as this may do you more harm than good. You must answer the specific question that has been set.
- **Time taken to write irrelevant material is time lost from another question.**

After the exam – try to avoid becoming involved in prolonged analyses with other students over the 'ideal' answers to the questions; after all, it is too late to change anything at this stage. Go for a walk, watch TV for a while, or do something else that helps you relax, so that you are ready to face the next exam with confidence.

Make sure your name is on each exam book and on all other sheets of paper, including graph paper, even if securely attached to your script, as it is in your interest to ensure that your work does not go astray.

Never leave any exam early. Most exams assess work carried out over several months in a time period of 2–3 h and there is always something constructive you can do with the remaining time to improve your script.

Practical exams: special considerations

The prospect of a practical examination may cause you more concern than a theory exam. This may be due to a limited experience of practical examinations, or the fact that practical and observational skills are tested, as well as recall, description and analysis of factual information. Your first thoughts may be that it is not possible to prepare for a practical exam but, in fact, you can improve your performance by mastering the various practical techniques described in this book. The principal types of question you are likely to encounter include:

- Manipulative exercises, often based on work carried out as part of your practical course (e.g. recrystallization, p. 92).
- Interpretation of spectra – either provided by the examiner or obtained during the practical examination.
- Numerical exercises, including the preparation of aqueous solutions at particular concentrations (p. 15) and statistical exercises (p. 271). General advice is given in Chapter 39.
- Data analysis, including the preparation and interpretation of graphs (p. 251) and numerical information, from data either obtained during the exam or provided by the examiner.
- Preparative exercises – making a compound which will be assessed for yield and purity.
- Analytical exercises where the composition of an unknown chemical must be discovered and quantified.

Practical reports

You may be allowed to take your laboratory reports and other texts into the practical exam. Don't assume that this is a soft option, or that revision is unnecessary: you will not have time to read large sections of your reports or to familiarize yourself with basic principles etc. The main advantage of 'open-book' exams is that you can check specific details of methodology, reducing your reliance on memory, provided you know your way around your practical manual. In all other respects, your revision and preparation for such exams should be similar to theory exams. Make sure you are familiar with all of the practical exercises, including any work carried out in class by your partner (since exams are assessed on individual performance). Check with the teaching staff to see whether you can be given access to the laboratory, to complete any exercises that you have missed.

The practical exam

At the outset, determine or decide on the order in which you will tackle the questions. A question in the latter half of the paper may need to be started early on in the exam period (e.g. a preparation requiring a 1h reflux in a 3h exam). Such questions are included to test your forward-planning and time-management skills, e.g. you may need to take samples for measurement of reaction rates at 5 minute intervals, which can be done while the preparative part of the examination is occuring.

Make sure you explain your choice of apparatus and experimental design. Calculations should be set out in a stepwise manner, so that credit can be given, even if the final answer is incorrect (see p. 261).

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