

UV-VIS Introduction

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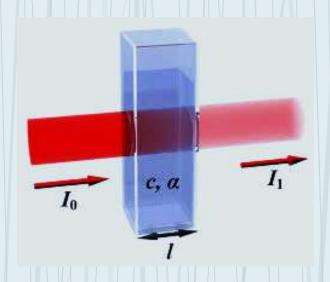
UV/VIS

- An analytical for measure the concentration
 of samples or samples components according
 to Beer's lambert Law depending on the ratio
 between incident and transmitted light
 through the cuvette.
- Absorbance is expressed as

$$A = \epsilon bc$$

where ϵ is a constant called absorptivity

Absorbance is directly proportional to the path length, b, and the concentration, c, of the absorbing molecule.





Technology

- UV-Visible spectroscopy is a mature technique capable of both quantitative and qualitative analysis of liquid, solid and gaseous samples.
- The technology can be applied to instrumentation capable of measuring micro-volume liquid samples less than 1 μL up to solid sample surfaces larger than the instrument performing the measurement.



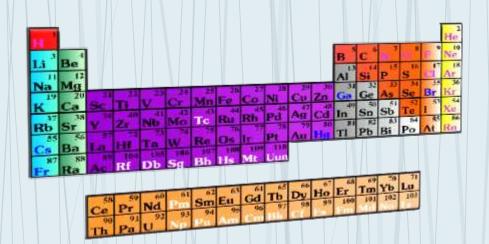


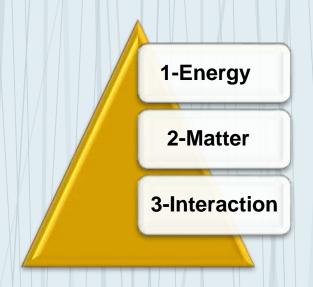
Introduction

Chemistry

Chemistry is the science of study the:-

- Interaction between
- Energy and
- Matter



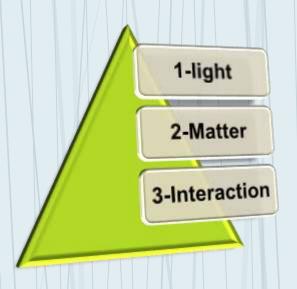


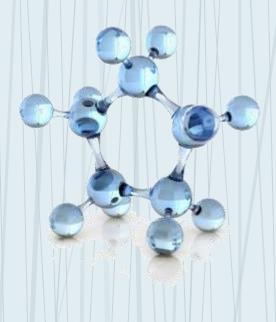


Spectroscopy

Spectroscopy is the science of study :-

- The interaction between
- Light and
- Matter

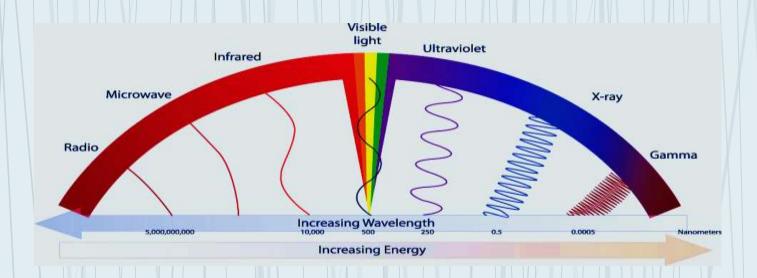






1. Light

Electromagnetic spectrum



- The spectrum is continuous and each region merges slowly into the next.
- The Electric and Magnetic fields are Oscillating in single planes at right angles to each others, the shorter the wavelength, the greater the energy.



UV/VIS Region

- The UV is the electromagnetic spectrum shorter than those of ordinary, vis ible violet light but longer than those of xrays.
- The human eye is only sensitive between approximately 380 and 780 nm and within this area we perceive the colors of the rainbow from violet through to red.

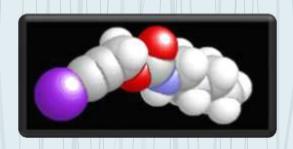
Region	Wavelength (nm)
Far ultraviolet	10-200
Near ultraviolet	200-380
Visible	380∤780
Near infrared	780-3000
Middle infrared	3000-30,000
Far infrared	30,000-300,000
Microwave	300,000-1,000,000,000



2. Matter

Tested samples





Water, Paints, Pigments, Polymers, Drugs
Food additives, Air polluents, Plasticizers, Rubber, Chemicals, Plastics

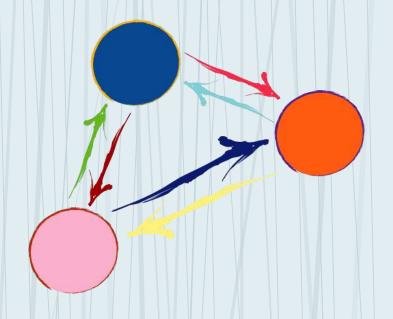


3. Interaction

- Atoms and molecules exist in a number of defined energy states or levels and a change of level requires the absorption or emission of an integral number of a unit of energy called a quantum.
- We have two types of interaction:

Atomic absorption

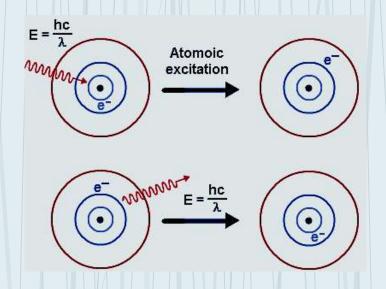
Molecular absorption





Atomic Absorption

- A type of Absorption where the absorption happen by the atoms.
- A free atoms absorb the light according the required energy for excitation.
- This type of absorption useful in the determination of metals concentration using atomic absorption and ICP.



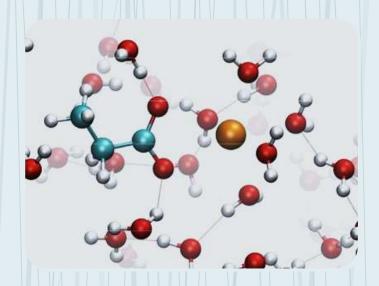


Molecular Absorption

- A molecule of any substance has an internal energy which can be considered as the sum of the energy of its electrons, Energy of molecule (E total)
- Molecules absorb light and its energy content will change :-

E total = 4 E

- 1. E electronic motion
- 2. E rotational motion
- 3. E translational motion
- 4. E Vibrational motion



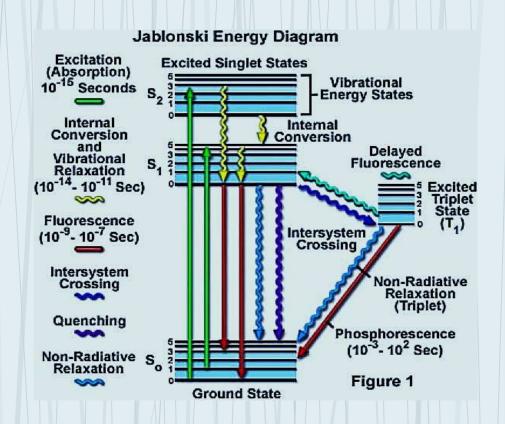


Jablonski Energy Diagram

- Some molecules when they absorb
 ultraviolet or visible light emit light
 when their electrons return from an
 excited state back down to the ground
 state.
- The energy of a photon absorbed or emitted during a transition from one molecular energy level to another is given by the equation

e=hv

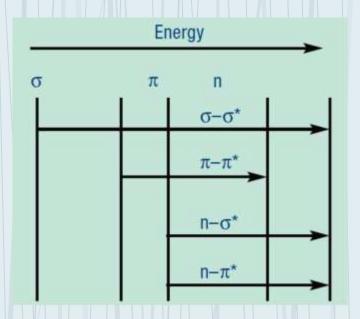
where h is known as Planck's constant and v is the frequency of the photon





Electronic Transition

- The absorption of UV or visible radiation corresponds to the excitation of outer electrons.
- There are three types of electronic transition which can be considered.
 - 1. Transitions involving π , α , and n electrons
 - 2. Transitions involving charge-transfer electrons
 - 3. Transitions involving *d* and *f* electrons (not covered in this Unit)



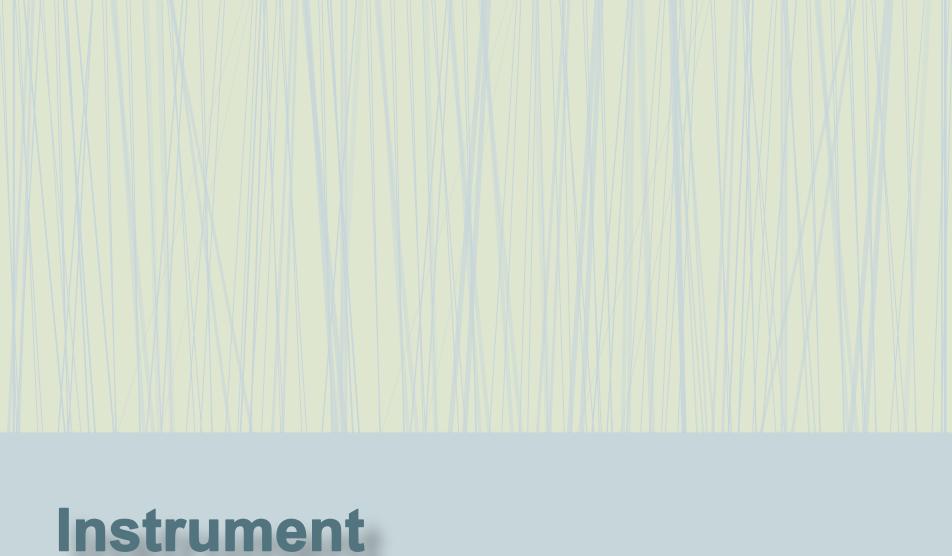


Relationship Bet. Light Absorption & Color

- A molecule or ion will exhibit absorption in the visible or ultraviolet region when radiation causes an electronic transition within its structure.
- The absorption of light by a sample in the ultraviolet or visible region is accompanied by a change in the electronic state of the molecules in the sample and color change

Color absorbed	Color observed	Absorbed radiation(nm)
Violet	Yellow-green	400-435
Blue	Yellow	435-480
Green-blue	Orange	480-490
Blue-green	Red	490-500
Green	Purple	500-560
Yellow-green	Violet	560-580
Yellow	Blue	580-595
Orange	Green-blue	595-605
Red	Blue-green	605-750





Spectrometer

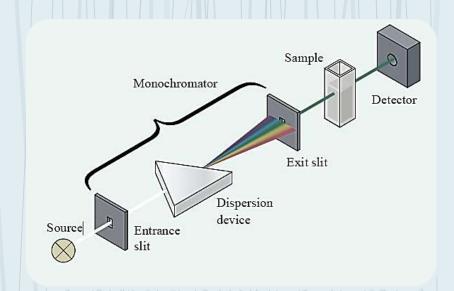
- A spectrophotometer is an instrument for measuring the transmittance or absorbance of a sample as a function of the wavelength of electromagnetic radiation.
- Single beam instrument.
- Double beam instrument.





Single Beam Spectrometer

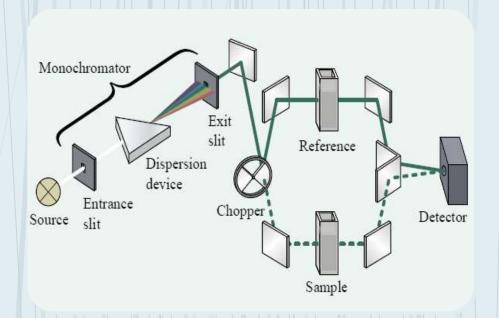
- by measuring the intensity of light reaching the detector without the sample (the blank) and comparing it with the intensity of light reaching the detector after passing through the sample.
- The blank and the sample are measured consecutively.
- Lamp drift can result in significant errors over long time intervals.





Double Beam Spectrometer

- Compensate for these changes in lamp intensity between measurements on blank and sample cuvettes.
- The chopper switches the light path between a reference optical path and a sample optical path to the detector.





Instrument Components

- 1. Light Source
- 2. Sample Chamber
- 3. Optical System
- 4. Detector





1. Radiation Sources

A source that generates a broad band of radiation.

1- UV source

Both deuterium and hydrogen lamps emit radiation in the range 160 - 375 nm.

Quartz windows must be used in these lamps, and quartz cuvettes must be used, because glass absorbs radiation of wavelengths less than 350 nm.

2- VIS. Source

The tungsten filament lamp is commonly employed as a source of visible light.

Lamps emit radiation in the range 380 - 780 nm.

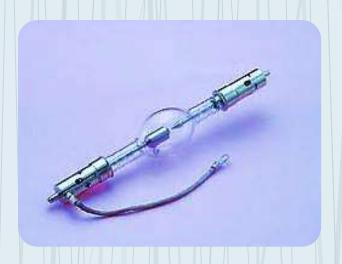






Xenon Lamp

- The wavelength range of 190-1100 nm.
- As the lamp reaches the end of its lifespan, the light output decreases, resulting in decreased sensitivity.
- The xenon gas inside the glass bulb is under extremely high pressure, the xenon lamp can explode.
- Xenon flash lamp is only on when taking measurements extending its lifetime





2. Sample Chamber

Cuvettes

Cuvettes The containers for the sample and reference solution must be transparent to the radiation which will pass through them.

Quartz or fused silica cuvettes are required for spectroscopy in the UV region.

These cells are also transparent in the visible region.

Silicate glasses can be used for the manufacture of cuvettes for use between 350 and 2000 nm.

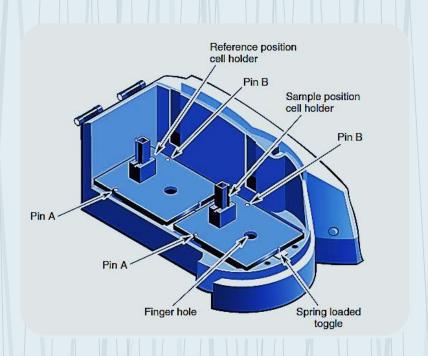






Manual Accessory

- Manual accessories are mounted on a common baseplate.
- This system ensures correct alignment of the accessory within the instrument automatically.
- Two standard rectangular cell holders.
- The sample (front) position and/or the reference (rear) position.





Automatic Accessory

- Two 7-Cell Changers can be installed in the sample and reference positions.
- Set up the Sample Beam cells.
- Set up the Reference Beam cell changer if used.
- the number of samples is greater than the number of positions in the cell changer, the software will pause and prompt for the next cell holder to be presented.







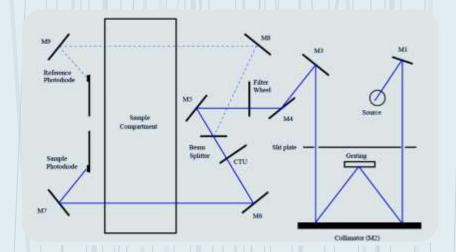
3. Optical System

Monochromator

A device which isolates a very narrow band of wavelengths of the light coming from a source. It consists of:

- An entrance slit
- A collimating lens
- A dispersing device (a prism or a grating)
- A focusing lens
- An exit slit

A dispersion devices cause different wavelengths of light to be dispersed at different angles, lenses or concave mirrors are used to relay and focus light through the instrument.





4. Detector

Detectors to measure the intensity of radiation. it should give:-

- Linear response over a wide range
- Low noise.
- High sensitivity.

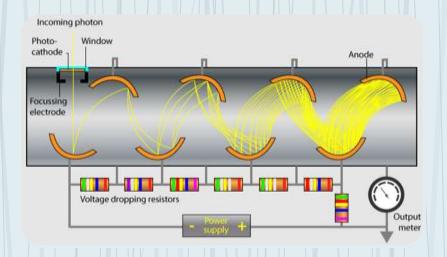




PMT Detector

The photomultiplier tube

- Is a commonly used detector in UV-Vis spectroscopy.
- It consists of a photo emissive cathode (a cathode which emits electrons when struck by photons of radiation), several dynodes (which emit several electrons for each electron striking them) and an anode.





Silicone Detector

- Silicon detectors measure the energy of an incoming photon by the amount of ionization it produces in the detector material.
- This material is high purity silicon with a very low leakage current.
- Silicon photodiodes for extended wavelength range to 1100 nm





Performance Verification

PV Tests

- PV Tests (Performance Verification Tests)
 application to check the instrument's
 performance with standardized tests.
- Results of the tests are compared to limit/tolerance values in order to reach a pass/fail decision.





Certified Reference Materials CRM

Test type	Reference material
Wavelength accuracy	Sample with sharp absorbance bands like holmium oxide glass or emission lines of internal or external lamps
Wavelength repeatability	As above; test at one of wavelengths defined above that is nearest to 500 nm
Photometric (ordinate) accuracy 1	Certified gray glasses or potassium dichromate solutions
Photometric (ordinate) accuracy 2	Certified gray glasses or potassium dichromate solutions
Stray light ratio	Stray light test solutions according to EP (European Pharmacopeia) or ASTM E-387
Noise	No sample or attenuator with defined absorbance
Resolution (spectral bandpass)	Toluene in n-hexane according to EP



Wavelength Accuracy

- This is the agreement between the value displayed on the spectrophotometer and the true value.
- Wavelength accuracy is a performance characteristic that describes the positioning accuracy of the monochromator and therefore, the accuracy of the wavelength scale.
- The exact position of the peak at 525 nm is measured and certified for each set of standards.





Photometric Accuracy

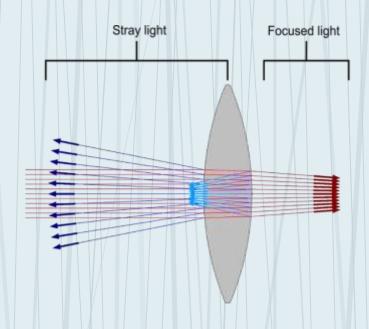
- The photometric accuracy performance test compares the photometric value (absorbance or %T) measured by your spectrophotometer to the well-known value of a standard.
- This test verifies that you are measuring the correct absorbance or percent transmittance of your sample.
- Two filters to test photometric accuracy at 10% and 50% transmission.





Stray Light

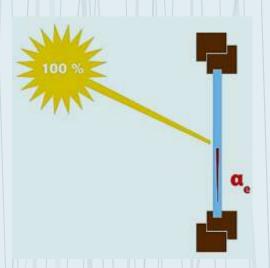
- Stray light is a performance test that measures the amount of unwanted light (light of a different wavelength than the selected wavelength) which reaches the detector.
- Each of the three stray light standards is essentially opaque at the test wavelength and highly transmitting at longer wavelengths.
- These filters readily reveal any stray energy in both the visible and UV regions at 220, 340, and 400 nm.





0% Transmittance

- The opaque 0%T Standard is used to test for light leaks, set the electronic zero offset (if applicable), and to check the 0%T control setting on your spectrophotometer.
- The 0%T standard can also be used with the appropriate Stray Light Standard to determine total instrumental stray light.

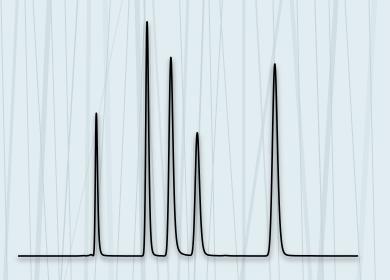




Techniques

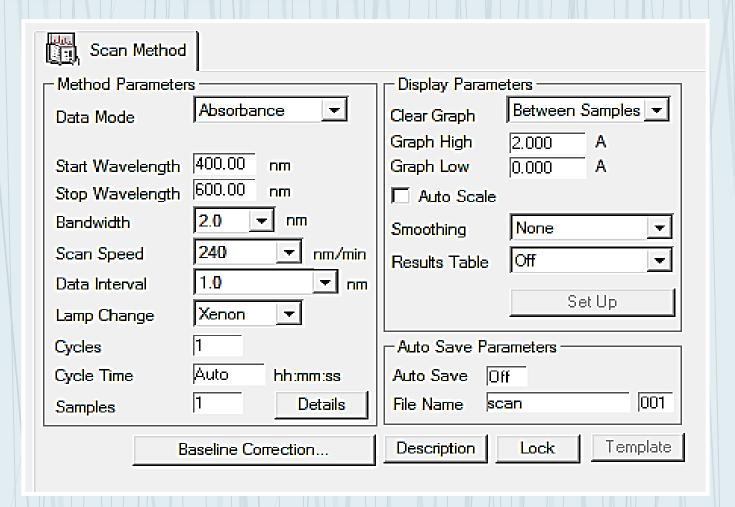
1.Scan

- Spectrum recording.
- of light that passes through the sample over a continuous range of wavelengths and plots a graph of the resulting spectrum.
- The starting and finishing wavelengths of the scan are both set.
- Select the appropriate Baseline Correction you wish to perform.
- Maximum wavelength range is determined by the working range of the spectrophotometer.





Scan Parameters





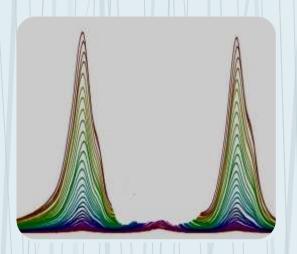
Definitions

No.	ltem	Definition			
1	Data Mode	The Data Mode enables you to select what will be used as the vertical axis when the spectrum is first plotted.			
2	Bandwidth	The Effective Spectral Slit width of the monochromator.			
3	Scan speed	A measure of how fast the monochromator changes the wavelength of the light falling on the sample.			
4	Data interval	The Data Interval is the difference in wavelength between successive measurement points.			
5	Lamp change	Control an instrument with a deuterium lamp installed to cover the UV wavelengths and a tungsten lamp to cover the visible range. It will not be available if the instrument has a xenon lamp installed,			
6	Cycle	Single measurement on a sample			
7	Baseline correction	The standard User Baseline Correction selection will be the 100%T .			



2. Fixed

- Recording spectrometer readings at different dedicated wavelengths and performing simple calculations
- In the Fixed application the instrument measures the amount of light passing through the sample at one or more wavelengths.
- Set the wavelengths at which the instrument will make measurements.





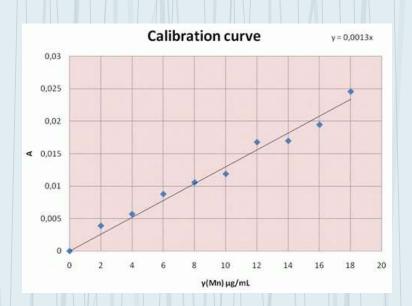
Fixed Parameters

Fixed Meth	ood	
Wavelengths — 1 550.00 2 OFF	3 OFF 5 OFF 4 OFF 6 OFF	7 OFF 9 OFF 8 OFF 10 OFF
Method Parame	Absorbance 🔻	Auto Save Parameters Auto Save Off
Fixed Mode Integ. Time	Nomal	File Name Fixed 001
Lamp Change	Xenon ▼ 2.0 ▼ nm	Description Lock Template
Bandwidth	1	
Cycle Time Samples	Auto hh:mm:ss Details	
Reference Wav	relength Correction	



3. Quantitative

- Quantifying sample concentrations via calibration, based on Lambert- Beers law
- The Quantification application is used to obtain quantitative concentration data on a single component from absorbance measurements.
- A valid calibration must be present before samples can be measured.
- Enter the number of standards that will be used.
- Once a calibration has been performed it can be saved as part of the method and will be available for use next time the method is loaded.





Quant. Parameters

Quant Calibration Method Quant Sample	Quant Sample Method
Method Parameters Quant Mode Single Wavelength Factor 1.0000 Wavelength 1 550.00 nm 1	Name Sample Actual Volume / ml
Wavelength 2 OFF nm Wavelength 3 OFF nm	High Limit OFF Conc Low Limit OFF Conc Auto Save Parameters
Integ. Time 0:00:01 hh:mm:ss Lamp Change Xenon ▼ Bandwidth 2.0 ▼ nm	Auto Save Off File Name quant 001
Standards 1 Replicates 1	Weight/Volume Correction Correction Method Weight 1.000 mg Method Volume 1.000 mL
[Description] Lock More	Description Lock Template



4. Rate

- The Rate application is used to measure the change in concentration of a reactant or product with time.
- The absorbance of the sample is measured repeatedly at a fixed wavelength over a period of time appropriate to the reaction being studied.
 The software then offers a variety of methods to calculate the rate of reaction from the variation of absorbance with time.
- Recording time dependent readings at a fixed wavelength and is dedicated for measuring rate curves.





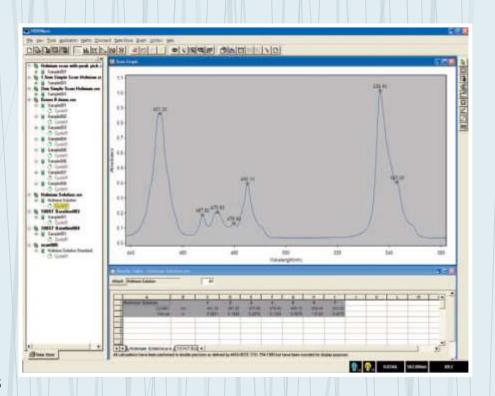
Rate Parameters

Method Param	eters		Display Parame	eters
Rate Mode	Serial	┰	Clear Graph	Between Batches 🔻
Data Mode	Absorbance	<u> </u>	Graph Mode	Delta Absorbance <u>▼</u>
Wavelength	340.00 nm		Range Slope	Auto
Data Points	241	♠ Auto	Auto Scale	
Duration	30.000 ss.sss	C Auto	Smoothing	None ▼
Interval	00.125 ss.sss	C Auto	Factor	1.000
Integ. Time	00.015 ss.sss	▼ Auto	Activity Units	1.555
Delay Time	00,000 ss.sss		Auto Save Par	
Lamp Change	Xenon ▼		File Name Tat	
Bandwidth	2.0 ▼ nm			4
Samples	1	More	Description	Lock Template



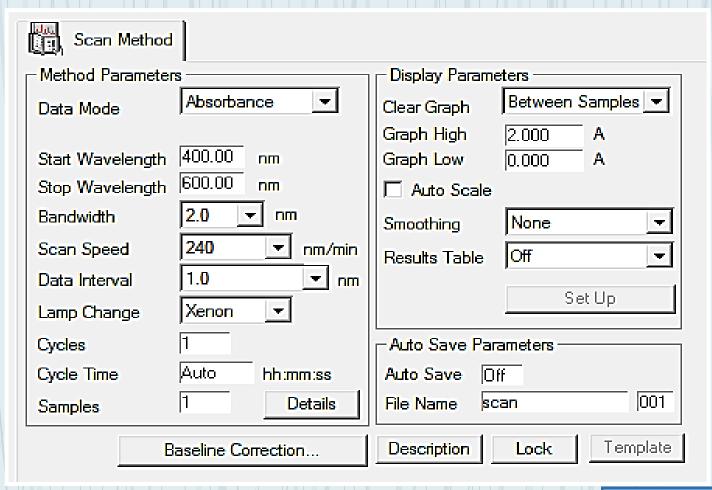
5. MCA

- It provides the ability to perform simultaneous quantitative analysis on samples for up to 20 components
- Standards and components are scanned,
 and each data point of the scan
 contributes to the calculation.
- A series of Standards, in which the concentrations of the components are known, is prepared and scanned.
- There must be at least as many standards as there are components to be analyzed.





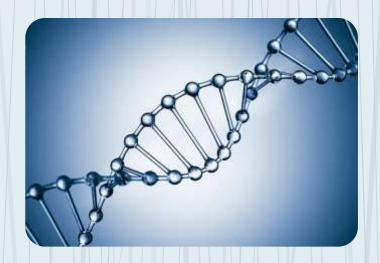
MCA Parameters





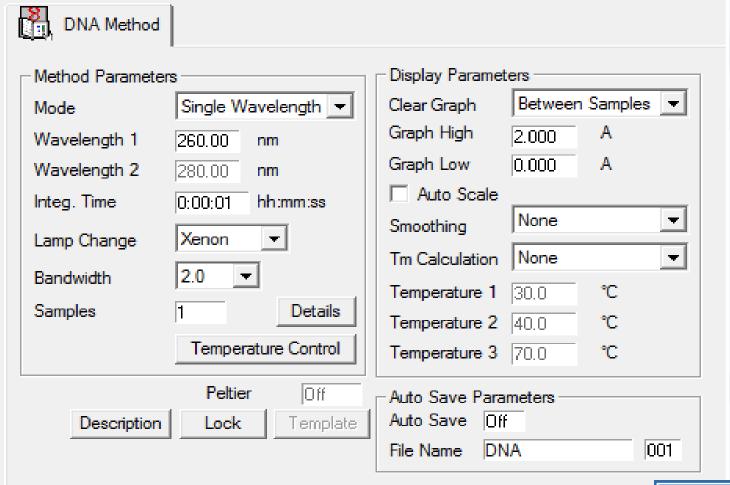
6. DNA

- During a DNA experiment, the sample is heated and Absorbance v Temperature data are collected at a suitable wavelength.
- Tm, the "melting point" of the DNA, may be calculated at run-time, or post-run via the Math menu.





DNA Parameters







Accessories

Rapid Mixing Accessory

- The Rapid Mixing accessory provides a very low dead time of 8 ms, giving you access to reactions that occur up to 1000 times faster than those visible to manual mixing experiments.
- The Rapid Mixing accessory provides a very low dead time of 8 ms, giving you access to reactions that occur up to 1000 times faster than those visible to manual mixing experiments.
- The Anaerobic accessory consists of a manifold, which is mounted over the base of the drive syringes and is purged with a steady stream of inert gas.



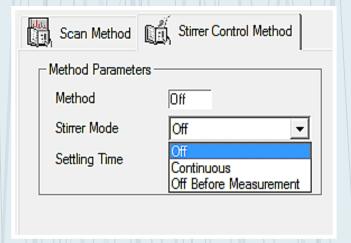


Stirrer Parameters

Off
 The stirrer is off

Continuous The cell is stirred continuously

- Off Before Measurement The stirrer is normally on but is switch off prior to and during measurement.
- A programmable settling time elapses before the measurement is made.





Air-Cooled Peltier

- The Air-cooled Peltier Accessory allows the cell to be thermostatted during measurements. It comprises a dedicated cell holder and an electronic control device, which is connected to the PC.
- The Air-cooled Peltier accessory delivers reliable temperature control.
- Wider temperature range from 0 to 110 °C
- Multiple cell efficiency for higher sample throughput.
- Designed for biologically relevant assays that require temperature control at 25°, 37°, 40° and 50 °C, the Aircooled Peltier accessory is ideal for the life science laboratory.



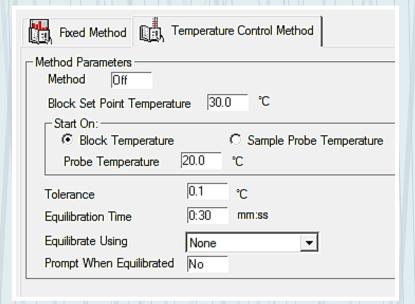


Temp. Control Parameters

- Method off not selected
- Tolerance Target Temperature ±

Tolerance

- Start on using block or probe
- The Equilibration Time permitted range and start a measurement.
- The Equilibrate Using sample or sample and reference.





Thermostatted Single Cell Holder

- Controls the temperature by circulation of liquid.
- Temperature range 40 150 °C.





nanoCell Accessory

 The nanoCell is useful for laboratories performing assays for nucleic acids,
 RNA/DNA, and protein concentrations.





Cell Changer

- Using a cell changer, you load the system
 with a batch of samples before starting
 the measurement.
- During the measurement the cell changer moves the required sample automatically into the sample beam of the spectrophotometer.
- This type of measurement procedure is advisable for time consuming analyses, e.g. rate measurements.

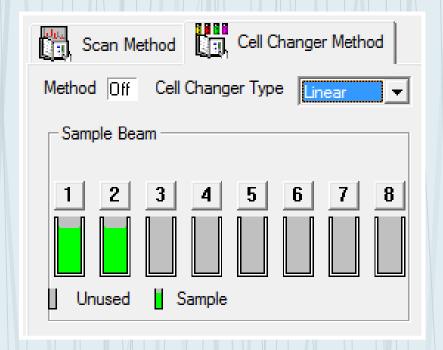






Cell Changer Parameters

- Method off not selected
- Cell changer type. When an instrument with a cell changer installed is connected, this field will automatically show the correct type.
- Each cell can contain a Sample (green) or a Reference (red), or not be used.





Sipper

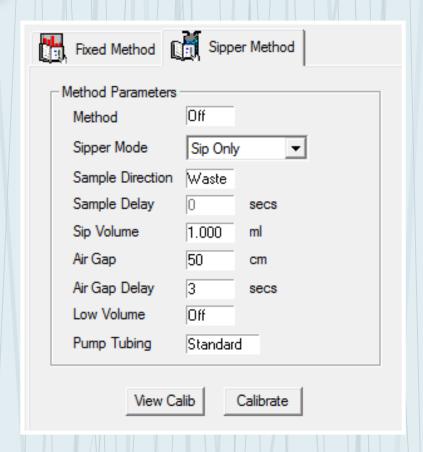
- In sipper operation the sipper pump transports the sample to the flow cell located in the sample beam. Then the measurement starts automatically.
- Therefore, there is no need to change and clean the cells.
- This operation mode is recommended for high sample throughput in routine analyses.





Sipper Parameters

- Method off not selected
- Sipper Mode. Choose between Sip Only, Sip and Run, Continuous.
- Sample Direction. When Waste is selected the sample is pumped to waste after it has been measured. Return sample is returned to the sample container.
- Sample Delay.
- The Sample time between the end of pumping the air gap and the start of sample measurement.
- The purpose of the Sample Delay is to allow time for the sample to settle down in the flowcell.



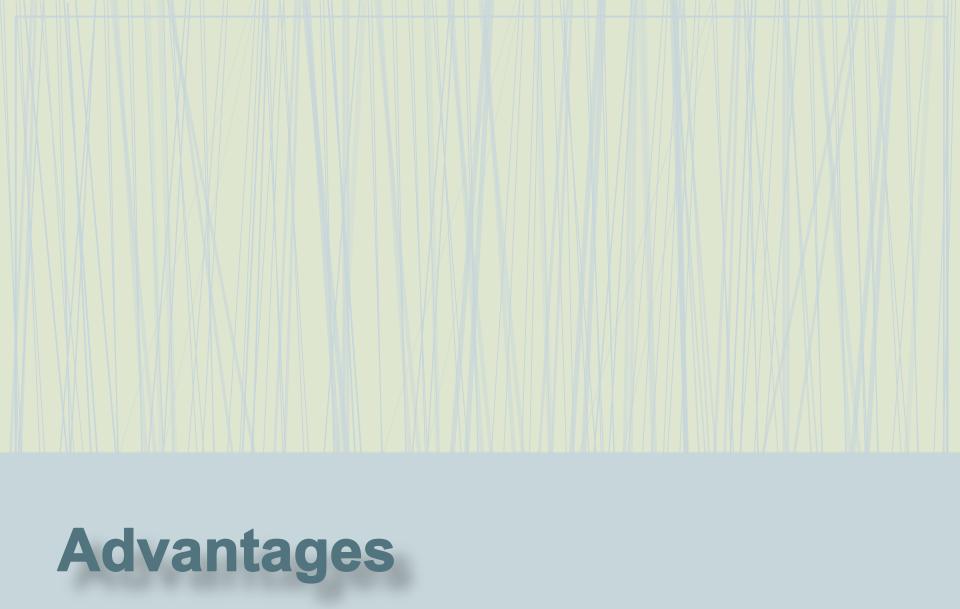


Diffuse Reflectance

- The accessory provides sample mounting configurations for performing both reflectance and transmission measurements.
- Using this accessory, you can easily measure a myriad of materials from solids to liquids.
- An integrating sphere that is coated with a highly thermoplastic material.
- perfect diffuser, chemically inert to most compounds, and maintains its high reflectance properties.







Advantages

- Advantages
- Low price of equipment
- Ability to determine wide range of analytes
- Sensitivity
- Ease of use test kits
- Most common analytical laboratory instrument tool
- Non-destructive quantitative and qualitative analysis
- Sample handling for the analysis of liquid, solid and gas materials
- Analysis volume: < 1 μL for liquids and ~ 1 mm for solids
- Heavily used in teaching, QA/QC and research laboratories
- Effective in measuring samples within high water concentration



Applications

Applications

- UV-Visible spectrophotometry is a common technique for the quantitative analysis of analytes in QA/QC, analytical testing and government regulatory laboratories.
- The fundamentals of the technique such as Beer's Law is taught in education.
- Mid-range to upper-end UV-Visible
 Spectrophotometers are commonly
 found in research laboratories including
 academic, industrial and government
 research facilities.





Few Of The Most Significant

- Nucleic acids (DNA/RNA) estimation and purity.
- Protein concentration determination.
- Researching enzyme and chemical kinetic reaction mechanisms.
- Quantitative analysis confirmation of specific molecules in industrial QC laboratories.
- % Label Claim confirmation in pharmaceutical and nutraceutical QA laboratories.
- Beverages including soft drinks, juices, beer and wine.
- Plastic/glass materials and finished goods including window glass, sunglasses, ski goggles, architectural glass, pharmaceutical bottles, and so on.
- Optical materials including mirrors, lenses, bandpass filters, cut On/Off filters, military optics, anti-reflective coatings, and so on.
- Color and appearance of products including pharmaceutical and food packaging,
 beverages and food products, textiles/fabrics, dyes, ink, pigments, paints, and so on.

