

Collecting and Preparing Samples

تلخيص

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ماجستير الكيمياء العضوية

شركة مياه الشرب و الصرف الصحي بالشرقية

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Booklet No. 15/2017



يهم كثير من الكيميائيين القائمين على تحاليل مياه الشرب و بخاصة فى المعامل الحاصلة على شهادات الجودة كالايزو I7025 و غيرها بتحسين طرق التحليل و من الممكن القول بصورة أخرى بتقليل مقدار الخطأ الكلى من خلال فقط الاهتمام بتقليل خطأ الطريقة المستخدم فى التحليل ويتجاهلون تقليل الخطأ الكلى من خلال تقليل خطأ سحب العينة . . . وفى هذا الكتيب المختصر توضيح لعدة امور من بينها الاجابة على اسئلة . . من اين نجمع العينة . . حجم العينة . . نوع العينة . . عدد مرات الجمع . . اوقات جمع العينة . . فضلا عن طرق سحب عينات السوائل و الغازات و المواد الصلبة باختصار . . . و طرق فصل العينة .

هذا العمل مجهود بشرى قد يعتريه بعض الخطأ او يحتاج الى تعديل

لذا نكون شاكرين لمن لديه تعديل او اكتشف خطأ او سهواً او اقتراح ان يتعاون معنا حتى تعم المنفعة و يكون العمل على الصورة التى ينبغى له ان يكون عليها.

للتواصل

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Sampling

st. deviation

$$\mu = \bar{X} + \frac{t_s}{\sqrt{n}} \rightarrow \text{st. dev. method} \quad (7-1)$$

True mean ← mean

Propagation of Random Error $\rightarrow S_o^2 = S_m^2 + S_s^2 \rightarrow \text{st. dev. for sampling} \Rightarrow (7-2)$

1:3 ratio of S_m / S_s is recommended for error in sampling to be less than 10%.

$\Rightarrow S_s$ larger than S_m

Sampling plane

نقطة أخذ العينات

لا ينبغي أن تكون نقطة أخذ العينات متجانسة

- ① From where within the target population should samples be collected
- ② what type of samples should be collected
- ③ what is the minimum amount of samples needed for each analysis.
- ④ How many samples should be analyzed.
- ⑤ How can the overall variance "deviation" be minimized.

① من أين يجب أخذ العينات ② نوع العينات ③ كم عدد العينات ④ كم عدد العينات التي يجب تحليلها ⑤ كيف يمكن تقليل التباين الإجمالي في العينات

⑥ where to sample the target population :-

is of concern is homogeneity. Homogenizing is impractical. Homogenization destroys information about the analyte's spatial or temporal distribution within the target population.

⑦ Random Sampling

A sample collected at random from the target population.

⑧ True random sample is difficult to obtain

⑧ The target population is divided into equal units, assign a unique no. to each unit, and use a random table to select the units from which to sample

محذرات

- ⊕ makes no assumptions about the target population, least biased approach to samples
- ⊕ requires more time & expense since a great no. of samples are needed.

Judgmental sampling

- * more biased than Random
- * fewer samples are required
- * encountered in many protocols in which the sample to be collected is specifically defined by the regulatory agency.

Samples collected from the target population using available information about

The analyte's distribution within the population

Systematic Sampling

⇒ $\mu_{\text{eff}} = \mu_{\text{spin}} + \mu_{\text{orb}}$

Samples collected from the target population at regular intervals in time or space.

نہ تعین population، کہ جس سے مطالعہ کیا گیا ہے D-O سے تعین کیا گیا ہے

Systematic: judgmental sampling

A hand-drawn diagram of a corn cob, showing a grid of kernels arranged in rows and columns. The diagram is labeled "5 ears of corn" below it.

systematic

جميع النظم $\rightarrow$ Mental.

- ④ Encountered in environmental studies

④ Composite sample.

④ Stratified sampling

Judgmental-random or stratified sampling.

یہاں population سے مراد ہے تمام افراد، strata یعنی تعاملات مع
 ہر stratum نظام random کہل stratum پہلی بار سے ہی مجموعہ میں

Stratified sampling: *

- The composition of each stratum is often more homogeneous than that of the entire target population.
- When true the sampling variance for each stratum is less than when the target population is treated as a single unit.

④ Convenience Sampling A sampling plan in which samples are collected because they are easily obtained.

what type of sample to collect?

- Grab sampling
- Composite sampling
- in situ sampling

④ Grab sample

A single portion of the target population is removed at a given time and location in space $\Rightarrow$ provides "snapshot" $\Rightarrow$ easily adapted to any of sampling scheme

$\rightarrow$ where to sample $\leftarrow$

④ Composite sample:-

several grab samples combined to form a single sample. after Thoroughly mixing.

④ Because information is lost when individual samples are combined it is normally desirable to analyze each grab sample separately.

* Determination a target population's average composition over time & space - also useful when a single sample cannot supply sufficient material for analysis.

④ need to remove a portion of the target population for analysis as a result neither type of sample can be used to continuously monitor a time-dependent change in the target population.

In situ sampling

an analytical sensor is placed directly in the target population allows continuous monitoring without removing individual grab samples
ex pH, Cond. electrodes: con line"

- ③ How much sample "چند نمونه لازم است؟"
④ to minimize sampling error $\Rightarrow$ grab sample must be of an appropriate size, not too small $\Rightarrow$ significant sampling error

too large $\Rightarrow$ may require more time, money to collect & analyze without providing a significant improvement in S_s

Sample containing particle A, B at fixed conc.

for sample containing n particles

$$n_A = np \rightarrow \text{probability of selecting particle of type A}$$

$$\text{Sampling st-dev } S_s = \sqrt{np(1-p)}$$

$$\text{relative st-dev of sampling } S_{s,r} = \frac{\sqrt{np(1-p)}}{np}$$

fixed sample size st-dev $\Rightarrow$ $n = \frac{1-p}{p} \times \frac{1}{S_{s,r}^2}$

$$n = \frac{1-p}{p} \times \frac{1}{S_{s,r}^2}$$

$S_{s,r}$ relative standard deviation

population 1×10^7 analyte 1% relative variance 1%

$$\therefore \text{probab. of analyte} = \frac{1 \times 10^7}{100} = 1 \times 10^5$$

$$n = \frac{1-p}{p} \times \frac{1}{S_{s,r}^2} = \frac{1 - (1 \times 10^{-5})}{1 \times 10^{-5}} \times \frac{1}{(0.01)^2} = 1 \times 10^8$$

$m \propto r^3$ 1×10^8 $m = 8$

particle $m = 8$ $m = 2^3$ 10 80

جمع کنند مقدار 10 و 80

(5)

∴ We can reduce the needed mass by crushing and grinding the particles to make them smaller.

* Many mixed "well-mixed" populations, in which the population's composition is homogeneous on the scale at which we sample, approximate binomial sampling statistics. Under these conditions the following relationship bet. the mass of randomly collected grab sample, m , and the percent relative standard deviation for sampling, R , is often valid.

$$m R^2 = K_s$$

$K_s \Rightarrow$ Sampling Const. equal to the mass of sample producing a percent relative standard deviation for sampling of $\pm 1\%$.

is evaluated by determining R using several samples of similar mass.

Once K_s is known the mass of sample needed to achieve a desired standard deviation for sampling can be calculated.

mass of cereal "g"	percentage Ash (w/w)
0.9956	1.34
0.9981	1.29
1.0036	1.32
1.0067	1.28

$\pm 2\%$ relative sampling st dev. needed sample m of K_s is $\pm 2\%$ relative st dev. Ash in cereal, well mass m to be determined

$$R = \frac{S_s}{\bar{X}} \times 100 = \frac{0.03194}{1.298} \times 100 = 2.46\%$$

relative st. dev. ash conc. 1.298

$$K_s = m R^2 \Rightarrow K_s = (1.0007 \text{ g}) (2.46)^2 \times 100 = 6.06 \text{ g}$$

mass needed to give ^{relative} st dev. of $\pm 2\%$ $m = \frac{K_s}{R^2} = \frac{6.06}{(2.46)^2} = 1.5 \text{ g}$

If we use 5 gm sample ∴ R "relative st. dev." = $\sqrt{\frac{K_s}{m}} = \sqrt{\frac{6.06}{5}} = 1.10\%$

and the absolute st dev $\Rightarrow R = \frac{S_s}{\bar{X}} \times 100 \Rightarrow S_s = \frac{R \bar{X}}{100} = \frac{1.1 \times 1.298}{100} = 0.00143$

⊛ when target population is segregated, or stratified, a more appropriate relationship which can be applied to both segregated & nonsegregated samples

$$S_s^2 = \frac{A}{mn_s} + \frac{B}{n_s}$$

$n \Rightarrow$ no. of samples to be analyzed

$m \Rightarrow$ mass of each sample

$A \Rightarrow$ homogeneity constant accounting for the random distribution of analyte.

$B \Rightarrow$ a segregation const. accounting for the nonrandom distribution of analyte in target population

انحراف معیار در نمونه‌ها و در جامعه هدف

در نمونه‌ها: S_s^2 (variance in samples) و در جامعه هدف: S^2 (variance in target population)

A, B یکسان یا یکسان نیستند؟ A و B یکسان نیستند

در نمونه‌ها: S_s^2 و در جامعه هدف: S^2 (variance in samples and target population)

$$\begin{array}{ccc} n = 15 & n = 10 & n = ? \\ m = 9 & m = 10 & m = 5 \\ S_s = 0.0183 & S_s = 0.0069 & S_s = 0.01 \end{array}$$

$$0.0183 = \frac{A}{(1 \times 9)} + \frac{B}{15} \quad (1)$$

$$0.0069 = \frac{A}{(10 \times 10)} + \frac{B}{10} \quad (2)$$

$$0.01 = \frac{0.228}{(5 \times n)} + \frac{0.0462}{n}$$

$$0.01 = S_s \text{ و } S = n$$

$$0.01 = \frac{0.228}{(m \times 5)} + \frac{0.0462}{5} \Rightarrow m = 60 \text{ gm.}$$

Minimizing The Overall Variance

1. The overall variance is the sum of the sampling variance and the method error variance.

$$S_o^2 = S_m^2 + S_s^2$$

2. The overall variance is the sum of the sampling variance and the method error variance.

3. The overall variance is the sum of the sampling variance and the method error variance.

no. of replicate analysis $(nr) = \frac{t^2 S_m^2}{e^2}$ method error

Significant level S_s & S_m are known

Overall error $e = t \left(\frac{S_s^2}{n_s} + \frac{S_m^2}{n_s nr} \right)^{1/2}$

relative method error, 0.4% = Sampling Variance S_s^2 is 0.05% when $(\alpha = 0.05)$ relative error is 0.07% =

① you collect 5 samples & analyzing each twice

② you collect 2 samples & " " Five times

2.26 = t value for 10 degrees of freedom

Overall error $e = 2.26 \left(\frac{0.4}{5} + \frac{0.07}{(5 \times 2)} \right)^{1/2} = 0.67\%$

Overall error $e = 2.26 \left(\frac{0.4}{2} + \frac{0.07}{(2 \times 5)} \right)^{1/2} = 1\%$

Sampling method error is 0.67% which is less than the method error. Strategy is to collect more replicates to reduce the overall error.

Implementing the sampling plan:-

involves three steps

① physically removing the sample from its target population.

② preserving the sample

③ preparing the sample for analysis.

Except for in situ sampling, the analysis of a sample occurs after removing it from the target population.

Why preservation $\Rightarrow$ Since the properties of the sample may change chemically or physically, and hence the sample will no longer represent the target population prop. So samples are often preserved before transport them to the lab. even when samples are analyzed in the field, preservation may still be necessary.

Gross sample = The initial sample, collected from the target population without any processing.
may be
Single or Composite
 $\rightarrow$ may be used to reduce the sample's particle size or to improve its homogeneity.

Solutions:-

Sample collection:- ex environmental sampling of waters and wastewaters, the chemical composition of surface waters, such as streams, rivers, lakes, estuaries, and oceans, is influenced by flow rate and depth.

⊗ Rapidly flowing shallow streams and rivers, and shallow (< 5 m) lakes are usually well mixed and show little stratification with depth $\Rightarrow$ Grab samples are conveniently collected by submerging a capped bottle below the surface and removing the cap.

⊗ The water-air interface which may be enriched with heavy metals or contaminated with oil is avoided when collecting the sample.

$\Rightarrow$ after the bottle is filled, the cap is replaced and the bottle is removed.

$\Rightarrow$ Slowly moving streams and rivers, lakes deeper than 5m, estuaries and oceans may show substantial stratification $\Rightarrow$ grab samples at greater depths are collected with a weighted sample bottle that is lowered to the desired depth - Once it has reached the desired depth, the sample bottle is opened.

~~allowing~~ allowed to fill, and closed before retrieving. Grab samples can be analyzed individually giving information about changes in the analyte's concentration with depth.

or
may be pooled to form a composite sample.

- wells used for collecting groundwater samples must be purged before the sample is collected. $\Rightarrow$ Since the chemical composition of water in the well-casing and in the adjacent matrix may be significantly different from that of the surrounding.

- These differences may result from contaminants introduced when drilling the well, or differences in the groundwater's redox potential when exposed to atmospheric oxygen.

- In general, wells are purged by pumping out a volume of water equivalent to several well-casing volumes or until the water's temperature, pH or specific conductance are constant.

- samples from municipal wastewater treatment plants & industrial discharge often collected as 24-h composites.

- samples are obtained using an automatic sampler that periodically removes individual grab samples.

Sample containers $\begin{matrix} \rightarrow \text{glass} \\ \leftarrow \text{plastic} \end{matrix}$

- | | |
|--|---|
| <ul style="list-style-type: none">Corning, Pyrex - borosilicatesterilizable, easy to cleaninert to all solutions except strong alkali. | <ul style="list-style-type: none">polyethylene, PP, "lower cost"lightweight, durable |
|--|---|

Disadvantage:- Cost, weight, likelihood of breakage. inexpensive except Teflon type

- when collecting samples for pesticides, oil and grease organics "because they interact with plastic surfaces"

$\Rightarrow$ easily absorb metal ions, plastic bottles are preferred when collecting for trace metals analysis. PE

Sample bottles

wide mouth

easy to fill & remove the sample.

narrow-mouth

used when exposing the sample to the container cap or the outside environment is undesirable.

⊗ Unless exposure to plastic is a problem, caps for sample bottles are manufactured from polyethylene.

⊕ When polyethylene must be avoided, the container cap includes an inert interior line of neoprene or Teflon.

⊕ Sample preservation - sample's chemical composition may change

as a result of chemical, biological, or physical processes.
 So preservation is recommended by controlling the solution pH, adding a chemical preservative, temperature, limiting its exposure to light or atmosphere.

⊕ The maximum holding time between preservation and analysis depends on the analyte's stability and the effectiveness of sample preservation.

Gases Sampling

① filling a stainless steel container or Tedlar/Teflon bag with a portion of the gas. using a pump to pull the gas into the container and after flushing the container for a predetermined time the container is sealed.

Advantages 1- Collecting more representative samples.

Disadvantages 2- tendency of some gases to adsorb to the container's wall, presence of analytes at concentration too low to detect with accuracy and precision.

→ Can be overcome with cryogenic cooling "gas $\Rightarrow$ converts to liquid"

Breakthrough Volume:- The volume of sample that can be passed through a solid sorbent before the analytes are no longer retained. (m^3/g_{pack})
 $g_{\text{pack}} \Rightarrow$ grams of sorbent.

Sample preservation:- "solid sorbent, filter, cryogenic cooling" samples are usually stable. ممكن في الفريزر

for solid sorbents:- gaseous compounds may be removed by
 ⊕ Thermal desorption or ⊕ extracting with solvent
 ⊕ if it is selective for a single analyte $\Rightarrow$ The difference in mass of sorbent determines analyte conc.
يعني مركبة واحدة في كل مرة
 الفريزر (المتغير بحدوده)

Solids

Other, big things

large particles "ores"
 small particles "soils, sediments, tablets, pellets, capsule, animal feed, sheet, materials "polymers and rolled metals, tissue samples"

Sample collection

- By ~~bottom~~ bottom grab sampler or with a corer $\Rightarrow$ sediments from the bottom of stream, rivers, lakes, estuaries, and oceans.

- By Grab sampler equipped with a pair of "jaws"

↳ "ease of use", collect a large sample"

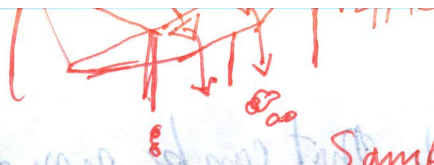
↳ Disadvantage $\Rightarrow$ tendency to lose finer grained sediment particles as water flows out of the sampler and loss of spatial information both laterally and with depth due to mixing of the sample.

- Cylindrical coring device. $\Rightarrow$ small surface area is sampled.

- Scoops & shovels $\Rightarrow$ soil samples at depths up to 30 cm.

depths greater than 30 cm $\Rightarrow$ samples obtained by digging a trench and collecting lateral samples with soil punch

Or an auger may be used to drill a hole to the desired depth and the sample collected with a soil punch.



Sample Thief

Sampling particulate matter

By the size of particles

particulate solids

as "Coals & ores"

random collect:

shovel

rattle

a trough containing even no. of compartments emptying on opposite sides of the rattle.

smaller particulate materials

"powders"

↓ by sample thief

allows materials to be collected simultaneously from several locations.

"Sampling a metal"

Sheet

random samples can be collected
By metal punch.

wire

→ cutting off

randomly pieces
of appropriate length

Bars or bricks

by ^{sawing} ~~sawing~~ ↓

by drilling through
the metal at
randomly selected
points and collecting
the "saw dust"

⇒ "Surface coating sampling"

By dissolving it with an appropriate solvent.

"Sampling biological tissue"

- By removing the entire organ which is then homogenized before smaller portions are taken for analysis.

- or several portions of tissue may be combined to form a composite sample. The composite sample is then homogenized and analyzed.

Sample preparation

reducing particle size → crushing & grinding

Ball mills, disk mills, and mortars & pestles

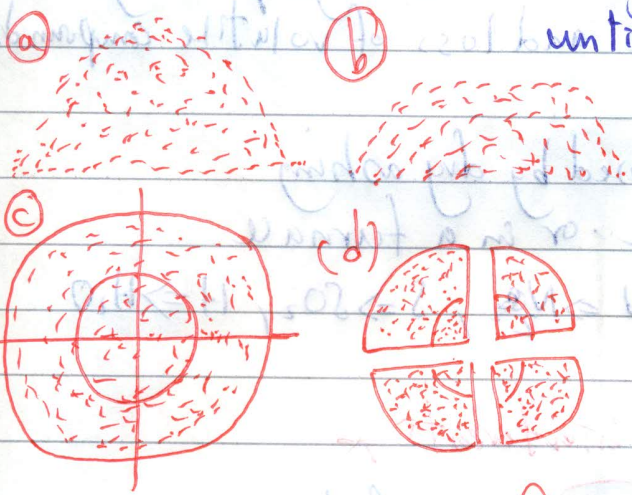
Disadvantages → increasing surface area → risk of losing volatile comp.

نقصانها → افزایش مساحت سطح → خطر از دست رفتن ترکیبات فرار
جدید ماده fresh ای که تازه است - که تازه است و هنوز تغییراتی در آن رخ نداده است

ای که تازه است "fresh"

"Laboratory sample" :- "Sample taken into the lab for analysis after processing the gross sample"

Coning & quartering :- The gross sample is piled into a cone, flattened, divided into four quarters and two diagonally opposed quarters are discarded. The remaining material is cycled through the process of coning and quartering until the desired amount of sample remains.



(a) پتہ جمع (کاروانہ) "پورے پتہ" کی شکل
(b) پتہ زرد "اکوٹ" "شکل"
(c) پتہ چھوٹا "ایک اربعہ" "تہہ"
(d) پتہ ازلہ، چھوٹے پتہ کی شکل "تہہ" کی شکل

Bringing the sample in solution :- Dissolving it using Dist H_2O , or methanol, chloroform, toluene or combination of solvent.

Digestion :- with an acid or base $\Rightarrow$ using an open container like beaker with hot plate $\Rightarrow$ disadvantage "losing volatiles" alternatively a closed containers are used for digestion using $\Rightarrow$ "microwave radiations" & vessels for this are made from Teflon or fused silica

- Thermally stable
- chemically resistant
- Transparent to microwave radiation
- withstanding elevated pressure.

- high temp "200-300 $^{\circ}C$ "
- pressure "40-100 bar"
- less time from hours in open container to 30 min in microwave digestion
- prevention of loss of volatile organic compds.

Microwave digestion

- inability to add reagents during digestion
- limitation on amount of sample that can be used "lg or less"
- safety concerns due to high P & temp. and corrosive reagents

General Theory of separation efficiency:

Goal of separation to remove analyte or interferent from matrix

⇒ So there must be at least one significant difference bet.

Chemical

physical

prop.

and selectivity is also required.

removing interferent completely ⇒ may result in partial loss of analyte
& To minimize loss of ~~analyte~~ ^{interferent} ⇒ interferent may be left behind

analyte recovery ⇒ $R_A = \frac{C_A}{(C_A)_0}$ → analyte conc. after separation
→ initial conc.

Interferent recovery ⇒ $R_I = \frac{C_I}{(C_I)_0}$

"R" recovery - The fraction of analyte or interferent remaining after separation
separation factor - a measure of the effectiveness of a separation at separating an analyte from an interferent

$$S_{I,A} = \frac{C_I / C_A}{(C_I)_0 / (C_A)_0} = \frac{R_I}{R_A}$$

Ideal separation ⇒ $[R_A = 1, R_I = 0, S_{I,A} = 0]$

In general ⇒ for quantitative analysis of trace analyte in the macro interferent ⇒ $S_{I,A} \approx 10^{-7}$
& for analyte & interferent in equal amounts $\approx 10^{-3}$

Recovery & separation factor & Error

$$E = \frac{S_{\text{samp}} - S_{\text{samp}}^*}{S_{\text{samp}}^*}$$

expected signal for an ideal separation when all analyte is recovered.

$$S_{\text{samp}}^* = K_A (C_A)_0$$

relative error

$$\therefore E = \frac{K_A (C_A + K_{A,I} C_I) - K_A (C_A)_0}{K_A (C_A)_0} \Rightarrow \frac{C_A + K_{A,I} C_I - (C_A)_0}{(C_A)_0}$$

$$= \frac{C_A}{(C_A)_0} - \frac{(C_A)_0}{(C_A)_0} + \frac{k_{A,I} \times C_I}{(C_A)_0} = (R_A - 1) + \frac{k_{A,I} \times C_I}{(C_A)_0}$$

$$\therefore R_I = \frac{C_I}{(C_I)_0} \therefore C_I = R_I (C_I)_0$$

$$\therefore E = (R_A - 1) + \left[\frac{k_{A,I} \times (C_I)_0 \times R_I}{(C_A)_0} \right]$$

... یہ کہ (R) سے زیادہ ہو جائے تو اس سے پہلے کہ analyte کو الگ کیا جائے گا۔
 ... یہ کہ (R) سے زیادہ ہو جائے تو اس سے پہلے کہ interferent کو الگ کیا جائے گا۔

* Classifying separation techniques

Basis of separation	separation technique
⊕ Size	- Filtration & dialysis & size-exclusion Chromat.
⊕ mass and density	- Centrifugation
⊕ Complex formation	- masking
⊕ change in physical state	- distillation, sublimation, recrystallization
⊕ Change in chemical state	- precipitation, ion exchange, electrodeposition, volatilization
⊕ partitioning between phases	- extraction, Chromatography.

(a) separation based on size:-

- Filtration

- Dialysis "a semipermeable membrane is used to separate the analyte and interferent, membrane usually constructed from cellulose, with pore size of 1-5 nm. The sample is placed inside a bag or tube membrane. Then placed in a container filled with a solution whose composition differs from that of the sample. due to concentration gradient a driving force is provide for passing particles through membrane. Dialysis used to purify proteins, hormones, and enzymes.

⇒ During kidney dialysis metabolic waste products, such as urea, uric acid, and creatinine, are removed from blood by passing it over a dialysis membrane.

Size-exclusion chromatography

or gel permeation, or molecular exclusion chromatography.

Column is packed with small $\approx 10\text{-}\mu\text{m}$, porous particles of cross-linked dextrin or polyacrylamide. $\Rightarrow$ greater cross linking $\equiv$ greater smaller pore size

"Sample + solvent" are pumped through the column at a fixed flow rate.

Particles too large to enter the pores are not retained and pass through the column at the same rate as the solvent.

Smaller particles, which penetrate more deeply into the pore structure take the longest time to pass through the column.

applications: polymer analysis, biochemistry, proteins separation.

Separation based on Mass or Density:

when analyte & interferent are different in the mass or density

Suspension $\Rightarrow$ centrifuge tube $\Rightarrow$ spun at high angular velocity (rpm)
rev (revolutions per minute rpm).

particles of a greater centrifugal force $\Rightarrow$ faster sedimentation rates $\Rightarrow$ bottom
when equal masses $\Rightarrow$ highest density have the greatest sedimentation rate.

Centrifugation $\Rightarrow$ Important for biochemistry for separating

⊕ Alternative approach to differential centrifugation: $\Rightarrow$

- Equilibrium density $\pm$ gradient centrifugation:

sample + solution with a preformed density gradient or solution "when centrifuged forms a density gradient". ex - CsCl, or ~~glucose~~ sucrose solutions.
During centrifugation the sample's components undergo sedimentation at a rate determined by their centrifugal force. Because the solution's density increase toward the bottom of the centrifuge tube, the sedimentation rate for each component decreases as it moves toward the bottom of the centrifuge tube.

$\Rightarrow$ when the component reaches a position where its density is equal to that of the solution the centrifugal force drops to zero and sedimentation stops.

⇒ each component is isolated as a separate band positioned where the density of the component is equal to the density of the solution. ⇒ mixture of proteins, RNA, and DNA can be separated

separation

Separation based on complexation reactions (Masking)

A. pseudo-separation method in which a species is prevented from participating in a chemical reaction by binding it with a masking agent in an unreactive complex.

masking agent - The reagent used to bind the species to be masked in an unreactive complex.

masking agent

species to be masked

SCN^-	Ag, Au, Cd, Co, Cu, Fe, Hg, Mn, Ni, Pd, Pt, Zn
SCN^-	Ag, Cd, Co, Cu, Fe, Ni, Pd, Pt, Zn
NH_3	Ag, Co, Cu, Fe, Pd, Pt
F^-	Al, Co, Cr, Mg, Mn, Sn, Zn
$\text{S}_2\text{O}_3^{2-}$	Au, Cd, Co, Cu, Fe, Pb, Pd, Pt, Sb
tartrate	Al, Ba, Bi, Ca, Ce, Co, Cr, Cu, Fe, Hg, Mn, Pb, Pd, Pt, Sb, Sn, Zn
oxalate	Al, Fe, Mg, Mn, Sn
thioglycolic acid	Cu, Fe, Sn

اذا تم عزل كل عنصر في حيزه الخاص فلا يتفاعل مع غيره في الكشف عنه

Separation Based on state

Physical → liquid-to-gas, solid-to-gas
Chemical → involve one or more chemical reactions.

Change in physical state

for liquid mixtures ⇒ Distillation ①

simple distillation ← ②

③ fractional distillation

separating volatile from nonvolatile liq.
- or liquids with boiling points that differ by more than 150°C .

- packing the distillation columns with high surface area material
ven steel sponge, glass beads.

④ Sublimation

solid sample, heated at temp & press. below triple point where solid vaporizes without passing through the liq. state. Then vapour is condensed to form liquid.

⑤ recrystallization

Change in chemical state changing chemical state of analyte or interferent

① $\text{SiO}_2 \Rightarrow$ converted to volatile SiF_4 by reacting with HF

② $\text{NH}_4^+ \Rightarrow$ can be separated by reacting with basics "pH of medium > 7 "
"removing volatile species from nonvolatile"

NH_3 produced can be removed by distillation.

③ precipitation, electrodeposition, ion-exchange.

→ pH-dependent solubility of metal oxides & hydroxides
→ are usually accomplished using strong acids, strong bases or $\text{NH}_3/\text{NH}_4\text{Cl}$ Buffer.

most metal oxides and hydroxides are soluble in hot conc. HNO_3 "although
few oxides " WO_3 , SiO_2 , and SnO_2 " remain insoluble"

ex det. of Cu in brass $\Rightarrow$ Sn interferent is removed by dissolving the sample
with strong acid $\Rightarrow$ insoluble residue of SnO_2 - removed by filtration.

→ most metals will precipitate as the hydroxide in the presence of conc.
 NaOH , metals forming amphoteric hydroxides, remain soluble in conc
 NaOH due to the formation of higher-order hydroxo-complex.

ex Zn^{2+} & $\text{Al}^{3+} \Rightarrow \text{Zn}(\text{OH})_3^-$, $\text{Al}(\text{OH})_4^-$
 $\text{Al}_2\text{O}_3 \xrightarrow{\text{conc. NaOH}} 2\text{Al}^{3+} + 6\text{OH}^- \rightarrow 2\text{Al}(\text{OH})_3 + 3\text{H}_2\text{O}$
 $\text{Al}(\text{OH})_3 + \text{OH}^- \rightarrow \text{Al}(\text{OH})_4^-$

→ pH of $\text{NH}_3/\text{NH}_4\text{Cl}$ ($\text{pK}_a = 9.24$) $\Rightarrow$ sufficient to ppt of most
metals as hydroxides. Alkaline earths, alkaline metals, will not
precipitate at this pH - in addition; metal ions that form soluble complexes
with NH_3 " Cu^{2+} , Zn^{2+} , Ni^{2+} , and Co^{2+} " also will not precipitate.

→ S^{2-} as a precipitating agent - used to separate metal ions
from the remainder of the sample matrix. This important for 2 factors

① most metal ions form insoluble sulfides
"except for alkaline earths and
alkaline metals"

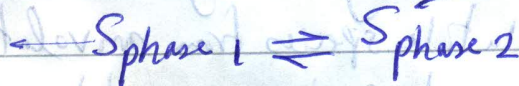
& solubilities of these
metal sulfides show a substantial
variation"

② since the conc. of S^{2-} is pH
dependent, control of pH was used
to determine which metal ions
would precipitate.

Separation based on partitioning Between phases:-

- ⊕ selective partitioning of the analyte or interferent between two immiscible phases.

phase containing
a solute



another phase.

at equilibrium

$$K_D = \frac{[S_{\text{phase 2}}]}{[S_{\text{phase 1}}]}$$

partition coefficient

" K_D "

An equilibrium constant describing the distribution of a solute between two phases; only one form of the solute is used in defining the partition coefficient.

$K_D > 1$

$\Rightarrow$ solute moves from phase 1 to phase 2

$K_D < 1$

$\Rightarrow$ remains in phase 1

- ⊕ a phase containing solutes is brought into contact with a second phase, and K_D is favorable for only one of the solutes, then a separation of the solutes may be possible.

- ⊕ The physical state of the two phases are identified when describing the separation process with the phase containing the analyte "sample" listed first e.g. liquid-solid - Gas-solid ~~contains the sample~~

Extraction between two phases:

Extraction:- The process by which a solute is transferred from one phase to a new phase.

useful for separations in which only one component has a favorable distribution ratio. "liquid-liquid", "liquid-solid", "gas-solid"

⊕ liquid-liquid extraction

using a separatory funnel & shaking to increase the surface area between the phases. when complete, the liquids are allowed to separate, with the denser phase settling to the bottom of separatory funnel.

- ⊕ may be done in the sample container by adding the extracting solvent when the sample is collected. e.g. "pesticides in water may be preserved for long periods by extracting into small volumes of hexane added to the sample in the field"

Solid-phase extraction:-

- Sample is passed through a cartridge containing solid particles that serve as the adsorbent material. For liquid samples the solid adsorbent is isolated in either a disk cartridge or a column.
- The choice of the adsorbent is determined by the properties of the species being retained & the matrix in which it is found.

Solid-phase microextraction:-

fused silica fiber is placed inside a syringe needle. The fiber which is coated with thin organic film such as poly (dimethylsiloxane) is lowered into the sample by depressing a plunger and exposed to the sample for a predetermined time. The fiber is then withdrawn into the needle and transferred to a gas chromatograph for analysis.

Gas-solid extraction

Sample is passed through a container packed with a solid adsorbent for analysis of organic compounds for carbon and hydrogen.

Sample is combusted in a flowing stream of O_2 , and the gaseous combustion products are passed through a series of solid-phase adsorbents that remove the CO_2 and H_2O .

Continuous extraction

⇒ when partition coefficient is unfavorable, a simple extraction will not be quantitative, instead the extraction is accomplished by continuously passing the extracting phase through the sample until a quantitative extraction is achieved.

Soxhlet extractor:-

The extracting solvent is placed in the lower reservoir and heated to its boiling point. Solvent in the vapor phase moves upward through the tube on the left side of the apparatus to the condenser where it condenses back to the liquid state. The solvent then passes through the sample which is held in a porous cellulose filter thimble, collecting the upper reservoir. When the volume of solvent in the upper reservoir reaches the upper end of the return tube, the solvent and any extracted components are siphoned back to the lower reservoir. Over time, the conc. of the extracted component in the lower reservoir increases.

Microwave assisted extraction $\Rightarrow$ microwave digestion & extraction

The sample is placed in a sealed digestion vessel along with the liquid extraction phase, and a microwave oven is used to heat the extraction mixture. The sealed vessel allows the extraction to take place at higher temperature & pressure thereby reducing the amount of time needed for quantitative extraction.

Soxhlet

extraction temp is limited to solvent boiling point^{ex}. acetone $\Rightarrow 56^\circ\text{C}$

microwave extraction

Temperature of over 150°C can be obtained when using acetone as the solvent.

Purge and trap:-

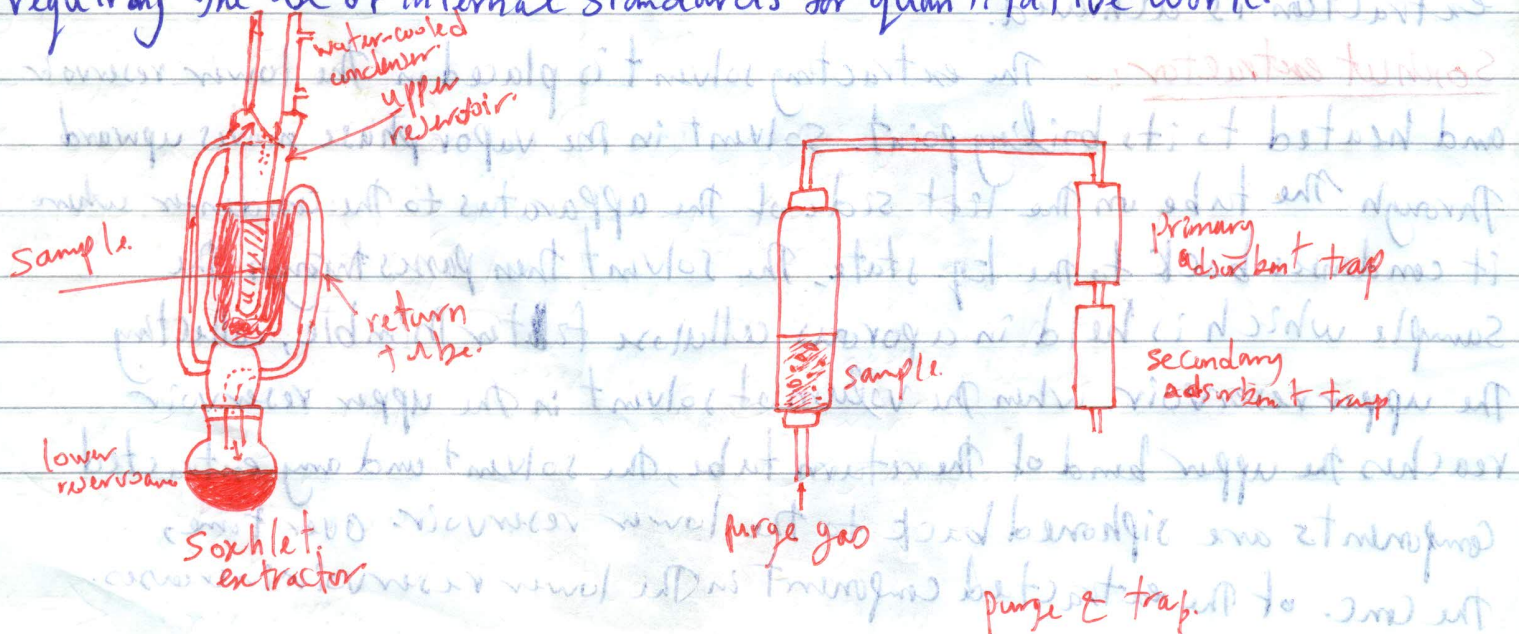
A technique for separating volatile organic analytes from liquid samples in which the analytes are subsequently trapped on a solid sorbent.

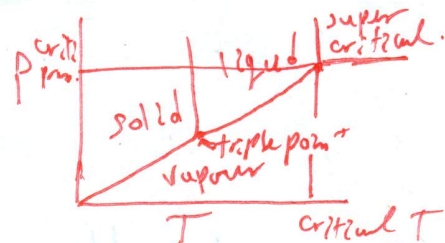
VOCs: volatile organic compounds. can be quantitatively removed from liquid samples by a liquid-gas extraction.

By passing an inert purging gas such as He, through the sample. The He removes the VOCs, which are then carried by He to a tube where they are collected on a solid adsorbent.

VOCs can then be removed from the trap for analysis by rapidly heating the tube while flushing with He.

- Recoveries for analytes using purge and trap may not be reproducible requiring the use of internal standards for quantitative work.





Continuous extraction using Supercritical fluids

A state of matter where a substance is held at a temperature and pressure that exceeds its critical temperature and pressure.

- properties of supercritical fluids are between those of gas & liquid.
- They are better solvents than gases $\Rightarrow$ better reagent for extractions.
- also viscosity of a supercritical fluid is significantly less than that of a liquid solvent $\Rightarrow$ allowing it to pass more readily through particulate samples.

ex Det. of total petroleum hydrocarbons (TPHs) in soils, sediments, and sludges with supercritical CO_2 .

3g sample in 10 ml stainless steel cartridge, supercritical CO_2 at pressure of 340 atm & T of 80°C is passed through the cartridge for 30 min at flow rate 1-2 ml/min. Petroleum hydrocarbons are collected by passing the effluent from the cartridge through 3 ml of Tetrachloroethylene at room temp. at this temp the CO_2 reverts to the gas phase and is released to atmosphere.

⊕ Chromatographic separation $\rightarrow$ stationary "fixed" phase (mobile phase)

continuously passing one sample-free phase "mobile phase" over a second sample-free phase that remains fixed or stationary. The sample is injected or placed into the mobile phase. separation is done by partitioning analytes between two phases.

Liquid-liquid extraction

Solute is partitioned between two immiscible phases, in most cases one of the phases is aqueous and the other phase is an organic solvent ex CH_2O , CHCl_3 .

- Because the phases are immiscible, they form two layers, with the denser phase on the bottom. The solute is initially present in one phase, but after extraction it is present in both phases. Efficiency of extraction is determined by the equilibrium constant for the solute's partitioning bet. the two phases.

⊕ Extraction efficiency is also influenced by any secondary reactions

acid-base

complexation equilibria

Partition Coefficient & Distribution Ratios:-

if the solute is initially in an aqueous phase and is extracted into an organic phase



The partition coefficient is

$$K_D = \frac{[S_{org}]}{[S_{aq}]}$$

$K_D \gg 1$ if large $\Rightarrow$ extraction into organic phase is favorable

Distribution ratio

A ratio expressing the total conc. of solute in one phase relative to a second phase all forms of the solute are considered in defining the distribution ratio (D)

$$D = \frac{[S_{org}]_{tot}}{[S_{aq}]_{tot}}$$

one form of solute $\therefore K_D = D$

form A, B of solute $\therefore K_D \neq D$

$$D = \frac{[S_{org}]_A}{[S_{aq}]_A + [S_{aq}]_B} \ll K_D = \frac{[S_{org}]_A}{[S_{aq}]_A}$$

Eq. extraction with no secondary reactions:-

in this case $D = K_D \Rightarrow \frac{[S_{org}]_{tot}}{[S_{aq}]_{tot}} = \frac{[S_{org}]}{[S_{aq}]}$

$\therefore (\text{Moles } aq)_0 = (\text{moles } aq)_1 + (\text{moles } org)_1$
 extraction no. $\nearrow$

Conc. of solute 'S' in aq. phase after extraction is

$$[S_{aq}] = \frac{(\text{moles } aq)_1}{V_{aq}}$$

Solute conc. in the organic phase

$$[S_{org}] = \frac{(\text{moles } org)_1}{V_{org}}$$

$[S_{org}]$ اس وقت کی مقدار ہے کہ ایک آپ ایک میں

$$[S_{org}] = \frac{(\text{moles } aq)_0 - (\text{mole } aq)_1}{V_{org}}$$

$$\therefore D = \frac{[S_{org}]}{[S_{aq}]} \Rightarrow D = \frac{(\text{moles } aq)_0 - (\text{moles } aq)_1 / V_{org}}{(\text{moles } aq) / V_{aq}}$$

$$D = \frac{[(\text{moles } aq)_0 V_{aq} - (\text{moles } aq)_1 V_{aq}]}{(\text{moles } aq) V_{org}}$$

Rearranging and solving for fraction of solute remaining in the aq. phase after one extraction

fraction of ext. in aq. phase

$$(q_{aq})_1 = \frac{(\text{moles } aq)_1}{(\text{moles } aq)_0} = \frac{V_{aq}}{D V_{org} + V_{aq}}$$

The fraction of present in the organic phase after one extraction

$$(q_{org})_1 = \frac{(\text{moles } org)_1}{(\text{moles } org)_0} = 1 - (q_{aq})_1 = \frac{D V_{org}}{D V_{org} + V_{aq}}$$

$$(\text{moles } aq)_0 = [S_{aq}]_0 \times V_{aq}, \quad [S_{aq}]_1 = \frac{(\text{moles } aq)_1}{V_{aq}}$$

$$[S_{org}]_1 = \frac{(\text{moles } org)_1}{V_{org}}$$

$$V_{org} \Rightarrow \frac{V_{aq} - (q_{aq})_1 V_{aq}}{(q_{aq})_1 D}$$

99.9% کا 99.9% $\Rightarrow (q_{aq})_1 \approx 0.001$

میں سے صرف V_{org} کے لئے D کے لئے V_{aq} کے لئے

If the volume of the aqueous and organic layers are the same for both extractions, then the cumulative fraction of solute remaining in the aqueous layer after two extractions, $(Q_{aq})_2$ is

$$(Q_{aq})_2 = \frac{(\text{moles } aq)_2}{(\text{moles } aq)_0} = (q_{aq})_1 (q_{aq})_2 = \left[\frac{V_{aq}}{DV_{org} + V_{aq}} \right]^2$$

for general

$$(Q_{aq})_n = \frac{(\text{moles } aq)_n}{(\text{moles } aq)_0} = \left[\frac{V_{aq}}{DV_{org} + V_{aq}} \right]^n$$

در دو استخراج با یک solvent (مثلاً) به نسبت 1:1 استخراج می‌کنیم و باقی‌مانده 99٪
باقی‌مانده در $(Q_{aq})_n$ باقی می‌ماند و $0.001 = V_{org}$

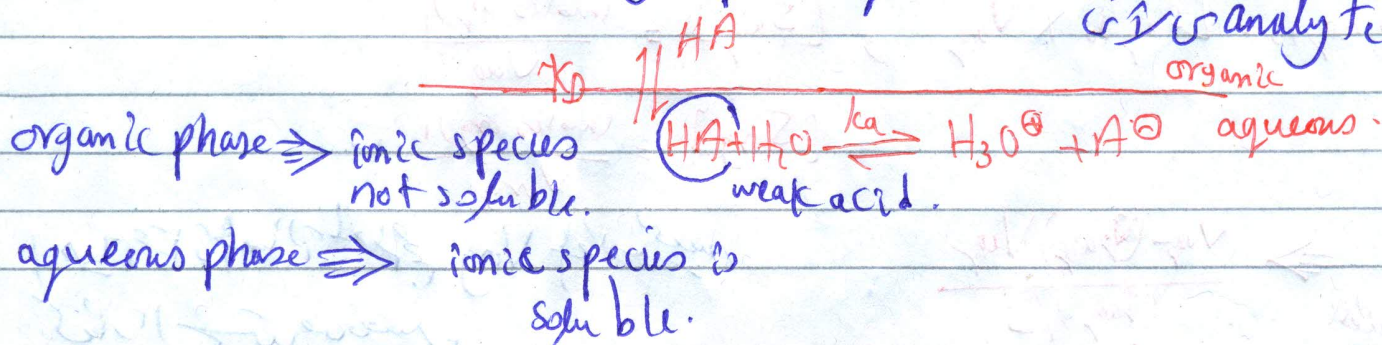
حاصل می‌شود که در هر مرحله 99٪ استخراج می‌شود و 1٪ باقی‌مانده می‌ماند.
در مرحله اول 99٪ استخراج می‌شود و 1٪ باقی‌مانده می‌ماند.
در مرحله دوم 99٪ از باقی‌مانده 1٪ استخراج می‌شود و 0.01٪ باقی‌مانده می‌ماند.
در مرحله سوم 99٪ از باقی‌مانده 0.01٪ استخراج می‌شود و 0.0001٪ باقی‌مانده می‌ماند.

برای تکرار استخراج solvent، توصیه می‌شود که در هر مرحله 99٪ استخراج می‌شود و 1٪ باقی‌مانده می‌ماند.
در مرحله اول 99٪ استخراج می‌شود و 1٪ باقی‌مانده می‌ماند.
در مرحله دوم 99٪ از باقی‌مانده 1٪ استخراج می‌شود و 0.01٪ باقی‌مانده می‌ماند.
در مرحله سوم 99٪ از باقی‌مانده 0.01٪ استخراج می‌شود و 0.0001٪ باقی‌مانده می‌ماند.

Liquid-liquid extraction involving Acid-Base equilibria

$$D = K_D$$

single phase equilibrium
organic phase $\Rightarrow$ ionic species not soluble.
aqueous phase $\Rightarrow$ ionic species soluble.



هو الى راجع التوزيع

$$K_D = \frac{[HA_{org}]}{[HA_{aq}]}$$

$$D = \frac{[HA_{org}]_{tot}}{[HA_{aq}]_{tot}} = \frac{[HA_{org}]}{[HA_{aq}] + [A_{aq}^-]}$$

∴ Since The position of acid, base equilibrium depends on pH

$$K_a = \frac{[H_3O^+][A_{aq}^-]}{[HA_{aq}]}$$

$$[A_{aq}^-] = \frac{K_a [HA_{aq}]}{[H_3O^+]}$$

$$D = \frac{[HA_{org}]}{[HA_{aq}] + \left(\frac{K_a [HA_{aq}]}{[H_3O^+]} \right)}$$

$$D = \frac{K_D}{\left(1 + \frac{K_a}{[H_3O^+]} \right)}$$

$$D = \frac{K_D [H_3O^+]}{[H_3O^+] + K_a}$$

$$(Q_{aq})_n = \left(\frac{V_{aq}}{D V_{org} + V_{aq}} \right)^n$$

هو الى راجع التوزيع

هو الى راجع التوزيع

extraction efficiency is better at more acidic pHs when HA is the predominate species in the aqueous phase.

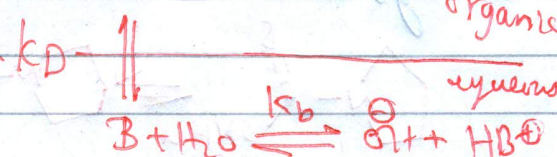
extraction efficiency is greatest for pHs more acidic than weak acid's pKa
decreases substantially at pHs more basic than pKa.

for bases

$$D = \frac{K_D K_a}{K_a + [H_3O^+]}$$

for the weak acids base's conjugated acid.

B



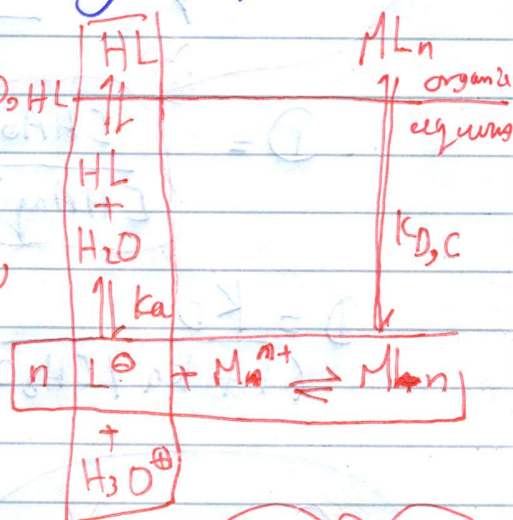
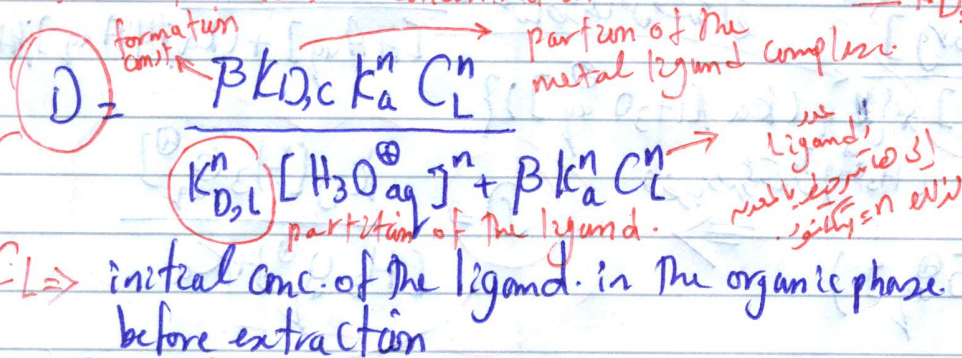
organic aqueous

Liquid-liquid extractions involving metal chelators.

- selective extraction of metal ions using chelating agent.
- why?? many chelating agents have a limited solubility in water or are subject to hydrolysis or air oxidation in aqueous solutions.
- aqueous phase. $\sim$ ~~very~~ organic solvent. $\Rightarrow$ chelating agent $\rightarrow$ ligand $\rightarrow$ M^{n+}

(aqueous phase + chelating agent) $\Rightarrow$ (metal-ligand complex) $\Rightarrow$ extracted in the organic phase.

If the ligand's concentration is much greater than the metal ion's concentration



distribution ratio

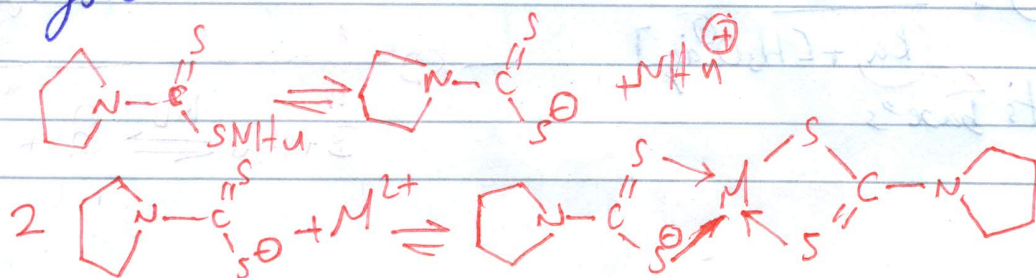
$$(Q_{aq})_n = \left(\frac{V_{aq}}{D V_{org} + V_{aq}} \right)^n$$

we need to know D to find Q_{aq}

ligands $\rightarrow$ like

- high degree of selectivity. & effect of pH ex 0% - 100% over a range of only 2 pH unit.

Ligand ammonium pyrrolidine dithiocarbamate (APDC) used as metal chelating agent. (Sample + APDC) mixed together, resulting metal-ligand complexes are extracted into methyl isobutyl ketone before analysis.



Separation vs. Preconcentration

Two frequently encountered problems

presence of matrix components
interfering with the analysis
of analytes

ربا قاتل قاتل (مضاد)
جميع مكونات الأداة، إضافة
إلى المواد الكيميائية الإضافية المستخدمة
في معالجة العينات analyte غير مرغوب فيه.

analyte's concentration
is too low.

Preconcentration :- The process of increasing an analyte's conc. before analysis.

