Ministry of Higher Education and Scientific Research
University of Baghdad
College of Science
Department of Biology

الكيمياء التحليلة –الماجستير-فرع البيئة

مدرس المادة : م. د. بهاء مالك عبد الكريم

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Lec 1

Chemistry

Chemistry

Chemistry is the study of matter, its chemical and physical properties, the chemical and physical changes it undergoes, and the energy changes that accompany those processes.

Major Areas of Chemistry

Chemistry is a broad area of study covering everything from the basic parts of an atom to interactions between huge biological molecules. Because of this, chemistry encompasses the following specialties.

Biochemistry

Organic chemistry

Inorganic chemistry

Analytical chemistry

Physical chemistry

Experimental Quantities

The quantities that are most often determined include mass, length, volume, time, temperature, and energy:

Mass

Mass describes the quantity of matter in an object. The terms *weight* and *mass*, in common usage, are often considered synonymous. They are not, in fact. Weight is the force of gravity on an object:

Weight = mass ×acceleration due to gravity

The common conversion units for mass are as follows:

1 gram (g) = 10^{-3} kilogram (kg) = $\frac{1}{454}$ pound (lb)

In chemistry, when we talk about incredibly small bits of matter

such as individual atoms or molecules, units such as grams and even micrograms are much too large. Similarly, an atom of a substance such as hydrogen is very tiny. Its mass is only 1.661×10^{-24} gram.

Volume

The standard metric unit of volume, the space occupied by an object, is the liter. A liter is the volume occupied by 1000 grams of water at 4 degrees Celsius (°C). The volume, 1 liter, also corresponds to:

1 liter (L) = 10^3 milliliters (ml) .The relationship between the liter and the milliliter is shown in Figure 2.

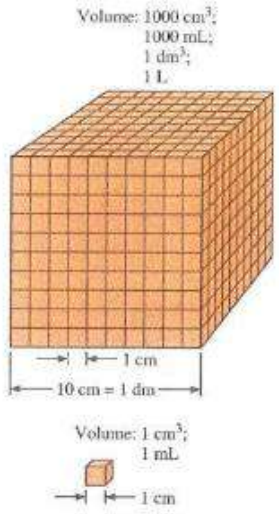
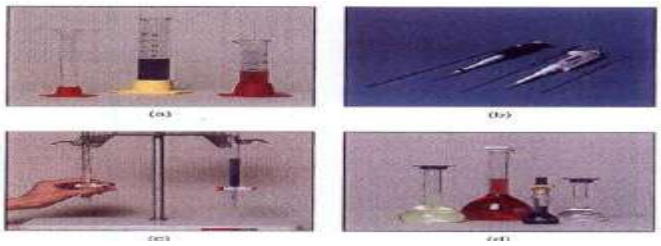


Figure 2 The relationship among various volum units

Typical laboratory glassware used for volume measurement is shown in Figure 3. The volumetric flask is designed to contain a specified volume, and the graduated cylinder, pipet, and burette dispense a desired volume of liquid.



Concentration

Concentration is a measure of the number of particles of a substance,

or the mass of those particles, that are contained in a specified volume. Concentration is a widely used way of representing mixtures of different substances

We will describe many situations in which concentration is used to predict useful information about chemical reactions.

Density and Specific Gravity

Both mass and volume are a function of the amount of material present. Density, the ratio of mass to volume,

$$d = \frac{mass}{volume} = \frac{m}{V}$$

In density calculations the mass is usually represented in grams, and volume is given in either milliliters (ml) or cubic centimeters (cm^3 or cc):

1 ml = 1 cm^3 = 1 cc

The unit of density would therefore be g/ml, g/cm^3, or g/cc.

Density is called the specific gravity, the ratio of the density of the object in question to the density of pure water at 4 C^o.

$$\text{Specific gravity} = \frac{\text{Density of object (g/ml)}}{\text{Density of water (g/ml)}}$$

Lec 2

Introduction to Analytical Chemistry

Introduction

Analytical chemistry is the science of obtaining, processing, and communicating information about the composition and structure of matter. In other words, it is the art and science of determining what matter is and how much of it exists.Analytical chemistry is science that concerned with the separation, identification, and determination of the relative amounts of the components making up a sample.

Applications of Analytical Chemistry

Analytical chemistry used in many fields:

· In ***medicine***, analytical chemistry is the basis for clinical laboratory tests which help physicians diagnosis disease and chart progress in recovery.

· In ***industry***, analytical chemistry provides the means of testing raw materials and for assuring the quality of finished products whose chemical composition is critical. Many household products, fuels, paints, pharmaceuticals, etc. are analysed by the procedures developed by analytical chemists before being sold to the consumer.

· ***Enviermental quality*** is often evaluated by testing for suspected contaminants using the techniques of analytical chemistry.

· ***Forensic analysis*** - analysis related to criminology; DNA finger printing, finger print detection; blood analysis.

· ***Bioanalytical chemistry and analysis*** - detection and/or analysis of biologicalvcomponents (i.e., proteins, DNA, RNA, carbohydrates, metabolites, etc.).

Classification of analysis

Qualitative analysis: An analysis in which we determine the identity of the constituent species in a sample.

Quantitative analysis: An analysis in which we determine how much of a constituent species is present in a sample.

Sample analysis

Analytes: The constituents of interest in a sample.

Matrix: All other constituents in a sample except for the analytes.

Classifying Analytical Techniques

Classical techniques

Mass, volume, and charge are the most common signals for classical techniques, and the corresponding techniques are:

1- Gravimetric techniques.

2- Volumetric techniques.

3- Coulometeric techniques.

Instrumental techniques

1- Spectroscopic methods - measuring the interaction between the analyte and electromagnetic radiation (or the production of radiation by an analyte).

2- Electroanalytic methods - measure an electrical property (i.e., potential, current,

resistance, amperes, etc.) chemically related to the amount of analyte.

3-Chromatographic analysis and analytical separation.

Methods for the expression of concentration

1-Mole: Symbol (mol)

Is defined as the quantity of given substance that contains as many molecules for formula units as the number of atoms in exactly

Number of moles for compounds = $\frac{\text{weight}}{\text{Molecular weight}}$

Note: Molecular weight of compounds is the sum of the atomic weight of all the atoms in the molecular formula of the compounds

For example: number of moles of (NH2)2CO = $\frac{\text{weight}}{(14+(2x1))x2+12+16}$

Number of moles of ion = $\frac{\text{wt}}{\text{Ionic weight}}$, for SO4 = $\frac{\text{weight}}{32+(4x16)}$

Number of moles of element = $\frac{\text{wt}}{\text{Atomic weight}}$, Ag = $\frac{\text{wt}}{108}$

2-Molarity: symbol (M)

When we dissolve a substance in a liquid, we call the substance (solute) and the liquid (solvent), so the molarity of solution defines the number of gram-molecular weight (or) moles of solute dissolved in 1 liter of solution, or the number of millimolecular weight in 1 millimeter of solution

$$M = \frac{number\ of\ moles\ of\ solute}{volumeof\ solution\ in\ liter}$$

$$Number\ of\ moles = \frac{wt}{M.wt}$$

For solid sustances

Thus : $M = \frac{wt}{Mwt} x \frac{1000}{Vol\ ml}$

Notes: (wt) means weight in gram unit (gm)

(vol) means volume of solution in milliter unit (ml)

For liquid substances

Also $M = \frac{Density\ or\ specific\ gravity\ x\ percentage\ x1000}{M.wt}$

Example: 1

What is the molarity of a solution containing (16 gm) CH3OH in 200 ml of solution? M.wt =32.

Sol.

$M=\frac{wt}{M\,wt}$ x $\frac{1000}{Vol}$

$=\frac{16}{32}$x $\frac{1000}{200}$

=2.5 mol/L

Example:2

Calculate the molarity of H2SO4 solution of specific gravity 1.198 , containing 27 % H2SO4 by by weight ?

At.wt. of H=1 , S=32 , O=16 .

Sol.

M.wt of H2So4 = (2x1)+32 +(4x16)=98 gm/mol

$M= \frac{Sp.gr\ \times\ \%\ \times 1000}{M.wt}$

$=\frac{1.198\times0.27\times1000}{98}$

=3.3 mol/L

3- <u>Normality</u>: symbol (N)

The normality of a solution expresses the number of milliequivalents of solute contained in 1 ml of the solution, or the number of gram equivalents contained in 1 liter.

$N= \frac{number\ of\ gram-equivalent\ weight\ of\ solute}{Volume\ of\ solution\ in\ liters}$

Number of gram-equivalent weight = $\frac{wt}{eq.wt}$

Note: (eq.wt) means equivalent weight. (Its unit is$\frac{gm}{gm.m.eq}$)

So, for solid substances

$$N=\frac{wt.(gm)}{eq.wt} \times \frac{1000}{vol.(ml)}$$

And, for liquid substances

$$N=\frac{specific\ gravity\ or\ density \times \% \times 1000}{eq.wt.}$$

Example :1

How many gram-equivalent of solute are contained in 0.5L of 0.2 N solution?

Sol.

$$N=\frac{number\ of\ gram-equivalent\ weight\ of\ solute}{Volume\ of\ solution\ in\ liters}$$

$0.2=\frac{x}{0.5}$ = 0.1 gm. Equivalent of solute

Example: 2

How many grams of solute are required to prepare 1 liter of 1N solution of NaCl , M.wt = 58.45 ?

Sol.

$$N=\frac{wt.(gm)}{eq.wt} \times \frac{1000}{vol.(ml)}$$

=58.45 gm.

Note: $N=n \times M$

Which, N=Normality

n= Valence number or equivalence number *

M=Molarity

*Valence number of an element is the number of atoms of hydrogen (or its equivalent) which one atom of the element combines with or displaces.

So, in NaCl: M.wt =eq.wt

But in H2SO4: eq.wt= M.wt/2 because there are 2 hydrogens (protons) which displaces

Example3

What will be the normality of 3 molar solution of calcium hydroxide $Ca(OH)_2$?

Solution:

M=$\frac{N}{n} = \frac{3}{2}$ = 1.5 N

Calculation of n

HCl=1 , H_2SO4=2 , H_3PO4=3, NaOH =1, $Ca(OH)_2$=2 , $Al(OH)_3$ =3, $CaCl_2$=2, Na_2SO4=2 , Na_2CO3=2, CaSO4=2

4-Percentage concentration:

The percentage composition of a solution can be expressed in several ways. Three of the common methods are defined as follows:

Weight percent =$\frac{weight\ of\ solute}{weight\ of\ solution}$ x100

Volume percent = $\frac{volume\ of\ solute}{volume\ of\ solution}$ x100

5-parts per million concentration :

For much diluted solution, the concentration is more conveniently expressed is part per million (ppm). This term is defined by equation:

ppm = $\frac{weight\ of\ solute\ (g)}{total\ weight\ of\ solution\ (g)}$ x 10^6 or mg/L

ppm = $\frac{volume\ of\ solute\ (ml)}{total\ volumeof\ solution\ (ml)}$ x 10^6

Thus, an aqueous solution containing 0.0003 % nickel by weight contains 3ppm of nickel by weight. How?

0.0003% means 0.0003 gm solute in 100gm solution.

So, ppm = $\frac{weight\ of\ solute}{weight\ of\ solution}$ x 10^6

= $\frac{0.0003}{100}$ x 10^6

=3 ppm

The relation between (ppm) and (molarity) is

ppm= molarity x molecular weight x 1000

The relation between (ppm) and (normality) is:

ppm = normality x equivalent weight x 1000

Example :

Aqueous solution of $NiCl_2$ with a concentration of 500 ppm, what is the molarity and normality of this solution? At.wt of : Ni=58.69 , Cl=35.5

Diluting solutions:

Example:

You are given a solution of 14.8 M NH_3 , How many milliliters of this solution do you require to give 100ml of 1M NH_3 when dilute ?

In this kinds of examples and if you need to get a diluted solution, follow the dilution equation:

$Mi \times Vi = Mf \times Vf$

$14.8 \times Vi = 1 \times 100$

$Vi = 6.76$ ml

Home work

Question: 45.57 mL of a solution is diluted to 63.40 mL. The diluted solution is found to have a concentration of 0.433 N. What was the concentration of the original solution?

Question: Calculate the weight of Calcium hydroxide present in 250 ml of two normal solution?

Question: If 40 grams of NaOH with an equivalent weight of 40 is dissolved in one liter of a solution, does the normality of the solution is one , give the calculation ?

Question: Calculate the molarity of 0.650 N HCl.

Question : What will be the normality of 3 molar solution of calcium hydroxide?

Lec 3

Fundamentals of Sample Pre-treatment for environmental analysis

Overview of Sample Preparation

- Sample Preparation for **Metal Analysis**
- Extraction for **SVOC and Non-VOC** from Liquid or Solid Samples
- **Derivatization** of Non-VOC for Gas Phase Analysis

Fundamentals of Sample Preparation for Environmental Analysis

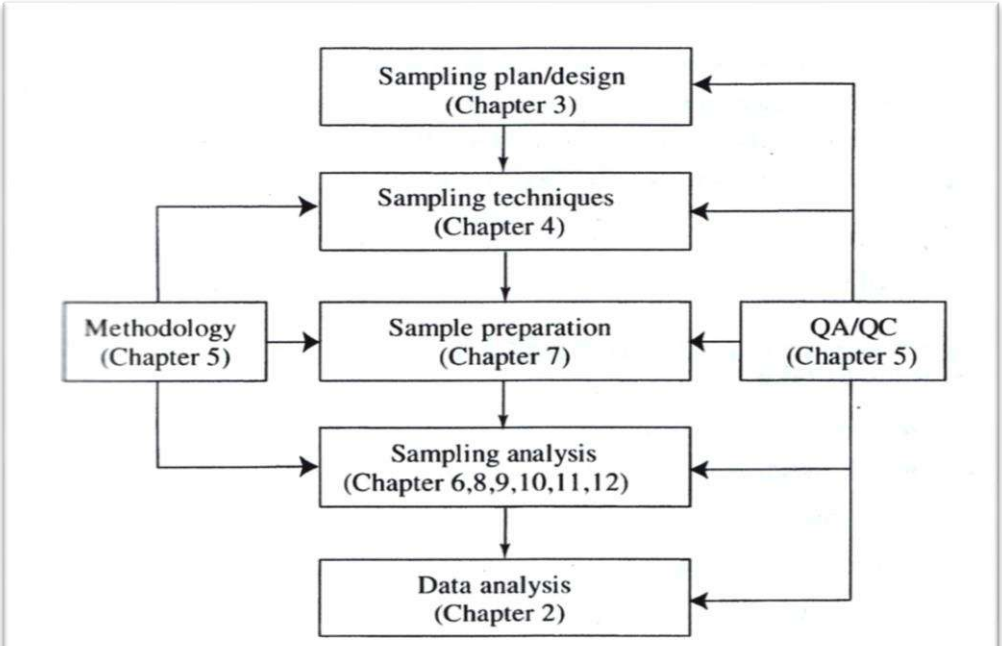
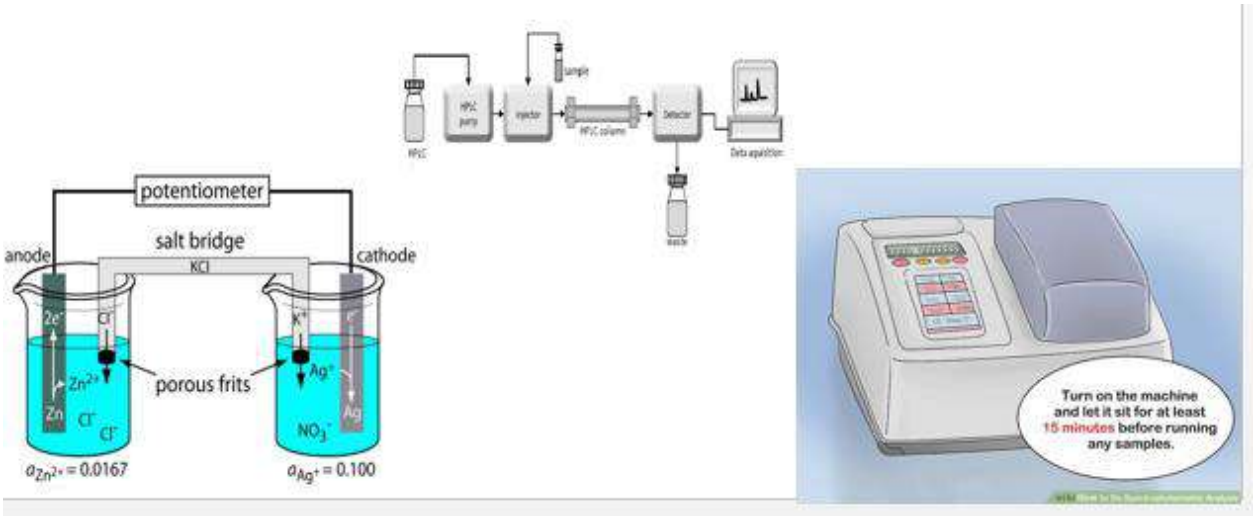


Figure 1.1. Environmental data acquisition process

Techniques used in sample analysis

- Spectrophotometric
- Chromatographic (HPLC & GC)
- Electro analytic and potentiometric



Very rarely can environmental samples be directly injected into an instrument without pretreatment.

- **Main categories:**
  - Digestion for metals
  - Extraction and post-extraction for SVOC's
  - Derivatization for non-VOCs
  - Preparation for VOC and air samples

Purpose of Sample Preparation

1. *To increase/decrease analyte concentration*.pre-concentration is needed for almost all trace analysis, dilution is used for the analysis of highly contaminated samples so the .concentration falls within the calibration range
2. *To remove **interfering chemicals***: major issue for trace organic compounds
3. *To change sample phase*: sample phase may be needed to be changed to fit the instrument
4. *To l**iberate** analyte from **sample matrix***: analyte species may be needed to be liberated from sample matrix
5. *To **modify chemical structure***: chemical **derivatization** is used to increase or decrease volatility for HPLC or GC analysis

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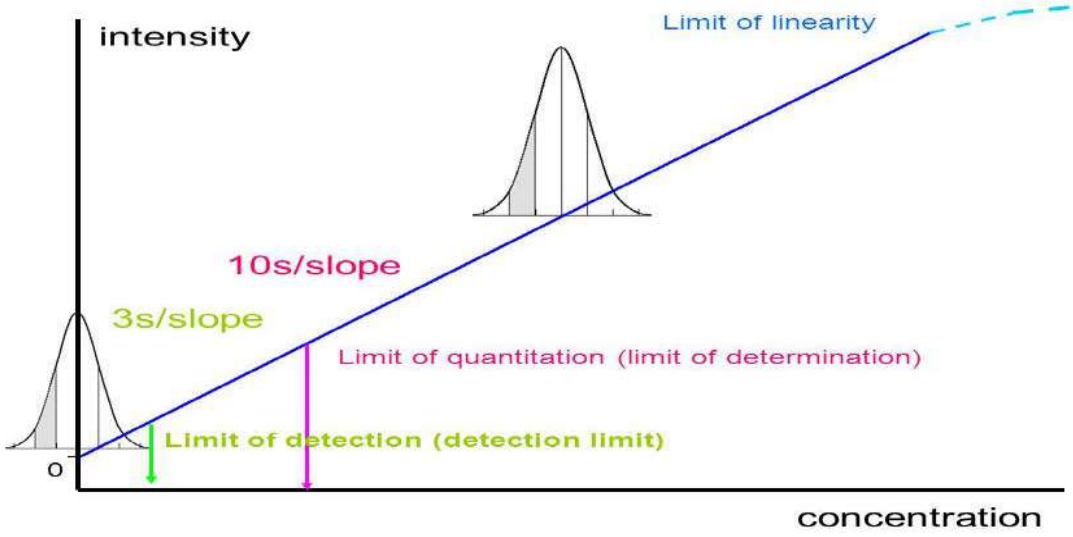


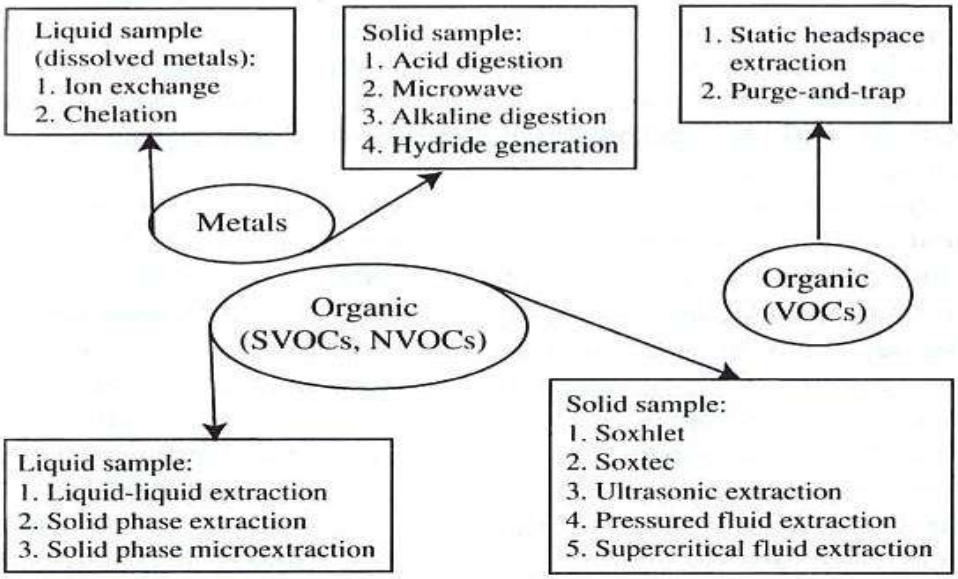


Figure 7.2 Types of sample preparation methods (VOCs = volatile organic compounds, SVOCs = semivolatile organic compounds, NVOCs = nonvolatile organic compounds)

Metals and Preparation Methods

Solid samples:

- *Total metal analysis* – **acid digestion** via hot-plate digestion or **microwave-assisted digestion**

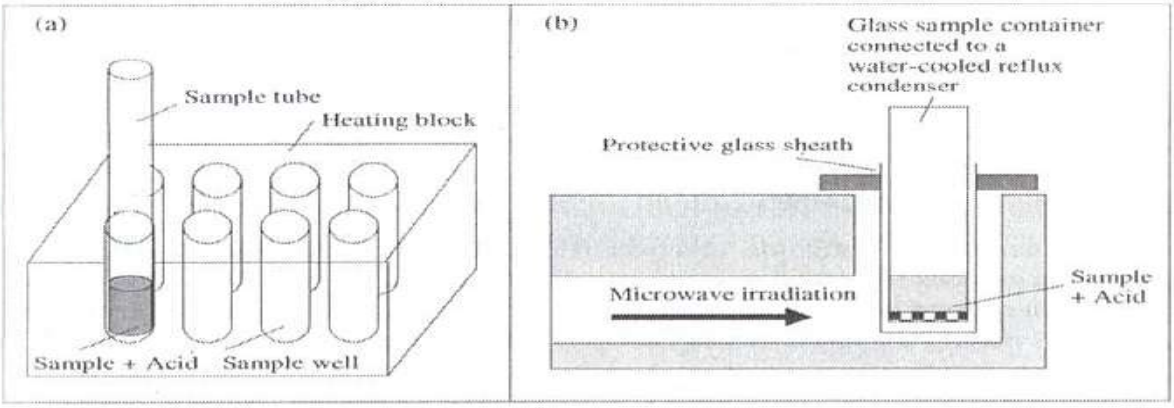


Figure 7.3 Schematic diagram of two common acid digestion apparatus: (a) heat block acid digestion system, (b) microwave-assisted acid digestion system. (Dean, JR, 2003, Methods for Enviromental Trace Analysis, © John Wiley & Sons Limited. Reproduced with Permission)

Extraction of SVOCs and nonvolatile compounds

- Liquid-Liquid extraction (LLE)
- Solid Phase Extraction (SPE)
- Solid Phase Micro Extraction (SPME)
- Soxhlet extraction
- Ultrasonic extraction
- Pressurized Fluid Extraction (PFE)

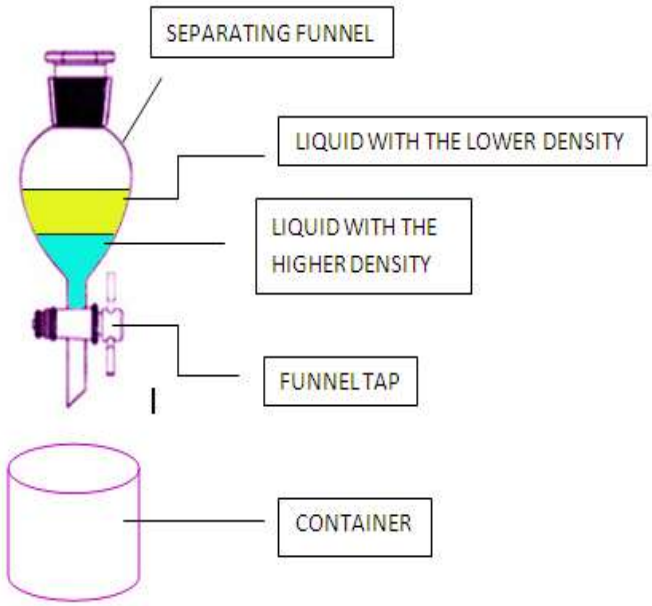
– Supercritical Fluid Extraction (SFE)

Lec 4

The Extraction of Semi volatile Organics from Liquids

Liquid-Liquid Extraction (LLE)

Partitioning analytes between water phase and organic phase



Distribution coefficient

- If a solute is in aqueous phase and is extracted into an organic phase:
- A solute S will distribute itself between two phases (after shaking/mixing).
- Ratio of [S] in the two phases will be constant.
- K_D – distribution coefficient

$$K_D = \frac{[S]_1}{[S]_2}$$

solvent 1 eg. organic solvent

solvent 2 eg. water

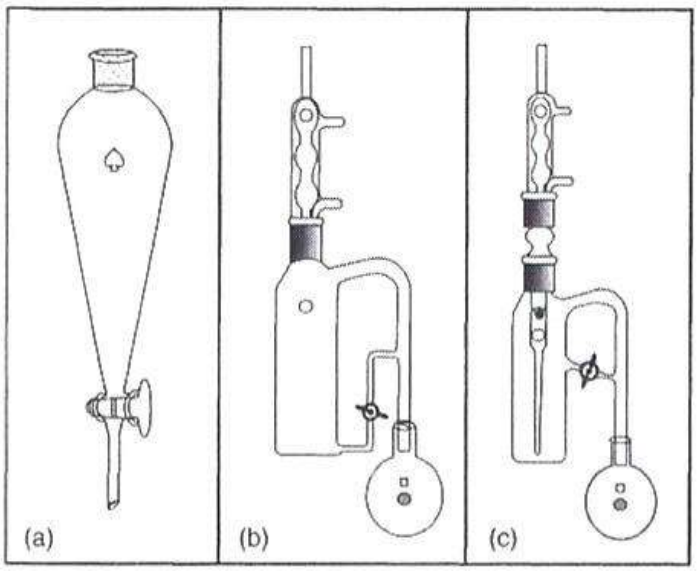


Figure 7.4 Liquid–liquid (L–L) extraction: (a) Conventional separatory funnel L–L extraction (Courtesy of Kimble Glass Inc.) (b) Continuous L–L, heavier than water extraction, and (c) Continuous L–L, lighter than water extraction (Courtesy of Kontes Glass Company)

Solid Phase Extraction (SPE)

- SPE retains analyte from a flowing liquid sample on solid sorbent, analyte is recovered via elution from the sorbent
- Phase types:
  - **Reverse phase**
  - **Normal phase**
  - **Ion exchange**
  - **Adsorption**

SPE techniques

Nonpolar:

Reverse phase C18 (octadecyl bonded silica) and C8 (octyl bonded silica) are most commonly used for hydrophobic
analytes

Polar:

Normal phase SPE uses cyanopropyl bonded, diol bonded, or amino propyl
bonded silica (used for polar analytes such as cationic compounds and organic
acids)

Electrostatic:

Ionic Exchange SPE is based on electrostatics uses quaternary amine, sulfonic acid, or carboxylic acid bonded silica

Adsorption type SPE uses unmodified materials such as alumina, Florosil, resins

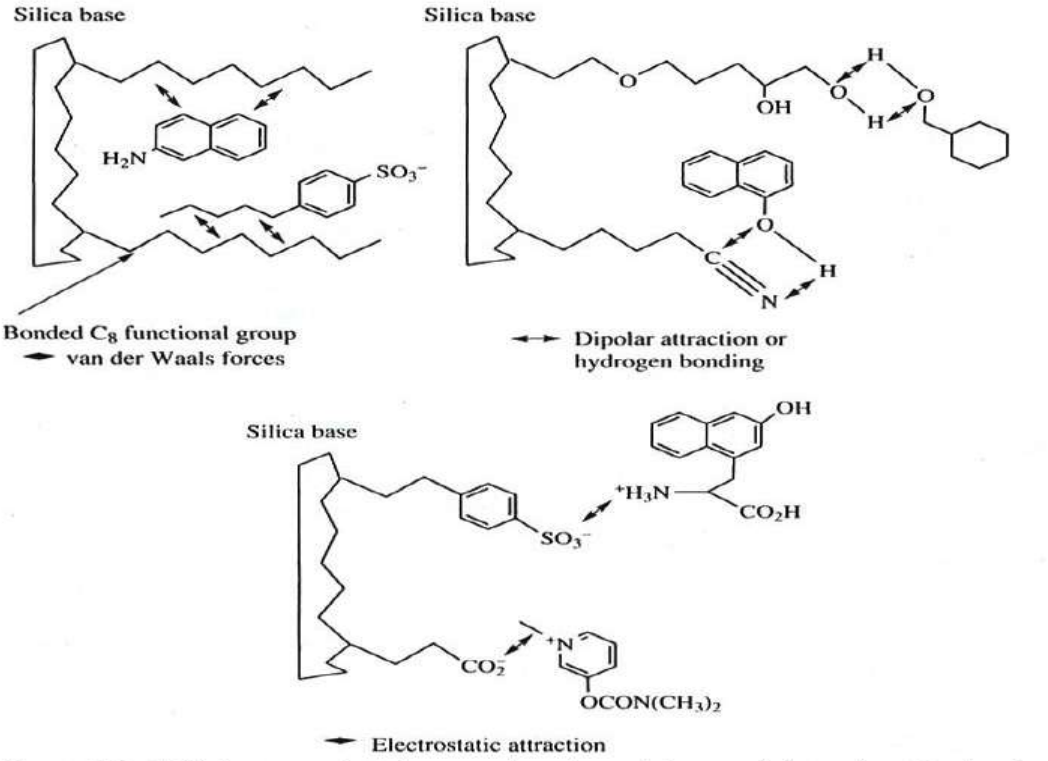


Figure 7.6 Solid-phase extraction using nonpolar, polar, and electrostatic interactions (Reprinted with permission from *American Laboratory*, 24(12):37–42. © 1992 by International Scientific Communications Inc.)

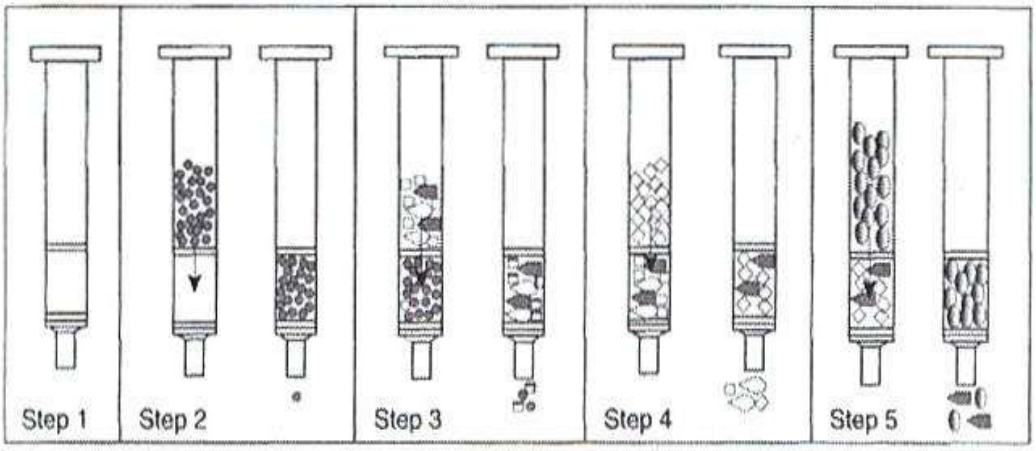


Figure 7.7 Five steps of solid phase extraction: (a) Select the proper SPE tube or disk from various commercially available SPEs, (b) Condition the SPE tube or disk, (c) Add the sample; (d) Wash the packing; (e) Elute the compounds of interest (Courtesy of Supelco)

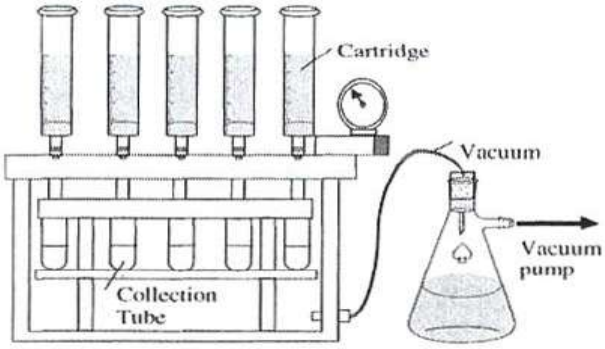


Figure 7.8 Vacuum manifold for solid phase extraction (SPE) of multiple cartridge units

(c) Automation is possible for SPE, resulting in reduced analytical cost, time, and labor.

Solid Phase Micro extraction (SPME)

- Based on a solvent-free **sorption-desorption** process
- SPME is a fused silica fiber coated with solid adsorbent
- Organic analytes absorb to the fiber, released by heating in GC port
- Combines extraction, concentration, and injection in one process

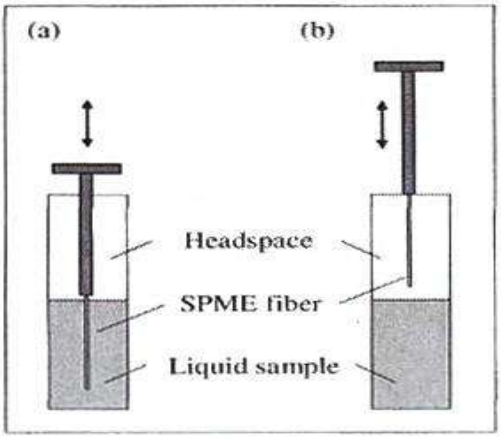


Figure 7.9 Two types of SPME applications in (a) liquid samples, and (b) headspace samples

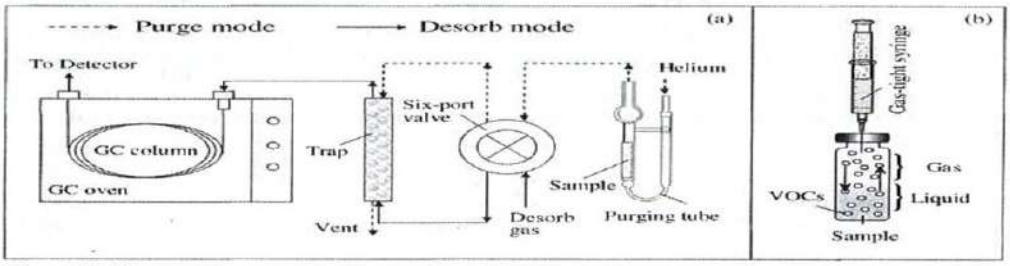
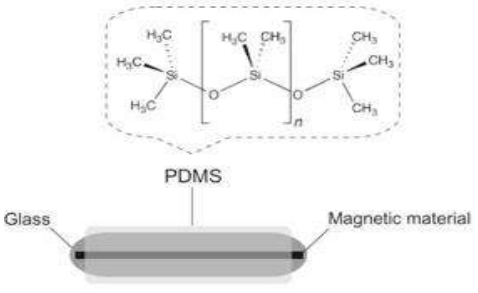
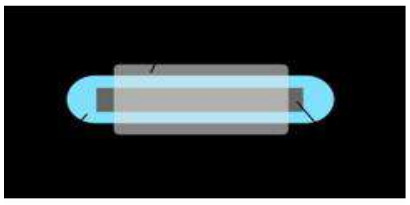


Figure 7.14 Sample preparation for VOCs: (a) Dynamic headspace extraction (purge-and-trap), and (b) Static headspace extraction (SHE)

Stir Bar Sorptive Extraction (SBSE)



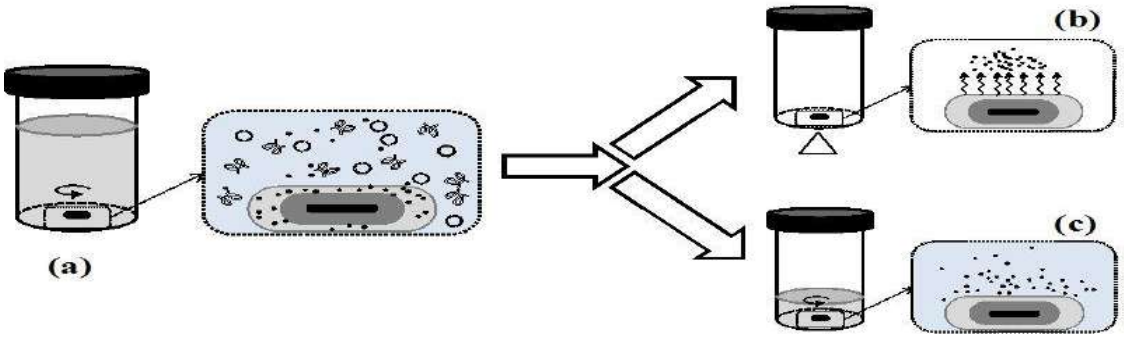


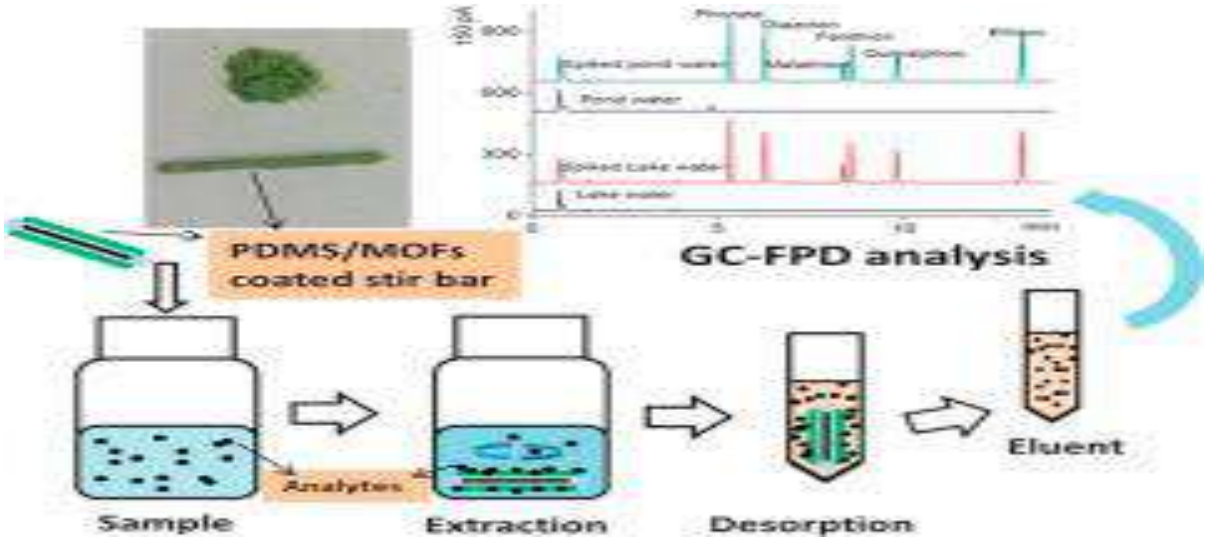
Polydimethylsiloxane (PDMS)

Materials used in SBSE

- Polystyrene- divinylbenzene (PS-DVB)
- octadecyl silica (ODS)

- cyclohexyl silica (CHS)





Lec 5

Extraction of Semi volatile Organic Compounds from Solid Matrices

Soxhlet and Automatic Soxhlet Extraction

- Dry solid sample is placed in a permeable cellulose ‘thimble’
- Extraction solvent in the flask is heated to boiling
- Vapors rise through the outer chamber and into the condenser
- Condense and drip down onto the extracting sample

- Extraction chamber with sample fills until it empties through the siphon arm into the flask below
- Continues until solution in Soxhlet chamber is same color as solvent

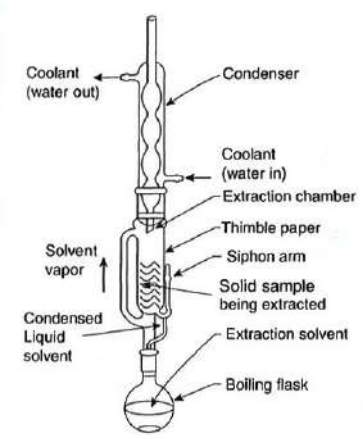
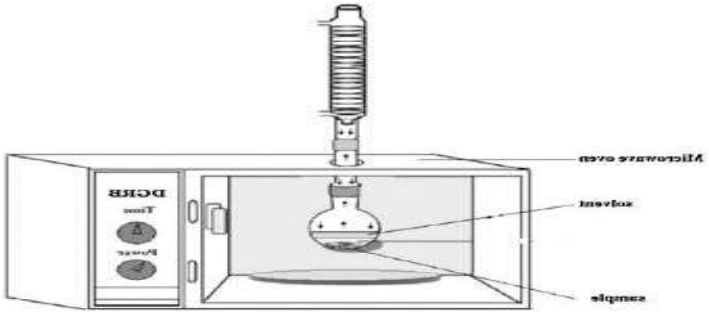
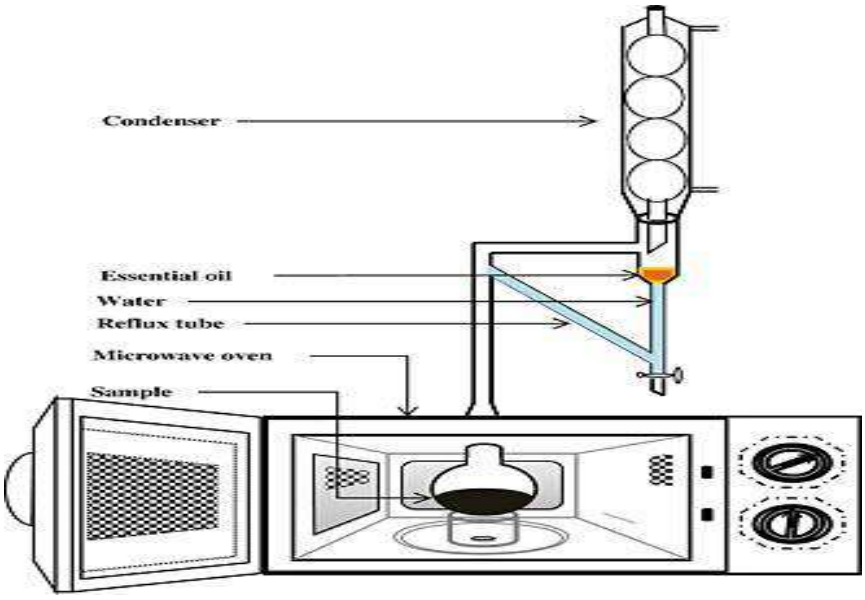


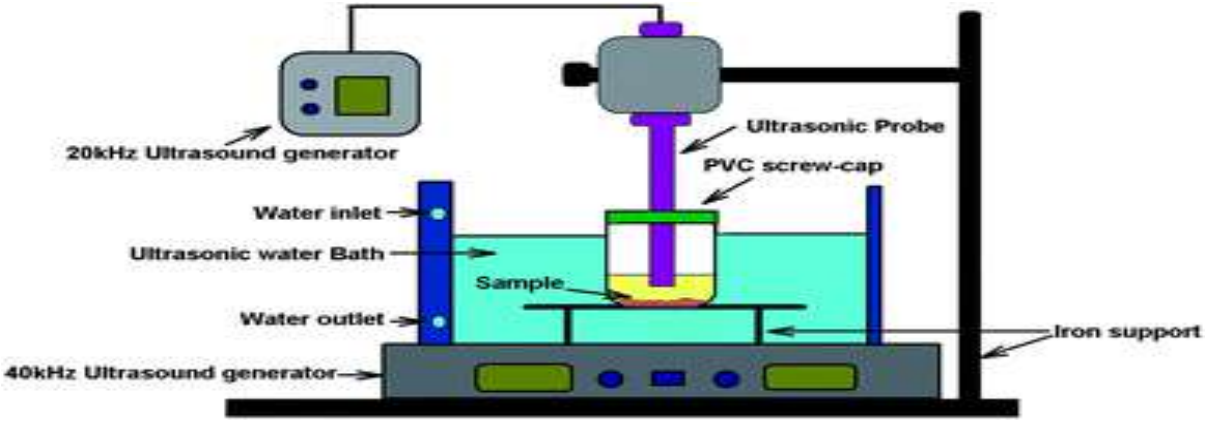
Figure 7.10 A schematic representation of a Soxhlet extractor

Microwave-assisted extraction (MAE)



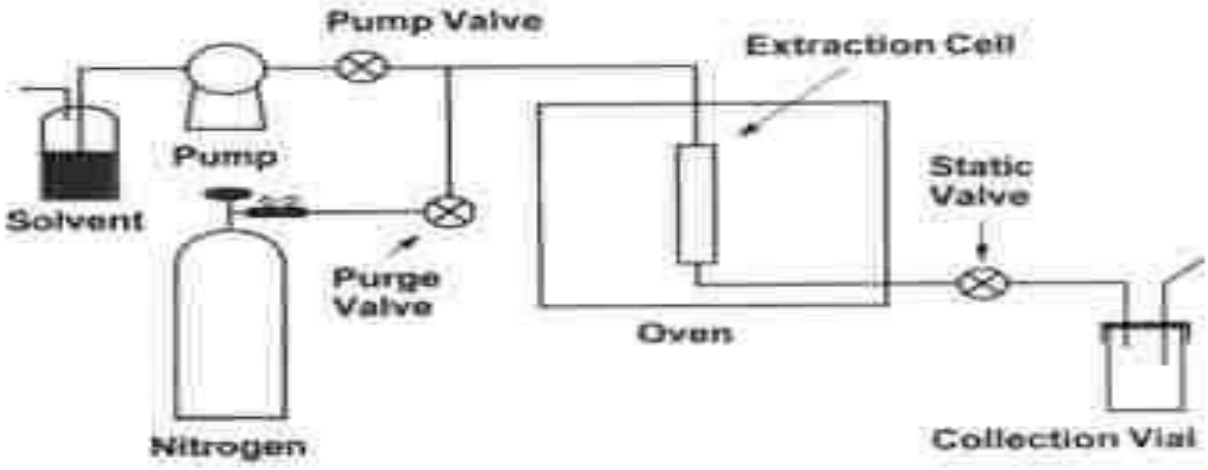


USE- Ultrasonic assistant extraction



Pressurized liquid/fluid extraction

- Also known as accelerated solvent extraction (ASE)
- Uses **elevated temperatures and pressures**
- Uses **less solvent and takes less time than Soxhlet**



Supercritical Fluid Extraction (SFE)

- **Extracting solvent is CO_2** in supercritical fluid state (SCF)
- SCF is defined as a substance above its critical temperature and pressure (highest temp. and pressure at which substance is a vapor and liquid in equilibrium)
- CO2 in SCF state has physical and thermal properties in-between pure liquid and gas forms

- Gas-like high mass transfer coefficient, liquid-like high solvent property
- High diffusivity of SCF allows it to penetrate porous solids
- SCF extractor

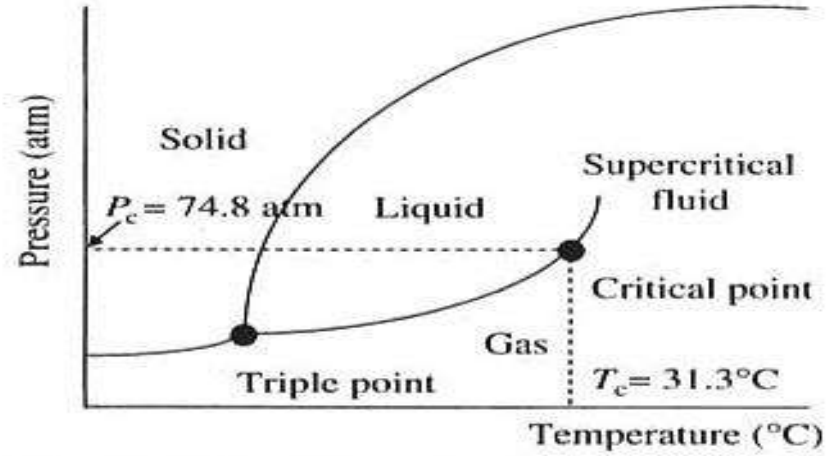


Figure 7.12 Schematic phase diagram of carbon dioxide (CO_2). P_c = critical pressure, T_c = critical temperature

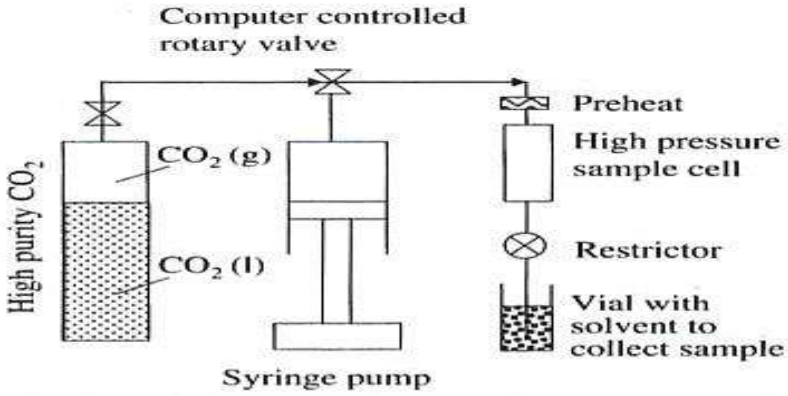


Figure 7.13 Schematic diagram of supercritical fluid extraction (SFE)

Table 7.1 Comparison of sample preparation techniques for SVOCs and VOCs

Extraction method	Application	Cost	Extraction time	Solvent usage	Simplicity	EPA method
Purge & trap	VOCs (L/S)	High	30 min	None	No	5030, 5035
Headspace	VOCs (L/S)	Low	30 min	None	Yes	3810, 5021
LLE	SVOCs, NVOCs (L)	Low	1 h	500 mL	Yes	3510/3520
SPE	SVOCs, NVOCs (L)	Medium	30 min	100 mL	Yes	3535
SPME	VOCs, SVOCs, NVOCs (L)	Low	30 min	None	Yes	None
Soxhlet/ Soxtec	SVOCs, NVOCs (S)	Low	4–24 h	250/50 mL	Yes	3540/3541
Ultrasonic	SVOCs, NVOCs (S)	Medium	10 min	200 mL	Yes	3550
SFE	SVOCs, NVOCs (S/L)	High	30 min	20 mL	No	3560/3561
PFE (ASE)	SVOCs, NVOCs (S)	High	15 min	25 mL	No	3545

LLE: liquid–liquid extraction; SPE: solid phase extraction; SPME: solid phase microextraction; SFE: supercritical fluid extraction; PFE: pressured fluid extraction; ASE: accelerated solvent extraction. VOCs: volatile organic compounds; SVOCs: semivolatile organic compounds; NVOCs: nonvolatile organic compounds. L: liquid samples; S: solid samples.

Derivatization of Non-VOC Gas Phase Analysis

- Many compounds of interest (particularly high MW compounds with polar functional groups) are difficult to analyze
- Derivatization transforms a chemical into a derivative
- Used for the following:

  (a) Increase volatility and decrease polarity
  (b) Increase thermal stability
  (c) Increase detection response
  (d) Improve separation

Derivatization of Non-VOC Gas Phase Analysis

- Common methods:
  - ***Silylation*:** replaces active hydrogens with trimethylsilyl (TMS) group [-Si(CH_3)$_3$]. Silyl derivatives are more volatile and thermally stable
  - ***Acylation***: adds an acyl group (RCO-), converts compounds with active hydrogens into esters, thioesters and amides. Reduces polarity of amino, hydroxyl, and thio groups
  - ***Alkylation*:** reduces polarity by replacing active hydrogens with an alkyl group (e.g. CH_3, $C2H_5$). Acidic hydrogens in carboxylic acids and phenols form esters, ethers, and amide
  - ***Esterification***: acid reacts with alcohol to form an ester with a lower bpt.

Lec 6

Ionic Equilibria

Aqueous solutions contain hydronium ions as well as hydroxide ions:

As we have seen, water is itself ionized to a very slight extent:

$2H_2O \longleftrightarrow H_3O^+ + OH^-$

H_3O^+ in the equation above represents the total hydrogen ion concentration in the solution from all sources, and OH^- represents the total hydroxide ion concentration.

Applying the equilibrium law to this equilibrium at constant temperature:

$$\frac{[H3O+][OH-]}{[H2O]2} = \text{constant (K)}$$

In dilute aqueous solutions the concentration of $[H_2O]$ is greater than the concentration of any other species.

Its concentration is substantially constant (around 55.5 mol/ L), so

$[H3O^+][OH^-] = K[H_2O]^2$

$[H3O+][OH-] = K_w$

Where (K_w) is called the ion product constant for water.

At 25 Cº the numerical value of this constant (1×10^{-14} mol^2/L^2). In pure water on in the presence of a solute that does not react to give H^+ or OH^- , the concentration of these two species are identical, and therefore there concentration must equal the square root of the ion- product constant: that is 1×10^{-7} mol/L at 25 Cº.

A solution in which the H_3O^+ and OH^- concentrations are the same is said to be neutral. The H_3O^+ and OH^- concentration of a neutral solution increase with temp. A solution is said to be acidic when the hydronium ion concentration exceeds that of the hydroxide ions; it is basic when the reverse is the case.

The hydrogen- ion exponent ((pH))

For many purposes, especially when dealing with small concentrations, it is cumbersome التثاقل to express concentrations of hydrogen and hydroxyl ions in terms of gram equivalents per liter. A very convenient method was proposed by Sorensen. He introduced the hydrogen- ion exponent (pH) defined by the relationship:

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

The quantity pH is thus the logarithm of the hydrogen- ion concentration with negative sign. This method has the advantage that all states of acidity and alkalinity between those of solutions molar with respect to hydrogen and hydroxyl ions can be expressed by a series of positive numbers between 1 and 14.

Thus a neutral solution with $[H^+]= 10^{-7}$ has a pH of 7.

$K_w = [H_3O^+][OH^-]$

$-\log K_w = - \log [H_3O^+] + (- \log [OH^-])$

$PK_w = pH + pOH = 14$

I----------I----------I

1 ← acid ↑ **7** → alkaline → 14

Neutral

Ex. Calculate the pH and pOH of a solution in which the H_3O^+ is $2\times10^{3-}$ M?

$pH = - \log [H_3O^+]$

$= - \log 2 \times 10^{3-}$

$= 2.7$

pOH= 14-2.7 = 11.3

Ex. Calculate the pH of 0.01 M solution of acetic acid ((the degree of dissociation is 12.5 %))

Dissociation %=$\frac{amount\ dissociated}{Initial\ concentration}$X100

[H_3O^+]= 12.5/100 × 0.01= 1.25 ×10^{3-}

PH= - log [H_3O^+]

= - log 1.25 × 10^{3-} = 2.903

Solutions of strong acids and strong bases bronsted- Lowry definition:

- A substance functions as an acid when it donated proton to a base.
- A substance functions as a base when it accepts proton from an acid.

Acids are proton donors, Bases are proton acceptors

These definitions imply acids and bases always react together.

The acid which has exactly one proton more than a particular base is called the **conjugate acid** of that base. Likewise, the base which has exactly one proton less than a particular acid is called the **conjugate base** of that acid Such as acid – base reactions:

a) In an aqueous solution of HCl functions as an acid, and water functions as a base:

$HCl + H_2O \longleftrightarrow H_3O^+ + Cl^-$

Acid Base conjugate Acid conjugate Base

The conjugate acid – base pairs are HCl/Cl^- and H_3O^+/H_2O

b) In an ammonia solution of NH_3 functions as a base and water functions as an acid:

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

Base Acid conjugate Acid conjugate Base

The conjugate acid – base pairs are NH_4^+ / NH_3 and H_2O / OH-

From reactions (a) and (b) we notice that water can function both as an acid and as a base. Such a substances are described as amphoteric.

$H_2O + H_2O \longleftrightarrow H_3O^+ + OH^-$

Acid Base conjugate Acid conjugate Base

Strength of acids and bases

Different acids hydrolyze in water to differing extents. The greater the extent of hydrolysis, the stronger is the acid or base.

Ex.: HCl, $HClO_4$, H_2SO_4, HNO_3 are stronger acids.

HCN, CH_3COOH, $H_2PO_4^-$ are weak acids.

(NaOH , KOH) ~~Strong~~ bases

(NH_3 , CN^-) ~~Weak~~ bases

The acidity constant may be used to compare acid strength's, so also one may define basicity constant for a base.

For example: $CH_3COOH = 10^{5-}$ (weak)

$HCl = 10^7$ (strong)

Calculation of pH or pOH of aqueous solution of strong acids or bases is straight forward, since the H_3O^+ or OH^- concentration can be calculated directly from the formal concentration of the solute. In such calculation the fraction of H_3O^+ and OH^- resulting from the dissociation of water is ordinarily vanishingly small and is not taken into account.

Ex. Calculate the pH and PoH of 0.05 M solution of HCl?

Since the acid is completely dissociated, the $[H_3O^+]$ is numerically equal to the molar concentration of HCl in the solution.

$[H_3O^+] = 0.05$

$pH = - \log [H_3O^+]$

$= - \log 0.05$

$= 1.3$

$pOH = 14 - 1.3$

$= 12.7$

Ex: Calculate the pH and pOH of a $3.2\times 10^{4-}$ M $Ba(OH)_2$ solution?

$Ba(OH)_2$ is stronger base containing 2 moles of OH^- for each formula weight of base.

Thus: $Ba(OH)_2 \longrightarrow Ba^{2+} + 2OH^-$

$[OH^-]= 2 \times 3.2\times 10^{4-}$

$= 6.4 \times 10^{4-}$ mol/l

$pOH = -\log [OH^-]$

$= -\log (6.4\times 10^{4-}) = 3.19$

$pH = 14-3.19$

$= 10.81$

Calculation of pH solution of a weak acids and bases

Weak acids and bases react incompletely with the solvent. As a consequence, the H_3O^+ or OH- concentration in such solutions will be less than the formal solute concentration.

Calculation of pH or pOH requires a numerical value for the equilibrium constant for the reaction of the substance with water.

When the weak acid HA is dissolved in water, the reaction:

$HA + H_2O \longleftrightarrow H_3O^+ + A^-$

Or $HA \longleftrightarrow H^+ + A^-$

is incomplete and the resulting equilibrium is described by the equation:

$K_a = \frac{[H+][A-]}{[HA]}$

Where K_a is the ionization constant or dissociation constant of the acid.

$[H^+] \rightleftharpoons [A^-]$

$K_a = \frac{[H+]2}{[HA]}$

$[H^+]^2 = K_a [\ HA]$

$[H^+] = \sqrt{Ka\ [HA]}$

Or $[H^+]= \sqrt{Ka \times Ca}$ Which C_a = concentration of acid

$pH= -\log H^+$

for bases : $B + H_2O \longleftrightarrow BH^+ + OH^-$

$K_b = \frac{[BH+][OH-]}{[B]}$

$[BH^+] = [OH^-]$

$K_b = \frac{[OH-]2}{[B]}$

$[OH^-]^2 = K_b[B]$

$[OH^-] = \sqrt{Kb\ [B]}$

Or $[OH^-] = \sqrt{Kb \times Cb}$ which C_b = concentration of base

$pOH = - \log [OH^-]$

Ex:

Calculate the hydronium ion concentration and pH of a $4\times 10^{2-}$M solution of formic acid, $K_a = 1.74\times 10^{4-}$?

$[H^+] = \sqrt{Ka \times Ca}$

$= \sqrt{\ } 1.74 \times 10^{4-} \times 4 \times 10^{2-}$

$= 2.64 \times 10^{3-}$ mol/L

$pH = - \log H^+$

$= - \log 2.64 \times 10^{3-} = ??$

Ex:

Calculate the pH of 0.075 m solution of NH_3 , $K_b = 1.86 \times 10^{5-}$

$[OH^-] = \sqrt{Kb \times Cb}$

$= \sqrt{\ } 1.86\times 10^{5-} \times 0.075$

$= 1.18\times 10^{3-}$ mol/L

$pOH = - \log [OH^-]$

$= - \log 1.18 \times 10^{3-}$

$= 2.93$

$PH = 14 - 2.93 = 11.07$

Relation between Ka and Kb :

Consider the hydrolysis reactions for the acid HA, and its conjugate base

A^- :

$K_a \longrightarrow HA + H_2O \quad H_3O^+ + A^-$

$K_b \longrightarrow A^- + H_2O \quad HA + OH^-$

The acidity and basicity constant are defined as:

$K_{a(HA)} = \frac{[H3O+][A-]}{[HA]}$

$K_{b(A)} = \frac{[HA][OH-]}{[A-]}$

Now the product:

$K_a \times K_b = \frac{[H3O+][A-]}{[HA]} \times \frac{[HA][OH-]}{[A-]}$

$K_a \times K_b = [H_3O^+][OH^-]$

Thus $K_a \times K_b = K_w$

Ex: Find the $K_b(NH_3)$ at 25 Cº, given $K_a(NH_4^+) = 6.3 \times 10^{10-}$

Since $K_b = K_w / K_a$

$K_b = 10^{14-} / (6.3 \times 10^{10-})$

$= 1.6 \times 10^{5-}$

Ex: Find the p $K_b(NS^-)$ at 25 Cº, given $pK_a(H_2S)=7.2$

Since $K_a \times K_b = K_w$

$pK_a + pK_b = pK_w = 14$

$pK_b = 14-7.2 = 6.8$

Lec 7

Hydrolysis:

Is the interaction between the ions of salt and the ions of water with the production of a weak acid or a weak base or of both a weak acid and a weak base.

Calculation of the pH of solutions of salts

Salts may be divided into four main groups:

1- Those derived from the strong acids and strong bases, e.g., (KCl)
2- Those derived from the weak acids and strong bases, e.g., (CH_3COONa).
3- Those derived from the strong acids and weak bases, e.g.,(NH_4Cl)
4- Those derived from the weak acids and weak bases, e.g.,(CH_3COONH_4).

When any of these is dissolved in water, the resulting solution may be neutral, acid, or alkaline according to nature of the salt.

Calculations:

1- Salts of strong acid and base:

These salts in aqueous solutions, the anions do not have any tendency to combine with the $[H^+]$ also the cations with the hydroxyl ions of water, since the related acids and bases are strong electrolytes. The equilibrium between H^+ and OH^- ions in water:

$H_2O \longleftrightarrow H^+ + OH^-$

$[H^+]= [OH^-] \longrightarrow pH= 7$

The solutions remain neutral.

2- Salts of weak acid- strong base:

The essential chemistry of the solubi/lization of the completely ionic solid sodium acetate CH_3COONa, in water and which endows its solution with a basic character (pH greater than 7) is formulated as:

$CH_3COONa \longrightarrow CH_3COO^- + Na^+$

$CH_3COO^- + H_2O \longleftrightarrow CH_3COOH + OH^-$

$K_h = K_b = \frac{[OH-][CH3COOH]}{[CH3COO-]}$

$K_b \times K_a = K_w$

$K_h \times K_a = K_w$

The end of the derivation yields:

$H^+ = \sqrt{\frac{kw\ ka}{[Salt]}}$

$PH = \frac{1}{2}[\ pK_w + pK_a + \log C]$

Which C= salt concentration.

3- Salts of weak base- strong acid:

Like ammonium chloride, its aqueous solution is acidic because of hydrolysis:

$NH_4Cl \longleftrightarrow NH_4^+ + Cl^-$

$NH_4^+ + H_2O \longleftrightarrow NH_3 + H_3O^+ \text{ or } H^+$

$K_h = \frac{[NH3][H+]}{[NH4+]}$

When the derivation is completed, we found this equation:

$H^+ = \sqrt{\frac{Kw[\ salt]}{Kb}}$

$pH = \frac{1}{2}[pK_w - pK_b - \log C]$

4-Salt of weak base- weak acid:

As an example (ammonium acetate).

Whether the final solution at equilibrium will be acidic, basic or neutral will obviously depend upon the relative strengths of the acid and base formed- that is, upon the respective values of K_a and K_b. When these are nearly identical- as in the present instant.($K_b(NH_3) = K_a\ (CH_3COOH) = 1.8 \times 10^{5-}$ at 25 C°).

We obtain a neutral solution pH = 7. When $K_a > K_b$ the solution will be

acidic; When $K_b > K_a$ the solution will be basic.

The equation of salt derived from weak acid and base is:

$pH = \frac{1}{2}[pK_w + pK_a - pK_b]$

Ex:

Calculate:

1- pH of 0.01 M solution of sodium acetate, $K_a = 1.82 \times 10^{5-}$

2- pH of 0.02 M solution of NH_4Cl, $K_b = 1.85 \times 10^{5-}$

3- pH of solution of ammonium format, $K_b(NH_3) = 1.8 \times 10^{5-}$,
$K_a(HCOOH) = 1.77 \times 10^{4-}$

Sol.

1- $pH = \frac{1}{2}[pK_w + pK_a + \log C]$ $pK_a = - \log K_a$

$= \frac{1}{2}[13 + (- \log 1.82 \times 10^{5-}) + \log 0.01]$

$= 8.37$

2- $pH = \frac{1}{2}[pK_w - pK_b - \log C]$ $pK_b = - \log K_b$ $=- \log 1.8 \times 10^{5-}$

$pH = \frac{1}{2}[14 - 4.74 - \log 0.02]$

$= 4.98$

3- $pH = \frac{1}{2}[pK_w - pK_b - pK_b]$

$= \frac{1}{2}[14 + 3.76 - 4.74]$

$= 6.51$

ملاحظه عامة: اذا كان التركيز بوحدات غم/ لتر, في هذه الحالة نقسم على الوزن الجزيئي ليكون بوحدات مول/ لترفمثلا اذا كان تركيز الملح = 0.6 غم / لتر فيجب ان نقسم على الوزن الجزيئي فيكون التركيز بوحدات مول / لتر.

$\frac{0.6\ gm/L}{M.wt(\frac{gm}{mol})}$ = mol/L/ or molarity

Buffer solutions:

Buffer solutions usually consist of solutions containing a mixture of weak

acid or base and its salt. Buffers are of great importance in chemistry because they have the property of resistance changes in pH both upon dilution and upon addition of strong acid and base. In order to understand buffer action, let us study first the equilibrium between a weak acid and its salt. The dissociation of a weak acid is given by:

$HA \longleftrightarrow H^+ + A^-$

$K_a = \frac{[H+][A-]}{[HA]}$

$[H^+] = \frac{Ka[HA]}{[A-]}$

$[H^+] = K_a(\frac{HA}{A-})$

Or $[H^+] = K_a(\frac{acid}{salt})$

$-\log H^+ = -\log K_a - \log(\frac{acid}{salt})$

$pH = pK_a + \log \frac{[salt]}{[acid]}$

Similarly for a mixture of a weak base of dissociation constant K_b and its salt:

$pOH = pK_b + \log \frac{[salt]}{[base]}$

How can buffer solution resist the changes in pH upon addition of strong acids or bases?

Let us take an acid buffer (CH_3COOH+ CH_3COONa)

or $\left.\begin{matrix} CH_3COOH \\ \\ CH_3COONa \end{matrix}\right\} \rightarrow$ $+HCl(H^+) \Leftarrow CH_3COOH$ weak acid(pH const.)

$+ NaOH(OH^-) \Leftarrow H_2O$ neutral (pH= 7).

If we add a small concentration of H^+ to such a solution, it will combine with the acetate ions from the salt to form un dissociated acetic acid.

Similarly, if a small concentration of hydroxyl ions be added, the latter

will combine with the hydrogen ions arising from the dissociation of the acetic acid and form unionized water; the equilibrium will be disturbed, and more acetic acid will dissociated to replace the hydrogen ions removed in this way.

Example 1:

Calculate the pH value of a liter of an aqueous solution concentration 6 gm of CH_3COOH (M.wt = 60.05) and 8.2 gm of CH_3COONa (M.wt = 82.05), $K_a = 1.8 \times 10^{5-}$.

Sol:

$pK_a = - \log K_a$

$= - \log 1.8 \times 10^{5-}$

$= 4.74$

$pH = pK_a + \log \frac{[salt]}{[acid]}$

$= 4.74 + \log \frac{[\frac{8.2}{82.05}]}{[6/60.05]}$

$= 4.74 + \log 0.1/0.1$

$= 4.74$

Example 2 :

To 50 ml of 0.1 N acetic acid. 10 ml of 0.2 N NaOH solutions have been added. Calculate the pH of the resulting solution, $K_a = 1.8 \times 10^{5-}$

Sol:

Before reaction:

(V×N)

(50 ml) × 0.1 g-meq./ ml)= 5 g- meq of CH_3COOH

(10 ml) ×0.2 g-meq./ ml)= 2g-meq. Of NaOH

After reaction

$CH_3COOH + NaOH \rightarrow CH3COONa + H_2O$

5g-meq 2g-mg 0 0

3g-meq 0 2g-meq

As a result of reaction between the acid and the base , 2g-meq. Of sodium acetate will be formed and an access of 3g-meq. of acetic acid will remain in the solution.

The resulting solution is a buffer since it contains a weak acid and its salt. (CH3COOH, CH3COONa).

The normality of CH3COOH after reaction, is no longer 0.1 since part of it has been converted to sodium acetate and also the volume has been increased to 60 ml.

$C_{(acid)}$ =3g-meq/60ml=0.05 g-meq/ml or 0.05N=0.05M

$C_{(salt)}$=2g-meq/60ml=0.033 g-meq/ml 0r 0.033N=0.033M

$pH=pK_a + \log \frac{Salt}{acid}$

$pH=4.74 + \log \frac{0.033}{0.05}$ $pK_a = -\log 1.8x10^{5-}$

$=4.74$

$=4.57$

Buffer Capacity: Is defined as the number of mmols of any acid or base that causes one milliliter of the buffer to undergo a one unit change in pH. The capacity of a buffer depends not only on the total concentration of the two buffer components but also on their concentration ratio.

Capacity of buffer solution when added strong acid is the number of millimoles of strong acid added to one milliliter of buffer solution to decrease the value of pH one unit.

Capacity of buffer solution when added strong base(beta value) is the number of millimoles of strong base added to one milliliter of buffer solution to increase the value of pH one unit.

Ex: Calculate: **a)** pH, **b**) capacity to acid added to solution contains 12 mmol of acetic acid (pK_a = 4.74) and 8 mmol of sodium acetate in 100 ml.

Sol: **a)** pH= pK_a + log C_s/C_a ⟹ pH= 4.74+ log $\frac{8/100}{\frac{12}{100}}$ ⟹ pH= 4.56

b)pH= 4.56- 1= 3.56

pH= pK_a+ log C_s/C_a ⟹ 3.56= 4.74+logC_s/C_a ⟹ - log C_s/C_a= 1.18

C_a/C_s= 15.1 ⟹ C_a =15.1 C_s

No. of mmol of total= No. mmol of acid + No. of mmol of salt

8+12 =(15.1) No. of mmol of salt + No. mmol of salt

20 = (15.1 +1) No.of mmol of salt

No. of mmol of salt= 20/16.1= 1.24= No. mmol of salt remained

No. mmol of acid added= No. mmol of salt- No.mmol of salt remained

No.mmol of acid added= 8- 1.24= 6.8

Capacity= $\frac{No.mmol\ of\ acid\ added}{Total\ volume\ of\ buffer\ solution}$ = 6.8/100

Lec 8

Chromatography

Chromatography is an analytical method that is widely used for separation, identification and determination of the chemical components in Complex mixtures.

Chromatography is the separation of the components of a mixture based on the different degrees to which they interact with two separate material phases.

One of the two phases is a moving phase (the mobile phase), while the other does not move (the stationary phase). The mobile phase can be either a gas or a liquid, while the stationary phase can be either a liquid or solid.

Chromatography is a physical method of separation in which the components to be separated are distributed between two phases.

Mobile phase (MP) is a liquid or gas that carries analyses through a liquid or solid stationary phase.

Stationary phase (SP) is a solid or immobilized liquid in chromatography upon which analytic species are partitioned during

passage of a mobile phase.
The two phases are hosen so that the components of the sample distribute mobile and stationary phases to varying degrees.
1- Components more strongly retained by the SP, move slowly with flow of MP.
2- Components weakly held by SP move rapidly.
3- UV detectors used or any suitable detector.
4- Separation based on affinity of SP with the sample.

Purpose of chromatography

1- **Analytical**-determine chemical composition of Sample (qualitative and quantitative).
2- **Preparative**-purify and collect one or more components of Sample.

Basic principles

All chromatographic methods require one static part (the stationary phase) and one moving part (the mobile phase). The techniques rely on one of the following phenomena*: adsorption; partition; ion exchange; or molecular exclusion.*

1. Adsorption

It has a Solid Stationary phase and a liquid or gaseous mobile phase. The different solutes (analytes) travelled different distances through the Solid, carried along by the Solvent Each solute has its own equilibrium between adsorption onto the Surface of the solid and Solubility in the Solvent, the least Soluble or best adsorbed ones travel more slowly. The result is a separation into bands containing different Solutes. Liquid chromatography using a Column containing silica gel or alumina is an example of adsorption chromatography (Fig.1). The solvent that is put into a column is called the eluent, land the liquid that flows out of the end of the column is called the eluate.

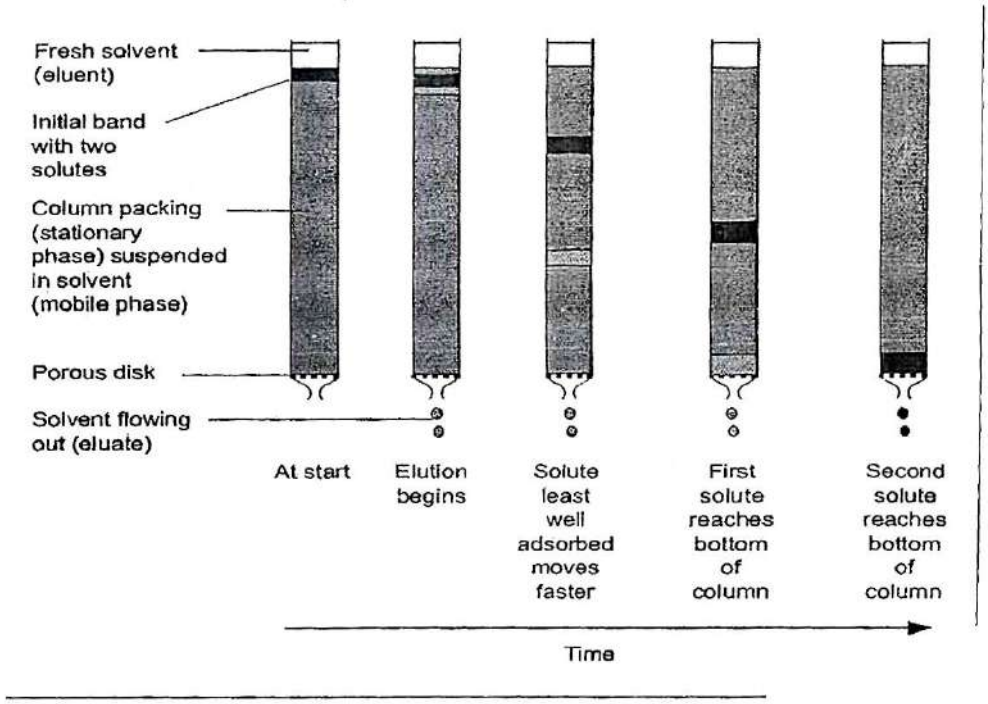


Figure 1 Adsorption chromatography using a column

2. Partition

In partition chromatography the stationary phase is a non-volatile-liquid which is held as a thin layer or film on the surface of an inert solid. The mixture to be separated is carried by a gas or a liquid as the mobile phase. The solutes distribute themselves between the moving and the Stationary phases, with the more soluble component in the mobile phase reaching the end of the chromatography Column first (Fig. 2). Paper chromatography is an example of partition chromatography.

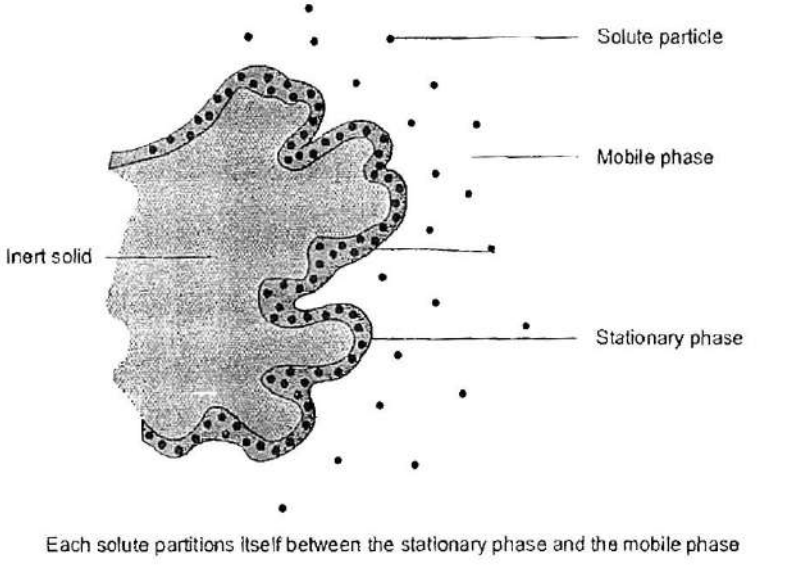


Figure 2 Partition chromatography

3-Ion exchange

Ion exchange chromatography is Similar to partition chromatography in that it has a coated Solid as the stationary phase) The coating is referred to as a resin, and has ions (either catians or anions, depending on the resin) covalently bonded to it land ions of the opposite charge are electrostatically bound to the surface When the mobile phase (always a liquid) is eluted through the resin the electrostatically bound ions are released as other ions are bonded preferentially (Fig. 3).

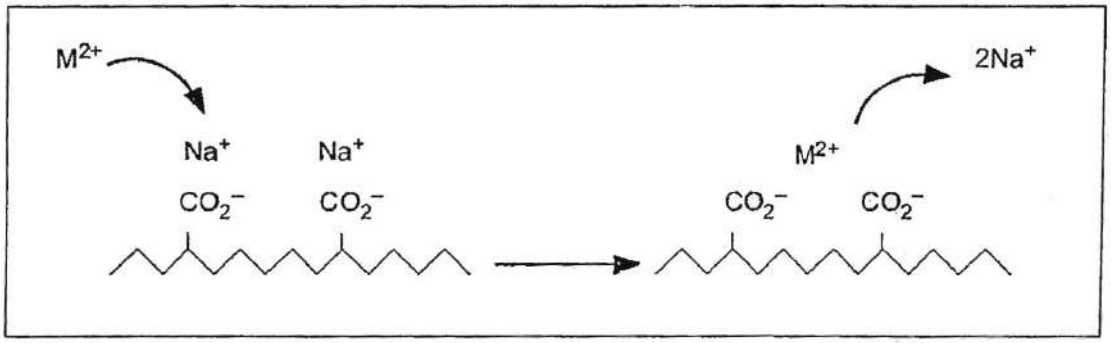


Figure 3 Ion exchange chromatography

4. Molecular exclusion - Size exclusion chromatography is a type of column chromatography. It has a little modification in that the column is filled or packed with a stationary phase which can act as molecular sieve. A porous material is used as stationary phase while a liquid as mobile phase.

Classification of chromatography methods is based on the nature of the two phases:

1. Gas-Liquid Chromatography (GLC)
The mobile phase is a gas and Stationary phase is a liquid.
2. Gas-Solid Chromatography (GSC)
The mobile phase is a gas and Stationary phase is a solid.
3. Liquid- Liquid Chromatography (LLC)
The mobile phase is a liquid and Stationary phase is a liquid.
4. Liquid-Solid Chromatography (LSC)
The mobile phase is a liquid and stationary phase is a Solid.

Chromatographic techniques

1. Paper chromatography

This is probably the first, and the simplest, type of chromatography. A drop of a Solution of a mixture of dyes or inks is placed on a piece of chromatography paper (or Filter paper) and allowed to dry. Paper chromatography works by the partition of Solutes between water in the paper fibers (stationary phase) and the Solvent (mobile phase) common solvents that are used include pentane, propanone and ethanol, in addition

a mixturees of solvents are also used. As each solute distributes itself (equilibrates) between the stationary and the mobile phase. A very important factor called the retardation factor or the retention ratio, and is given the symbol R or R_f is calculated:

$$\text{Retention ratio} = \frac{\text{distance moved by solute}}{\text{distance moved by solvent}} = R_f$$

So a component can be identified from its retention ratio (Fig. 4).

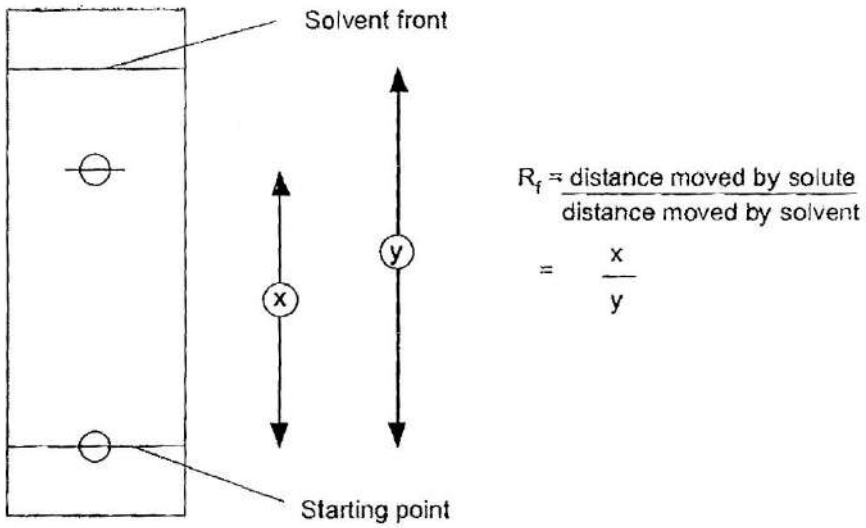


Fig. 4: Paper chromatography

2-Thin laver chromatography (TLC)

TLC is a type of planar chromatography. Principle of TLC is similar to other chromatographic methods:

1. The separation depends on the relative affinity of compounds towards stationary and the mobile phase.
2. TLC is similar to paper chromatography, but the stationary phase is a thin layer of a solid such as alumina of silica supported on an inert base such as glass, aluminum foil or insoluble plastic.
3. The mixture is spotted' at the bottom of the TLC plate and allowed to dry. The plate is placed in a closed vessel Containing solvent (the mobile phase) so that the liquid level is below the spot.
4. TLC has advantages over paper chromatography in that its results are more reproducible, and that separations are very efficient.
5. This technique is usually done in a close vessel to ensure that the

atmosphere is saturated with solvent vapour and that evaporation from the plate is minimised before the run is complete)
6. The plate is removed when the solvent front approaches the top of the plate and the position of the solvent front recorded before it is dried (this allows the R_f value to be calculated).

3-Liquid chromatography (LC)

Liquid chromatography uses a liquid as mobile phase. The stationary phase is usually an inert solid such as silica gel ($SiO_2.XH_2O$), alumina ($Al_2O_2.XH_2O$) or Cellulose Supported in a glass column.
1. Silica is slightly acidic, and readily adsorbs basic Solutes.
2. Alumina is slightly basic and strongly adsorbs acidic solutes.
3. Other stationary phases that can be used include magnesia, $MgO.xH_2O$ (Good for separating un Saturated Organic compounds).
4. Dextran (a polymer of glucose) Cross-linked with propan-1, 2, 3-triel (Glycerol, $CH_2OHCHOHCH_2OH$), which is sold as Sephadex and can separate Compounds Such as purines.
A wide range of Solvents are used in this technique. A more elaborate variation on liquid chromatography is high performance liquid chromatography (HPLC).

High- performance Liquid Chromatography (HPLC)

HPLC is a form of liquid Chromatography to Separate Compounds that are dissolved in Solution. The efficiency of a separation increases if the particles in the stationary phase are made Smaller. This is because the Solute can equilibrate more rapidly between the two phases.
Consequently, high pressure has to be applied to the solvent to force it through the Column, therefore:
HPLC instruments consist of

- A reservoir of mobile phase.
- A pump.
- An injector.
- A separation Column.
- A detector.

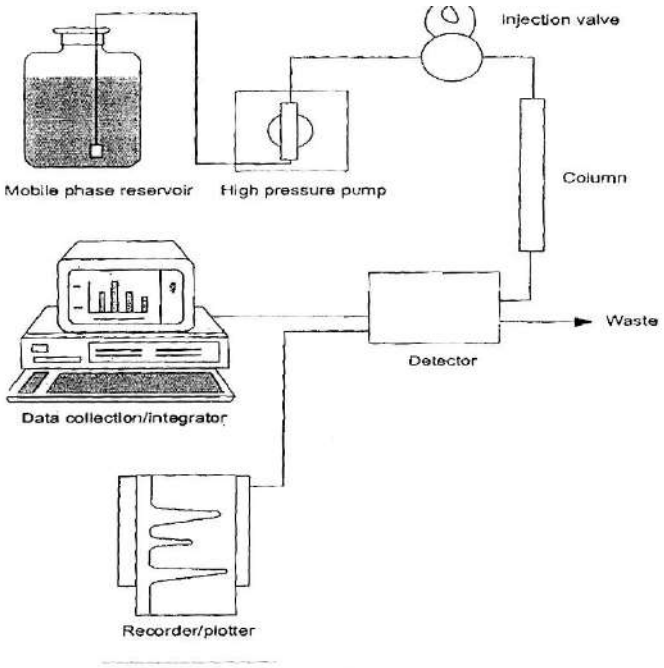


Fig.5: HPLC

Principles of HPLC

1-HPLC (Fig.5) is column chromatography in which the stationary phase is made up of small particles and the mobile phase is forced through the particles by high pressure.

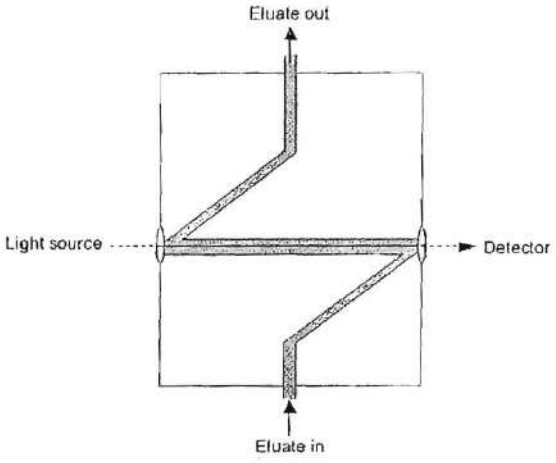
2. The stationary phase normally consists of uniform porous silica particles of diameter of $10^{-6} – 10^{-9}$m. The surface pores having a diameter of 10^{-8} 10^{-9}. (This gives the solid a very high surface area). The stationary phase particles are packed into the HPLC column.

3. Columns are typical 10-30 cm long, with an internal diameter of 4 mm with applied pressures u to $10MNm^{-2}$ .The high pressures involved mean that the instrumentation has to be very strong, and the plumbing is usually constructed from stainless steel.

4. Sample volumes are small $5–20mm^3$ is usually sufficient.

5. The passage of solutes through HPLC is achieved by changing the composition of the mobile phase

6. The Solutes in solution are analysed as they leave the column. Most compounds separated by HPLC absorb ultraviolet light. The eluate is passed along a small cell so that ultraviolet radiation can be passed through the liquid.



A micro cell for identifying solutes in the liquid leaving an HPLC column

Normal Phase vs revers Phase

If the stationary phase is more polar-than the mobile phase, the separation is deemed normal phase. If the stationary phase is less polar than the mobile phase, the separation is reverse phase.

What is HPLC used for?

Separation and analysis of chemical and biological compounds that are non- volatile.

Typical non-volatile compounds are:

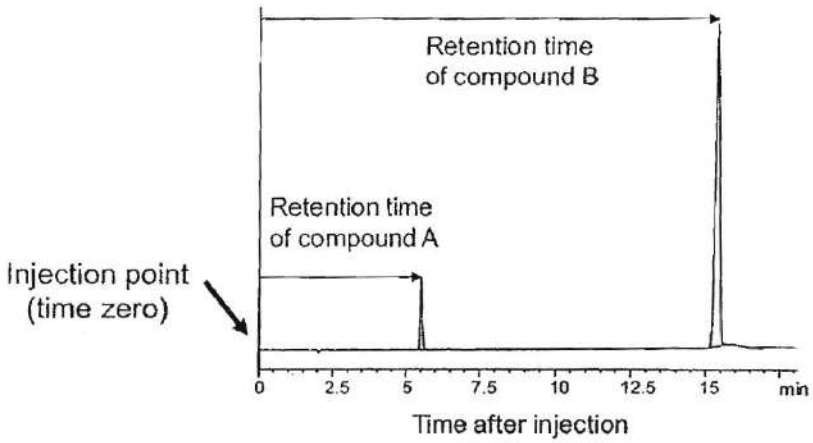
- Pharmaceuticals like aspirin, ibuprofen, or acetaminophen.
- Salts like sodium chloride and potassium phosphate
- Proteins like egg white or blood protein.
- Organic chemicals like polymers (e.g. polystyrene, polyethylene)
- Heavy hydrocarbons like asphalt or motor oil.
- Many natural products such as herbal medicines, plant extracts
- Thermally unstable compounds such as trinitrotoluene (TNT), enzymes

What is HPLC used for?

The identification (ID) of individual compounds in the sample;

• The most common parameter for compound ID is its retention time (the time it takes for that specific compound to elute from the Column after injection);

• depending on the detector used, compound ID is also based on the chemical structure, molecular weight or some other molecular parameter.

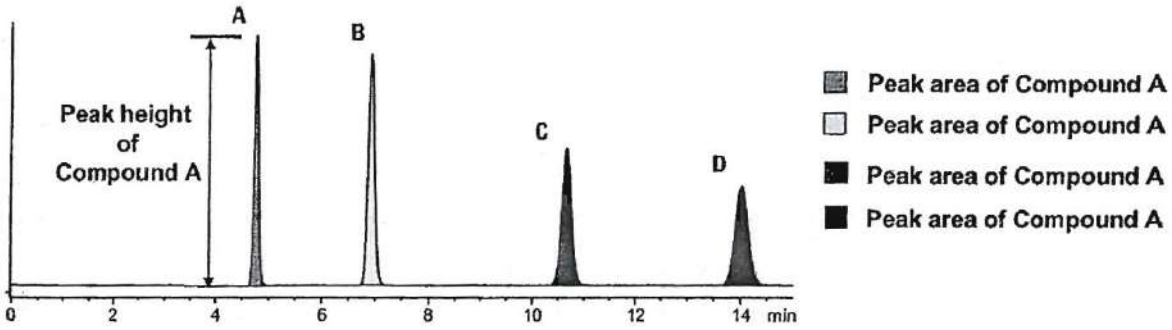


2-Quantitative Analysis

The measurement of the amount of a compound in a sample (concentration); meaning, how. Much is there?

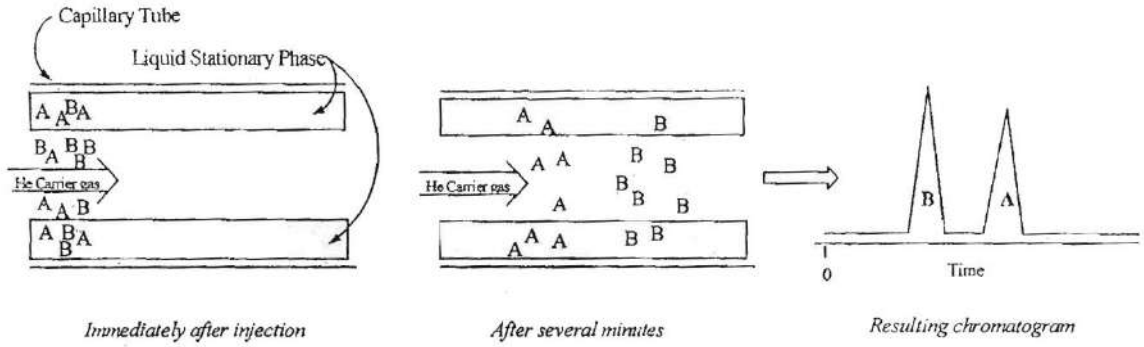
There are two main ways to interpret a chromatogram (i.e. perform quantification):

1. Determination of the peak height of a chromatographic peak as measured from the baseline:
2. Determination of the peak area (see figure below): in many cases, there is a linear relationship between the height or area and the amount of sample.



4- Gas Chromatography

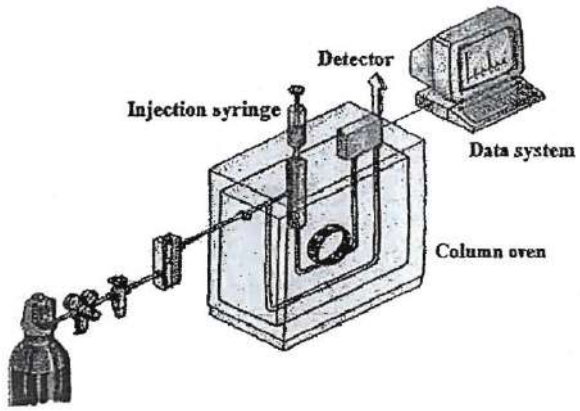
Most gas chromatography procedures utilize a liquid stationary phase, or **Gas chromatography** is a chromatographic technique that can be used to separate volatile organic Compounds.



A gas chromatography consists of a flowing mobile phase, an injection port, a separation column containing the stationary phase, and a detector. The organic compounds are separated due to differences in their partitioning behavior between the mobile gas phase and the stationary phase in the column.

GC instruments consist of:

- Flowing mobile phas
- Injection port
- Separation column (the stationary phase)
- Oven
- Detector



1-Mobile phases are generally inert gases such as helium, argon, or nitrogen.

2- The injection port consists of a rubber Septum through which a syringe needle is inserted the sample. The injection port is maintained at a higher temperature than the boiling point of the least volatile component in the sample mixture. Since the partitioning behavior is dependent on temperature.

3-The separation Colum is usually contained in **a thermostat-controlled oven.** Separating components with a wide range of boiling points is accomplished by starting at a low oven temperature and increasing the temperature over time to elute the high-boiling point components. Most Columns contain a liquid stationary phase on a Solid support

4- Detector is designed to generate an electronic signal when a gas other than the carrier gas elutes from the column.

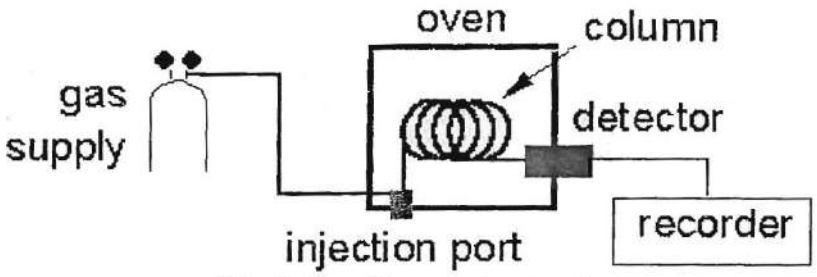


Fig.4: Gas Chromatography

GC Columns

GC columns are of two designs:

1- Packed columns are typically lass on stainless steel coil (typically 1-5 m total length and 5 mm inner diameter) that is filled with the stationary phase.

2- Capillary columns are a thin d-silica (purified silicate glass) capillary (typically 10-100 m total length and 250 m inner diameters) that has the stationary phase coated packed columns but are more easily overloaded by too much sample. on the inner surface. Capillary columns provide much higher separation efficiency than packed columns but are more easily overloaded by too much sample.

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Instrumental Methods

Spectrochemical Methods

Spectrochemical methods have provided the most widely used tools for the explanation the structure of molecular species-as well as the quantitative and qualitative determination of both organic and inorganic compounds.

Spectroscopy referred to a branch of science in which light (that is, visible radiation) was resolved into its components wave lengths to produce spectra.

Spectroscopy use of absorption, emission or scattering of electromagnetic radiation by atoms or molecules to qualitative or quantitative study the atoms or molecules or to study physical process.)

There are five types of analytical spectroscopy:

- Absorbance
- Fluorescence and Phosphorescence
- Emission (atomic with flames, res, sparks)
- Chemiluminescence and Bioluminescence
- Scattering.

Optical instruments are Spectroscopic devices that employ ultraviolet, visible and infrared radiation most of these instruments are made up of five components:

1. A stable source of radiant energy.
2. A wavelength selector that permits the isolation of a restricted wavelength reign.
3. One or more sample containers.
4. A radiation detector
5. A radiation detector
6. A signal processor and readout

Energy states of chemical species

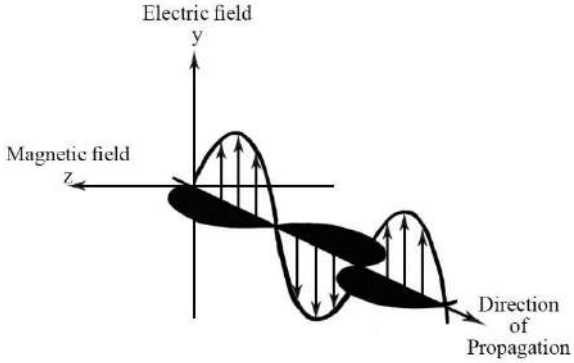
The interaction of radiation with matter can cause redirection of the radiation and/or transitions between the energy levels of the atoms or molecules

A transition from a lower energy level to a higher energy level with transfer of energy from the radiation field to the atom or molecule is called absorption.

A transition from a higher energy level to a lower energy level is called emission if energy is transferred to the radiation field or nonradiative decay if no radiation is emitted. Redirection of light due to its interaction with matter is called scatterin and may not occur with transfer of energy, i.e., the scattered radiation has a slightly different or the same wavelength.

Electromagnetic radiation (EM radiation or EMR)

EMR is a form of energy emitted and absorbed by charged particles which exhibits wave-like behavior as it travels through space. EMR has both electric and 1 magnetic field components, which oscillate in phase perpendicular to each other and perpendicular to the direction of energy and wave propagation.



EMR is made up of packets of energy called photons (or quanta).The energy of a photon depends upon the frequency of the radiation and is given by

$$E = h\nu = \frac{hc}{\lambda}$$

E = energy of the photon (ergs)
h =Planck's constant =6.626×10^{-34} j s
v = frequency (s^{-1} or Hz)
c=velocity of light (c = 3×10^{10}cm s^{-1})
λ = wavelength (cm).
Working ranges of the UV-Vis spectra, including: (UV, 200-380 nm and Vis, 380-780 nm)

Light is a form of electromagnetic radiation. When it falls on a substance, three things can happen:
• The light can be reflected by the substance
• It can be absorbed by the substance
• Certain wavelengths can be absorbed and the remainder transmitted or reflected.

Absorption Spectrometry

Absorption spectrometry is based on the absorption of photons by the analyte. The beam of light consists of a stream of photons, when a photon encounters an analyte molecule (the analyte is the molecule being studied), the analyte will absorb the photon. This absorption reduces the number of photons in the beam of light, thereby reducing the intensity of the light beam.

A spectrophotometer is employed to measure the amount of light that a sample absorbs. The instrument operates by passing a beam of light through a sample and measuring the intensity of light reaching a detector.
Absorption spectrometry, including:

- Molecular absorption spectrometry.
- Atomic absorption spectrometry.

An important principle of spectrophotometry is that "every substance absorbs or transmits certain wavelengths of radiant energy but not other wavelengths". For example, chlorophyll always absorbs red and violet light, while it transmits yellow, green, and blue wavelengths. The transmitted and reflected wavelengths appear green- the color your eye "sees."

A solution contains copper ions is blue because it absorbs the complementary color yellow from white light and transmits the remaining blue light (Table 1). The absorption or transmission of specific wavelengths is characteristic for a Substance, and a spectral analysis serves as a "fingerprint" of the compound.

Table 1: Colors of different wavelength regions

Wavelength absorbance(nm)	Color absorbed	Color observed Complement
380-435	Violet	Yellow-green
435-480	Blue	Yellow
480-490	Blue-green	Orange
490-500	Green-blue	Red
500-560	Green	Purple
560-580	Yellow-green	Violet
580-595	Yellow	Blue
595-650	Orange	Blue-green
650-750	Red	Green-blue

Transmittance, Absorbance and The Beer-Lambert law

We define **transmittance (T)** as the ratio of the amount of light transmitted to the amount of light that initially fell on the Surface.

P° is the radiant power from the source and, P is the radiant power transmitted by the sample.

$$T = \frac{P}{P^\circ} = \frac{\text{intensity of transmitted light}}{\text{Intensity of transmitted light}}$$

$$T\% = \frac{P}{P^\circ} \times 100$$

Absorbance (A) is defined as the negative logarithm of the transmittance, and you will note that absorbance and transmittance bear an inverse relationship.

$$\text{Absorbance} = -\log T = -\log \frac{P}{P^\circ}$$

$$A = 2 - \log \%T$$

Beer-Lambert law, "for monochromatic absorbance is directly proportional to the path length b through the medium and the concentration c of the absorbing species these relation shins are given by

$$A = a\,b\,c$$

Where

- A is a dimensionless number.
- A the proportionality co`nstant, is called the absorptivity. It is a constant for a given Substance, provided the temperature and wavelength are constant. It has units of L/g • cm.

- B and c have the usual units of length (cm) and concentration (gL).

Absorptivity depending on:
1-Nature of substance. 2-Wnavelength. 3- Path length of radiation in solution.
4-Type of solvent.
Note:
If b and c have the units of length (cm) and concentration (mol/L), the absorptivity, (a) is called the molar absorptivity and is given the special symbol δ Thus,
$A = \delta b c$
Where δ has the unit $L\ mol^{-1}\ cm^{-1}$

Limitations to Beer's Law

The linearity of the Beer-Lambert law is limited by chemical and instrumental factors. Causes of nonlinearity include:
√ Deviations in absorptivity coefficients at high concentrations (>0.0IM) due to electrostatic interactions between molecules in close proximity.
√ Scattering of light due to particulates in the sample.
√ Changes in refractive index at high analyte concentration.
√ Shifts in chemical equilibria as a function of concentration.
√ Non-monochromatic radiation.
√ stray light.

Instrument Designs for Molecular UV/Vis Absorption

A UV-Vis absorption instrument is made up of the usual components including:
1. A Source
2. Wavelength selector
3. Sample container
4. Radiation transducer
5. Signal processor

Two types of instrumento that are used for absorption measurements with UV, visible, or infrared radiation:
• Photometers
• Spectrophotometers

Spectrophotometers employ a grating or a prism monochromator to provide a narrow band of radiation for measurements Photometers, in contrast, use an absorption filter or an interference filter for this purpose.

1-Photometers

Photometers use filters to provide wavelength selection. The diagram of simple single-beam photometer used for measurement in visible region shown in Figure 1.

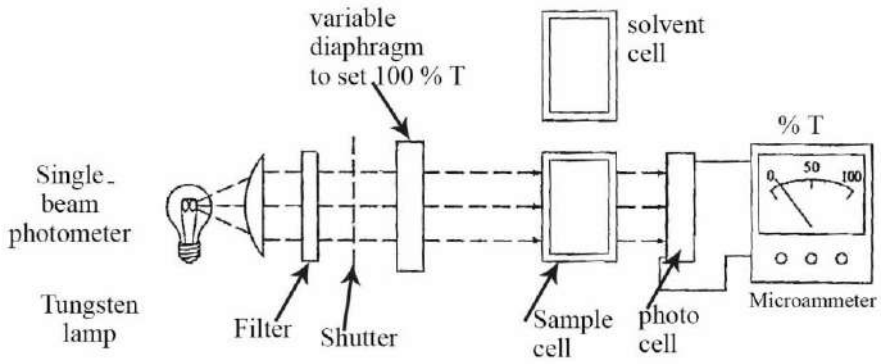


Figure1: Single-beam photometer for absorption measurements in visible region.

This kind of photometer has a single optical path between the source and detector. The filter is placed between the source and sample to prevent the sample from decomposing when exposed to high-energy radiation. The detector is usually a photovoltaic cell and the source is a tungsten halogen lamp. Photometers are ordinarily supplied with several filters, each of which transmits a different portion of the visible spectrum.

The instrument is calibrated to 0%T while using a shutter to block the source radiation from the detector, after removing the shutter, the instrument is calibrated to 100%T using an appropriate blank. The blank is then replaced with the sample, and its transmittance is measured. The photometer must be recalibrated whenever the filter is changed.

Photometers offer advantages of:

1- Low-cost
2- Simplicity
3- Ruggedness
4- Portability
5- Ease of maintenance

A disadvantage of a photometer is that it cannot be used to obtain an absorption spectrum.

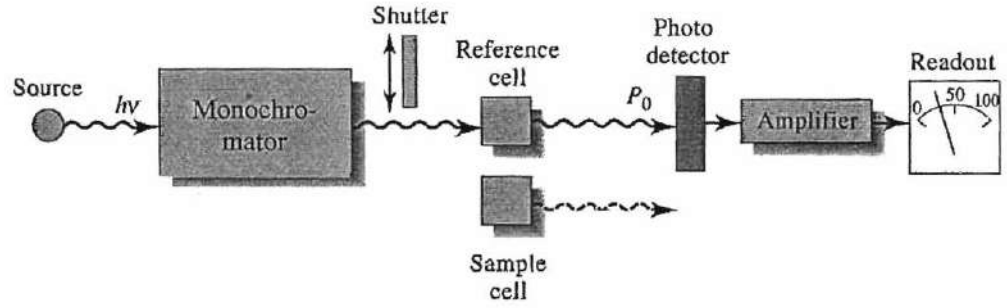
2-Spectrophotometers

Instruments using **monochromators** for wavelength selection are called spectrometers. In absorbance spectroscopy, where the transmittance is a ratio of two radiant powers, the instrument is called a **spectrophotometer.**

Spectrophotometric configurations:

1- Single- beam spectrophotometer

The simplest spectrophotometer is a single-beam instrument equipped with a fixed wavelength monochromator, the schematic diagram for which is shown in Figure 2.



(a)
.2007Thomoon Higher Education

Figure2: Single beam fixed wavelength spectrophotometer

A single-beam spectrophotometer measures the relative light intensity of the beam before after a test sample is inserted. Two sources are used, a tungsten halogen and a deuterium, lamp where a mechanism for source selection should be available. The wavelength selector is a grating or prism monochromators and the detector is usually a photomultiplier tube.
Single-beam spectrophotometer offers advantages of:
- Can have a larger dynamic range
- Are optically simpler and more compact.
- Easier to maintain and slightly less costly to operate.
- High throughput and hence high sensitivity.

In the other side the limitations of this instrument are:

- Are not practical for recording spectra (adjusting the Wavelength and recalibrating are time consuming).
- The accuracy is limited by the stability of its source and detector over time.

2-Double-beam spectrophotometer

A double beam spectrophotometer compares the light intensity between two light paths, one path containing a reference sample and the other the test sample

(a) Double-beam (in time) spectrophotometer

In this instrument (Figure 3) beam is split in two, but measured by same detector and "in-time" because the beam appears in 2 places over one cycle in time.

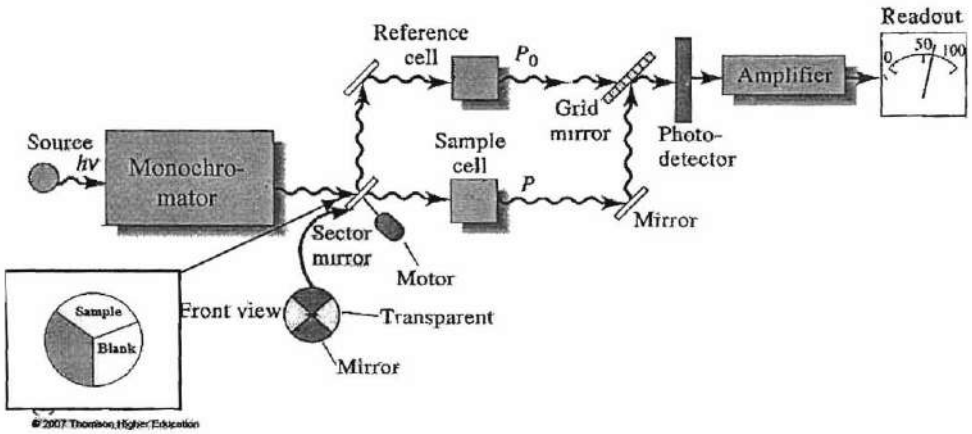


Figure3: Double- beam (in time) spectrophotometer

The instrument is equipped with interchangeable-deuterium/tungsten Sources, a reflection grating monochromator, and photodetector. The beam splitter is a motor driven circular disk of chopper that is divided into three segments,

- one of which is transparent,
- The second reflecting.
- And the third opaque.

With each rotation, the detector receives three signals, the first corresponding to Po, the second to P, and the third to the dark current. The resulting electrical signals are then processed electronically to give the transmittance or absorbance on a readout device.

Note:

In some kinds of double-beam instrument the beams are separated in time by a rotating Sector mirror. The motor-driven sector mirror is made up of pie shaped segments, half of which are mirrored and half of which are transparent. The mirrored sections are held in place by blackened metal frames that periodically interrupt the beam and prevent its reach the detector.

(b) Double-beam (in space) spectrophotometer

In this kind of double-beam spectrophotometer beam is split into two paths and measured by matched detectors). It is “in space” because two beams are always present in space.

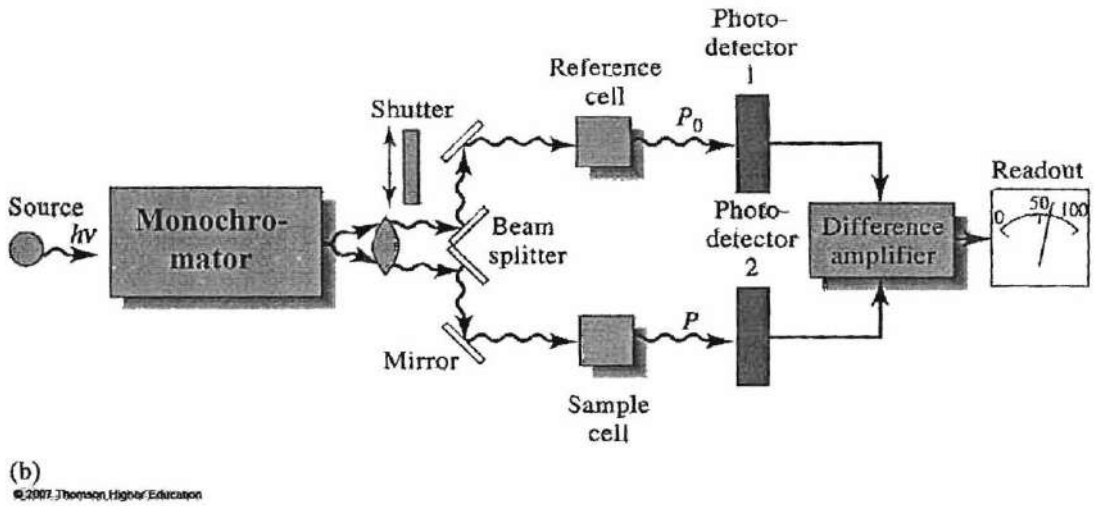


Figure 4: Double-beam (in space) spectrophotometer

Figure 4 illustrates a double-beam-in space instrument in which two beams are formed in space by a V-shape mirror called a beam splitter. One beam passes through Solution to a photodetector, and the second simultaneously traverse the sample to a Second, matched photodetector.

The two outputs are amplified, and their ratio is determined electronically and displayed by read out device. The detector is usually a pair of photomultiplier tubes connected to a difference amplifier and the wavelength selector is a grating or prism monochromators. Double-beam instruments are more versatile than single-beam instruments, being useful for both quantitative and qualitative analyses, they are however, more expensive.

3-Multichannel spectrophotometer

The most widely used multichannel instrument is that based on photodiode array detector) (Figure 5). A linear photodiode array consists of multiple detectors, or channels, allowing an entire spectrum to be recorded in as little as 0.1s.

The heart of these instruments is an array of several hundred silicon diode detectors that are fabricated side by side on a single silicon chip (1 to 6 cm in length). The chip contains a capacitor and electronic Switch for each diode.

After passing through the analyte solution, the radiation is focused on an entrance slit and then passes onto the Surface of reflecting grating and dispersed. The linear photodiode array is situated at the grating's focal plane, with each diode recording the radiant power over a narrow range of wavelengths.

Instrument advantages:

1- Speed of data acquisitions, which collect several spectra for single sample.

2- The system contains in moving parts; the wavelength reproducibility

from scan to scan is very high.

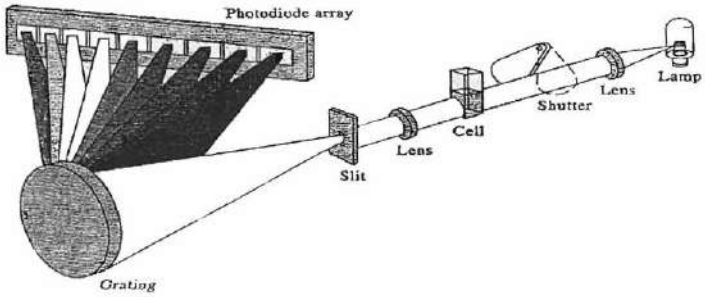


Figure5: Multichannel spectrophotometer

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Atomic Absorption Spectroscopy

Atomic absorption spectrometry is an analytical technique for the quantitative determination of chemical elements employing the absorption of optical radiation (light) by free atoms in the gaseous state. The technique makes use of the wavelengths of light specifically absorbed by an element.

How it Works the process of atomic absorption is illustrated in Figure 1.

Atomic Absorption Process

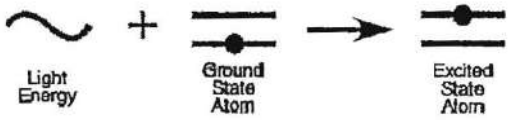


Figure 1. Atomic Absorption Process

The "ground state" atom absorbs light energy of a specific wavelength as it enters the "excited state." As the number of atoms in the light path increases, the amount of light absorbed also increases. By measuring the amount of light absorbed, a quantitative determination of the amount of analytic can be made. The use of special light Sources and careful selection of wavelengths allow the specific determination of individual elements.

Steps in the experiment

- The analytic is prepared in aqueous solution.
- The solution is aspirated into the flame, using the nebulizer.
- The solvent evaporates.
- The gaseous analytic decomposes, and some of it is converted to gaseous atoms.
- The gaseous atoms absorb radiation from the hollow cathode lamp.
- The absorbance of the sample is determined by comparing 1 (when no analytic is present in the flame) and I_{trans}.

Instrumentation

There are five basic components of an AA instrument:

1. The light source that emits the spectrum of the element of interest (hollow cathode lamp).
2. An "absorption cell" in which atoms of the sample are produced (flame, graphite furnace.....etc).
3. A monochromatic.
4. A detector, which measures the light intensity and amplifies the signal.
5. A display that shows the reading.

There are two basic types of atomic absorption instruments: single beam and double-beam (Fig. 2 and 3).

In single-beam AAS, the light source emits a spectrum specific to the element of which it is made, which is focused through the sample cell into the monochromator. The light source must be electronically modulated or mechanically chopped to differentiate between the light from the source and the emission from the sample cell.

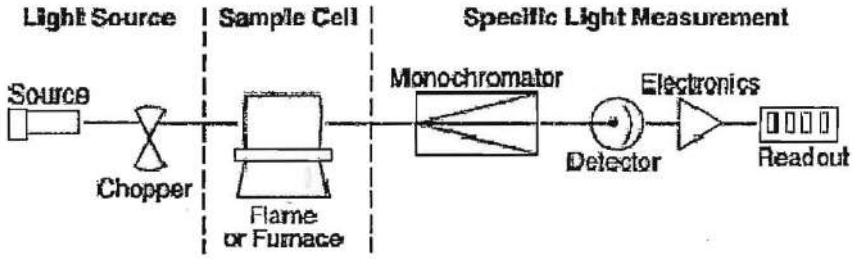


Figure 2. Single-Beam Atomic Absorption Spectrometer

In a double-beam AAS, the light from the source lamp is divided into a Sample beam, which is focused through the sample cell, and a reference beam, which is directed around the sample cell. In a double-beam system, the readout represents the ratio of the sample and reference beams.

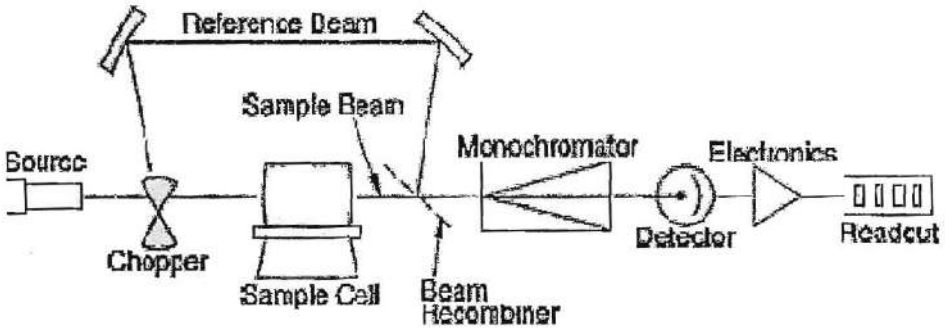


Figure 3. Double-Beam Atomic Absorption Spectrometer

Atomization

The most important difference between a spectrophotometer for atomic absorption and one for molecular absorption is the need to convert

the analyte into a free atom. The process of converting the analyte in solid, liquid, or solution form to a free gaseous atom is called atomization.

Two general methods of atomization are used:

1- Flame atomization

2- Electrothermal atomization.

A few elements are atomized using other methods.

Flame Atomizers

In flame atomization the sample is first converted into fine mist (aerosol) consisting of Small droplets of solution. This is accomplished using a nebulizer assembly similar to that shown in the insetto Figure 4.

The sample is aspirated from its container into a spray chamber by passing a high-pressure stream consisting of one or more combustion gases. The impact of the sample with the glass impact bead produces anaerosol mist.

The aerosol mixes with the combustion gases in the spray chamber before passing to the burner where the flames thermal energy desolates the aerosol mist to a dry aerosol of small, solid particles. Energy (volatilizes the particles, producing a vapor consisting of free atom.

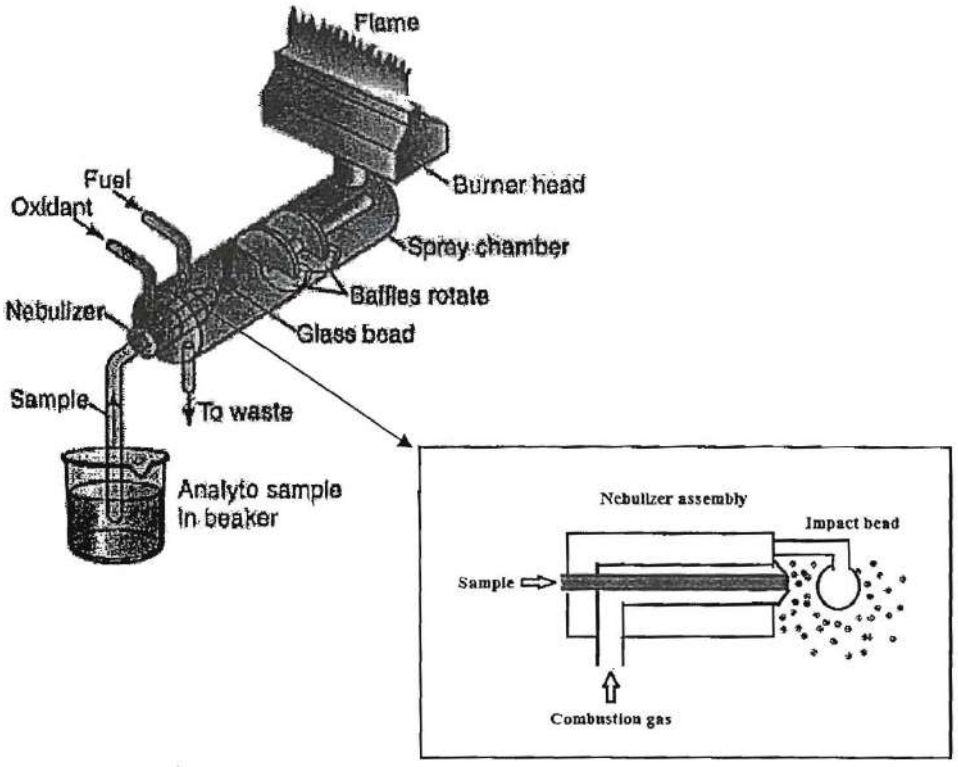
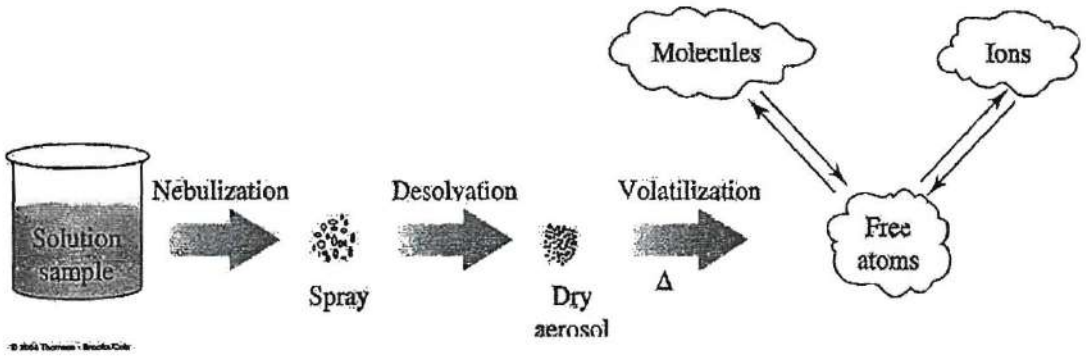


Figure 4: Atomization assembly equipped with spray chamber and slot burner. The inset shows the nebulizer assembly



Flames

Thermal energy in flame atomization is provided by the combustion of a fuel oxidant mixture. Common fuels and oxidants and their normal temperature ranges are:

1- Hydrogen-air (2000-2100°C).
2- Acetylene-air (2100-2400°C).
3- Acetylene-nitrous oxide (2600-2800°C).
4- Acetylene - Oxygen (3050-3150°C).

Samples introduction

The most common means for introducing samples into a flame atomizer is continuous aspiration, in which the sample is continuously passed through the burner while monitoring the absorbance. Continuous aspiration is sample intensive; typically requiring 2-5 mL of Sample. Flame microsampling provides a means for introducing a discrete sample of fixed volume and is useful when the volume of sample is limited or when the sample's matrix is incompatible with the flame atomizer. Flame microsampling is accomplished using a micropipette to place (50-250 p.L of Sample in a Teflon funnel connected to the nebulizer. **Advantages and disadvantages of flame atomizers**

Advantages are:

- Fast analysis (10-15 s per sample per element).
- Very good precision.
- No or moderate interferences that can be easily corrected.
- Easy automation of the measurement.
- Low cost.

A significant disadvantage to flame atomizers is that the efficiency of atomization may be quite poor. This may occur for two reasons:

1. The majority of the aerosol mist produced during nebulization consists of droplets that are too large to be carried to the flame by the combustion gases. Consequently, as much as 95% of the sample never reaches the

flame.
2. The large volume of combustion gases significantly dilutes the sample and reduce sensitivity.

Electrothermal Atomizers

A significant improvement in sensitivity is achieved by using resistive heating in place of a flame. A typical electrothermal atomizer, also known as a Graphite furnace, consists of a cylindrical graphite tube approximately 1-3 cm in length, and 3-8 mm in diameter (Figure 5). Graphite furnace is an electrothermal atomizer that relies on resistive heating to atomize samples.

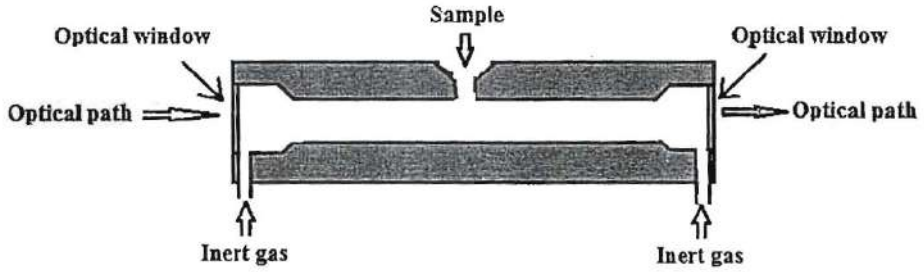


Figure 5: Electrothermal analyzer

Components of Electrothermal Atomizers

1- A graphite tube which is housed in an assembly that seals the ends of the tube with optically transparent windows.
2- A continuous stream of inert gas passage to Protecting the graphite tube from oxidation.
3- A power supply is used to pass a current through the graphite tube.
4- A Small diameter hole located at the top of the tube used for injection the samples (5. and 50 uL) from an automated micropipette. The tube is heated electrically by passing a current through it in a preprogrammed series of steps.
1. Drying evaporation of solvent (30-40 seconds at 150°C).
2. Drive off any volatile organic material and convert the sample to ash (30 seconds at 600 °C).
3. Vaporize and atomize elements (2000–2500 °C for 5-10 seconds).
During this heating Cycle the graphite tube is flushed with argon gaS to prevent the tube burning away. In electro thermal atomization almost 100% of the sample is atomized. This makes the technique much more sensitive than flame AAS.

Advantages and disadvantages of electro thermal atomizers

Advantages are:

- High sensitivity and good detection limits by trapping the gaseous analyte in the Small volume of the graphite tube.

- Trace analysis and microanalysis liquid Samples even when organic matrix is present (beverages, milk, blood, plasma etc.) in addition to the analysis of powdered Solids or Suspensions of Solid samples.

Disadvantages are:

.A lot of interferences مور

Slow analysis.

s Expensive equipment.

Miscellaneous Atomization Methods

1-Hydride Generation Atomic Absorption (HGAA) Spectroscopy

A few elements may be atomized by a chemical reaction that produces a volatile product. Elements such as As, Se, Sb, Bi, Ge, Sn, Te, and Pb form volatile hydrides when reacted with NaBH4 în acid, An inert gas carrier the volatile hydrides to either a flame or to a heated quartz observation tube (must be heated to dissociate the hydride into free atoms (AS^o) and (Se^o)) situated in the optical path.

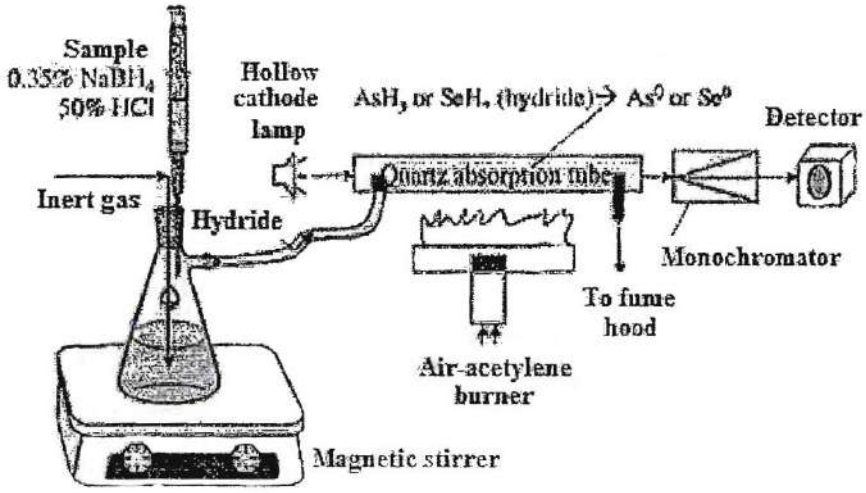


Figure 8: A Hydride Generation and atomization system

2- Cold Vapor Atomic Absorption (CVAA) Spectroscopy

Mercury (Hg) (and also the ionic forms Hg22' and Hg2) is determined by the Cold-vapor method in which it is reduced to elemental mercury with Strong reducing agent SnCl2. The Volatile Hg is carried by an inert gas to an unheated observation tube Situated in the instrument's optical path.

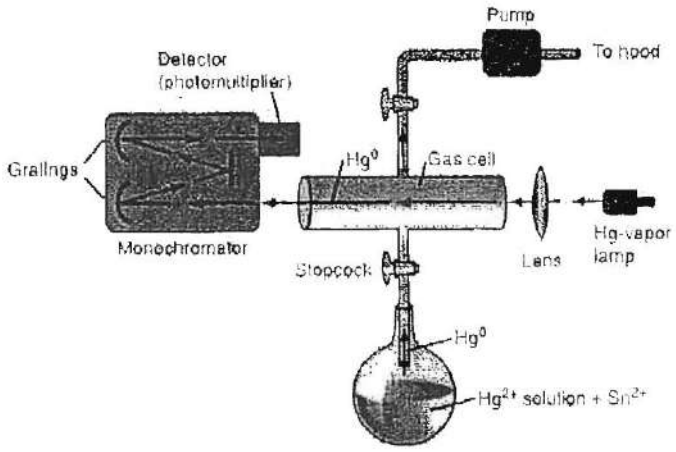


Figure 9,5 Schematic diagram of Cold vapor mercury analyzer (Girard. El Principles of Environmental chemistry, 2005, Jones and Battle e publishers, Sudubury, MA. WWW.jbpub.com Reprinted with permission.)

Analytical Applications of Atomic Absorption Spectrometry (AAS)

AAS is used for the determination of all metal and metalloid elements. Nonmetals cannot be determined directly because their most Sensitive resonance lines are located in the vacuum UV region of the spectrum. Neither flame nor furnace Commercial atomizers can be operated in a vacuum. It is possible to determine some nonmetals indirectly taking advantage of the insolubility of some compounds. For example, chloride ion can be precipitated as insoluble Silver chloride by adding a known excess of Silver ion in Solution (as Silver nitrate). The Silver ion remaining in Solution can be determined by AAS and the chloride ion Concentration Calculated from the change in the Silver ion Concentration.

Light Sources in Atomic Absorption

The Source for atomic absorption is a hollow cathode lamp(HCL) consisting of a Cathode and anode enclosed within a glass tube or cylinder filled with a low pressure of A Ne At (Figure 9).The glass cylinder has a quartz or UV glass window for optimum transmittance of the emitted radiation. Hollow

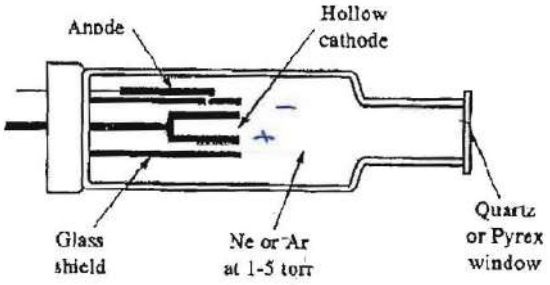


Figure9: Hollow cathode lamp

When a potential is applied across the electrodes, the filler gas is

ionization. The positively charged ions Collide with the negatively charged cathode, dislodging, "sputtering," atoms from the Cathode's Surface. Some of the Sputtered atoms are in the excited State and emit radiation characteristic of the metal from which the Cathode was manufactured.

Preparing the Sample

Samples in Solid form are prepared for analysis by dissolving in an appropriate solvent when the sample is not soluble, it may be digested, either on a hot plate or by microwave, using HNO_3, H_2SO_4, or $HClO_4$. Liquid samples may be analyzed directly or may be diluted or extracted if the matrix is incompatible with the method of atomization. Serum samples, for instance, may be difficult to aspirate when using flame atomization and may produce unacceptably high background absorbance when using electro thermal atomization. A liquid-liquid extraction using an organic solvent containing a chelating agentis frequently used to concentrate analyses. Dilute solution of Cd^{2+} , Co^{2+} Cu^{2}+, Fe^{3}", Pb^{2+}, Ni^{2+} and Zn^{2}+, for example, can be concentrated by extracting with a solution of ammonium pyrimidine dithiocarbamate in methyl isobutyl ketone.