CHM 301 COURSE CONTENT AND ALLOCATION AMONG INSTRUCTORS

1. **Analytical separations**

   i. Solvent Extraction; pH effect; Extraction with metal chelator

   ii. Types of chromatography (Ion-Exchange Chromatography)

2. **Molecular spectroscopy and flame methods**

   i. Molecular spectra and regions of emr

   ii. Wavelength selectors, cells, detectors

   iii. Quantitative laws of Absorption

   iv. Use of Absorbance Measurements for Quantitative Analysis

   v. Absorbance Measurements and Location of Titration End points

   vi. Determination of Ligand:Metal ratio in a complex

   vii. Deviations from Beer's law

   viii. Use UV radiation in a Qualitative work

3. **Luminescence, Nephelometry and Turbidimetry**

   i. Instrumentation

   ii. Uses for Qualitative work

4. **Atomic Spectra and Spectra Line Widths**

   i. Apparatus

   ii. Radiation sources

   iii. Hollow Cathode Lamps, Cells, Flames & Furnaces

   iv. Quantitative Applications of Atomic Spectroscopy

   v. Interference studies in Atomic Spectra

5. **Analytical Automation**

   i. Automatic titrators

   ii. pH-stat- and process control.

Texts:

1. Pecsok, R. L *et al*. (1976). Modern Methods of Chemical Analysis, 2nd Edition, John Wiley and Sons, NY.

2. Harris D. C. (2007). Quantitative Chemical Analysis, 7th Edition, W.H. Freeman and Company.

3. Skoog, D. A., Holler, F. J. and Crouch, S. R. (2007). Principles of Instrumental Analysis, 6th Edition, Thomson Brooks/Cole, Canada.

4. Garry D. Christian. (©2004, 2008). Analytical Chemistry, 6th Edition, Wiley-India.

INTRODUTION TO SPECTROMETRY

Spectrometric methods are a large group of analytical methods that are based on atomic and molecular spectroscopy. ***Spectroscopy is the science that deals with the interactions of various types of radiation with matter***, usually between electromagnetic radiation and matter, but could also include interactions between matter and other forms of energy such as acoustic waves and beams of particles (ions and electrons).

Spectrometry and spectrometric methods refers to the measurement of the intensity of radiation with a photoelectric transducer or other types of electronic devices.

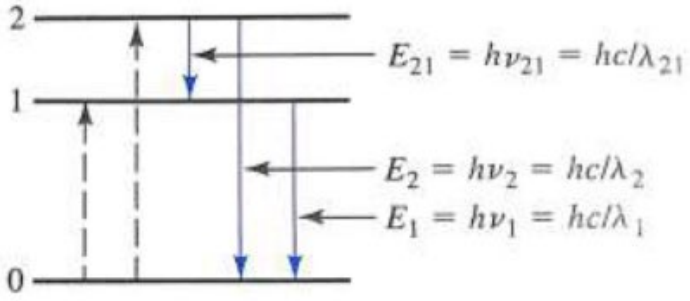
Energy States of Chemical Species

Some of the important postulates of quantum theory that can be used to explain interactions of radiation and matter are:

1. Atoms, ions and molecules can exist only in certain discrete states, characterized by definite amount of energy.
2. When a species changes its state, it absorbs or emits an amount of energy exactly equal to the energy difference between the states.
3. When atoms, ions or molecules absorb or emit radiation in making the transition from one energy state to another, the frequency ν or the wavelength λ of the radiation is related to the energy difference between the states by the equation:

$$E_{1\_} E_0 = h\nu = \frac{hc}{\lambda}$$

where E_1 is the energy of the higher state, E_o is the energy of the lower state, C is the speed of light and h is the Planck's constant (6.62608×10^{-34} JS).



NOTE:

For atoms or ions in the elemental state, ***the energy of any given state arises from the motion of electrons around the positively charged nucleus***; ***hence, the various energy states are called electronic states***. Molecules also have quantized vibrational states that are associated with the energy of interatomic vibrations and quantized rotational states that arise from the rotation of molecules around their centres of mass.

The lowest energy state of an atom or molecule is its ***ground state***, while their higher energy states are termed ***excited states***. Usually at room temperature, chemical species are regarded to be in their ground state.

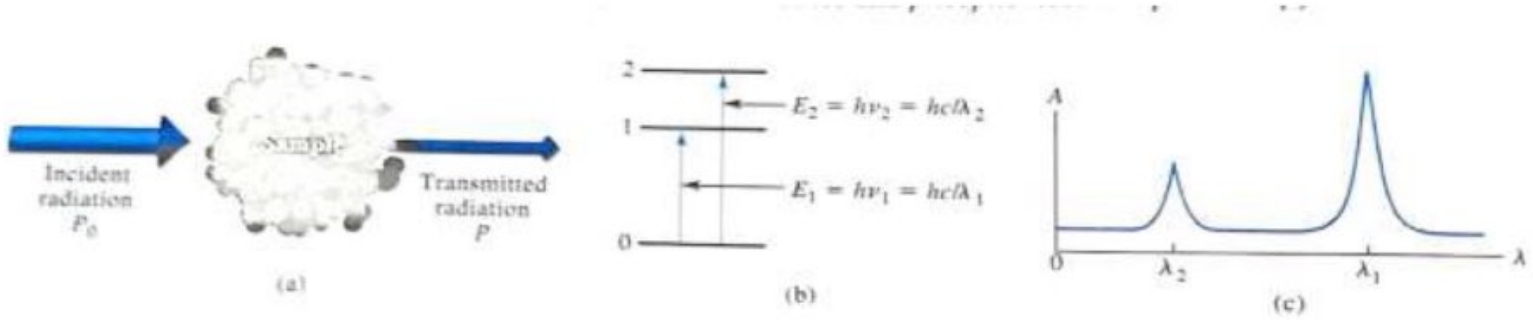
Acquiring Information About an Analyte

When a sample/an analyte is stimulated by applying energy in the form of heat, electrical energy, light, particles, or a chemical reaction, some of the analyte species undergo a transition from their ground state to the excited state. Information about the analyte's identity and concentration is acquired

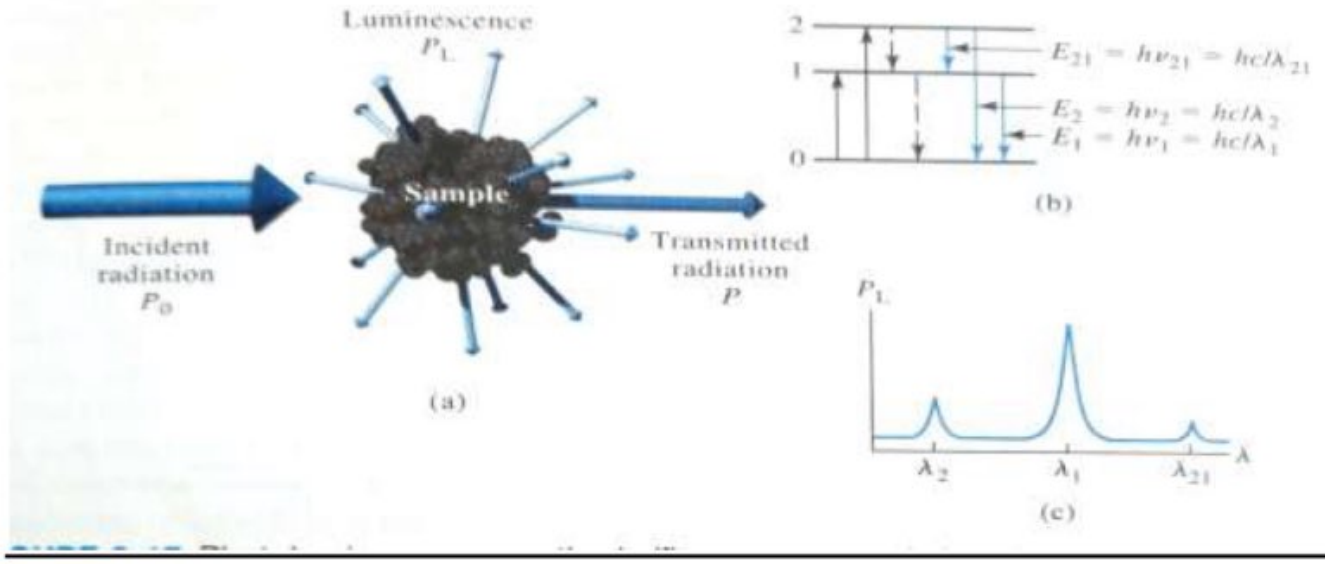
by measuring the electromagnetic radiation emitted as it returns to the ground state or by measuring the amount of electromagnetic radiation absorbed or scattered as a result of excitation. The results of such a measurement are often expressed graphically by a spectrum, which is a plot of the emitted radiation as a function of frequency or wavelength.

When the sample is stimulated by the application of an external electromagnetic radiation source, the radiation can be reflected, scattered or absorbed.

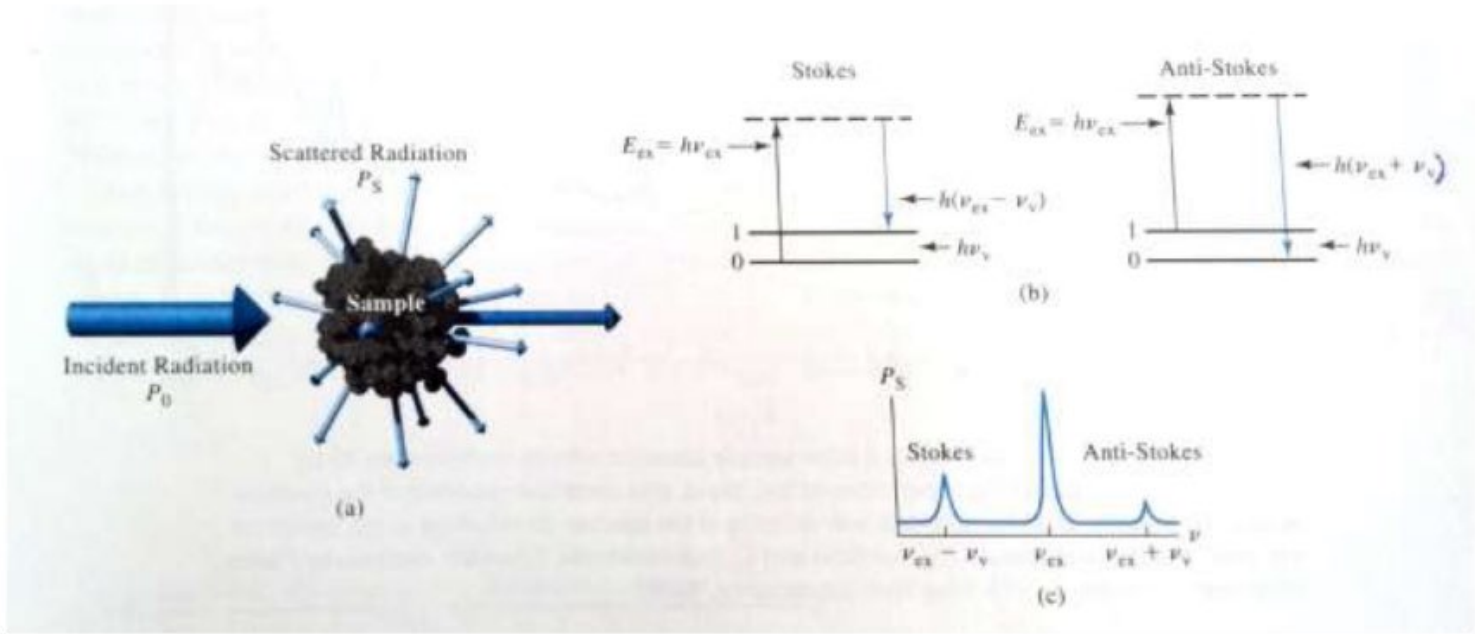
Absorption Methods: *Radiation of incident radiant power P_o can be absorbed by the analyte, resulting in a transmitted beam of lower radiant power P. For absorption to occur the energy of the incident beam must correspond to one of the energy differences shown in (b) and the resulting absorption spectrum is shown in (c).*



Photoluminescence (Fluorescence and Phosphorescence) Methods: *Fluorescence and Phosphorescence result from absorption of electromagnetic radiation and then dissipation of the energy emission of radiation (a).In (b), the absorption can cause excitation of the analyte to state 1 or state 2. Once excited, s energy can be lost by emission of a photon (luminescence, shown as solid lines) or by nonradiative processes (dashed lines). The emission occurs over all angles, and the wavelengths emitted (c) correspond to energy difference between levels.* The major distinction between fluorescence and phosphorescence is the time scale of emission, with fluorescence being prompt and phosphorescence being delayed.



Scattering of Radiation: *When radiation is scattered, the interaction of the incoming radiation with the sample may be elastic or inelastic. In elastic scattering, the wavelength of the scattered radiation is the same as that of the source radiation. The intensity of the elastically scattered radiation is used to make measurements in nephelometry and turbidimetry, and particle sizing.*



Classes of Spectroscopy

There are two classes of spectroscopy, namely: ***emission spectroscopy*** and ***absorption spectroscopy***.

Emission Spectroscopy involves methods in which the stimulus is the heat or electrical energy. **Chemiluminescence Spectroscopy** refers to the excitation of the analyte by a chemical reaction. In both cases, measurement of the radiant power emitted as the analyte returns to the ground state can give information about its identity and concentration. In photoluminescence spectroscopy, the emission of the photons is measured after absorption. Fluorescence and phosphorescence spectroscopy are the most important forms of photoluminescence.

In absorption Spectroscopy, we measure the amount of light absorbed as a function of wavelength. This can give both qualitative and quantitative information about the analyte.

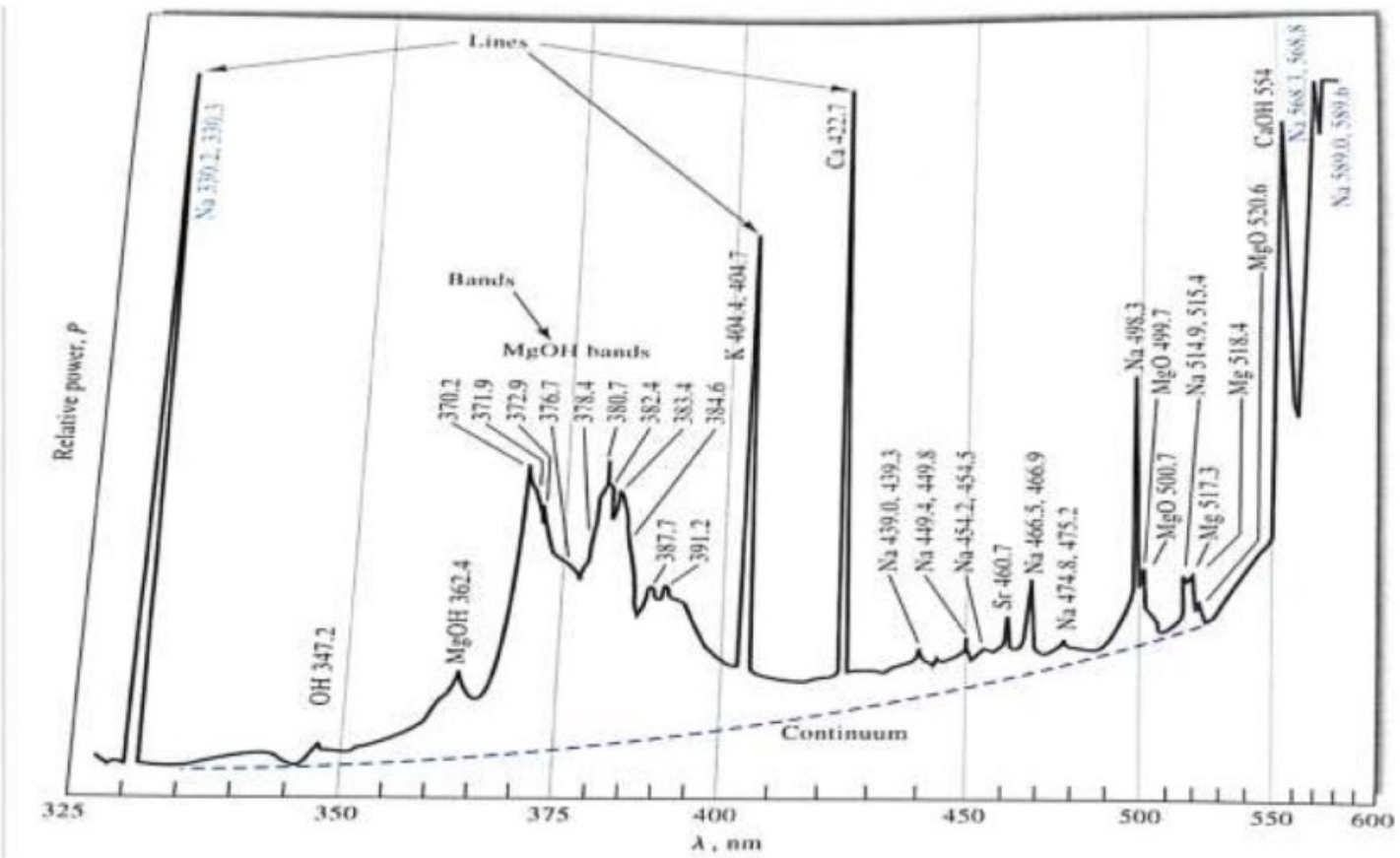
Emission of Radiation

Electromagnetic radiation is produced when excited particles (atoms, ions or molecules) relax to lower energy levels by giving up their excess energy as photons. Excitation can be brought about by:

1. bombardment with electrons or other elementary particles, which generally leads to the emission of X- radiation;
2. exposure to an electric current, an ac spark, or an intense heat source (flame, dc arc, or furnace), producing UV, Visible, or IR radiation;
3. irradiation with a beam of electromagnetic radiation, which produces florescence radiation; and
4. an exothermic chemical reaction that produces chemiluminescence.

Radiation from an excited source is conveniently characterized by means of an emission spectrum, which is a plot of the relative power of the emitted radiation as a function of wavelength or frequency.

Diagram of a Typical Emission Spectrum



The emission spectrum above was obtained by aspirating a brine solution into an oxyhydrogen flame which produced **three types of spectra**, namely: ***lines***, ***bands***, and a ***continuum***.

Line Spectra

Line spectra in the UV and Visible regions are produced when the radiating species are individual atomic particles that are well separated in the gas phase. The individual particles in a gas behave independently of one another, and the spectrum consists of a series of sharp lines with widths of about 10^{-5} nm (10^{-4} Å). In the diagram above, lines for gas-phase Na, K, and Ca are identified.

Band spectra

Band spectra consist of a series of closely spaced lines that are not fully resolved by the instrument used to obtain the spectrum. Bands arise from the numerous quantized vibrational levels that are superimposed on the ground-state electronic energy level of small molecules (or gaseous radicals) such as OH, MgOH and MgO as illustrated in the diagram above.

Continuum spectra

A continuum radiation is produced when solids are heated to incandescence. Thermal radiation of this kind, which is called black-body radiation, is characteristic of the temperature of the emitting surface rather than the material of which that surface is composed.

General Designs of Optical Instruments

Instruments for the UV, Visible, and IR regions have enough features in common that they are often referred to as optical instruments even though the human eye is not sensitive to UV or IR wavelengths. Optical spectroscopic methods are based on six phenomena as listed below:

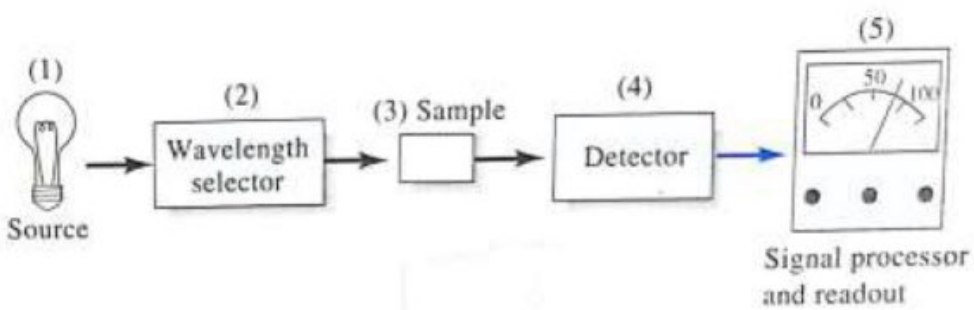
(1) Absorption
(2) Fluorescence
(3) Phosphorescence
(4) Scattering
(5) Emission
(6) Chemiluminescence.

Although the instruments for measuring each differ in configuration, most of their basic components are remarkable similar. Also, the required properties of these components are the same regardless of whether they are applied to the UV, Visible, or IR portion of the spectrum.

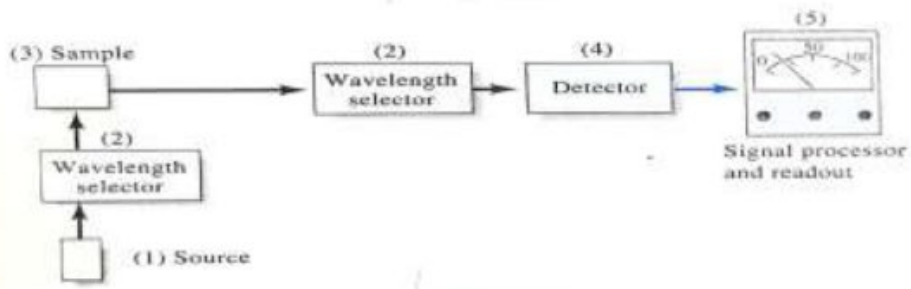
Typical spectroscopic instruments contain the following five components: (1) Stable sources of radiant energy; (2) Transparent container for bolding the sample; (3) Device that isolates a restricted region of the spectrum for measurement; (4) Radiation detector (which converts radiant energy to a usable electrical signal); and (5) Signal processor and readout (which displays the transduced signal on a meter scale, a computer screen, a digital meter, or another recording device).

Arrangement of Components of Instruments for:

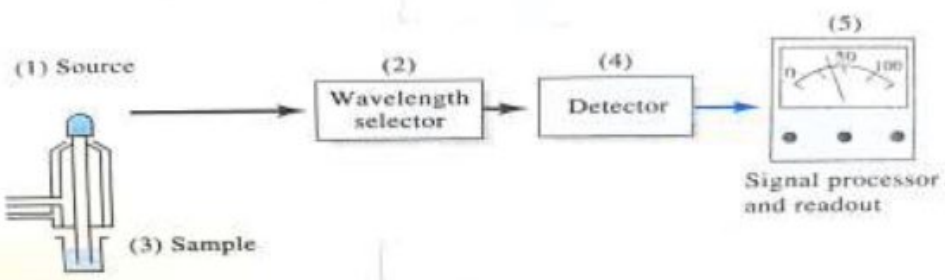
(a) Absorption measurements:



(b) Fluorescence/phosphorescence measurements:



(c) Emission Spectroscopy



Note that components (1), (4) and (5) are arranged in the same way for each type of measurement.

Atomic Absorption Spectrophotometry (AAS)

Atomic absorption spectrophotometry (AAS) as a technique is closely related to flame emission spectrometry because they each use a flame as the atomizer.

Principle

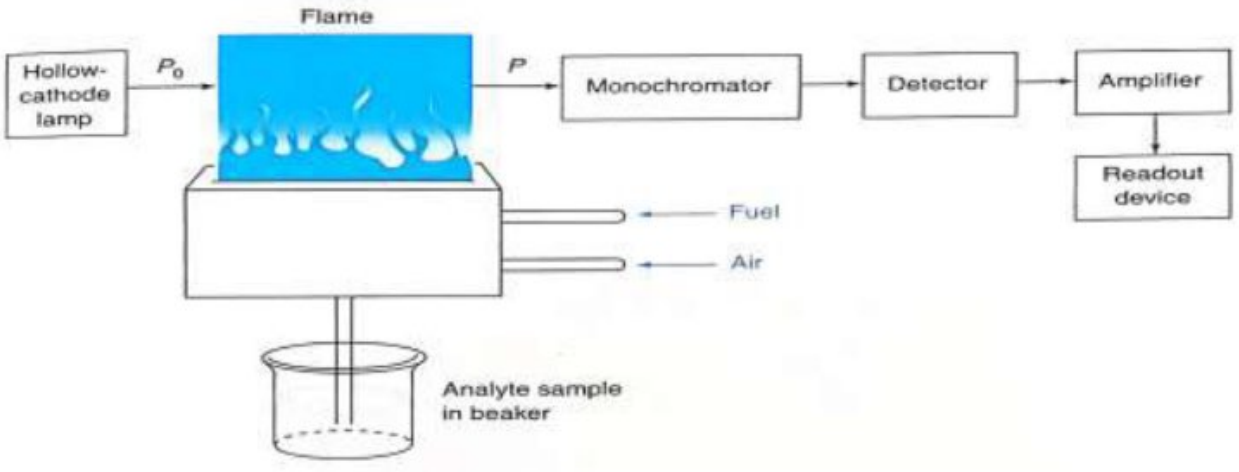
The sample solution is aspirated into a flame, and the sample element is converted to atomic vapour in the flame. Here, some are thermally excited by the flame, but most remain in the ground state. These ground-state atoms can absorb radiation of a particular wavelength that is produced by a special source made from that element. The wavelengths of radiation given off by the source are the same as those absorbed by the atoms in the flame. The absorption follows Beer's law. That is, the absorbance is directly proportional to the concentration of the atomic vapour in the flame and to the path length in the flame. The concentration of atomic vapour is directly proportional to the concentration of the analyte in the solutions being aspirated.

The procedure used is to prepare a calibration curve of concentration in the solution versus absorbance. The major disadvantage of making measurements using atomic absorption is that a different source is required for each element.

Instrumentation

The requirements for AAS are a light source, a cell (the flame), a monochromator, and a detector.

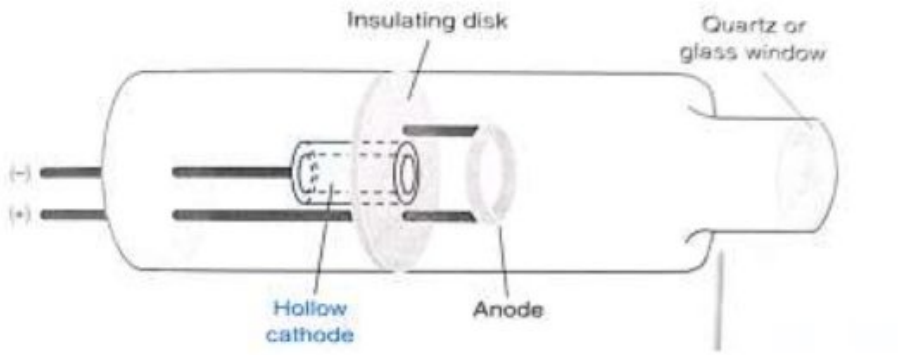
Schematic diagram of Atomic Absorption Instrumentation



This is for a double-beam instrument that measures the ratio of Po/P. The flame is placed between the source and the monochromator. The source beam is alternately sent through the flame and around the flame by the chopper. The detector measures these alternately and the logarithm of the ratio is displayed. The detector amplifier is tuned to receive only the radiation modulated at the frequency of the chopper, and so, d.c. radiation emitted by the flame is discriminated against. Double beam instruments are required for background correction using deuterium continuum lamps.

Sources

The source used almost exclusively in AAS is a hollow-cathode lamp (HCL). This is a sharp-line source that emits specific (essentially monochromatic) wavelengths. It consists of a cylindrical hollow cathode made of the element to be determined or an alloy of it, and a tungsten anode. These are enclosed in a glass tube usually with a quartz window since the lines of interest are often in the UV region.



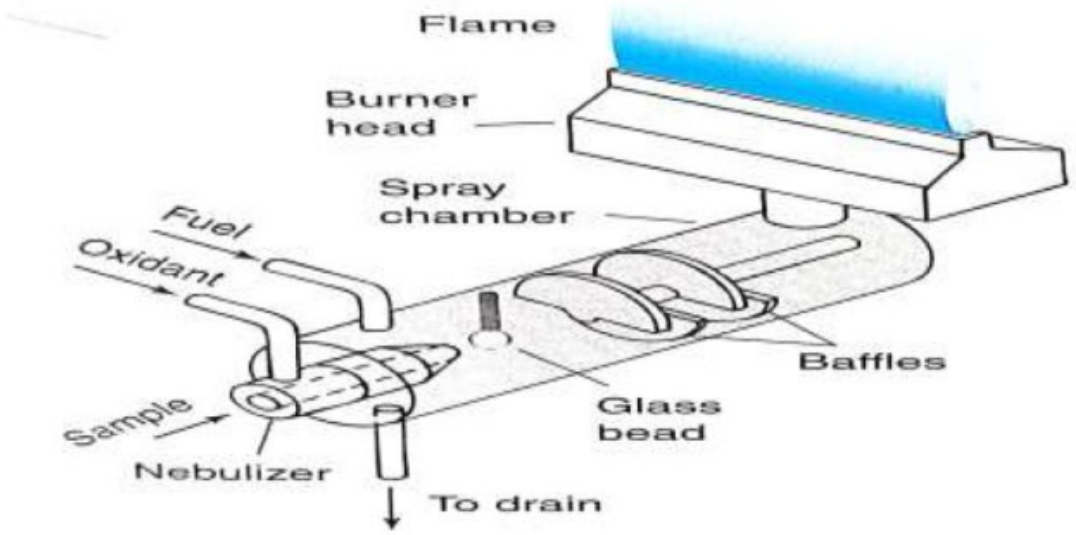
The tube is under reduced pressure and filled with an inert gas such as argon or neon (at 1 – 5 torr). A high voltage is impressed across the electrodes, causing the gas atom to be ionized at the anode. These positive ions are accelerated towards the negative cathode. When they bombard the cathode, they cause some of the metal to "sputter" and become vaporized. The vaporized metal is excited to higher electronic levels by continued collision with the high-energy gas ions. When the electrons return to the ground state, the characteristic lines of that metallic element are emitted. These emitted lines are passed through the flame and can become absorbed by the test element because they possess just the right energy to result in the discrete electronic transitions. The most strongly absorbed line is often (but not always) the one corresponding to the most probable electronic transition from the ground state to the lowest excited state. This is called the resonance line.

It is sometimes possible to use an alloy of several elements for the hollow cathode, and with such lamps, the lines of all of elements are emitted. These are the so called multielement hollow-cathode lamps and can be used as a source for usually two or three elements. They may exhibit shorter lifetimes than do single-element lamps due to selective volatilization of one of the elements from the cathode with condensation on the walls of the lamp.

Burners

The burner used in most commercial instruments is the premix chamber burner, sometimes called the laminar-flow burner. The fuel and support gasses are mixed in a chamber before they enter the burner.

Schematic diagram of a premix burner



The sample solution is aspirated through a capillary by the Venturi effect using the support gas (air) for the aspiration. The sample is broken into a fine spray (aerosol) at the tip. This is the usual process of nebulization. As much as 90% of the (larger) droplets condense and drain out of the chamber, leaving only 10% to enter the flame. The atomization efficiency of the (10%) portion of the sample that enters the flame is high because the droplets are finer.

Flames: The most widely used flames for atomic absorption are the air-acetylene flame and the nitrous oxide-acetylene flame with premix burners. The latter high temperature flame is not required and may even be detrimental for many cases in atomic absorption because it will cause ionization of the gaseous atoms. However, it is very useful for "refractory elements" i.e. those elements that tend to form heat-stable oxides in the air-acetylene flame. The air-acetylene and other hydrocarbon flames absorb a large fraction of the radiation at wavelength below 200 nm and an argon-hydrogen-entrained air flame is preferred for this region of the spectrum for maximum detectability. It is used for elements such as arsenic (193.5 nm) and selenium (197.0 nm). A nitrous oxide-acetylene flame offers an advantage in this region of the spectrum when danger of molecular interference exists.

Interferences: The two types of interferences encountered in atomic absorption methods are broadly classified as **spectra** interferences and **chemical** interferences.

Spectra Interferences

(i) These arise ***when the absorption or emission of an interfering species either overlaps or lies so close to the analyte absorption or emission*** that resolution by the monochromator becomes impossible. For such an interference to occur, the separation between the two lines would have to be less than about 0.1 Å. For example, a vanadium line at 3082.11 Å interferes in the determination of

aluminium based on its absorption line at 3082.15 Å. The interference can be easily avoided by observing the aluminium line at 3092.7 Å instead.

(ii) Spectra interferences also result from the ***presence of combustion products that exhibit broadband absorption or particulate products that scatter radiation***. Both reduce the power of the transmitted beam and lead to positive analytical errors. Interference caused by scattering may also be a problem when the sample contains organic species or when organic solvents are used to dissolve the sample. Here, incomplete combustion of the organic matrix leaves carbonaceous particles that are capable of scattering light.

(iii) Spectra interference because of ***scattering by products of atomization*** is most often encountered when concentrated solutions containing elements such as Ti, Zr and W, which form refractory oxides, are aspirated into the flame. Scattering of the incident beam results as metal oxide particles with diameters greater than the wavelength of light are formed.

Chemical Interferences

Chemical interferences are caused by: (i) stable compound formation, (ii) ionization, (iii) matrix effects, (iv) molecular absorption, and (v) background absorption.

(i) Stable compound formation. This leads to incomplete dissociation of the substance to be analyzed when placed in the flame. Another possibility is formation of refractory compounds in the flame which fail to dissociate into the constituent atoms e.g. determination of calcium in the presence of sulphate or phosphate, and the formation of stable refractory oxides of Ti, V and Al.

(ii) Ionization. Ionization of the ground state gaseous atoms within a flame, $M = M^{+} + e^{-}$, will reduce the intensity of the emission of the atomic spectra lines in FES, or it will reduce the extent of absorption in AAS.

(iii) Matrix effects. These are predominantly physical factors which will influence the amount of sample reaching the flame, and are related in particular to factors such as the viscosity, density, surface tension and volatility of the solvent used to prepare the test solution.

(iv) Molecular absorption. For example, in an acetylene-air flame, a high concentration of NaCl will absorb radiation at wavelength in the neighbourhood of 213.9 nm, which is the wavelength of the major zinc resonance line; hence NaCl would represent an interference in the determination of Zn under such conditions.

(v) Background absorption. The difference between the net absorption of the analyte atoms and the measured gross absorbance is called background absorbance. This is a nonanalyte signal that occurs when anything else in the sample (the matrix) reduces the intensity of radiation from the source as a result of scattering or molecular absorption, in a way that is indistinguishable from the analyte. In

other words, background absorption occurs when part of the radiation is absorbed by molecules or lost owing to scattering which results in measuring a higher gross absorbance.

Overcoming Chemical Interferences

The following factors can help to overcome *chemical interferences*:

1. Increase in flame temperature
2. Use of a releasing agent
3. Extraction of the analyte
4. Adding of an excess of an ionization suppressant
5. Adoption of matrix matching
6. Incorporation of a background correction facility, e.g. deuterium arc background correction, Zeeman background correction, Smith-Hieftje system.

ANALYTICAL AUTOMATION

In the environmental and clinical laboratories, the analyst often handles a large number of samples and process a vast amounts of data. To increase the load capacity of the laboratory, instruments are available that will automatically perform many or all of the steps of an analysis.

There are two basic types of automation equipment: ***automatic*** devices and ***automated*** devices. Automatic devices perform specific operations usually at the measurement steps in an analysis. For example, an automatic titrator will stop a titration at the end point, either mechanically or electrically, upon sensing a change in the property of the solution. On the other hand, automated devices control and regulate a process without human intervention, through mechanical and electronic devices that are regulated by means of feedback information from a sensor(s). For example, an automated titrator may maintain a sample pH at a present level by addition of acid or base as a drift from the set pH is sensed by a pH electrode. Such an instrument is called a pH-stat.

Generally, automatic instruments improve the analyst's efficiency by performing some of the operations done manually, while automated instruments control a system based on the results of the analysis.

Automated Instruments

The measurement devices may be classed as continuous or discrete (batch) instruments.

 # Continuous Instruments/Analyzers

The continuous instrument constantly measures some physical or chemical property of the sample and yields an output that is a continuous (smooth) function of time.

Process control instruments operate by means of a control loop, which consists of three primary parts:

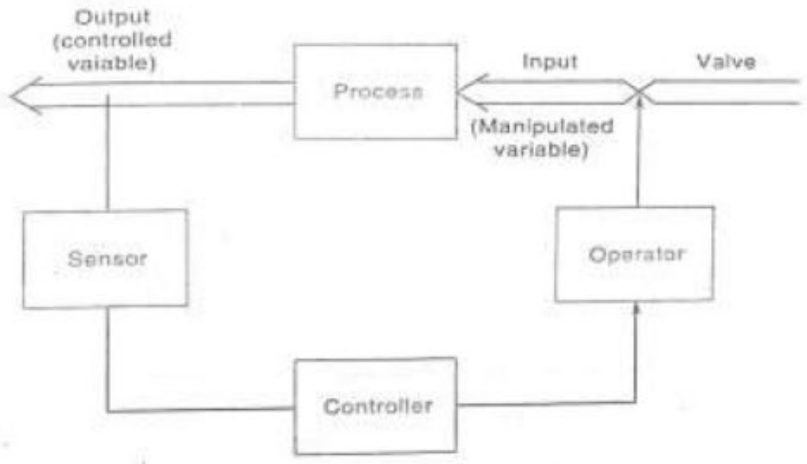
1. A sensor: a measuring device that monitors the variable being controlled.

2. A controller: compares the measured variable against a reference value (set point) and feeds the information to an operator.

3. An operator: activates some device such as a valve to bring the variable back to the set point.

The control loop operates by means of a feedback mechanism.

A feedback control loop



 # Discrete Instruments/Analyzers

In discrete (or batch) analyzers, a batch sample is taken at selected intervals and then analyzed, with the information being fed to the controller and operator. Discrete measurements are made when the sensing instrument requires discrete samples, as in a chromatograph, or a flow injection analyzer.

Instrument used in Automated process Control

Usually, the instruments used are the more robust ones designed for unattended operation, with minimal skills required for operation. However, selecting the measurement technique, the suitable instrument and obtaining reliable data are areas that require proven skills. The choice is often dictated by cost and applicability to the problem. The most widely used methods include:

Spectrophotometry: to measure colour, UV or IR absorption, turbidity, film thickness, etc.

Electrochemistry: primarily potentiometry, for the measurement of pH and cation and anion activity.

Gas and Liquid Chromatography: especially in the petrochemical industry where complex mixtures from distillation towers are monitored.

Spectrophotometric and other measurements are often rapidly made using flow injection analysis.

Automatic Instruments

Automatic instruments are not feedback control devices, but rather, are designed to automate one or more steps in an analysis. Automatic instruments relieve the analyst from several operations. The precise nature of automatic operations improves the precision.

Automatic instruments will perform one or more of the following operations:

i. Sample pickup
ii. Sample dispensing
iii. Dilution and reagent addition
iv. Incubation
v. Placing of the reacted sample in the detection system
vi. Reading and recording the data
vii. Processing of the data.

Instruments that provide only a few of these steps, primarily electronic data processing, are called semi-automatic instruments.

Automatic instruments may be classified as follows:

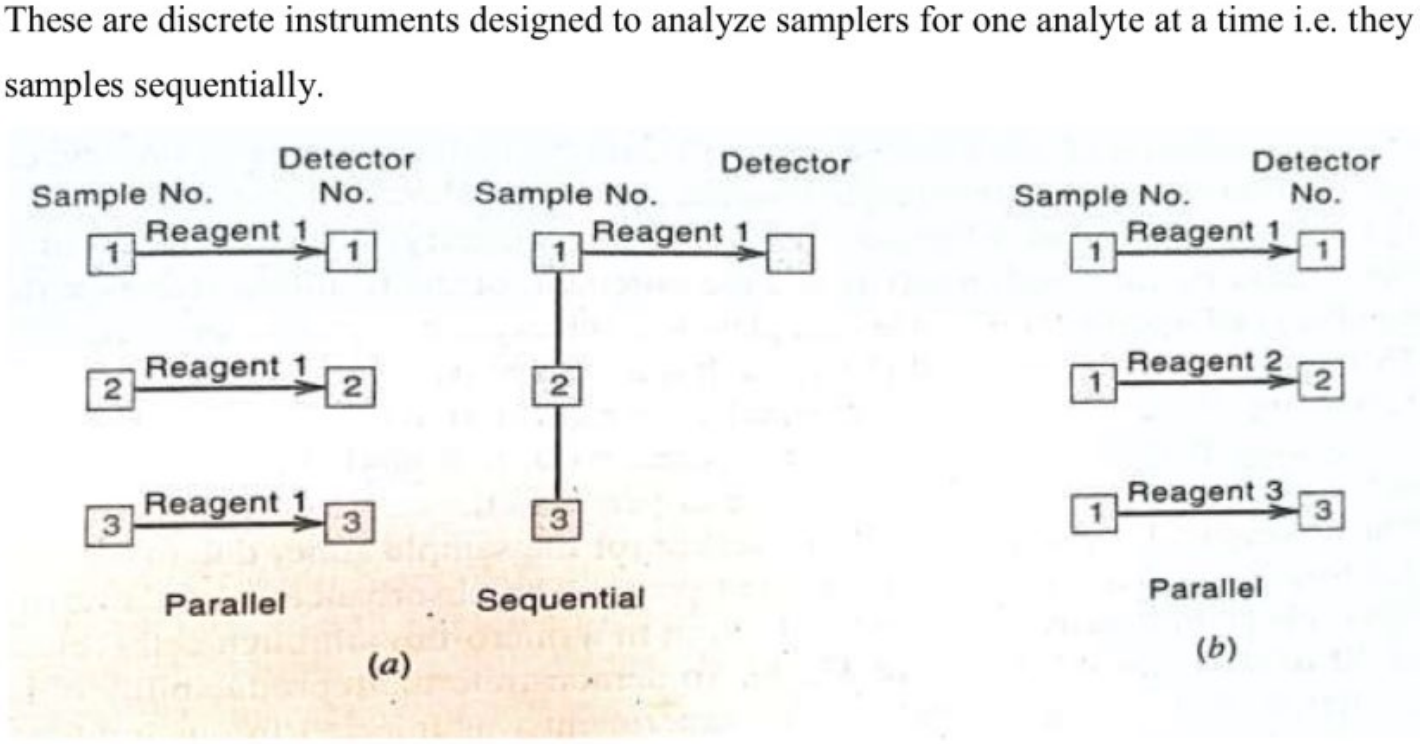
1. Discrete sampling instruments

In discrete sampling, each sample undergoes reaction (and usually measurement) in a separate curvet or chamber. These samples may be analyzed sequentially or in parallel

Discrete Samplers

Batch instruments (Single-channel analyzers)

These are discrete instruments designed to analyze samplers for one analyte at a time i.e. they analyze samples sequentially.



a = Single channel (batch) ***b*** = Multichannel

Batch instruments that analyze the samples in parallel, that is, simultaneously rather than sequentially, can analyze a large number very quickly and they can readily be changed to perform different analyses. Discrete analyzers may also analyze separate aliquots of the same sample (one in each angle) in parallel for several different analytes. These are **multichannel analyzers**.

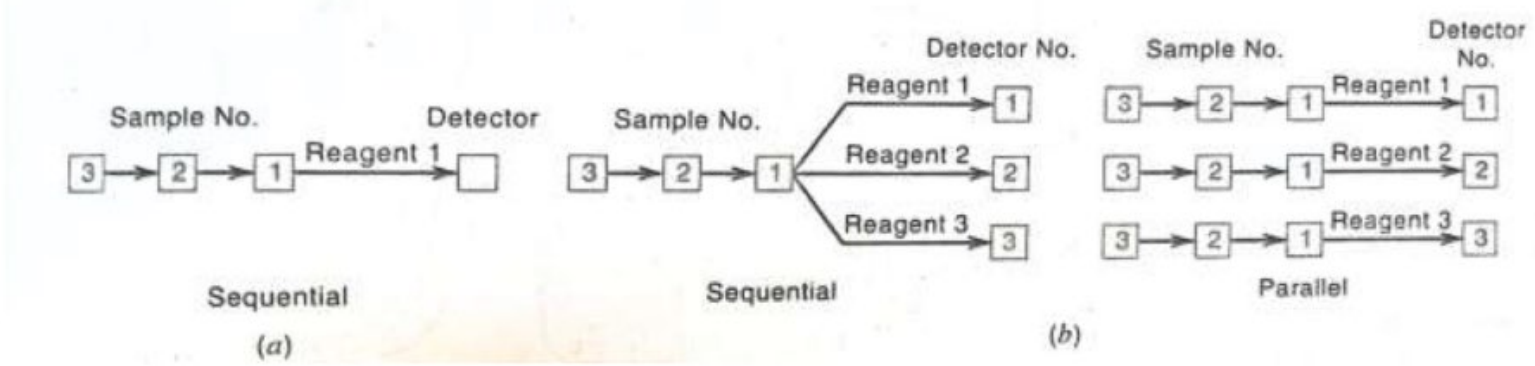
2. Continuous flow sampling instruments

In continuous-flow sampling, the samples flow sequentially and continuously in a tube, perhaps being separated by air bubbles. They are each sequentially mixed with reagents in the same tube at the same point downstream and then flow sequentially into a detector. Discrete samples have the advantage of

minimizing or avoiding cross contamination between samples. Continuous-flow instruments require fewer mechanical manipulations and can provide very precise measurements.

Continuous-flow samplers

Continuous-flow instruments may also be single-channel (batch) instruments that analyze a continuous series of samples sequentially for a single analyte, or they may be multichannel instruments in which the samples are split of one or more points downstream into separate streams for analyses of different analytes. Sometimes, separate aliquots of samples may be taken with separate streams in parallel.



Continuous-flow analyzers: (a) Single channel (batch); (b) Multichannel

Principles of Flow Injection Analysis (FIA)

Flow injection analysis is based on the injection of a liquid sample into a moving, non-segmented continuous carrier stream of a suitable liquid. The injected sample is transported toward a detector during which the reagent mixes with the sample in the flowing stream by diffusion controlled processes. In the process, a chemical reaction occurs and the detector continuously records the changes in some physical parameters (e.g. absorbance, electrode potential, etc.) of the analytes as the sample material passes through the flow cell.

A key feature of FIA is that all samples are sequentially processed in exactly the same way during passage through the analytical channel. Hence, what happens to one sample happens in exactly the same way to any other sample.

pH-Stat-Titration

This is an automated titration that uses a highly precise motor-driven piston burette to dose titrant in extremely small increments, e.g. 0.001 mL. pH-Stat-Titration refers to a situation in which the pH of the reactive media of reaction kinetics is kept stationary. It consists of the controlled addition of dilute acid or alkaline solutions to maintain a constant pH system where a pH affecting reaction is taking place. Under these conditions, the titration rate is proportional to the reaction rate.

The study of the kinetics of a chemical reaction is one of the simplest and quickest ways of measuring the efficiency of active ingredients in drugs for pharmaceutical industry, e.g. the measurement of

enzyme kinetics and activity of antacid products. Also, in organic and mineral chemistry, kinetics of dissolution of fertilizers or solubility of an additive to cattle feed to test its digestibility can be monitored.

In the vast majority of reaction kinetics, the major concern has to do with production or consumption of H_3O^+ or OH^- ions. Their speed of formation depends on the operating conditions, particularly the pH of the reactive media. A pH-stat study involves the following:

- determining an optimum value for the pH of the studied reaction;
- keeping the pH constant by adding a reagent to neutralize H_3O^+ or OH^- ions; and
- calculating the kinetics of the studied reaction based on the consumption of reagent required to keep the pH constant over time.

Although always called pH-stat, this technique also applies to other ions, cations or anions, whenever an electrochemical sensor is present. In this case, the pH electrode is replaced by an ion-selective or metal electrode.

Theory of pH-Stat Titration

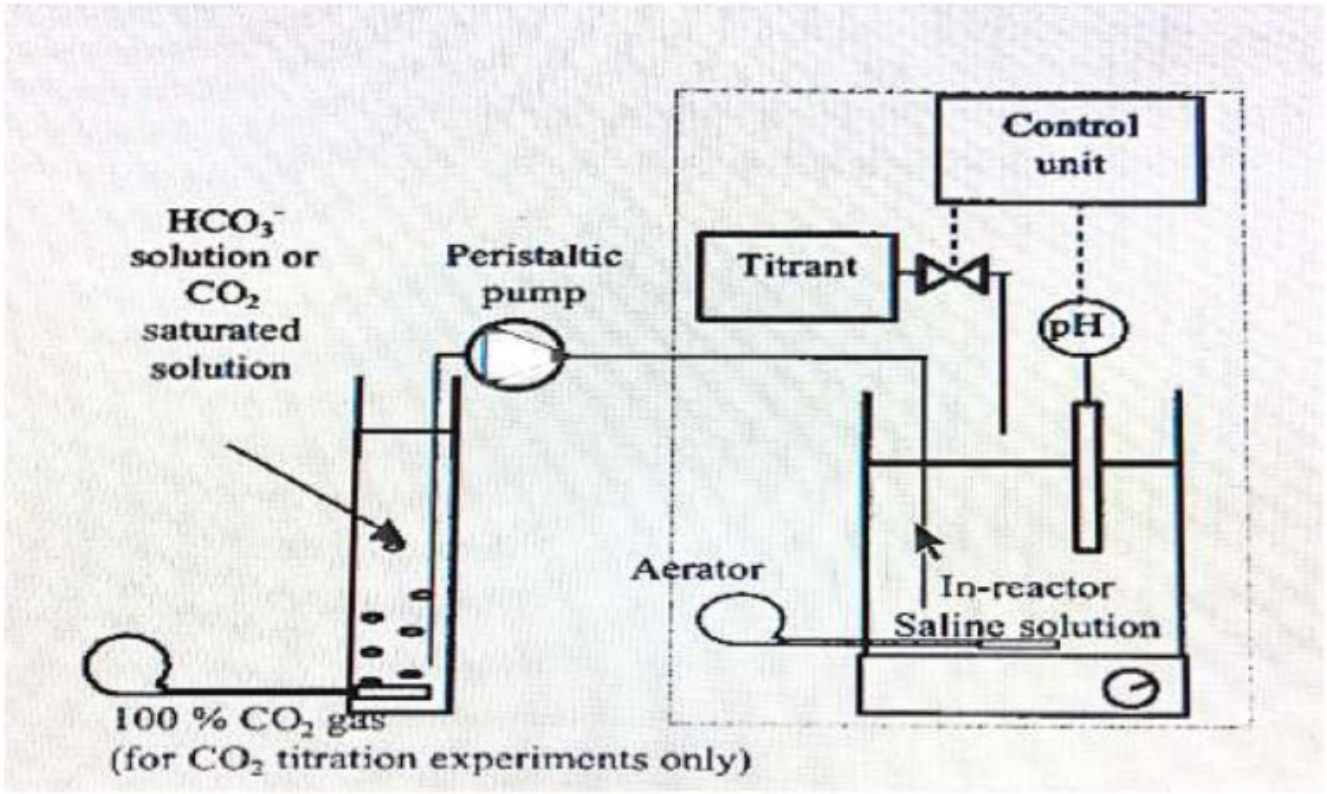
The pH-stat titration technique consists of the controlled addition of dilute acidic or alkaline solutions to maintain a constant pH in systems where a pH affecting reaction is taking place. Under these conditions, the titration rate is proportional to the reaction rate. This technique is applicable to any biological or physico-chemical reaction affecting the proton concentration, i.e. any reaction converting neutral substrates into acidic or basic products, or acid or basic substrates into neutral products.

There are several bioreactions of environmental interest which affect the pH of biological suspensions. So far, research on pH-stat titrations has been mainly focused on the assessment of protons producing or consuming reactions e.g. nitrification and denitrification. However, production or consumption of H_3O^+ or OH^- is not the only way to affect the pH in a reactor. For instance, any reaction involving the $CO_2/HCO_3^-/CO_3^{2-}$ equilibria, such as reactions producing or consuming CO_2 or HCO_3^-, also result in a pH variation and can be monitored by pH-stat titration. Heterotrophic degradation of organic substrates produces CO_2, acetoclastic methanogenesis produces HCO_3^-, growth of chemolitotrophic substrates produces CO_2, while the **anaerobic ammonia oxidation** (ANAMMOX) process consumes CO_2 and produces bicarbonate.

The pH-Stat Unit

This consists of bioreactor, where the biomass is maintained at a constant temperature and mixed at a constant rate, a pH-meter, and a titration control unit.

Diagram of a pH-stat bioreactor unit



According to the pH signal, a control unit activates the addition of an appropriate titrant (a diluted acid or alkaline solution) to maintain the pH constant at a pre-set value (pH_{stat}). The system is thus a fed-batch reactor with liquid inflow (the titrant added) and no liquid outflow. Gas/liquid mass-transfer processes, such as gas sparging or gas production by the biomass, may also play a significant role. When gas sparging is present, the system is defined as open; whereas in a closed system, no liquid/gas mass transfer takes place.

In the bioreactor, a generic reaction takes place which converts substrate **S** into product **P**, with the concomitant production of protons, carbon dioxide, bicarbonate, and carbonate. These products affect the $CO_2/HCO_3^-/CO_3^{2-}$ equilibria, and thus, the suspension pH.

$$\mathbf{s}S \rightarrow P + \mathbf{b}HCO_3^- + \mathbf{c}CO_2 + \mathbf{d}CO_3^{2-} + \mathbf{h}H^+$$

where b, c, d and h are positive when the corresponding chemical species is produced, or negative when it is consumed. Coefficient **s** for the substrate is always positive.

Applications of pH-Stat

A. In the laboratory, the instrument is used to:

(i) monitor the kinetics by using small volumes of titrant;

(ii) store the reagent consumption curve versus time in order to make the necessary calculations;

(iii) measure the activity of certain effervescent drugs e.g. antacids;

(iv) monitor the activity of enzymes such as lipase, cholinesterase, or trypsin;

(v) study the efficiency of certain toothpastes by measuring the precipitation kinetics of dicalcium phosphate;

(vi) study the complexing power of various organic constituents of soil;

(vii) determine the dissolution or precipitation kinetics of various minerals;

(viii) monitor the digestibility of various milk proteins; and

(ix) measure the activity of yeast in food products.

B. In the pilot plant (preparatory chemistry)

Here it is necessary to work with a regulation system that can:

(i) monitor large volumes of titrant over long periods (over 12 hours). This can be achieved either by using peristaltic pumps or by a dual-burette system working alternately (second burette operational during refill of first) allowing permanent control of the process;

(ii) use two competing reagents (in separate burettes) in order to be able to monitor a process liable to evolve in different directions versus time.

SEPARATION METHODS OF ANALYSIS

If it were possible to identify or quantitatively determine any element or compound by simple measurement, no matter what it's concentration or the complexity of the matrix, separation technique would be of no value to the analytical chemist.

But most techniques fall short of this ideal, because of the interference with the required measurement by other constituents of the sample. Many techniques for separating and concentrating the species of interest have just been devised. Such techniques are in at exploiting differences in physicochemical properties between the various components of mixture volatility; solubility, charge, molecular size, shape, and the polarity are the most useful. In this respect, a change of phase has occurred during distillation or formation of new phase as in precipitation, can provide a simple means of isolating a desired component.

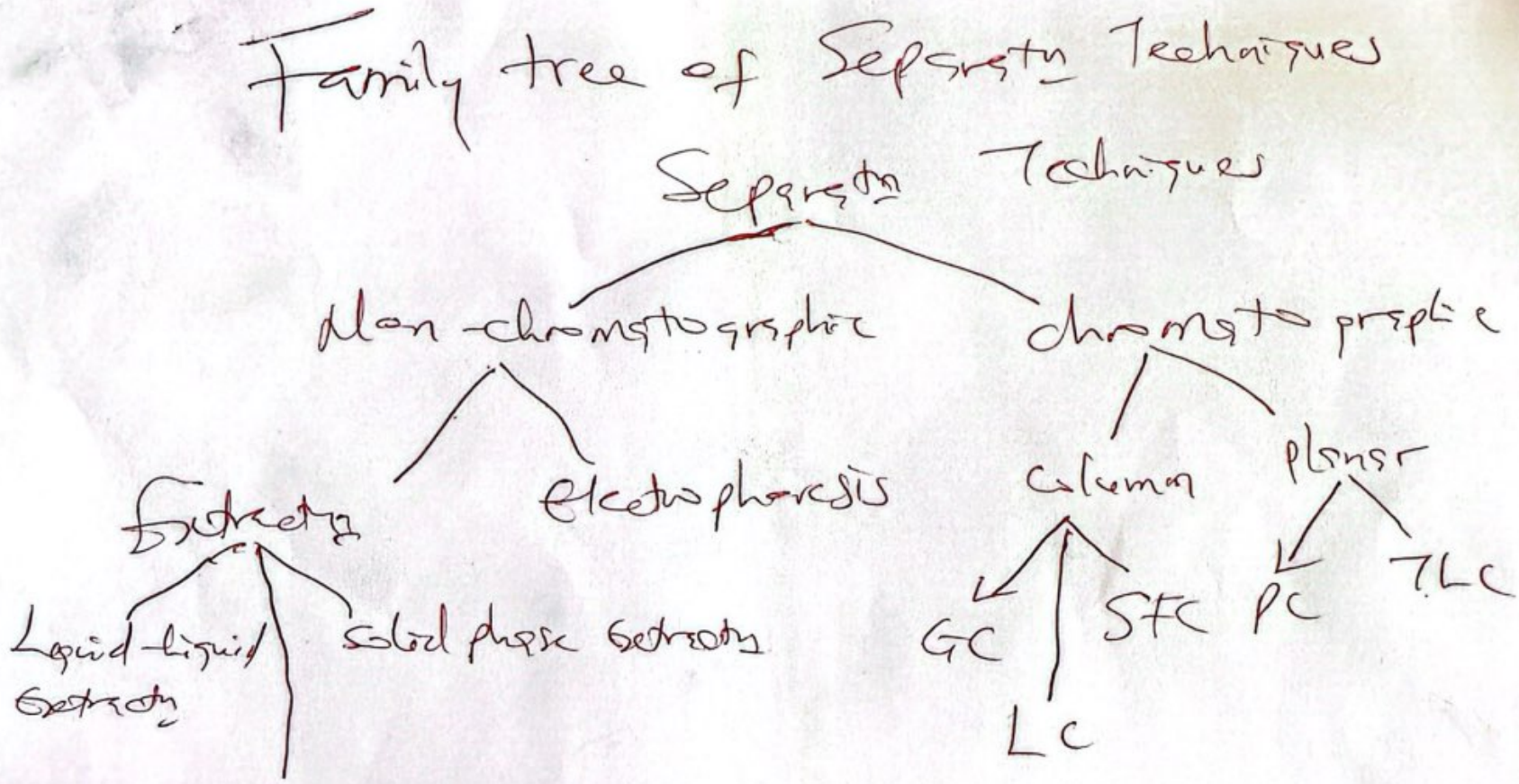
Usually, more complex separation procedures are required for multi component sample most depend on the selective transfer of materials between two immiscible phases. The most widely used techniques and the phase system associated with them are summarized as follows:

Classification of separation techniques

S/NO	Technique	Phase system
1.	Solvent extraction	Liquid - liquid
2.	Gas chromatography	Gas - liquid
3.	Liquid chromatography	Liquid - liquid Liquid – solid
4.	Thin - layer chromatography	Liquid – solid Liquid - liquid
5.	Ion – exchange and gel – formation chromatography	Liquid - solid Liquid - liquid
6.	Supercritical fluid chromatography and	Supercritical fluid - liquid or solid – liquid.
7	Electrophoresis	liquid

All separation techniques involve one or more achieve chemical equilibrium. Consequently, the degree of separation achieved can vary greatly according to experimental condition to a large extent; attainment of optimal condition to a large extent, attainment of optimal condition has to be approached empirically

Family tree of Separation Techniques



rather than by the application of a rigid theory in the following section which deals with solvent extraction, chromatography and electrophoresis. The minimum theory necessary for an understanding of the basic principle is presented.

SOLVENT EXTRACTION

Solvent extraction is a selective transfer of ug to g in quantity between 2 immiscible phases (liquid). Separation is based on solubility differences and selectivity is achieved by pH control and complexation.

Separating funnel is used for batch extraction and special glass apparatus, soxhlet (for cont. extraction). Batch methods are rapid, simple and versatile.

Disadvantages of batch method.

(1) It sometimes requires large quantities of organic solvents.

(2) Poor resolution of mixture of organic components.

THEORY

Solvent extraction is sometimes called liquid extraction. It involves the selective transfer of a substance from one liquid phase to another e.g. aqueous solution of iodine and sodium chloride is shaking with ccl4 (carbon tetra-chloride) and the liquid allowed to separate. Most of the iodine will be transferred to ccl4 layer and will sodium chloride will remain in aqueous layer. The extraction of soxhlet is governed by Nernst partition or distribution law which states that at equilibrium, a given solute will always be distributed between two essentially immiscible liquid in the same proportion.

$$\frac{[A]\ \text{organic}}{[A]\ \text{aqueous}} = K_D \qquad \text{(same temperature and pressure)}$$

Where [ ] represents the concentration and K_D is "partition coefficient" and it is independent of the concentration of the solute.

If $A_{x_0} \longrightarrow A_{aq}\ {}_{x_1} + A_{org}\ {}_{x_0 - x_1}$

$$K_D = \frac{[A_{org}]}{[A_{aq}]} = \left(\frac{x_0 - x_1}{V_{org}}\right) \Big/ \frac{x_1}{V_{aq}} = \left(\frac{x_0 - x_1}{x_1}\right) \Big/ \left(\frac{V_{org}}{V_{aq}}\right)$$

$$K_D\left(\frac{V_{org}}{V_{aq}}\right) = \frac{x_0 - x_1}{x_1} = \frac{x_0}{x_1} - 1$$

$$1 + K_D\left(\frac{V_{org}}{V_{aq}}\right) = \frac{x_0}{x_1}$$

$$x_1 = \frac{x_0}{1 + K_D\left(\frac{V_{org}}{V_{aq}}\right)} = x_0\left[\frac{1}{1 + K_D\frac{V_{org}}{V_{aq}}}\right]$$

Note:

(1) constant Temp & Pressure should be assumed or maintained

(2) 'A' must exist in the same form in both phases. Equilibrium is established when the chemical potential (free energy) of the solute in the two phases are equal and is usually achieved within a few minutes of shaking. The value of K_D is a reflection of the relative solubilities of the solute in the two phases.

The value of K_D breaks down if 'A':

1. dissociates
2. polymerizes
3. form complexes with other components e.g. solvents.

Analytically, the total amount of solute present in each case is better described in distribution 'D',

Where $D = \frac{[C_A]_o}{[C_A]_{eq}}$ CA = amount of solute

If there is no interaction, $D = K_D$.

Efficiency of Extraction.

It depends on the magnitude of D or K_D and on the relative volumes of the liquid phases

The % of extraction is given by:

$$E = \frac{100D}{[D + \frac{Vaq}{Vo}]}$$

For equal volumes of aqueous and organic solvent,

$$E = \frac{100D}{[D+1]}$$

If D is large i.e. tending towards 100, a single extraction may affect virtually quantitative transfer of the solute, whereas with small values of D, several extractions would be required. The amount of solute remaining in aqueous solution is readily calculated for any number of extractions with equal volumes of organic solvents from the following equation.

$$[C_{aq}]_n = C_{aq}\left[\frac{Vaq}{(DVo+Vaq)}\right]^n$$

Where $[Caq]_n$ is the amount of solute remaining in aqueous phase.

Vaq = the volume of aqueous solvent after n extraction
Vo = the volume of organic solvent after n extraction.

If the value of D is known, the equation above is useful in determining the outmost condition for quantitative transfer of material.

For example, the complete removal of 0.1g of iodine from $50cm^3$ of an aqueous solution of iodine and sodium chloride is carried out using $CC1_4/H_2O$; the value of D was given as 85.

Calculate the efficiency using:

1. $25cm^3$ of CCl_4 once
2. Batch extraction three times.

Which method out of the two would be most efficient?

1. $\{Caq\}1 = 0.1\left\{\frac{50}{(85 \times 25)+ 50}\right\}^n$

$= 0.0023$

% efficiency $= \frac{0.1-0.0023}{0.1} \times 100$

$= 97.7\%$

2. $\{Caq\}3 = 0.1\left\{\frac{50}{(85 \times \frac{25}{3})+ 50}\right\}^3$

$= 2.86 \times 10^{-5}$

% efficiency $= \frac{0.1-2.86s10^{-5}}{0.1} \times 100$

$= 99.9\%$

It is therefore clear that extracting several times with small volumes of organic solvent is more efficient than one extraction with large volume.

CHROMATOGRAPHY

Analytical chemists have few tools as powerful as chromatography to measure distinct analytes in complex samples. The power of chromatography comes from its ability to separate a mixture of compounds, or "analytes", and determine their respective identity (chemical structure) and concentration. Chromatography can be divided into three basic types that include gas, liquid, and supercritical fluid chromatography. This mobile phase is then forced through a stationary phase, which is fixed in place in a column or on a solid surface. The two phases are chosen so that the components of the sample distribute themselves between the mobile and stationary phase to varying degrees. Liquid chromatography can further be divided into ion exchange, separations based on size, and even extended to gel based electrophoretic techniques.

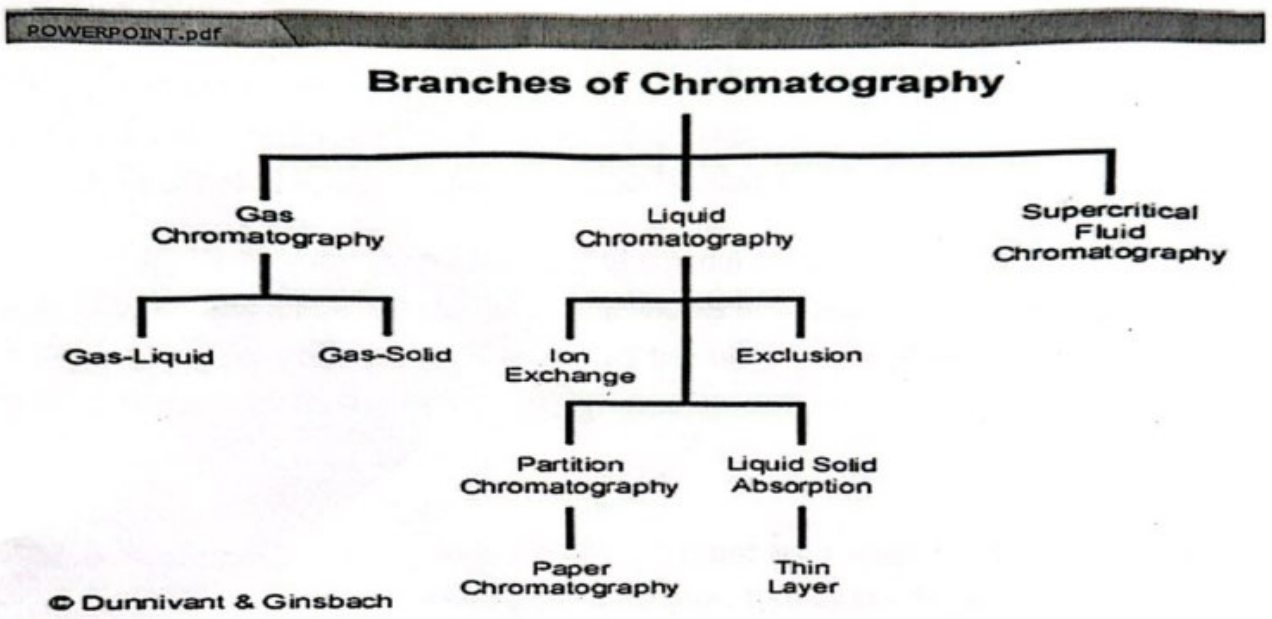


Figure 1: Categories of Chromatography

In general, each type of chromatography is comprised of two distinct steps: chromatography (or separation of individual compounds in distinct elution bands) and identification (detection of each elution band). During a chromatographic separation solute molecules are continually moving back and forth between the stationary and mobile phases. The rate of migration of each solute is therefore determined by the proportion of time it spends in the mobile phase, or in other words by its distribution ratio.

Characterization of Solutes

The process whereby a solute is transferred from a mobile to a stationary phase is called sorption.

As already described, the rate of movement of a solute is determined by its distribution ratio defined as

$$D = \frac{\text{Cstationary phase}}{\text{Cmobile phase}}$$

The larger the value of D, the slower will be the progress of the solute through the system, and the components of a mixture will therefore reach the end of a column or the edge of a surface in order of increasing value of D. In column methods, a solute is characterized by the volume of mobile phase required to move it from one end of the column to the other, known as the retention volume, V_R, it is defined as the volume passing through the column between putting the sample on the top of the column and the emergence of the solute peak at the bottom. It is given by the equation:

$V_R = V_M + kV_M$

Where V_M is the volume of mobile phase in the column (*the dead or void volume*) and k is the retention factor which is directly proportional to D but takes account of the volume of each phase. Sometimes k is used to characterize a solute rather than V_R.

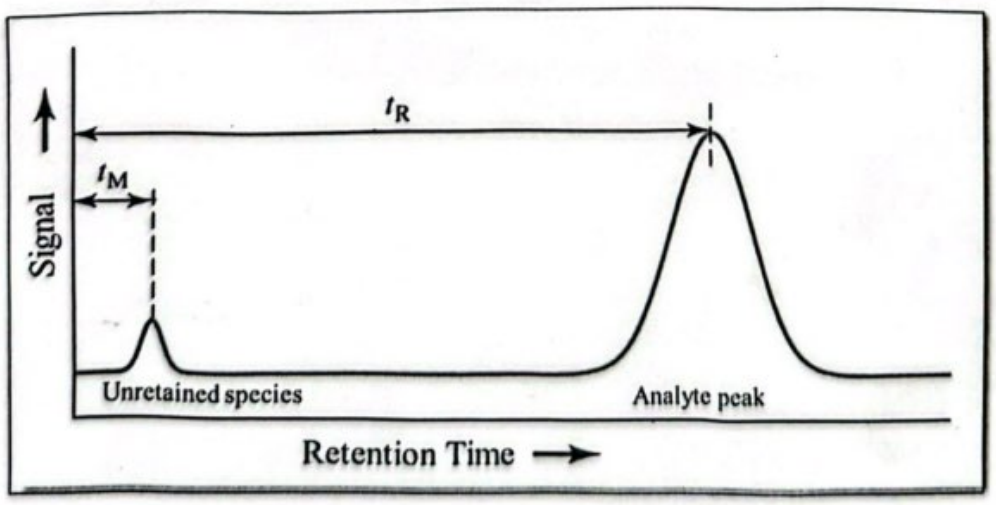
If $k = 0$, then $V_R = V_M$ and the solute is eluted without being retarded or retained by the stationary phase. Large values of k, which reflect large values of D, result in very large retention volumes, and hence long retention times. At a constant rate of flow of mobile phase F, V_R is related to the retention time, t_R by the equation

$V_R = Ft_R$

If the flow of mobile phase is monitored by a detector and recorder/VDU system, such as is used in gas and high-performance liquid chromatography, then T_R can be used as a measure of V_R

Retention time (t_R): is the time it takes after sample injection for the analyte to reach the detector.

Dead time (t_M): is the time for an unretained species to reach the detector.



The retention factor, or capacity factor, is an important parameter that is widely used to describe the migration rates of solutes on columns.

For a solute A, the retention factor (k') is defined as:

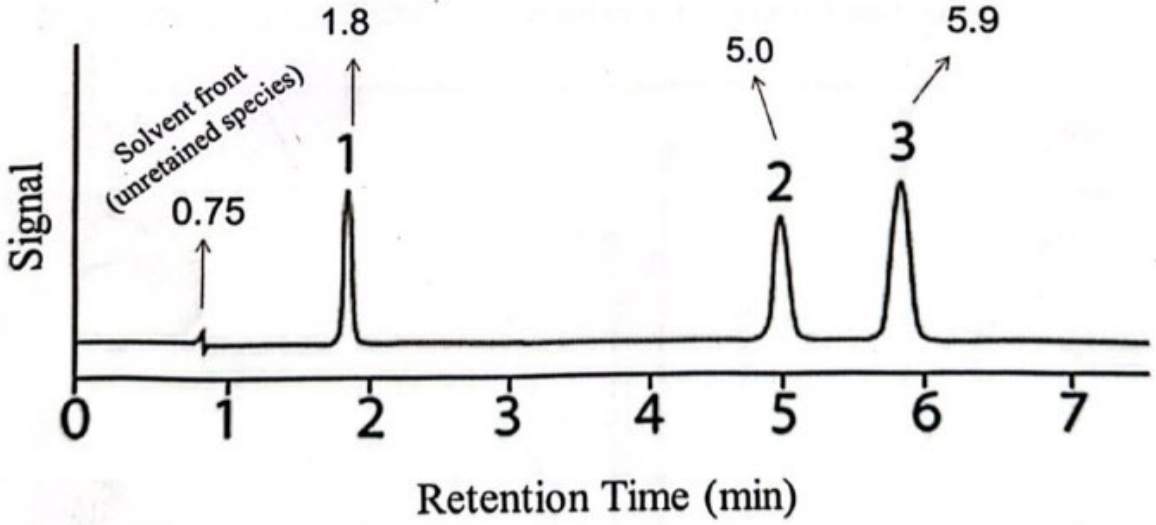
$$k'A = \frac{t_R - t_M}{t_M}$$

t_R and t_M can be readily obtained from the chromatogram

$$2 \leq k'A \leq 10$$

- When the retention factor for a solute is less than 1.0, elution occurs so rapidly that accurate determination of the retention times is difficult.
- When the retention factor is larger than perhaps 20 to 30, elution times become inordinately long
- Ideally, separations are performed under conditions in which the retention factors for the solutes in a mixture lie in the range between 2 and 10.

Example 1: Calculate the retention factor (') for the peaks 1, 2 and 3 in the chromatogram shown below.



Solution

$$k'_A = \frac{t_R - t_M}{t_M}$$

$$k'_1 = \frac{t_{R,1} - t_M}{t_M} = \frac{5.1 - 1.7}{1.7} = 2.00$$

$$k'_{11} = \frac{t_{R,11} - t_M}{t_M} = \frac{25.5 - 1.7}{1.7} = 14.0$$

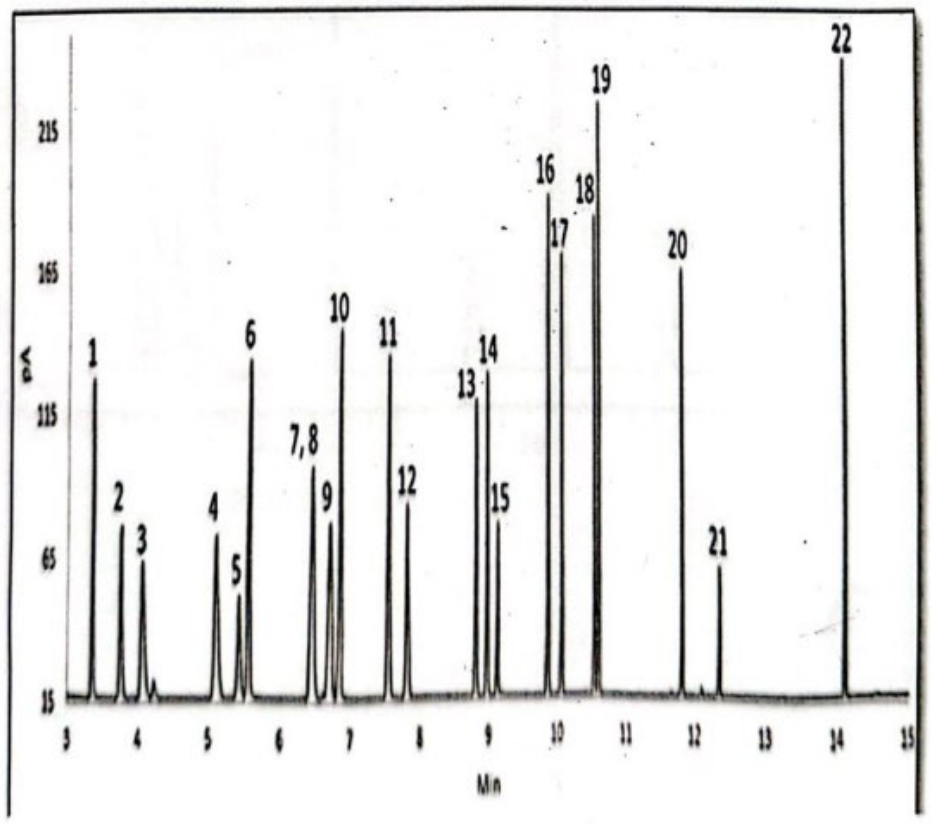
Conclusion

Since $k'1$ is equal to 2.00, peak 1 is suitable for quantitation. However, since $k11'$ *is larger than* 10, peak 11 is not.

Chromatograms

If a detector that responds to solute concentration is placed at the end of the column and its signal is plotted as function of time, a series of peaks is obtained. Such a plot, called a chromatogram, is useful for both qualitative and quantitative analysis. The positions of peaks on the time axis may serve to identify the components of the sample (qualitative analysis). The area under the peak (or peak height) provides a quantitative measure of the amount of each component.

An example of a chromatogram



Peaks	Peaks
1. Ethanol	13. Methyl methacrylate
2. Allyl chloride	14. n-Propyl acetate
3. Acetone	15. 2-Chloroethanol
4. Acrylonitrile	16. 4-Methyl-2-pentanone
5. Vinyl acetate	17. Ethyl methacrylate
6. Allyl alcohol	18. n-Butyl acetate
7. Ethyl Acetate	19. 2-Hexanone
8. Methyl acrylate	20. 1-Bromo-4-fluorobenzene
9. 2-Butanone	21. Pentachloroethane
10. Propargyl alcohol	22. Nitrobenzene
11. Isobutyl alcohol	
12. Isopropyl acetate	

1

$$k'_A = \frac{t_R - t_M}{t_M}$$

$$k'_1 = \frac{t_{R,1} - t_M}{t_M} = \frac{1.8 - 0.75}{0.75} = 1.40$$

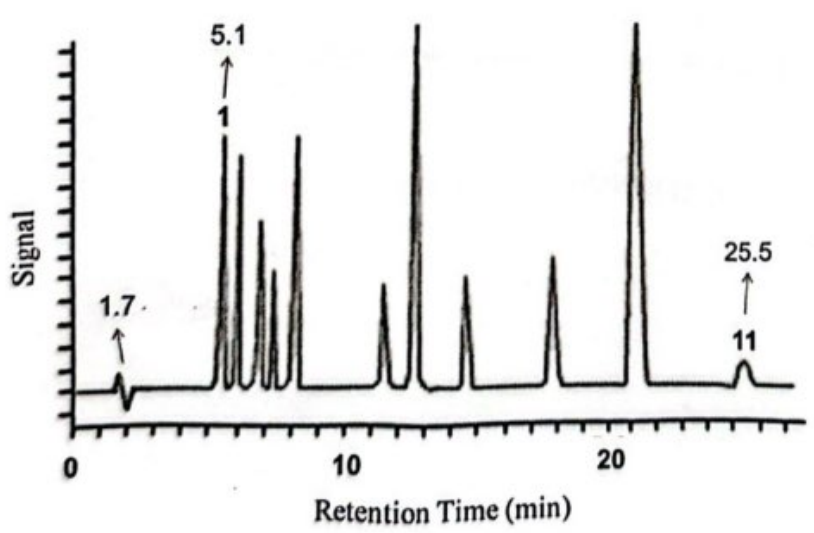
$$k'_2 = \frac{t_{R,2} - t_M}{t_M} = \frac{5.0 - 0.75}{0.75} = 5.67$$

$$k'_3 = \frac{t_{R,3} - t_M}{t_M} = \frac{5.9 - 0.75}{0.75} = 6.87$$

Conclusion

Since all of *k'* values for 2 and 3 lie in the preferred range of 2-10, the peaks are suitable for quantitation. However, peak 1 is not.

Example 2: Calculate the retention factor for the peaks 1 and 11 in the chromatogram shown below. Comment on the quality of those peaks for quantitation.



Solution

In paper and thin-layer chromatography, the separation process is halted at a stage which leaves the separated components *in situ* on the surface in the form of spots. The rate at which a solute has moved is then determined by its retardation factor, R_f, which is defined as

$$R_f = \frac{\text{distance travelled by the centre of the solute spot}}{\text{distance travelled by the front of the mobile phase}}$$

It is inversely related to D and cannot be greater than 1. Distances are measured from the point of application of the sample. As both t_R and R_f are related to D they will depend on the conditions under which a chromatogram is run.

Chromatographic techniques are based on four different sorption mechanisms, namely surface adsorption, partition, ion-exchange and exclusion.

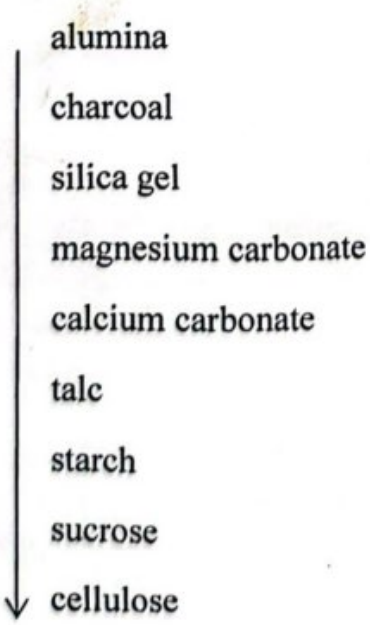
Stationary and Mobile Phases

There is a very wide choice of pairs of liquids to act as stationary and mobile phases. It is not necessary for them to be totally immiscible, but a low mutual solubility is desirable. A hydrophilic liquid may be used as the stationary phase with a hydrophobic mobile phase or vice versa.

Stationary Phase

Almost any polar solid can be used, the most common choices being silica gel or alumina. A selection of stationary phases is listed in Table 1 in order of decreasing adsorptive power. Silica gel and alumina are highly polar materials that adsorb molecules strongly. They are said to be active adsorbents. Activity is determined by the overall polarity and by the number of adsorption sites.

Table 1: Adsorbents used in column chromatography



The nature of stationary phase and its degree of activity is determined by the nature of the sample.

Mobile Phase

The eluting power of a solvent is determined by its overall polarity, the polarity of the stationary phase and the nature of the sample components. Table 2 lists some widely used solvents in order of their eluting power, this being known as an eluotropic series.

Table 2: An example of an eluotropic series

Solvent	UV cut off mm	R1 25°C	Viscosity C_p	Solvent polarity (P'. Partion –based)	Solvent polarity (ε^0, adsorption-based)
n-hexane	190	1.372	0.30	0.1	0.01
cyclohexane	200	1.423	0.90	-0.2	0.04
carbon tetrachloride	265	1.457	0.90	1.6	0.18
toluene	285	1.494	0.55	2.4	0.29
benzene	280	1.498	0.60	2.7	0.32
methylene chloride	233	1.421	0.41	3.1	0.42
n-propanol	240	1.385	1.9	4.0	0.82
tetrahydrofuran	212	1.405	0.46	4.0	0.57
ethyl acetate	256	1.370	0.43	4.4	0.58
iso-propanol	205	1.384	1.9	3.9	0.82

Eluotropic series: Such series are useful for determining necessary solvents needed for chromatography of chemical compounds. Normally such a series progresses from non-polar solvents, such as n-hexane, to polar solvents such as methanol or water

Detectors

The purpose of a detector is to monitor the carrier gas as it emerges from the column and respond to changes in its composition as solutes are eluted. Ideally, a detector should have the following characteristics:

- Rapid response to the presence of a solute
- A wide range of linear response
- High sensitivity
- Stability of operation

Most detectors are of the differential type that is their response is proportional to the concentration or mass flow rate of the eluted component. The signal from the detector is fed to a chart recorder, computing integrator or VDU screen via suitable electronic amplifying circuitry

Introduction: chromatographic techniques are used widely across scientific disciplines and in industry for multiple applications, some of which may even be overlapping. Broadly classified into planar and column chromatography, the general principle involves the separation of components (i.e. analytes of interest) of a mixture (of widely varying constitution e.g. peptides, lipids, pigments etc.) based on their relative solubility in, and hence movement through "phases" i.e. mobile and stationary, used in the technique. Further, chromatography is divided into gas and liquid based on the nature of the mobile phase. When the mobile phase is liquid then the technique is referred to as "liquid chromatography" and when the mobile phase is gaseous then as "gas chromatography" (GC). The idea of using gas chromatography as an analytical tool was originally introduced by Martin and Synge in the 1940s who then demonstrated its utility by separating a mixture of fatty acids using GC. Gas chromatography is a highly sensitive technique that is routinely used in industrial laboratories for quantification of compounds, quality control and in forensic laboratories for detection of many chemicals that are present in extremely small amounts.

The mobile phase in gas chromatography is an inert gas that does not interact with the analytes in the mixture to be separated. If the gas phase moves over a solid stationary phase, then it is called "gas solid chromatography". If the gas moves over a non-volatile liquid phase coated over a support, then it is referred to as "gas-liquid chromatography".

Principle of gas chromatography

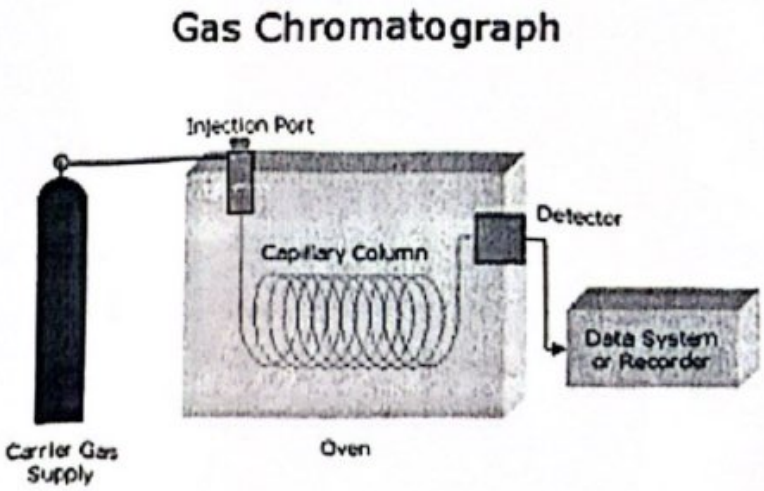
The underlying premise of separation using GC is based on the presence of analytes that are volatile (or modified i.e. *derivatized* to be volatile) at the temperatures at the point of injection. These analytes are typically dissolved in a solvent, and small amounts (typically in microliters) are injected into the gas chromatograph through an inlet. The separation will depend how strongly the compounds in the injected sample interact with the stationary phase. A stronger interaction means a longer interaction with the stationary phase and hence a longer time to migrate through the column and exit the column.

Each of the components of the sample leaves the column at a certain time called its "*retention time*". The exit of analytes from the column is perceived by a detector and converted into electrical signals for each of the analytes exiting the column which are visible as peaks on the recorded output of the process. This output is called the "*chromatogram*". There are many kinds of detectors(D) used in gas chromatography e.g. electron capture (ECD), flame ionization (FID), thermal couple (TCD), Nitrogen-Phosphorus (NPD) and mass spectrometry (MS).

The gas chromatographic system:

Overview: the system consists of an injection port, an oven containing the column which may operate isothermally or maybe temperature programmed, an exit port, and a detector that detects the analytes coming out of the column. In addition, gas cylinders for maintaining the flow of the mobile phase i.e. gas, are also connected to the oven containing the column.

Figure 1: Schematic diagram of a gas chromatographic system



Components:

Carrier gas: is the medium in which the sample mixture is carried along the column, and flows out. The carrier gas should be inert, should allow a sufficient detector response and from a practical perspective, also be low-cost. The gases usually selected as carrier gases are helium or nitrogen although some other gases have also been used. Certain types of detectors such as the

Flame Ionization Detector also need a source of combustion for the detector to function (i.e. the flame to be lighted). The gas used for this is hydrogen.

The injection port is where the sample is introduced into the column (i.e. the column head). In modern gas chromatographs, the port is usually heated so that sample injection and vaporization can take place simultaneously. The sample volumes typically injected are in the range of a few microliters through a rubber septum and into the vaporization chamber. Depending on whether the whole sample is needed (uncommon) for analysis or only a small amount is needed, the injection can be split less or split by means of a splitter that directs excess sample to a waste collector.

Column: the column is the heart of the gas chromatography based separation process. There are hundreds of types of columns available with varying chemical composition and lengths. They are broadly categorized into "packed" and "capillary". In the initial decades of analytical gas chromatography, **packed columns** were used, in which a glass or metal (mostly stainless steel but also aluminium, copper, polytetrafluorethylene) column tubing is packed with small spherical inert supports (e.g. diatomaceous earth). The liquid phase adsorbs onto the surface of these beads in a thin layer. Packed columns have higher sample capacity[1]. However, packed columns are usually less efficient and have lower resolution as compared to capillary columns. They are often used for preparative work and gas analysis. Their inner diameters range from 1.5 mm to 6 mm. A category of micro-packed columns are also available. They are micro pore tubes having inner diameters ranging from 0.3-1 mm and at lengths varying from 1 to 15 m, packed with particles 0.007-0.3 mm in diameter.

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[1] The amount of sample that can be applied to a column without overloading.

The other kind of columns, called "**capillary columns**" are more in use nowadays and consist of a tubing, the walls of which are coated with the stationary phase or an adsorbent layer, which supports the liquid phase. Stationary phase thickness is typically 0.25 microns. Capillary columns have a higher efficiency and hence better peak separation than packed columns. Capillary columns are

categorized into "The Wall Coated Open Tubular (WCOT)" columns, "Support Coated Open Tubular" (SCOT) and the "Porous Layer Open Tubular (PLOT)" columns. Capillary columns have inner diameters ranging from 0.10 to 0.20 mm and lengths including 10 m, 15m, 30m, 50 m, upto 15m depending on the inner diameter. A very efficient, popular type of WCOT column is the fused- silica wall-coated (FSWC) open tubular column that is chemically very inert sample sizes
The columns are typically packed or coated with solid material which could perform the role of adsorbent (Gas Solid chromatography), in which case the separation takes place by adsorption, or the solid material could be support for stationary phase (which is a non-volatile liquid), as in Gas Liquid Chromatography, coated on a granular material, in which case the separation takes place by absorption.

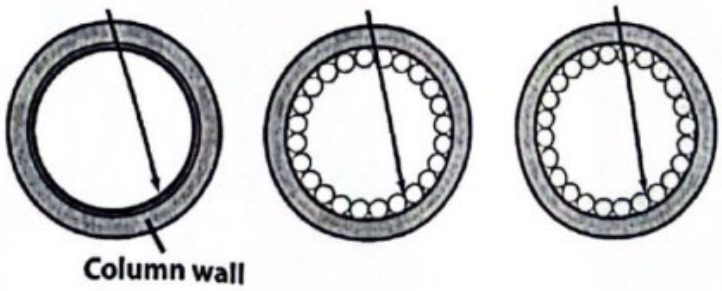
A vast array of solid and liquid stationary materials has evolved for use over time, numbering into hundreds. These are sometimes naturally occurring materials such as Kieselguhr (diatomaceous earth based, that contain polysilicic acid as hydrated amorphous silica with a porous structure and varying amounts of metal oxides of Fe, Al, Mg, Ca, Na, K) or synthetically created polymers such as Squalane, teflon. If a liquid is chosen as the stationary phase, it should dissolve all the components of the samples differentially, be practically non-volatile at the temperature of the column, be chemically inert and have high thermal stability. The liquid may be non-polar (e.g. silicone oils methylsilicone type), polar (polyethylene glycol, silicone oils with cyanopropyl groups) or of intermediate polarity (e.g. phenyl methyl silicone phase, dinonyl phthalate). The organic compounds are also empirically classified based on their chromatographic separation from Class I (very polar) to Class V (non-polar).

The suitability of the packing/coating material of the column for analysis of the sample mixture will depend on the chemical (and occasionally, structural) nature of the analytes in the mixture so as to allow an interaction between the stationary phase so that retention times vary as per the respective degrees of interaction between the analyte and stationary phase. In case the analyte is

polar, the stationary phase should be polar and if the analyte is non-polar, then a non-polar/less-polar stationary phase should be chosen. Since compounds vary in their polarity in the same mixture, a compromise is often made while choosing the stationary phase.

The stationary phases on the GC columns need to be conditioned and activated by allowing the mobile phase to flow over the column before the samples for analysis are applied onto the column for separation. Also, while using the columns, care must be taken to understand the robustness of the column chemicals with regard to its thermal stability and also the effect of certain corrosive chemicals that may poison the column (e.g. oxygen, chromic acid, potassium hydroxide, perfluoroacids). To minimize changes of such damage, a guard column can be installed ahead of the main column. Sometime trimming a few feet of the initial part of the column can remove the damaged section.

Fig 2: Types of GC columns and their stationary phases:



[redacted]:

Analysis: The quantitative analysis of a given component is based upon evaluating the chromatographic peak, which is triangle-shaped when columns with filling are used; its surface is measured and divided by the total surface, in different percentages for different types of detectors. For capillary columns with good resolution, the signal takes the shape of straight lines and calculating the composition of the mixture is done in the order of succession, dividing each

individual line by the total number of lines and using an adequate calibration curve, drawn upon determinations of known compounds

Mixture components can be identified by comparison using chromatographic standards – that are pure substances or known mixtures of components. In these cases, universal detectors may be used. For unknown mixtures, mass spectrometry is recommended.

Detectors: A wide range of detectors are available that are linked to gas-chromatography systems and are of varying sensitivities and applicationThe background levels (i.e. noise) and interferences are very low because the carrier gases used in GC are transparent to most detectors. Some of the detectors available are able to detect a wide range of samples down to picogram limits. The key attributes of a detector are sensitivity [which is indicated by the limit of detection [2] (LOD)], selectivity[3] and dynamic range[4]. Finally, the detector may respond to the concentration of an analyte passing through it or to the mass of the analyte passing through it. The former is called *concentration-sensitive* detector and the latter, *mass sensitive detector*. One of the detector types linked to the GC instrument, the *thermal conductivity detector* (TCD), is the most commonly used *concentration-sensitive detector.*

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[2] This is the minimum quantity of material than can be distinguished from background

[3] the ratio of the amount of a compound that does not contain the selected property that generates the same signal as a compound with the selected functionality

[4] the usable (operating) range over which the detector will generate a changing signal as the amount of analyte changes

Characteristics of a good Detector

1. Rapid response to the presence of a solute
2. A wide range of linear response
3. High sensitivity
4. Stability of operation

The list below describes the most common types of detectors used in gas chromatography:

Type of detector	Type of response	Response characteristic	Destructive	LOD
Flame Ionization Detector	Universal to C*	Mass	Yes	1 pg/s
Thermal Conductivity Detector	Universal	Concentration	No	500 pg/ml
Electron Capture Detector	Selective	Concentration	No	5 fg/s
Nitrogen Phosphorous Detector	Selective	Mass	Yes	1 pg N/s
Flame Photometric Detector	Selective	Mass	Yes	0.1-1pg P or S /s
Mass Spectrometry	Both	Mass	Yes	.25 to 100 pg
Atomic Emission Detector	Both	Mass	Yes	0.1 ng-1 pg/s

*Carbon.

Applications of gas chromatography:

Gas chromatography has many applications, both quantitative and qualitative. These include:

a. Environmental analysis: pesticides' analysis in water/vegetables, vehicle emissions

b. Clinical medicine: blood alcohol, drugs (nicotine, opioids)

c. Forensic medicine: explosives

d. Consumer goods quality control

e. Food analysis: fatty acid composition, flavor components of edible products

f. Petrochemicals: petrol composition; solvent purity, gas refineries

Factors to be considered during GC method development to optimize time of separation and optimum resolution[5]:

- **Carrier gas flow rate** : an increase in flow rate shortens retention times but may decrease resolution
- **Temperature program heating rates**: very high column temperatures lead to loss of resolution; optimum separation and retention time are usually achieved with temperature gradients.
- **Column length , column diameter**: A longer column and thinner diameter improve resolution

- **Thickness of the stationary phase**: Standard thickness in capillary columns is 0.25 microns; thicker stationary phases increase sample capacity and retention time as well as improve resolution of early peaks.
- **Sample volumes**: the sensitivity of capillary column based gas chromatography also limits the sample volume that can be applied to the column; overloading the column can lead to tailing of peaks and poor resolution.

[1] Resolution is a measure of how well two elution peaks can be differentiated in a chromatographic separation.

Disadvantages

Samples must be volatile and thermally stable below about 400°C; most commonly used detectors are non-selective; published retention data are not always reliable for qualitative analysis.

Mass Spectrometry Detectors: Mass Spectrometer (MS) detectors are most powerful of all gas chromatography detectors. In a GC/MS system, the mass spectrometer scans the masses continuously throughout the separation. When the sample exits the chromatography column, it is passed through a transfer line into the inlet of the mass spectrometer. The sample is then ionized and fragmented, typically by an electron-impact ion source. During this process, the sample is bombarded by energetic electrons which ionize the molecule by causing them to lose an electron due to electrostatic repulsion. Further bombardment causes the ions to fragment. The ions are then passed into a mass analyzer where the ions are sorted according to their m/z value, or mass-to-charge ratio. Most ions are only singly charged. The Chromatogram will point out the retention times and the mass spectrometer will use the peaks to determine what kind of molecules exists in the mixture. GC/MS units are advantageous because they allow for the immediate determination of the mass of the analyte and can be used to identify the components of incomplete separations. They are rugged, easy to use and can analyze the sample almost as quickly as it is eluted. The disadvantages of mass spectrometry detectors are the tendency for samples to thermally degrade before detection and the end result of obliterating the entire sample by fragmentation.

Flame Ionization Detectors: Flame ionization detectors (FID) are the most generally applicable and most widely used detectors. In a FID, the sample is directed at an air-hydrogen flame after exiting the

column. At the high temperature of the air-hydrogen flame, the sample undergoes pyrolysis, or chemical decomposition through intense heating. Pyrolized hydrocarbons release ions and electrons that carry current. A high-impedance picoammeter measures this current to monitor the sample's elution. It is advantageous to use FID because the detector is unaffected by flow rate, noncombustible gases and water. These properties allow FID high sensitivity and low noise. The unit is both reliable and relatively easy to use. However, this technique does require flammable gas and also destroys the sample.

Thermal Conductivity Detectors: Thermal conductivity detectors (TCD) were one the earliest detectors developed for use with gas chromatography. The TCD works by measuring the change in carrier gas thermal conductivity caused by the presence of the sample, which has a different thermal conductivity from that of the carrier gas. Their design is relatively simple, and consists of an electrically heated source that is maintained at constant power. The temperature of the source depends upon the thermal conductivities of the surrounding gases. The source is usually a thin wire made of platinum, gold. The resistance within the wire depends upon temperature, which is dependent upon the thermal conductivity of the gas. TCDs usually employ two detectors, one of which is used as the reference for the carrier gas and the other which monitors the thermal conductivity of the carrier gas and sample mixture. Carrier gases such as helium and hydrogen has very high thermal conductivities so the addition of even a small amount of sample is readily detected. The advantages of TCDs are the ease and simplicity of use, the devices' broad application to inorganic and organic compounds, and the ability of the analyze to be collected after separation and detection. The greatest drawback of the TCD is the low sensitivity of the instrument in relation to other detection methods, in addition to flow rate and concentration dependency.

Electron-capture Detectors: Electron-capture detectors (ECD) are highly selective detectors commonly used for detecting environmental samples as the device selectively detects organic compounds with moieties such as halogens, peroxides, quinones and nitro groups and gives little to no response for all other compounds. Therefore, this method is best suited in applications where traces quantities of chemicals such as pesticides are to be detected and other chromatographic methods are unfeasible. The

simplest form of ECD involves gaseous electrons from a radioactive emitter in an electric field. As the analyte leaves the GC column, it is passed over this emitter, which typically consists of nickle-63 or tritium. The electrons from the emitter ionize the nitrogen carrier gas and cause it to release a burst of electrons. In the absence of organic compounds, a constant standing current is maintained between two electrodes. With the addition of organic compounds with electronegative functional groups, the current decreases significantly as the functional groups capture the electrons. The advantages of ECDs are the high selectivity and sensitivity towards certain organic species with electronegative functional groups. However, the detector has a limited signal range and is potentially dangerous owing to its radioactivity. In addition, the signal-to-noise ratio is limited by radioactive decay and the presence of O2 within the detector.

Atomic Emission Detectors: Atomic emission detectors (AED), one of the newest additions to the gas chromatographer's arsenal, are element-selective detectors that utilize plasma, which is a partially ionized gas, to atomize all of the elements of a sample and excite their characteristic atomic emission spectra. AED is an extremely powerful alternative that has a wider applicability due to its based on the detection of atomic emissions. There are three ways of generating plasma: microwave-induced plasma (MIP), inductively coupled plasma (*ICP*) or direct current plasma (DCP). MIP is the most commonly employed form and is used with a position able diode array to simultaneously monitor the atomic emission spectra of several elements.

CHM 301 – INSTRUMENTATION AND ANALYTICAL CHEMISTRY I

TOPICS: i. Molecular Spectroscopy and Flame Methods
ii. Luminescence, Nephelometry and Turbidimetry

Lecturer: ***Prof. F. M. Adebiyi***

MOLECULAR SPECTROSCOPY

- **Spectroscopy** is the use of absorption, emission, or scattering of electromagnetic radiation by atoms (e.g Fe) or molecules (e.g NO_2) or molecular ions (e.g NO_3^- & SO_4^{2-}) to qualitatively or quantitatively study the atoms or molecules or to study physical processes e.g turbidity of water.
- The interaction of radiation with matter can cause redirection of the radiation and/or transitions between the energy levels of the atoms or molecules.
- **Absorption** is a transition from a lower level to a higher level with transfer of energy from the radiation field to the atom or molecule. A transition from a higher level to a lower level is called **Emission**, if energy is transferred to the radiation field or non-radiative decay; while redirection of light due to its interaction with matter is called **scattering** which may or may not occur with transfer of energy i.e the scattered radiation has a slightly different or the same wavelength.
- **Electromagnetic Spectrum**

Spectroscopy/spectrophotometry is mainly concerned with the following regions of the spectrum

Spectrum region	Wavelength range
Ultraviolet (UV)	185 t0 400nm
Visible	400 to 760nm
Infrared (IR)	0.76µm to 15µm

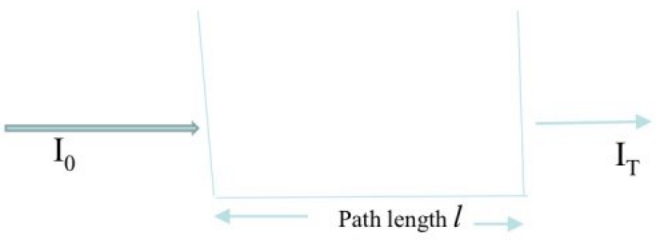
Colorimetry is concerned with the visible region of the spectrum.

Visible regions

Spectrum region	Wavelength range (nm)	Types of transition
Violet	400-450	Outer electrons
Blue	450-500	Outer electrons
Green	500-570	Outer electrons
Yellow	570-590	Outer electrons
Orange	590-630	Outer electrons
Red	630-760	Outer electrons
Other electromagnetic spectrum regions		
Gamma rays	$<10^{-12}$pm	nuclear
x-rays	1nm-1pm	inner electron
near IR	2.5nm- 760nm	outer electron & molecular vibration
Microwaves	1mm-25μm	molecular vibration & electron spin
Radio waves	>1mm	nuclear spin flips

Beer- Lambert Law

- When a monochromatic or heterogeneous light (electromagnetic radiation) is passed through an absorbing medium (gas, liquid or solution), some is reflected (I_R), some absorbed (I_A) and the rest is transmitted (I_T); i.e
- $I_O = I_R + I_A + I_T$
- Where I_O is the incident radiation.
- By using a control cell containing ideally a non-absorbing solvent, compensation is made for any loss by reflection and any absorption by the solvent. Thus, for the absorbing substance only:
- $I_0 = I_A + I_T$
- When radiation such as UV, visible or IR passes through matter and interacts with it, there are laws governing their behaviour. Suppose monochromatic light of initial intensity I_O strikes a sample of concentration c and thickness *l* as shown in the figure below:



Absorption of light by a sample

- The amount of light absorbed will depend on I_O and c across the whole of the sample, so that when we integrate we find that we get the Beer- Lambert Absorption law
- $A = \log_{10} (I_O/I_T) = \varepsilon cl$ **(Note: ε is pronounced as epsilon)**
- Where A is the absorbance, I_T the transmitted intensity; Ɛ the molar absorptivity of the sample at this wavelength; c the concentration; and l the path length through the sample.
- If we use SI units, Ɛ will be expressed in m^2mol^{-1}, which is very logical since it represents the effective interacting area of the molecules of the sample. In older units c was expressed in mol/dm^3 (molarity) and l in cm, so that the older values of Ɛ are one-tenth (1/10) of the SI values.
- The experimental measurements are usually made in terms of transmittance (T) which is defined as:
- $T = (I_T/I_O)$
- Where I_T is the light intensity after it passes through the sample and I_O is the initial light intensity. The relationship between A and T is
- $A = -\log_{10} T$
- $= - \log_{10} (I_T/I_O)$
- Modern absorption instruments can usually display the data as either transmittance, %-transmittance or absorbance. An unknown concentration of an analyte can be determined by measuring the amount of light that the sample absorbs and applying Beer- Lambert law. If the absorptivity coefficient is not known, the concentration can be determined using a working curve of absorbance versus concentration from standards.
- Specific absorption coefficient (or absorbancy index) is the absorbance per unit path length and unit concentration.

$\mathcal{E}s = Acl$ or $I_T = I_O \times 10^{-\mathcal{E}cl}$

Molar absorption coefficient or molar absorptivity (formerly the molar extinction coefficient), $\mathcal{E}s$ is the specific absorption coefficient for a concentration of 1mol l^{-1} and a path length of 1cm. i.e $\mathcal{E} = A/cl$

LIMITATIONS OF THE BEER- LAMBERT LAW

The linearity of the Beer- Lambert law is limited by chemical and instrumental factors. Causes of non- linearity include:

i. Deviations in molar absorption coefficients at high concentrations (> 0.01M) due to electro-static interactions between molecules in close proximity.

ii. Scattering of light due to particulates in the sample

iii. Fluorescence or phosphorescence of the sample

iv. Changes in refractive index at high analyte concentration

v. Shifts in chemical equilibria as a function of concentration

vi. Non- monochromatic radiation. Deviations can be minimized by using a relatively flat part of the absorption spectrum such as the maximum of an absorption band.

vii. Stray light.

Question- If 1cm of a solution with 3.75 g of a compound of Relative Molecular Mass (RMM) 126 g per 1000 cm^3 in aqueous solution lets through 30 % of the incident light of wavelength 60 nm, calculate its molar absorptivity (Ɛ).

Answer

$A = \log_{10}(100/30) = 0.523$

$C = \text{mass/RMM} = 3.75/126$

$= 0.0298\ moldm^{-3}$

$= 29.8\ molm^{-3}$

$l = 1cm = 0.01m$

$Ɛ = A/Cl = 0.523/(29.8 \times 0.01)$

$= 1.76\ m^3mol^{-1}$

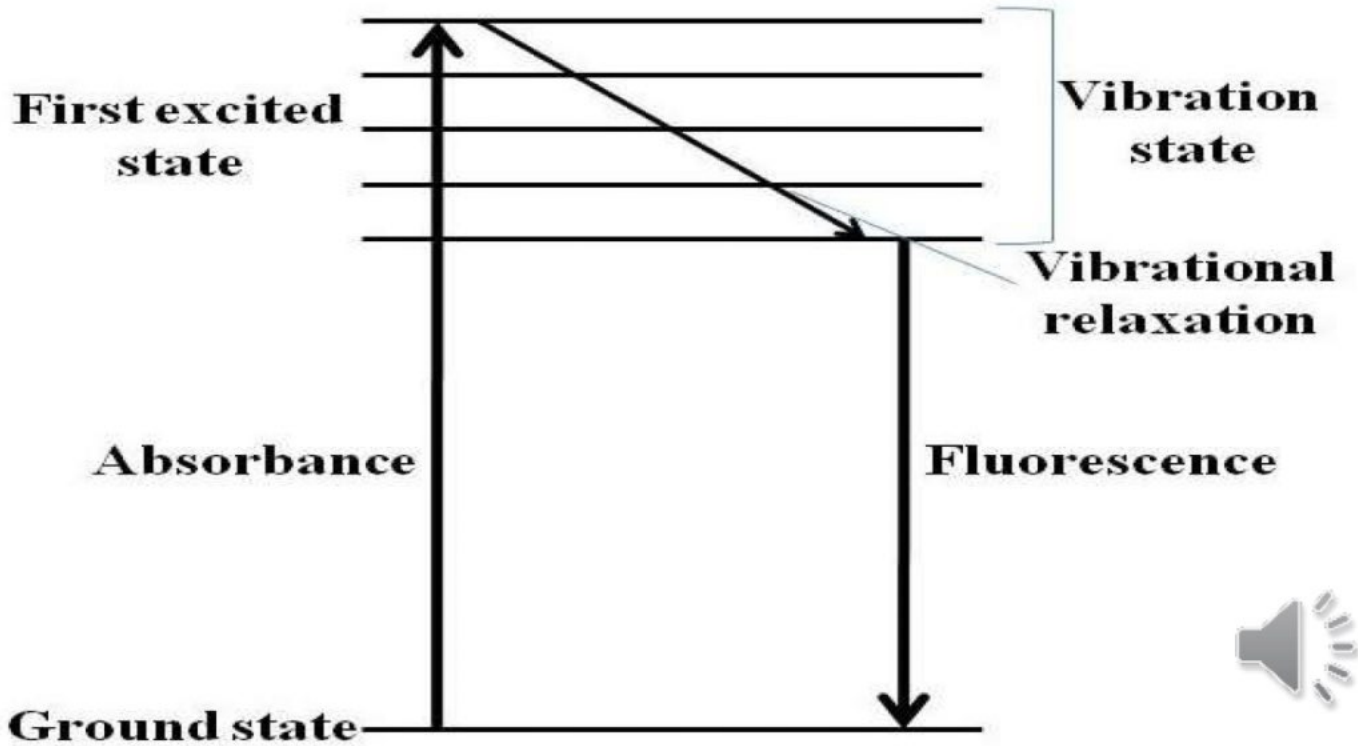
Or $Ɛ = 0.523/(0.0298 \times 1)$

$= 17.6\ dm^3mol^{-1}cm^{-1}$

MOLECULAR LUMINESCENCE SPECTROSCOPY

- Several chemical species are photoluminescent; i.e. they can be excited by electromagnetic radiation and re-emit radiation of the same or longer wavelength. This photoluminescence can be classified as fluorescence or phosphorescence depending upon the life time of the excited state.
- **PHOSPHORESCENCE**
- This is based upon the nature and intensity of light emitted by molecules in the triplet state. The lifetimes of phosphorescence vary from 10^{-2} seconds to 100 seconds or more.
- On account of their **long life** they are subjected to deactivation process and when the substance is dissolved in a rigid medium, phosphorescence emission can usually be observed. It is unique in regard to frequency, lifetime, quantum yield and vibrational pattern.
- Phosphorescence is a luminescence process in which a molecule undergoes a transition from the triplet to the ground state. Phosphorescence quantum efficiency can be increased by cooling the solution to a lower temperature (-77K).

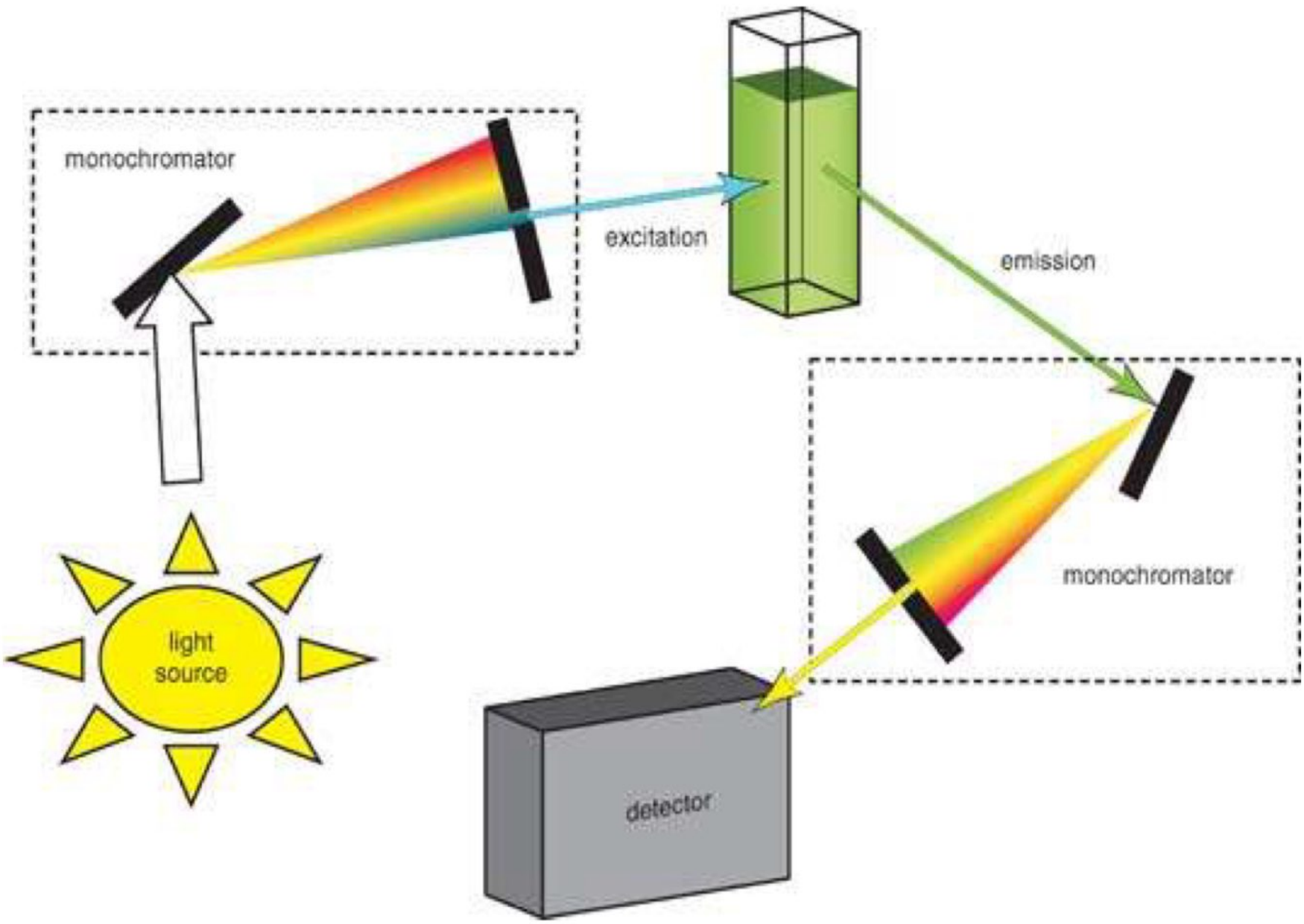
- **FLUORESCENCE**
- This is an analytically important emission process in which atoms or molecules are excited by the absorption of a beam of electromagnetic radiation. The excited species then relax to the ground state, giving up the excess energy as photons.
- With fluorescence, luminescence steps immediately ($<10^{-6}$ seconds) after irradiation is discontinued.
- Thus, the lifetimes for fluorescence are very short: viz 10^{-6}- 10^{-9} seconds. Exciting radiations and re-emitted radiations are either in the visible or UV regions. They may sometimes be in high energy X- ray regions.
- Fluorescence is a process involving the emission of light from any substance in the excited states. Generally speaking, fluorescence is the emission of electromagnetic radiation (light) by the substance absorbed the different wavelength radiation. Its absorption and emission is illustrated in the Jablonski diagram (*the figure in the next slide*).



Jablonski diagram of fluorescence

- In principle, a fluorophore is excited to higher electronic and vibrational states from ground state after excitation. The excited molecules can relax to lower vibrational state due to the vibrational relaxation and, then further return to the ground state in the form of fluorescence emission.

- **Instrumentation of spectrofluorometers**
- Most spectrofluorometers can record both excitation and emission spectra. They primarily consist of four parts: light sources, monochromators, optical filters and detector (*Schematic diagram is on the next slide*).
- **Light Sources**
- Light sources that can emit wavelength of light over the UV and visible ranges can provide the excitation energy. There are different types of light source, including arc and incandescent xenon (Xe) lamps, high-pressure mercury (Hg) lamps, Xe-Hg arc lamps, low pressure Hg and Hg-Ar lamps, pulsed xenon lamps, quartz-tungsten halogen (QTH) lamps, LED light sources, etc. The proper light source is chosen based on the application involved.
- **Monochromators**
- Prisms and diffraction gratings are the two mainly used types of monocharomators, which help to get the experimentally needed chromatic light with a wavelength range of 10 nm. Typically, the monocharomators are evaluated based on dispersion, efficiency, stray light level and resolution.
- **Optical Filters**
- Optical filters are used in addition to monochromators in order to further purifying the light. There are two kinds of optical filters. The first one is the colored filter, which is the most traditional filter and is also divided into two catagories, which are - monochromatic filter and long-pass filter. The other one is thin film filter that is the supplement for the former one in the application.
- **Detector**
- An alloy of indium gallium arsenide (InGaAs) array is the standard detector used in many spectrofluorometers. It can provide rapid and robust spectral characterization in the near-IR.



Schematic representation of a fluorescence spectrometer

Possible errors in fluorimetry and phosphorimetry:

These are:

- **i.** The quantum efficiency of a luminescent process must be the same and reproducible. If such quantum efficiency decreases, it leads to the phenomenon of quenching.
- **ii.** Heavy atoms and paramagnetic species affect intersystem crossing (ISC) and quantum efficiency, especially in fluorimetry.
- **iii.** Oxygen contributes its paramagnetism and leads to quenching.
- **iv.** A drift or change in source intensity and position of the cell lead to wrong measurement.
- **v.** The inner filter effect which originates due to difference in intensity of fluorescence leads to wrong measurements.

- **Merits of fluorimetry and phosphorimetry:** Advantages of fluorimetry as well as phosphorimetry in quantitative analysis are as follow:
- **i.** The methods are selective and have no spectral interference; if such interference is present it can be mitigated by the right choice of both excitation and luminescence wavelength.
- **ii.** The methods are sensitive. Time resolution is of importance in phosphorimetry, due to its long life-time. This also helps to eliminate scattering from the sample.
- **Advantage of fluorimetry over phosphorimetry:** In comparison to fluorimetry, phosphorimetry is however not commonly used in chemical analysis due to the complexity of instrumentation and the need to cool the sample to get reproducible results.

- **APPLICATIONS OF FLUORIMETRY**

This technique has diverse applications in medicine, forensic and environmental levels in addition to inorganic and organic analysis.

i. In medicine e.g. drugs like quinine can be analysed at nanogram levels

ii. In environmental chemistry, atmospheric carcinogens belonging to polynuclear aromatic hydrocarbons like 3-4 benzopyrine which are formed by incomplete combustion of fuel and cigarette tar can be analysed by fluorimetry.

iii. The inorganic analysis of metals can be done by fluorimetry.

iv. Fluorescent probes - This method is used to qualitatively and quantitatively detect biological compounds present in low concentration.

v. Protein structure - There are several intrinsic fluorophores present in proteins, such as tryptophan, tyrosine, phenylalanine among others. The fluorescence of a protein is determined by all its amino acids, solvent properties, and presence of co-factors, such as NAD, FAD. Thus, this method can help in determining the protein structure.

v. Fluorescence-activated cell sorting (FACS) - This is method used to sort a mixture of cells based on the differences in fluorescent properties.

- **Factors that affect fluorescence spectroscopy**

a. Molecular rigidity

- Fluorophores with rigid structures are preferred for performing fluorescence spectroscopy, as they have reduced vibrations and reduced probability of transitioning to triplet state. Fluorescein and eosin have rigid structures and are strongly fluorescent, while phenolphthalein is non-rigid and non-fluorescent.

b. Solvent polarity

- The polarity of the solvent can also dictate the degree of fluorescence. The fluorescence of the structures can decrease in the presence of heavy atoms in the solvent.

c. Dissolved oxygen

- Dissolved oxygen in the solvent can also affect the fluorescence by reducing the emission intensity. This is achieved by photochemical oxidation of fluorophore. The paramagnetic properties of oxygen can also lead to quenching of fluorescence.

d. pH

- pH can affect the fluorescent properties. One such example is aniline, which exists as a cation at lower pH and as anion at higher pH. In both instances, the fluorescence is lost. .

e. Quenching

- Quenching refers to decrease in the fluorescence intensity. This may be caused by absorption of fluorescence by the solution or absorption of the fluorescent material itself. The latter effect is called self-quenching.

- **TURBIDIMETRY AND NEPHELOMETRY**
- These refer to the peculiar spectral properties of dispersion and may be taken as the ratio of light reflected to the light incident upon a dispersion. The intensity of light reflected by a suspension is a function of concentration when the other conditions are constant. The method of measurement of turbidity fall under three classes:

a. measurement of the ratio of the intensity of scattered light to the intensity of incident light;

b. measurement of the intensity of transmitted light to that of the incident light and,

c. measurement of the extinction effect i.e. the depth at which a target disappears beneath the layer of a turbid medium.

Turbidimetry involves the measurement of transmitted light, and is directly proportional to the concentration and depth of the dispersion. It also depends upon the colour. For smaller particles the Tyndall ratio is proportional to the cube of the particle size and is inversely proportional to the fourth power of the wavelength.

The principle of absorption spectroscopy can be used in turbidimetry wherein absorption due to the suspended particles is measured while in nephelometry, the scattering of light by a suspension is measured.

- Although the methods are not so precise they have practical utility. It will be seen that in much measurement accuracy depends upon the size and shape of particles. Hence any instrument used for absorption spectroscopy can be used for turbimetry whereas the nephelometer needs a receptor at right angles to the light path. Nephelometric methods are less frequently used in inorganic analysis. At a higher concentration, absorption varies directly with the concentration. This however does not hold good at low concentrations e.g. colloidal copper ferrocyanide and heavy metal sulphides. The solubility of the suspension should be slight. A protective colloid like gelatin is used to obtain a non-stable and uniform dispersion of the colloidal substance.
- The turbidity produced by suspension is given by an expression
- $S = \log \frac{Po}{P} = \frac{Kbcd^3}{\delta^4 \alpha \lambda^4}$
- Where S = turbidance; Po= intensity of incident radiation; λ= wavelength; P= intensity of transmitted radiation; c= concentration; b= thickness of a layer of sample; d= average diameter of a particle and δ, K are both constant. This expression holds good for a dilute solution. In a monochromatic radiation, α, K, d, λ are constant hence the above equation reduces to
- $S \propto \alpha bc$ or $S = Kbc$
- This expression is similar to Beer's law.

- All the laws governing turbidimetric determinations are also applicable to *nephelometric* measurements. The only point of difference in nephelometry is that the light source and receptor are at right angles. In both turbidimetry and nephelometry one must use a uniform suspension. It is possible to obtain particles with a uniform physical character. It is necessary to control the concentration of the two ions which produce the precipitate. The ratio of concentrations of the two solutions to be mixed should be controlled. The manner and order of mixing should be observed and the time of mixing should be standardised. The amount of protective colloids present should be properly controlled and the temperature carefully noted. For best results during nephelometric measurements, turbidity should be homogeneous with a low density, turbidities should have the same dispersion. A constant degree of turbidity should be maintained through the entire process of measurement and the results should be definitely reproducible.

Applications of turbidimetic and nephelometric techniques

- The applications of both turbidimetic and nephelometric techniques are numerous e.g. in water pollution studies the amount of sulphate in water is measured by nephelometry as barium sulphate. Previously, the trbidity of water was measured by a Jackson candle turbidimeter in JU-units but nowadays it is measured by a turbidimeter in NTU units. Ammonia from water can be determined with Nessler's reagent by turbidimetry. Similarly phosphate can be determined as phosphomolybdate. The amount of selenium or tellurium in a sample can be determined by nephelometry by reduction with stannous chloride as the blue sols. Other determinations include colloidal sulphur, acetone, pepsin, proteins and trypsin. Other examples of the use of light scattering methods are determination of silver as chloride suspension, gold as colloidal gold, calcium as oxalate suspension, potassium suspension with sodium cobaltnitrite, sodium with zinc uranylacetate and tellurium with dihydrogen sodium phosphite.

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