KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University)

(Established Under Section 3 of UGC Act 1956)

Coimbatore – 641 021.

DEPARTMENT OF CHEMISTRY

Class : M.Sc Chemistry
Subject : Physical Methods in Chemistry
Subject Code : 17CHP303
Semester / Year : II/III

SYLLABUS

Semester -III

17CHP303 PHYSICAL METHODS IN CHEMISTRY (INSTRUMENTATION) 4H 4C

Instruction Hours/week:L: 4 T:0 P:0 Marks: Internal:40 External: 60 Total:100

Course Objectives

1. To learnt about the various methods involved in analytical techniques like thermal analysis, electron microscopy and circular dichorism.
2. To learn about the ESR spectroscopy.
3. To understand different chromatographic methods.

Course Outcome

1. Understood the separation techniques like Column, Paper, Thin Layer and Chromatography and HPLC.
2. Known about the stages of thermal degradation patterns of materials using TGA and DTA techniques and various types of electron spectroscopy.
3. Known about Circular Dichroism and Optical Rotatory Dispersion
4. Understood the concepts in ESR and Flame emission spectroscopy.

UNIT – I

Chromatography: Theory, instrumentation, basic principles & application in the chemical analysis of the following – columns, paper, thin layer and ion exchange-gel permeation-HPLC applications in chemical analysis-gas chromatography**.**

UNIT – II

Thermal analysis: Differential thermal analysis DTA and differential scanning calorimetry DSC - basic principles - thermo gravimetric analysis.

Electron spectroscopy: ESCA XPS: Principle, chemical shifts - description of ESCA spectrometer, X-ray sources, samples, analysis, detectors and recording devices-applications.

Auger electron spectroscopy AES and Ultra-Violet photo electron spectroscopy UPS/PES principles and applications.

UNIT – III

Circular Dichroism and Optical Rotatory Dispersion: Basic principles -cotton effects-octants rule –axial halo ketone rule-application of ORD and CD. Tyndal Scattering-turbidimetry and nephelometry-applications. Atomic absorption spectroscopy.

UNIT – IV

ESR spectroscopy: Theory - derivative curves - g shift - hyperfine splitting-isotropic and anisotropic systems-zero field splitting and Kramer degeneracy. Identification of free radicals – applications to copper complexes.

UNIT – V

Flame Emission Spectroscopy: Introduction, flames and flame spectra, flames temperature, chemical reaction in flame and flame background. Flame photometers, Flame spectrophotometers, photosensitive detectors, single beam and double beam instruments, calibration curve, errors in flame photometers, applications.

SUGGESTED READINGS:

Text Books:

1. Gopalan, V., Subramanian, P. S., & Rangarajan, K. (2003). *Elements of Analytical Chemistry*. New Delhi: S. Chand and Sons.
2. Usharani, S. (2002). *Analytical Chemistry*. Chennai: MacMillan India Ltd.
3. Sharma, B. K. (2005). *Instrumental Methods of Chemical Analysis* (24th Edition). Meerut: Krishna Prakashan Media (P) Ltd.
4. Ewing, G. W. (1988). *Instrumental Methods of Chemical Analysis* (III Edition). Singapore: McGraw Hill International Edition.
5. Banwell, C.N., & McCash, E.M. (2010). *Fundamentals of Molecular* and *Spectroscopy.* McGraw-Hill Education (India) Pvt. Limited
6. Jag Mohan. (2007). *Organic Spectroscopy: Principles and Applications* (II Edition). New Delhi: Narose Publishing House.

Reference Books:

1. Chatwal, G. R., & Anand, S. K. (2015). *Instrumental Methods of Chemical Analysis* (V Edition). New Delhi: Himalaya Publishing House.
2. Drago, R .S. (1965). *Physical Methods in Inorganic Chemistry*. New York: Reinhold Publishing Corporation.
3. Skoog, D. A., & West, D. M. (2004). *Fundamentals of Analytical Chemistry* (VIII Edition). Singapore: Thomson Book Store.
4. Svehla, G. (2002). *Vogel's Qualitative Inorganic Analysis* (VII Edition). Singapore. Pearson Education.
5. Skoog, D.A., Holler, J. & Crouch, S.R. (2009). *Instrumental Analysis* (India Edition). Cengage Learning India Private Limited, New Delhi.

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LECTURE PLAN

DEPARTMENT OF CHEMISTRY

Staff In charge : Dr.M.R.Ezhilarasi
Class : M.Sc Chemistry
Subject : Physical Methods in Chemistry
Subject Code : 17CHP303
Semester / Year : II/III

S.No	Lecture Duration Period	Topics to be covered	Support Material/Page Nos
	UNIT I		
1	1	**Chromatography**- Introduction, Basic principles	T1: 3-20
2	1	Column Chromatography	T1:96-112
3	1	Paper Chromatography	T1: 225-240
4	1	Thin Layer Chromatography	T1: 241-267
5	1	Ion exchange Chromatography	T1:123-160
6	1	Gel Permeation Chromatography	T1:161-178
7	1	High Performance Liquid Chromatography	T1:286-385
8	1	Detectors in HPLC	T1: 286-385
9	1	Gas Chromatography	T1:180-221
10	1	Recapitulation and discussion of important questions	
	Total No of hours planned for Unit I-10		

	UNIT-II		
1	1	**Thermal Analysis:** Differential thermal analysis	T1: M 318-329
2	1	Differential scanning calorimetry	W1
3	1	Thermo gravimetric analysis	T1: M 308-318
4	1	Thermometric titrations	T1: M 329- 335
5	1	**Electron Spectroscopy:** Basic principles	T1: S 588
6	1	X-ray Photoelectron spectroscopy (XPS) or Electron spectroscopy for chemical analysis (ESCA). Principles of ESCA or XPS	T1: S 590-594
7	1	Instrumentation and applications of ESCA or XPS	T1: S 594-600
8	1	Auger electron spectroscopy	T1: S 602-606
9	1	Ultraviolet Photoelectron spectroscopy	T1: S 600-602
10	1	Recapitulation and discussion of important questions	
	Total No of hours planned for Unit II-10		
	UNIT-III		
1	1	**Circular dichroism and optical rotator dispersion:** Introduction	T2: 473-475
2	1	Circular birefringence and circular dichroism	T2:475-477
3	1	ORD , Cotton effect	T2: 477-479
4	1	Axial haloketone rule, The octant rule and its applications	T2:479-480, 480-485,
5	1	Tyndal scattering or Light scattering	T1: S 576-577
6	1	**Nephelometry and Turbidimetry**- Introduction, Theory, Instrumentation	T1: S 577-581
7	1	Applications of Turbidimetry and Nephelometry	T1: S 581-582
8	1	Atomic absorption spectroscopy	T1: S 383-389
9	1	Recapitulation and discussion of important questions	

	Total No of hours planned for Unit III-9		
	UNIT-IV		
1	1	**ESR spectroscopy:** Introduction	T1: S 737-739
2	1	Factors affecting the g-value	T1: S 739-740
3	1	Hyperfine splitting- Isotropic and anisotropic coupling constant	T1: S 746-751
4	1	The spin Hamiltonian	T1: S 751-752
5	1	Zero field splitting and Kramer degeneracy	T1: S 760-762
6	1	Identification of free radicals	T1: S 754
7	1	Instrumentation-ESR spectrometer	T1: S 743-745
8	1	Recapitulation and discussion of important questions	
	Total No of hours planned for Unit IV-8		
	UNIT-V		
1	1	**Flame Emission Spectroscopy:** Introduction. Principle	T1: S 421-423
2	1	Flame and Flame spectra	T1: S 423-424
3	1	Flame temperature, chemical reactions	T1: S 424-427
4	1	Effect of organic solvents in flame spectra, Flame background	T1: S 428-430
5	1	Instruments-Single beam and double beam instruments-flame spectrophotometers	T1: S 430-434
6	1	Calibration curve	T1: S 434-437
7	1	Errors in flame photometry, Applications	T1: S 437-447
8	1	Recapitulation and discussion of important questions	
9	1	Previous year ESE question paper discussion	
10	1	Previous year ESE question paper discussion	
11	1	Previous year ESE question paper discussion	

	Total No of hours planned for Unit V-11	
Total planned hours	48	

SUPPORTING MATERIALS:

Text Books:

T1: Sharma.B.K.(2012) Instrumental methods of chemical analysis (28th Edition). Meerut: Krishna Prakashan Media (p) Ltd

T2: Jag mohan (2007). Organic Spectroscopy: Principles and Applications (II Edition). New delhi: Narose publishing house

Website:

W1: www. http// differential scanning calorimetry

KAHE

Lecture Notes

UNIT-I

SYLLABUS

Chromatography: *Theory, instrumentation, basic principles & application in the chemical analysis of the following – columns, paper, thin layer and ion exchange-gel permeation-HPLC applications in chemical analysis-gas chromatography.*

Chromatography:

Introduction: The molecules present in biological system or in synthetic chemistry are produced through a series of reactions involving intermediates. As discussed in previous lecture, at any moment of time biological organism has major fraction as desired product but has other compounds in minute quantities. The minor species present in a product is always referred as "impurities" and these compounds need to separate from desired product for biotechnology applications. **How two molecules can be separated from each other?** To answer this question we can take the example of three molecules given in Figure 1. These 3 molecules (benzene, phenol, aniline) are similar to each other but have distinct physical and chemical properties which can be used as a criterion to separate them. The physical and chemical properties which can be use to separate molecules are-

Physical Properties

1. Molecular weight

2. Boiling point (in case both are liquid, as in this case)

3. Freezing point

4. Crystallization

5. Solubility

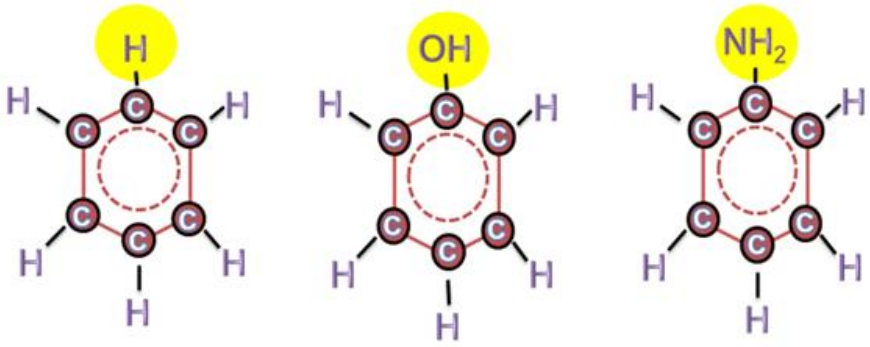
6. Density

Chemical Properties

1. Functional Group, for example, phenol has –OH where as aniline has NH2.

2. Reactivity towards other reagent to form complex

Now for example you have a mixture of compound 1 (benzene) and compound 3 (Aniline) and you would like to purify benzene rather than aniline. In this situation, you can take the physical and chemical properties of benzene into the account and isolate it from the mixture.



Name	Benzene	Phenol	Aniline
Molecular formula	C_6H_6	C_6H_6O	$C_6H_5NH_2$
Molar mass (g mol^{-1})	78.11	94.11	93.13
Density	0.8765 g cm^{-3}	1.07 g cm^{-3}	1.0217 g ml^{-1}
Melting point (°C)	5.5	40.5	-6.3
Boiling point (°C)	80.1	181.7	184.13

Figure 1: Chemical Structure and physical Properties of benzene, phenol and aniline.

Principle of Seperation: How a physical or chemical property will allow to isolate a particular substance? The mixture of compound 1 and 3 is shown in Figure 2 and assume if we are using boiling point as a criteria to isolate them. As we will heat the mixture there will two phase forms, one liquid phase and other is vapor phase. The molecules of compound 1 and 3 will distribute between these two phases and as the temp is near to boiling point of compound 1, more amount of 1 will be present in vapor phase than liquid phase. Whereas more number of compound 3 will be in liquid phase. Eventually as this process will continue, at the end two molecules will get separated from each other. The distribution coefficient (Kd) to describe the distribution of compound 1 between two phase A and B is as follows:

$$Kd = \frac{\text{Concentration in Phase A}}{\text{Concentration in Phase B}}$$

Similarly one can also exploit other physical & chemical parameters as well. With each and every physical and chemical parameter the molecule present in the mixture will distribute as per their behavior in each parameter.

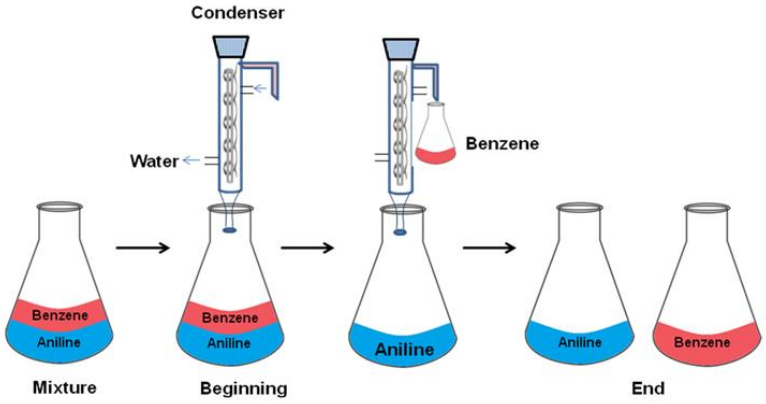


Figure 2: Distribution of molecules during distillation.

Chromatography: The purpose of chromatography is to separate a complex mixture into individual component exploiting the partition effect which distribute the molecules into the different phases. As discussed above, a distribution of a molecule between two phases A and B is given by a distribution coefficient, Kd. In most of the chromatography techniques, phase A is stationary phase or matrix and phase B is mobile phase or buffer.

Column Chromatography: In column chromatography, a stationary phase is filled into a cylindrical tube made up of glasss or steel. The mixture of analyte is loaded on the top and it runs from top to bottom. **How Kd is exploited in column chromatography ?** Assume two molecules, X and Y with a Kd value of 1 and 9 and they are traveling through a column with water as mobile phase as given in Figure 3. As they will travel, X and Y will partition between stationary phase and mobile phase. As there is a huge difference in Kd, Y will be associated with the matrix and remain on the top of the column where as X will move along the water. At the end of chromatography, X will come out first whereas Y will come out last.

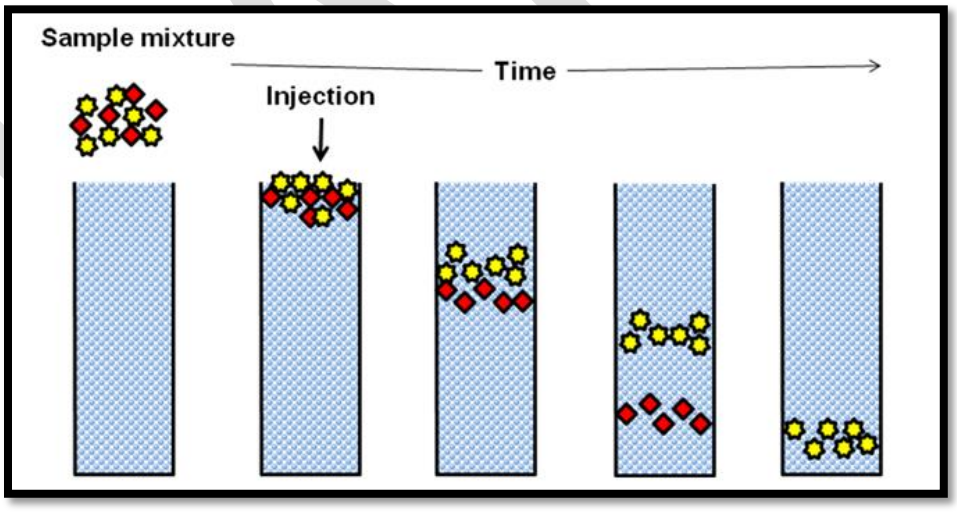


Figure 3: Seperation of two molecules on a column.

Chromatogram: The plot of elution volume along with the absorbance is known as chromatogram as given in Figure 28.4. The volume or time it takes for a analyte to come out from the column is known as retention volume or time. The chromatogram may have separate peaks (A and B) or peaks (C and D) with overlapping base, these peaks are called fused peaks.

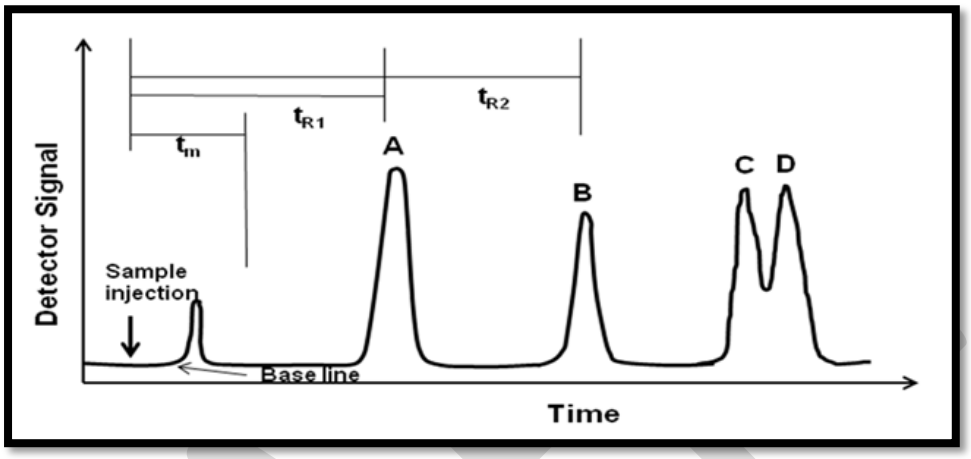


Figure 4: A typical chromatogram.

Resolution: The ability of a chromatography column to separate two analyte peak from another is known as resolution. It is defined as the ratio of difference in retention time between two peaks and average of base of peak width. It is given by

$$Rs = \frac{\Delta tR}{Wav}$$

When Rs=1, the separation of two peaks is 97.7% and a column with Rs more than 1.5 considered good. The number of distribution events governs the ability of a column to separate the two analytes. In another words, resolution is directly proportional to the number of distribution events. In column chromatography, each thin plain of column matrix participate in distribution of molecule. Assume height of a distribution plain is **H** and length of a column is **L**, hence number (**N**) of distribution plain in a column is given by,

$$N = \frac{L}{H}$$

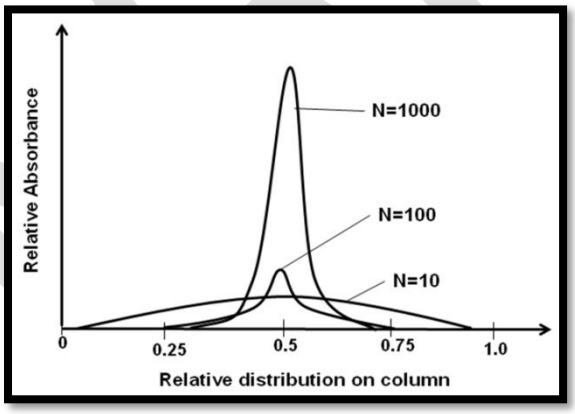
$$N=16\ (t_R/W)^2$$

$$N=5.54\ (t_R/W_{1/2})^2$$

Hence, Number of distribution plain in a column is controlling two parameters:

(1) As number of distribution plain will go up, it will allow the analyte to travel for longer period of time, consequently it will increase the distance between two peaks.

(2) As number of distribution plain will go up, it will reduce the width of the base of peak, as a result the peaks will be more sharp. A representative example, how number of distribution plain affects the base of the peak is given in Figure 5. As the number is increasing, the peak width is decreasing. Hence, number of distribution is an indirect way to measure the column efficiency, higher N number is desirable for better separation.



High Pressure Liquid Chromatography: Pressure limit more than 50-350 bar. A typical polysaccharide bead is not appropriate to withstand high pressure during HPLC. Hence, in HPLC silica based beads are recommended. Due to high pressure and smaller size of the silica beads gives

higher number of theoretical plates. This gives HPLC superior resolving power to separate complex biological samples.

3. Mixer: A mixer is required to mix the buffer received from both pumps to form a linear or step gradient.

4. Column: A column made up of glass or steel.

5. Detector: The elution coming out from column goes to the online monitoring system to test the presence of the analyte based on different properties. There are different types of detectors are known in chromatography such as UV-Visible detector etc.

6. Fraction Collection- The eluent can be collected in different fractions by a fraction collector.

7. Recorder: The profile of eluent with respect to the measured property in a detector can be plotted in the recorder.

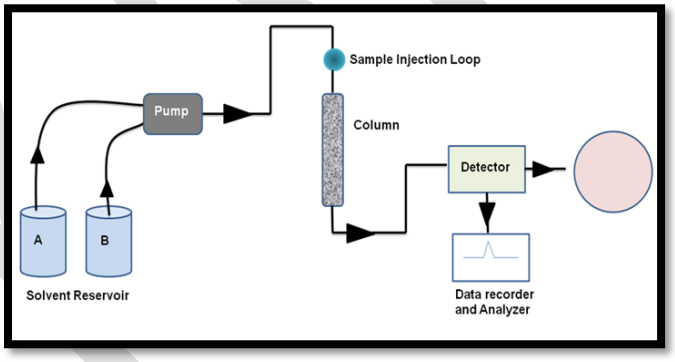


Figure 6: Different components of a chromatography system.

Different forms of chromatography:

Partition Chromatography: In this form of chromatography, an analyte distribute themselves into two phases, liquid stationary and mobile phase. The major advantage of this chromatography is that

it is simple, low cost and has broad specificity. It is further divided into liquid-liquid chromatography and bonded-phase liquid chromatography. The example of this chromatography is cellulose, starch or silica matrix.

Adsorption Chromatography: In this form of chromatography, matrix molecule has ability to hold the analyte on their surface through a mutual interaction due to different types of forces such as hydrogen bonding, electrostatic interaction, vander waal etc. The examples are ion-exchange chromatography, hydrophobic interaction chromatography, affinity chromatography etc.

Paper chromatography

Paper chromatography has proved to be very successful in the analysis of chemical compound and lipid sample in particular. In paper chromatography, the sample mixture is applied to a piece of filter paper, the edge of the paper is immersed in a solvent, and the solvent moves up the paper by capillary action.

Components of the mixture are carried along with the solvent up the paper to varying degrees, depending on the compound's preference to be adsorbed onto the paper versus being carried along with the solvent. The paper is composed of cellulose to which **polar water molecules** are adsorbed, while the solvent is less polar, usually consisting of a mixture of water and an organic liquid.

The paper is called the stationary phase while the solvent is referred to as the mobile phase. In order to obtain a measure of the extent of movement of a component in a paper chromatography experiment, we can calculate an "**Rf value**" for each separated component in the developed**chromatogram**. An Rf value is a number that is defined as: distance traveled by component from application point.

Nature of the paper:

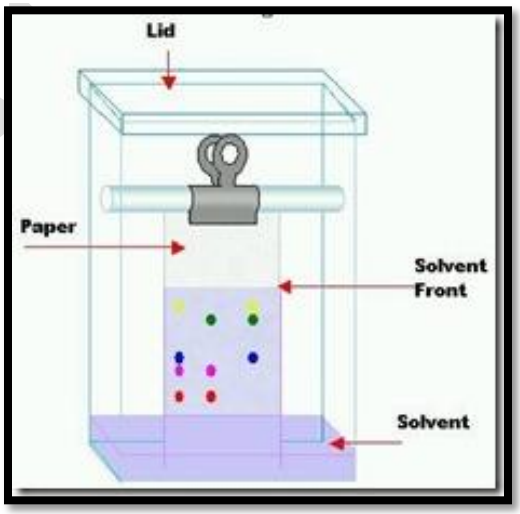
The paper commonly used consists of highly purified cellulose. Cellulose, a homopolysaccharide of glucose. Contains several thousand anhydro-glucose units-linked through oxygen atoms. The paper exhibits **weak ion exchange and adsorptive properties.**Modified forms of paper have been produced in which the paper has been impregnated with *alumina, silica gel, and ion-exchange resin*etc.

The chemical composition of whatmann filter paper no: 1 is: a-cellulose (98 to 99%), b-cellulose (0.3 to 1%), Pentosans (0.4 to 0.8%), Ash (0.07 to 0.1%) & ether soluble matter (0.015 to 0.1%).

Apparatus:

The apparatus required for paper chromatography are

1. Support for paper
2. Solvent trough
3. Airtight chamber
4. Whatmann filter paper number 1
5. Capillary tubes
6. Samples – Amino acids (or) Pigments
7. Solvents
8. Platinum loop

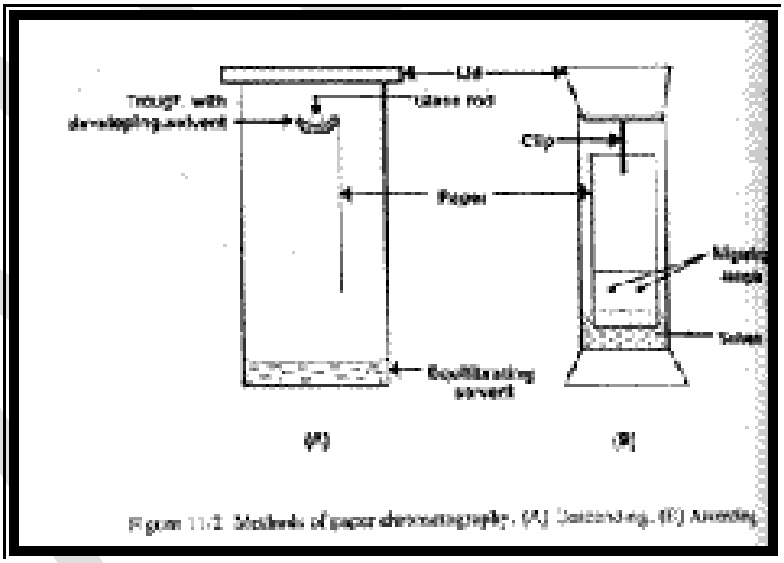


Paper development:

There are two main techniques, which may be employed for the development of paper Chromatograms.

1. *Ascending techniques*
2. *Descending techniques*
3. *Radial development*
4. *Two-dimensional chromatography*

1) Ascending techniques:



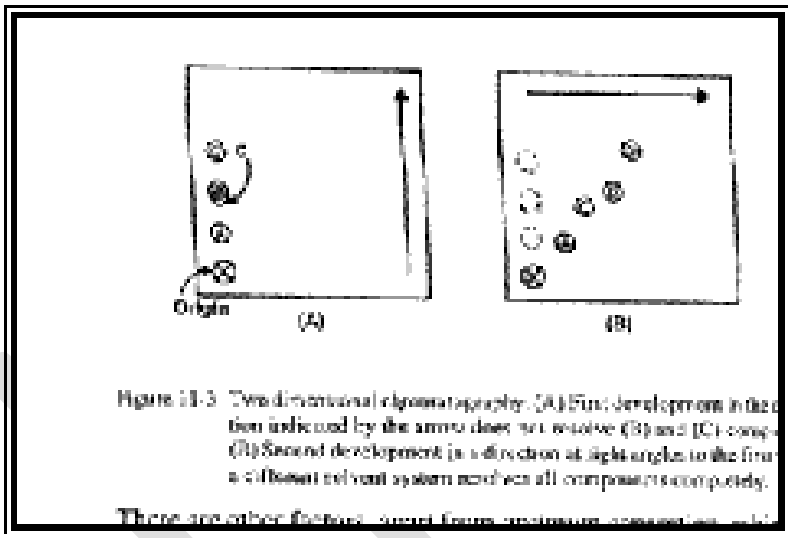
The filter paper is then dried and equilibrated by putting it into on airtight cylindrical jar, which contains an aqueous solution of a solvent. The most widely applicable solvent mixture is n-**B**utanol: **A**cetic acid: **W**ater (4:1:5), which is abbreviated as **BAW**.

The sheet of paper is supported on a frame with the button edge in contact with a trough with solvent. The arrangement is contained in an airtight tank lined with paper saturated with the solvent to prove a constant atmosphere and separations are carried out in a constant temperature room. Thus, the solvent will ascend into the paper this process is, therefore, termed "**Ascending Chromatography**"

2) Descending techniques:

The end of the filter paper may be put into the solvent mixture contained in a narrow trough mounted near the top of the container. In this chromatography, the solvent will descend into the paper and this process is then termed "Descending Chromatography".

This method is convenient for compounds, which have similar Rf values since the solvent drips off the bottom of the paper, thus giving a wider separation.



3) Two dimensional chromatography (3D):

The mixture is separated then the first solvent, which should be volatile: then after drying, the paper is turned through 90^0 and separation is carried out in the second solvent.

After locating the migrated unknown sample along with standard known sample, a map is obtained and comparing their position with a map of known compounds can identify compounds.

Locating the compounds:

Strip is removed when the solvent has migrated over most of the available space. The distance to which the solvent has run is marked. In most cases, the completed Chromatogram is colorless with no indication of the presence of any compounds. Such a chromatogram is said as "Undeveloped" for locating the various compounds.

The filter paper strip is first dried, then sprayed with *0.5% Ninhydrin in acetone* and at least heated for a few minutes at 80 to 100^0 C. the reaction occurs and the colored spots appear at the sites of the amino acids, such as Chromatogram is now called "Developed".

In paper chromatography, the stationary cellulose phase is more polar than the mobile organic phase.

Large non-polar side chain amino acids

→Leucine, Isoleucine
→Phenylalanine, Tryptophan
→Valine, Methionine
→Lysine

} Migrate faster in **n-butanol: Acetic acid: Water (4:1:5)**

Shorter non-polar side chains

→Proline, Alanine
→Glycine

} Migrate faster than large non-polar side chain amino acids

Polar side chains

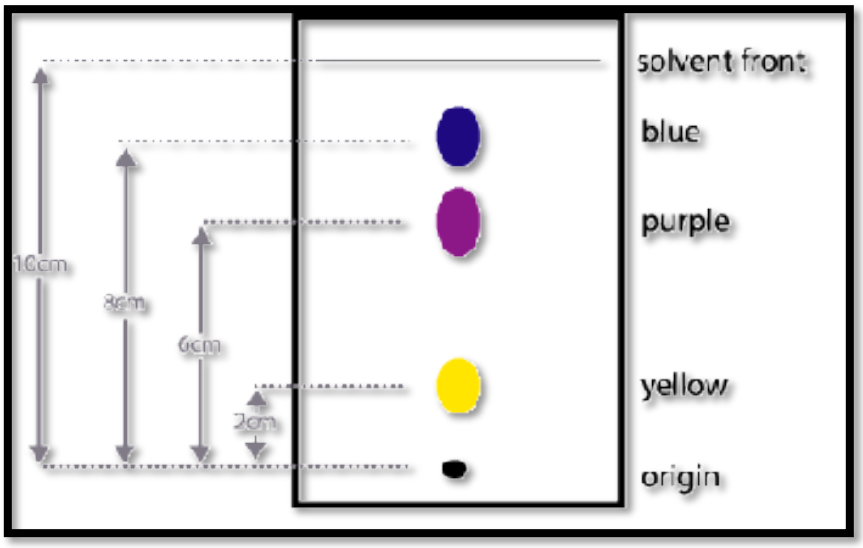
→Threonine, Glutamic acid,
→Serine,Arginine,
→Aspartic acid, Histidine
→Lysine,Cysteine

} Migrate faster than large non-polar side chain amino acids

Identifying the compounds:

The ratio of the distance travelled by a component (i.e. amino acid) to that travelled by the solvent front, both measured from the marked point of the application of the mixture, is called the "Resolution front (Rf)" value for that component.

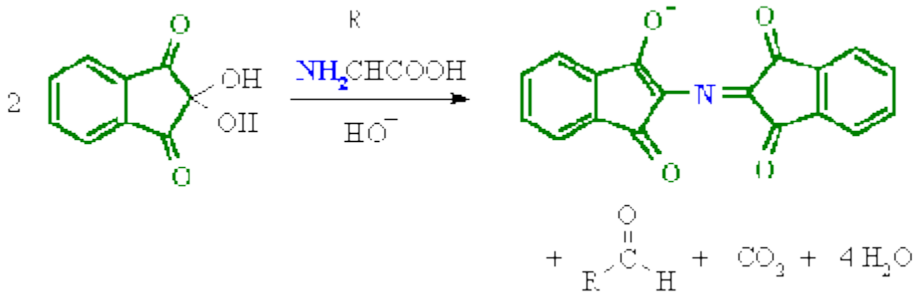
$$Rf = \frac{\text{Distance from origin run by the compound}}{\text{Distance from origin run by the solvent}}$$



Detection:

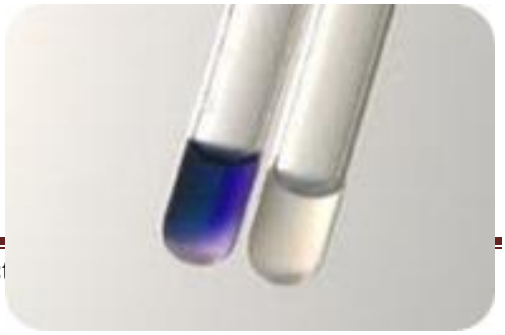
The filter paper strip may be sprayed with ninhydrin and heated so that the colored spots indicating the location of amino acids may develop. The color densities of these spots may be measured with a recording transmittance (or) reflectance photometer device.

- Ninhydrin test or Ninhydrin reagent



Ninhydrin test:

- Amines (including α-amino acids) react with ninhydrin to give a coloured product.
- It can be used qualitatively (*e.g.* for chromatographic visualization) or quantitatively (*e.g.* for peptide sequencing).
- The α-amino acids typically give a **blue-purple product**.



- Proline, a secondary amine, gives a **yellow-orangeproduct**.
- The test is sensitive enough that ninhydrin can be used for the visualization of fingerprints.

Applications of Paper Chromatography:

By using this technique

1. To check the control of purity of pharmaceuticals,
2. To the detection of adulterants,
3. To detect the contaminants in foods and drinks,
4. To the study of ripening and fermentation,
5. To the detection of drugs and dopes in animals & humans
6. To the analysis of cosmetics
7. To the analysis of the reaction mixtures in biochemical labs.

Thin Layer chromatography

The thin layer chromatography technique is an analytical chromatography to separate and analyze complex biological or non-biological samples into their constituents. It is most popular for monitoring the progress of a chemical reaction or estimation of a substance in a mixture. It is also one of the popular technique for testing the purity of a sample. In this method, the silica or alumina as a stationery phase is coated on to a glass or aluminium foil as thin layer and then a sample is allowed to run in the presence of a mobile phase (solvent). In comparison to other chromatography techniques, the mobile phase runs from bottom to top by diffusion (in most of the chromatography techniques, mobile phase runs from top to bottom by gravity or pump). As sample runs along with the mobile phase, it get distributed into the solvent phase and stationery phase. The interaction of sample with the stationery phase retard the movement of the molecule where as mobile phase

implies an effective force onto the sample. Supose the force caused by mobile phase is F_m and the retardation force by stationery phase is F_s, then effective force on the molecule will be (F_m-F_s) through which it will move (Figure 37.1). The molecule immobilizes on the silica gel (where, F_m=F_s) and the position will be controlled by multiple factors.

1. Nature or functional group present on the molecule or analyte.

2. Nature or composition of the mobile phase

3. Thickness of the stationery phase.

4. Functional group present on stationery phase.

If the distance travelled by a molecule on TLC plate is D_m where as the distance travelled by the solvent is D_s, then the retardation factor (R_f) of molecule is given by:

$$\mathbf{Rf} = \frac{\mathbf{Distance\ travelled\ by\ substance\ (Dm)}}{\mathbf{Distance\ travelled\ by\ solvent\ (Ds)}}$$

R_f value is characterstic to the molecule as long as the solvent system and TLC plate remains unchanged. It can be used to identify the substance in a crude mixture.

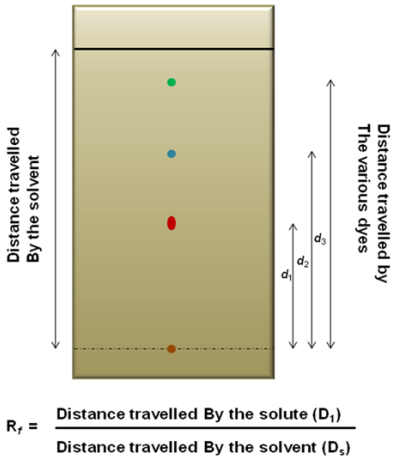


Figure 37.1: Principle of thin layer chromatography.

Operation of the technique- Several steps are required to perform a thin layer chromatography to analyze a complex samples. These preparatory and operational steps are as follows:

Thin Layer Chromatography Chamber- Thin layer chromatographjy chamber (rectangular or cylindrical) is made up of transparent non-reactive material, mostly glass (Figure 37.2). It is covered from top with a thick glass sheet and the joints are sealed with a high vacuum grease to avoid loss of solvent vapor. All three sides of the chamber is covered with a whatman filter paper to uniformly equilibrate the chamber. A solvent system is filled in the chamber and it is allowed to humidify the chamber with the solvent vapor. It is important for uniform running of solvent front during TLC.

Preparation of TLC plate- A silica slurry is prepared in water and spread on the glass or alumina sheet as a thin layer and allowed to dry. It is baked at 110^0C for 1hr in a hot air oven and then the plate is ready for TLC. The layer is thin (~ 0.1-0.25 mm) for analytical application and thick (0.4-2.1 mm) for preparative or bio-assay purposes.

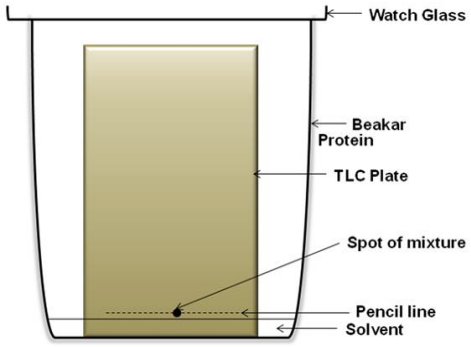


Figure 37.2: Thin Layer chromatography chamber

Spoting: The events involved in spotting is given in Figure 37.3. A line is draw with a pencil little away from the bottom. Sample is taken into the capillary tube or in a pipette. Capillary is touched onto the silica plate and sample is allowed to dispense. It is important that depending on the thickness of the layer, a suitable volume should be taken to apply. Spot is allowed to dry in air or a hair dryer can be used instead.

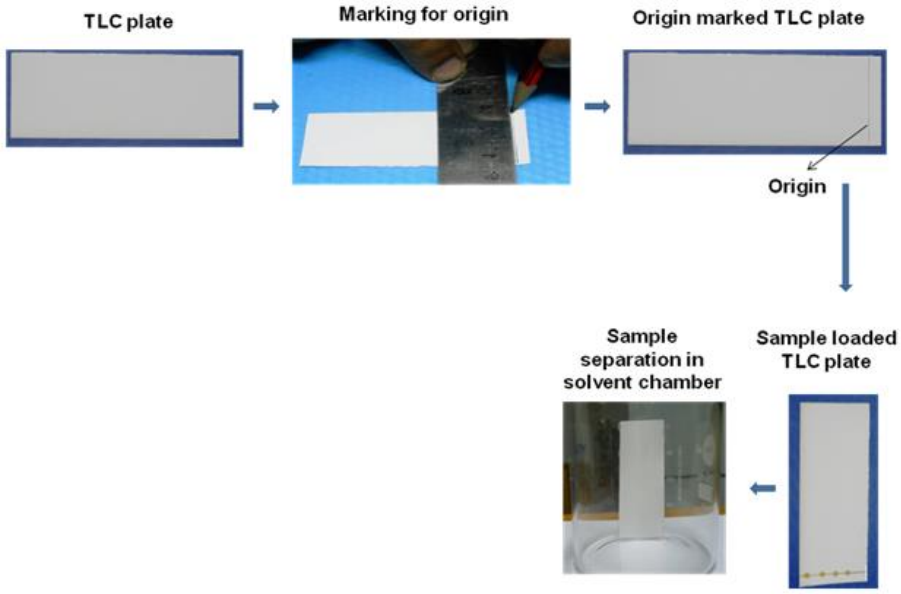


Figure 37.3: Events in spotting during thin layer chromatography.

Running of the TLC: Once the spot is dried, it is placed in the TLC chamber in such a way that spot should not be below the solvent level. Solvent front is allowed to move until the end of the plate.

Analysis of the chromatography plate- The plate is taken out from the chamber and air dried. If the compound is colored, it forms spot and for these substances there is no additional staining required. There are two methods of developing a chromatogram-

Staining procedure- In the staining procedure, TLC plate is sprayed with the staining reagent to stain the functional group present in the compound. Forx. Ninhydrin is used to stain amino acids.

Non-staining procedure- In non-staining procedure spot can be identify by following methods-

1. **Autoradiography-** A TLC plate can be placed along with the X-ray film for 48-72 hrs (exposure time depends on type and concentration of radioactivity) and then X-ray film is processed.
2. **Fluorescence-** Several heterocyclic compounds give fluorescence in UV due to presence of conjugate double bond system. TLC plate can be visualized in an UV-chamber (Figure 37.4) to identify the spots on TLC plate.

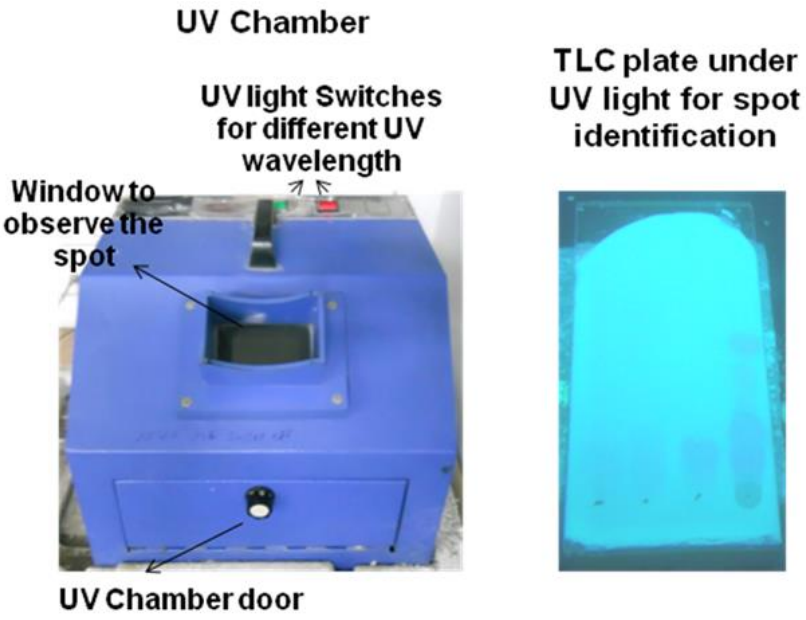


Figure 37.4: UV-Chamber and UV illuminated TLC plate.

Technical troubles with thin layer chromatography-

1. **Tailing effect-** In general sample forms round circular spot on the TLC plate. It is due to the uniform movement of the solvent front through out the plate. But in few cases instead of forming a spot, a compound forms a spot with long trail or rocket shape spot (Figure 37.5). it is due to few reasons as given below:

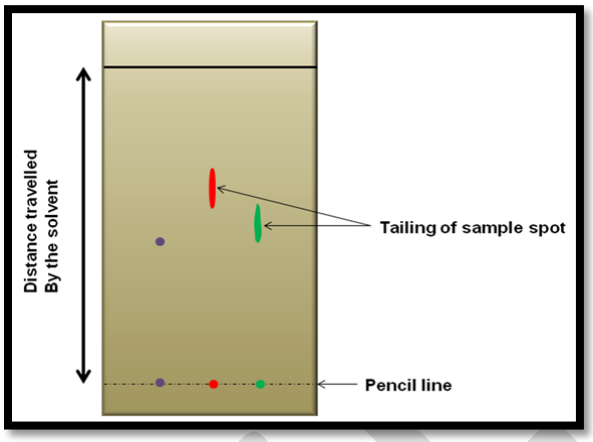


Figure 37.5: Tailing effect in thin layer chromatography.

A. Over-loading- if the sample is loaded much more than the loading capacity of the TLC plate, it appears as spot with trail or rocket shape spot. A diluted sample can be tested to avoid this.

B. Fluctuation in temp or opening of chamber- If there will be fluntuation in temperature or solvent saturation in the chamber (due to opening of the chamber during running), it disturb the flow of solvent front and consequently cause spot with trail. it can be avoided by maintaining a uniform temperature and the opening of the chamber should be minimized especially during running.

2. No movement of sample- In few cases, a sample doesn't move from the spot after the run is completed. These problems are common with high molecular weight substances such as protein or chemicals with large number of functional group. In this case, a change in polarity or pH of solvent system can be explored to bring the compound into the solvent front so that it run on silica plate to get resolved.

3. Movement is too fast- In few cases, the movement of a compound is too fast and does not give time to interact with the matrix to resolve into individual compounds. In this case, a change in polarity of solvent system can be explored to retard the running of the sample.

Applications of Thin layer Chromatography

1. Composition analysis of biomolecules/synthetic preparation

2. Quality testing of compound.

3. Identification of impurities in a sample

4. Progress of chemical reaction

5. Estimation of biomolecules

6. Bio-assay

Ion exchange chromatography

Ion exchange chromatography: Ion-exchange chromatography is a versatile, high resolution chromatography techniques to purify the protein from a complex mixture. In addition, this chromatography has a high loading capacity to handle large sample volume and the chromatography operation is very simple.

Principle: This chromatography distributes the analyte molecule as per charge and their affinity towards the oppositively charged matrix. The analytes bound to the matrix are exchanged with a competitive counter ion to elute. The interaction between matrix and analyte is determined by net charge, ionic strength and pH of the buffer. For example, when a mixture of positively charged analyte (M, M^{+}, M^{-1}, M^{-2}) loaded onto a positively charged matrix, the neutral or positively charged analyte will not bind to the matrix where as negatively charged analyte will bind as per their relative charge and needed higher concentration of counter ion to elute from matrix (Figure 29.1).

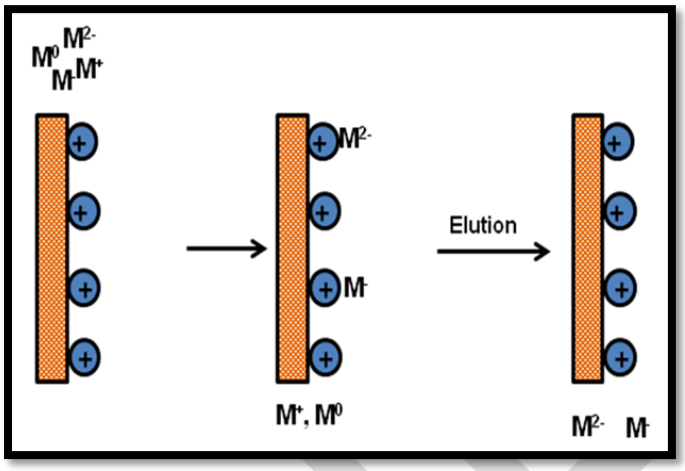


Figure 29.1: Affinity of analytes (M, M^{+}, M^{-1}, M^{-2}) towards positively charged matrix.

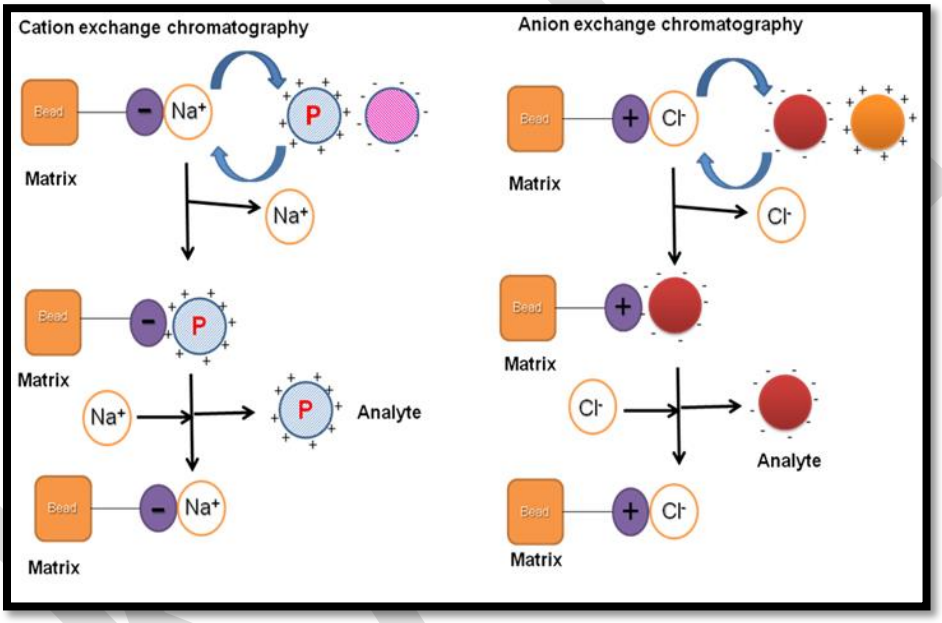
The matrix used in ion-exchange chromatography is present in the ionized form with reversibly bound ion to the matrix. The ion present on matrix participitate in the reversible exchange process with analyte. Hence, there are two types of ion-exchange chromatography:

1. Cation exchange chromatography- In cation exchange chromatography, matrix has a negatively charged functional group with a affinity towards positively charged molecules. The positively charged analyte replaces the reversible bound cation and binds to the matrix (Figure 29.2). In the presence of a strong cation (such as Na^{+}) in the mobile phase, the matrix bound positively charged analyte is replaced with the elution of analyte. The popular cation exchangers used are given in Table 29.1.

2. Anion Exchange chromatography- In anion exchange chromatography, matrix has a positively charged functional group with a affinity towards negatively charged molecules. The negatively charged analyte replaces the reversible bound anion and binds to the matrix (Figure 29.2, B). In the presence of a strong anion (such as Cl^{-}) in the mobile phase, the matrix bound negatively charged analyte is replaced with the elution of analyte. The popular anion exchangers used are given in Table 29.1.

Table 30.1: List of selected Ion-exchange matrix

S.No	Name	Functional Group	Type of Ion-exchanger
1	Carboxyl methyl (CM)	-OCH2COOH	Cation Exchanger
2	Sulphopropyl (SP)	-OCH2CH2CH2SO3H	Cation Exchanger
3	Sulphonate (S)	-OCH2SO3H	Cation Exchanger
4	Diethylaminoethyl (DEAE)	-OCH2CH2NH (C2H5)2	Anion Exchanger
5	Quaternary aminomethyl (Q)	-OCH2N(CH3)3	Anion Exchanger



1. pI value and Net charge- The information of a pI will be allow you to calculate the net charge at a particular pH on a protein. As discussed above, a cation exchange chromatography can be use below the pI where as an anion exchange chromatography can be use above the pI value.

2. Structural stability- 3-D structure of a protein is maintained by electrostatic and vander waal interaction between charged amino acid, Π-Π interaction between hydrophobic side chain of amino acids. As a result, protein structure is stable in a narrow range around its pI and a large deviation from it may affect its 3-D structure.

3. Enzymatic activity- Similar to structural stability, enzymes are active in a narrow range of pH and this range should be consider for choosing an ion-exchange chromatography.

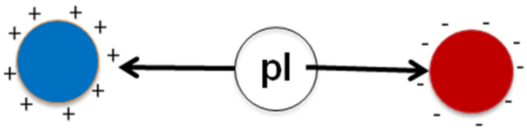


Figure 29.3: Change of charge with respect to the pI.

Operation of the technique- Several parameters needs to be consider to perform ion-exchange chromatography (Figure 29.4).

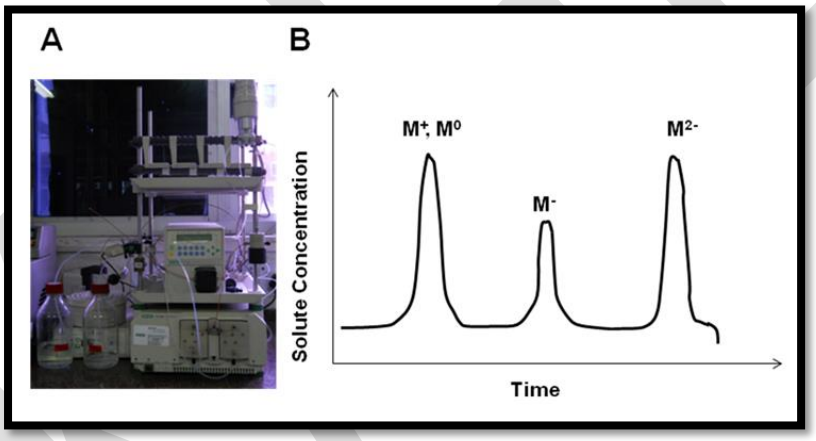


Figure 29.4 : Operation of the Ion-exchange Chromatography. (A) Chromatography system to perform gradient elution of analytes to give an (B) elution profile.

1. Column material and stationary phase- Column material should be chemically inert to avoid destruction of biological sample. It should allow free low of liquid with minimum clogging. It

should be capable to withstand the back pressure and it should not compress or expand during the operation.

2. Mobile Phase- The ionic strength and pH are the crucial parameters to influence the property of the mobile phase.

3. Sample Preparation- The sample is prepared in the mobile phase and it should be free of suspended particle to avoid clogging of the column. The most recommended method to apply the sample is to inject the sample with a syringe.

4. Elution- There are many ways to elute a analyte from the ion-exchange column. (1) Isocratic elution (2) Step-wise gradient (3) Continuous gradient either by salt or pH (4) affinity elution (5) displacement chromatography

5. Column Regeneration- After the elution of analyte, ion-exchange chromatography column require a regeneration step to use next time. column is washed with a salt solution with a ionic strength of 2M to remove all non-specifically bound analytes and also to make all functional group in a iononized form to bind fresh analyte.

Applications of Ion-exchange chromatography

1. Protein Purification- In the previous lecture we have already discussed how protein can be purified using ion-exchange chromatography.

2. Protein-DNA interaction- Ion-exchange column is used as a tool to study interaction between DNA and a particular protein. DNA is negatively charged and has strong affinity towards anion exchange chromatography. A schematic figure to depict the steps involved in DNA-protein interaction is given in (Figure 30.1). In this approach, anion exchange matrix is incubated with the DNA and allowed it to bind tightly. Excesss DNA is washed from the column. Now the pure protein is passed through the DNA bound beads, followed by washing with the buffer to remove

unbounded proteins. Now the DNA is eluted from the matrix either by adding high salt concentration or with denaturating condition. Now the fractions are tested for the presence of DNA and protein. Eluted protein is analyzed in the SDS-PAGE and DNA is in agarose. As a control, protein is also added to the matrix without DNA to rule out the possibility of protein binding directly to the matrix. If protein will have a affinity towards DNA, they both will comes out from the column at the same time and should give similar pattern in the elution profile. It could be possible that high salt may break interaction between DNA and protein, in such situation protein will comes out first followed by DNA. Besides this ion-exchange chromatography approach still be able to answer whether the DNA-protein are interacting with each other or not.

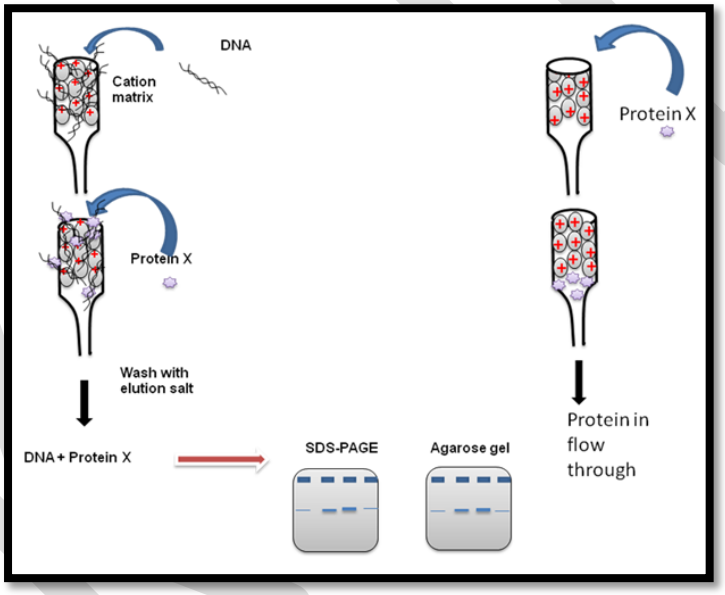


Figure 30.1: DNA-Protein interaction.

3. Softening of water- Ground water has several metals such as Ca^{2+}, Mg^{2+} and other cationic metals. Due to presence of the metal, hard water creates problem in industrial settings. Ion-exchange chromatography is used to remove the metals present in the water through an exchange of matrix bound Na^{+} (Figure 30.2). Calcium or magnesium present in the hard water has more affinity

towards the matrix and it replaces with matrix bound sodium ions. The schematic presentation of water softening is given in Figure 30.3. A cation exchanger matrix with bound sodium is packed in the column and the hard water containing calcium, magnesium is passed

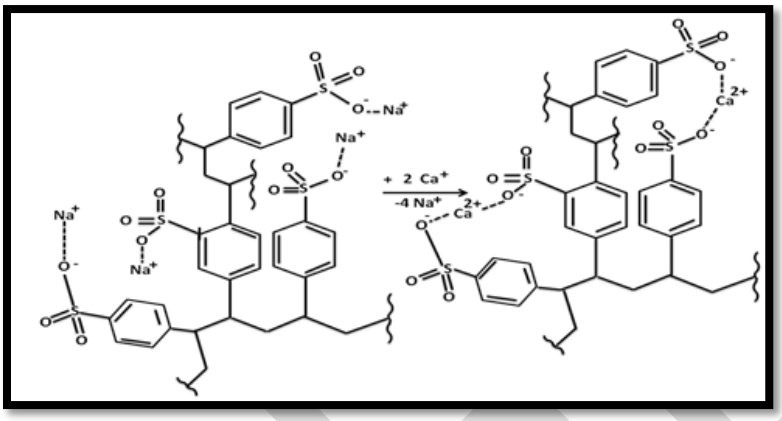


Figure 30.2: Mechanism of metal exchange during water softening.

through the column. In this process, calcium present in the solution preferentially migrate from the solution to the matrix where as sodium ion present on the matrix migrate into the solution. The matrix can be use for softening of the water until it has bound sodium ions. Once sodium ions are exhausted, matrix can be regenerated by flowing a solution of sodium chloride or sodium hydroxide. The calcium/magnesium bound to the matrix comes out in the solution and can be dumped into the sewage.

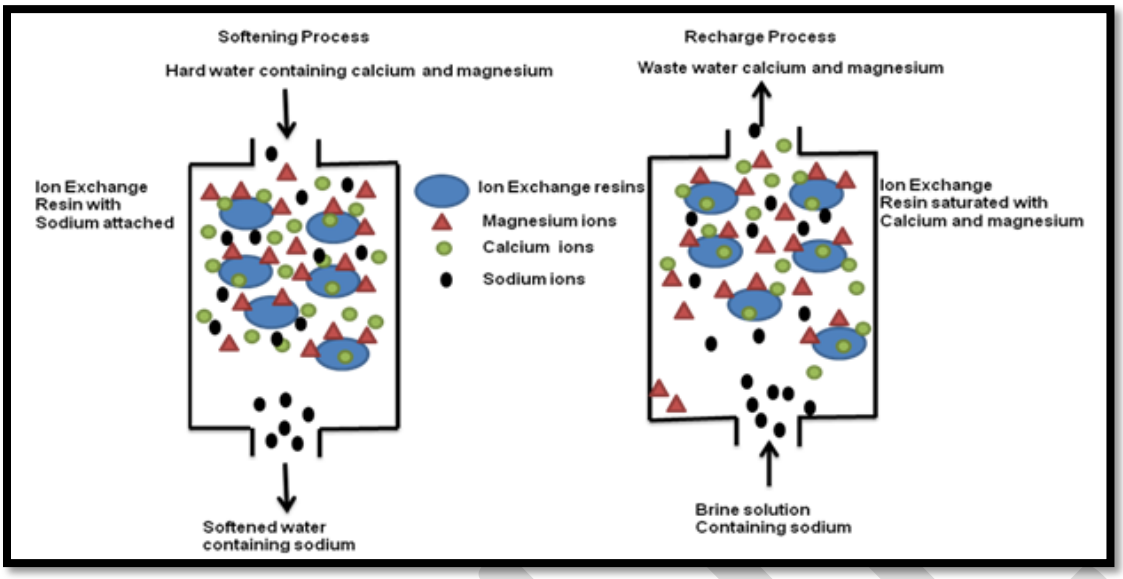


Figure 30.3: Softening of water by a cation exchanger matrix column. [NEEDS RECONSTRUCTION].

4. Protein kinase assay- Protein kinase are class of enzyme responsible for transfer of phosphate group on the substrate molecule. In the protein kinase assay, a radioactive substrate (preferable a radioactivity on carbon) was incubated with the enzyme protein kinase, $MgCl_2$ and non-radioactive ATP. A negative control is also been included where enzyme protein kinase is absent from the assay mixture. Reaction mixture from negative control and experimental will be loaded on two separate cation exchange chromatography columns to bind unphosphorylated substrate from the reaction mixture where as phosphorylated radioactive substrate is present in the flow through. The radioactive count of the flow through was measured using a liquid scintillation reagent (Figure 30.4).

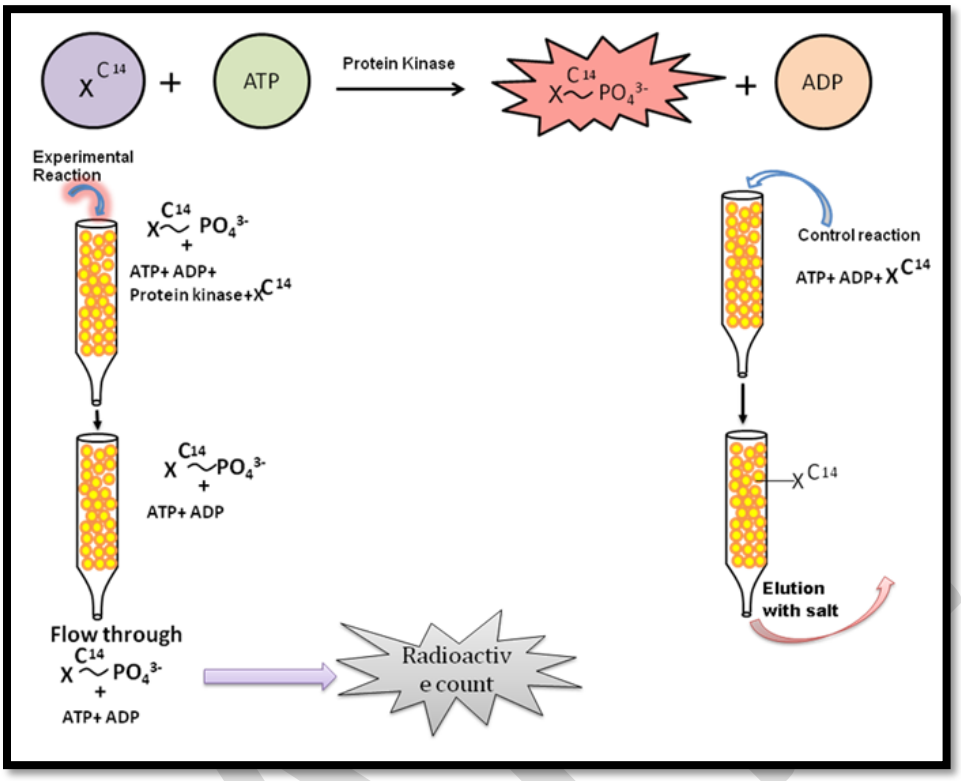


Figure 30.4: Protein kinase assay using ion-exchange chromatography.

5. Purification of rare earth metals from nuclear waste- Ion-exchange matrix is used to isolate and purify rare earth metals such as uranium or plutonium. The first process to isolate uranium in large quantities was developed by frank spedding. Ion-exchange beads are also found suitable to recover uranium from the water coming out of the nuclear power plant. Uranium binds to the matrix through the ion-exchange process. The uranium bound bead is sent to the processing unit where uranium is isolated from the beads to form **'yellow cake'** and stored in drum for further processing. The ion-exchange beads can be reused in the ion-exchange facility.

6. Concentrating a sample- A ion-exchange bead can be used to bind the analyte from a diluted solution and then sample can be eluted in smaller volume to increase the concentration.

Gel Filtration Chromatography

Introduction: This chromatography distributes the protein or analyte, based on their size by passing through a porous beads. The first report in 1955 described performing a chromatography column with swollen gel of maize starch to separate the protein based on their size. '**Porath and Floidin**' coined the term "gel filtration" for this chromatography technique separating the analytes based on molecular sizes. Since then the chromatography technique evolved in terms of developed of different sizes beads to separate protein of narrow range, as well as performing the technique in aqueous and non-aqueous mobile phase. The beads used in gel filtration chromatography is made up of cross linked material (such as dextran in sephadex) to form a 3-D mesh. These 3-D mesh swell in the mobile phase to develop pores of different sizes (Figure 32.1). The extent of cross linking controls the pores size within the gel beads.

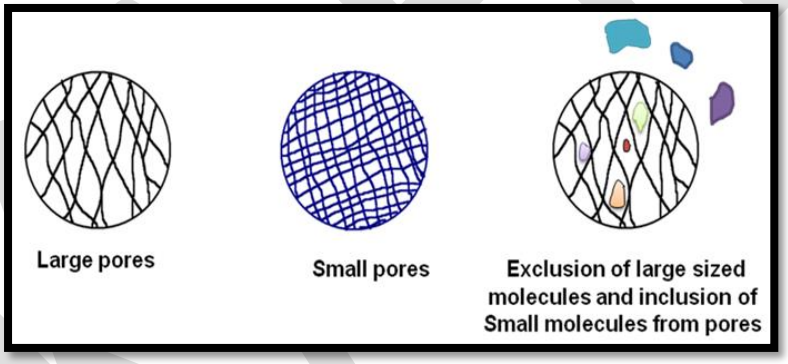


Figure 32.1: Gel Filtration Matrix has Beads with different pore sizes.

Principle: The principle of the chromatography technique is illustrated in Figure 32.2. The column is packed with the beads containing pores to allow entry of molecules based on their sizes. Smallest size in the inner part of pore followed by gradual increasing size and largest molecule excluded from entering into the gel. The separation between molecules occur due to the time they travel to come out from the pores. When the mobile phase pass through the column, it takes protein along with it. The small molecules present in the inner part of the gel takes longer flow of liquid (or time)

and travel longer path to come out where as larger molecules travel less distance to come out. As a result, the large molecule and small molecule get separated from each other. A schematic gel filtration chromatogram is given in Figure 32.3.

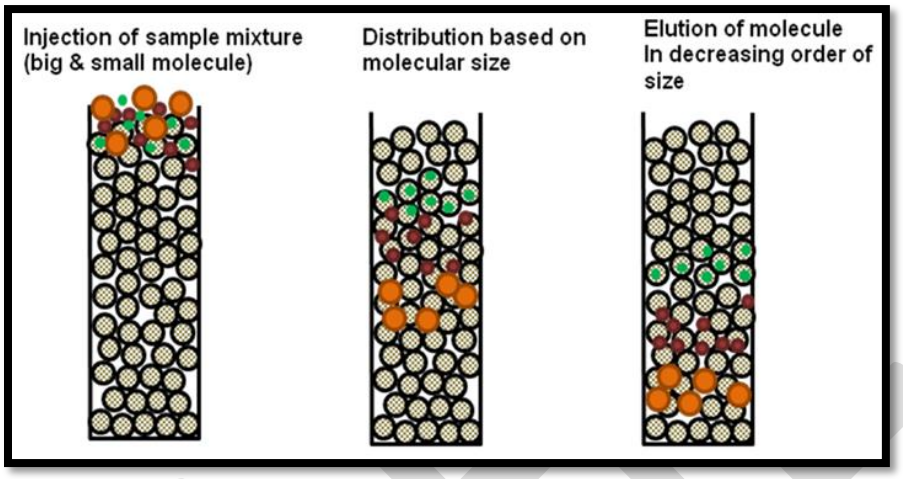


Figure 32.2: Principle of Gel Filtration Chromatography.

Suppose the total column volume of a gel is V t and then it is given by-

$$V_t = V_g + V_i + V_o \quad \text{.....Eq (32.1)}$$

V_g is the volume of gel matrix, V_i is the pore volume and V_o is the void volume. The volume of mobile phase flow to elute a column from a column is known as elution volume (V_e). The elution volum is related to the void volume and the distribution coefficient Kd as given below

$$V_e = V_o + KdV_i \quad \text{.....Eq (32.2)}$$

$$Kd = \frac{Ve - Vo}{Vi} \quad \text{.....Eq (32.3)}$$

Kd is the ratio of inner volume available for an analyte and it is independent to the column geometry or length. As per relationship given in Eq 32.3, three different type of analytes are possible:

1. Analyte with **Kd=0** , or Ve=Vo, these analytes will be completely excluded from the column.

2. Analyte with **Kd=1** or Ve=Vo+Vi, these analytes will be completely in the pore of the column.

3. Analyte with **Kd>1** , in this situation analyte will adsorb to the column matrix.

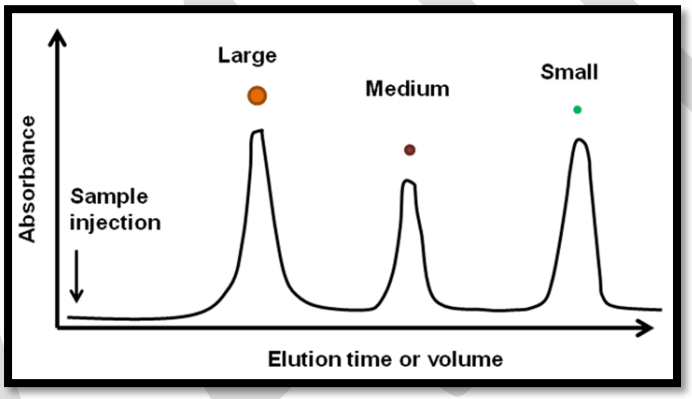


Figure 32.3: A typical Gel Filtration Chromatogram.

Choice of matrix for gel filtration chromatography- The choice of the column depends on the range of molecular weight and the pressure limit of the operating equipment. A list of popular gel filtration column matrix with the fractionation range are given in Table 32.1.

Operation of the chromatography-

1. Column packing- The column material is allowed to swell in the mobile phase. It is poured into the glass tube and allow the beads to settle without trapping air bubble within the column. Once the

matrix is settled to give a column, it can be tested for presence of air channel and well packing by flowing a analyte with Kd=1, it is expected that the elution volume (Ve) in this case should be $V_o + V_i$.

2. Sample Preparation- The sample is prepared in the mobile phase and it should be free of suspended particle to avoid clogging of the column. The most recommended method to apply the sample is to inject the sample with a syringe.

3. Elution- In gel filtration column, no gradient of salt is used to elute the sample from the column. The flow of mobile phase is used to elute the molecules from the column.

4. Column Regeneration- After the analysis of analyte, gel filtration column is washed with the salt containing mobile phase to remove all non-specifically adsorb protein to the matrix. The column is then equiliberated with mobile phase to regenerate the column. The column can be store at 4°C in the presence of 20% alchol containing 0.05% sodium azide.

Table 32.1: List of popular gel filtration matrix

S.No	Name of the matrix	Fractionation Range (Daltons)
1	Sephadex G10	Upto 700
2	Sephadex G25	1000-5000
3	Sephadex G50	1500-30,000
4	Sephadex G100	4000-150,000
5	Sephadex G200	5000-600,000
6	Sepharose 4B	60,000-20,000,000
7	Sepharose 6B	10,000-4,000,000

Figure 30.4: Protein kinase assay using ion-exchange chromatography.

Determination of native molecular weight of a protein using gel filtration chromatography

The molecular weight and size of a protein is related to the shape of the molecule and the relationship between molecular weight (M) and radius of gyration (R_g)is as follows-

$$Rg \propto M^a \quad \text{.....Eq 32.4}$$

here "a" is a constant and it depends on shape of the molecule, a=1 for Rod, a=0.5 for coils and a=0.33 for spherical molecules.

The set of known molecular weight standard protein can be run on a gel filitration column and elution volume can be calculated from the chromatogram (Figure 32.4). A separate run with the analyte will give elution volume for unknown sample. Using following formula, Kd value for all standard protein and the test analyte can be calculated.

$$Kd = \frac{Ve - Vo}{Vi}$$

A plot of Kd versus log mol wt is given in Figure 32.4, B and it will allow us to calculate the molecular weight of the unknown analyte.

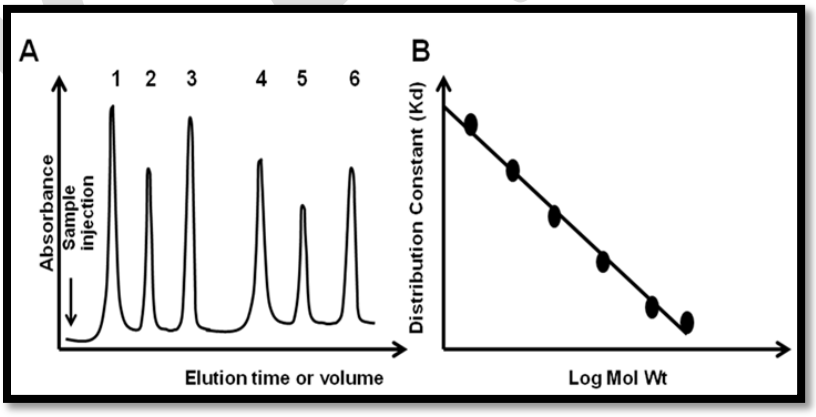


Figure 32.3: Determination of molecular weight by gel filtration chromatography. (A) Gel

filtration chromatogram with the standard proteins (1-6), (B) Relationship between distribution constant (Kd) and Log Molecular weight.

Oligomeric status of the protein- Native molecular weight determination by gel filtration in conjugation with the SDS-PAGE can be used to determine the oligomeric status of the protein.

$$\text{Oligomeric Status} = \frac{\text{Molecular weight (Gel Filtration)}}{\text{Molecular weight (SDS} - \text{PAGE)}}$$

Studying protein folding- Protein is madeup of the different types of amio acid residues linked by the peptide bond. As soon as peptide chain comes out from the ribosome, it folds into the 3-D conformation directed by the amino acid sequence, external environment and other factors. Protein structure has multilevel organization; Primary structure (sequence of protein), secondary (α-helix, β-sheet and turn), tertiary and quaternary structure. When protein is incubated with the increasing concentration of denaturating agents (such as urea), it unfolds the native structure into the unfolded extended conformation following multiple stages. The different protein conformation forms during unfolding pathway has distinct hydrodynamic surface area and it can be use to follow protein folding-unfolding stages with the gel filtration chromatography. The details of the experimental setup is given in the Figure 33.1. Protein is incubated with different concentration of urea (0-8M) for 8-10hrs at 37°C. A gel filtration column is equiliberated with the buffer containing urea (same as in incubation mixture) and the incubation mixture is analyzed. As the concentration of denaturating agent is increasing, protein will unfold with an increase in hydrodynamic surface area. As a result, protein peak shifts towards left. At highest concentration of denaturant, protein unfolds completely and mostly appear in void volume.

Studying protein-ligand interaction- Gel filtration chromatography separates the molecules based on their size. Ligand binding to the protein induces conformational changes, result into the change in size or shape (Figure 33.2). In addition, ligand is small in size where as protein-ligand complex is big and may appear at a distinct place in the column. In step 1, a gel filtration column is equiliberated with the buffer and elution profile of ligand is recorded. Now column is equliberated with the buffer containing ligand molecule. As the concentration of ligand is increased, protein binds ligand and form a larger complex with an increase in hydrodynamic surface area. As a result, protein peak shifts towards left. As the concentration of ligand will

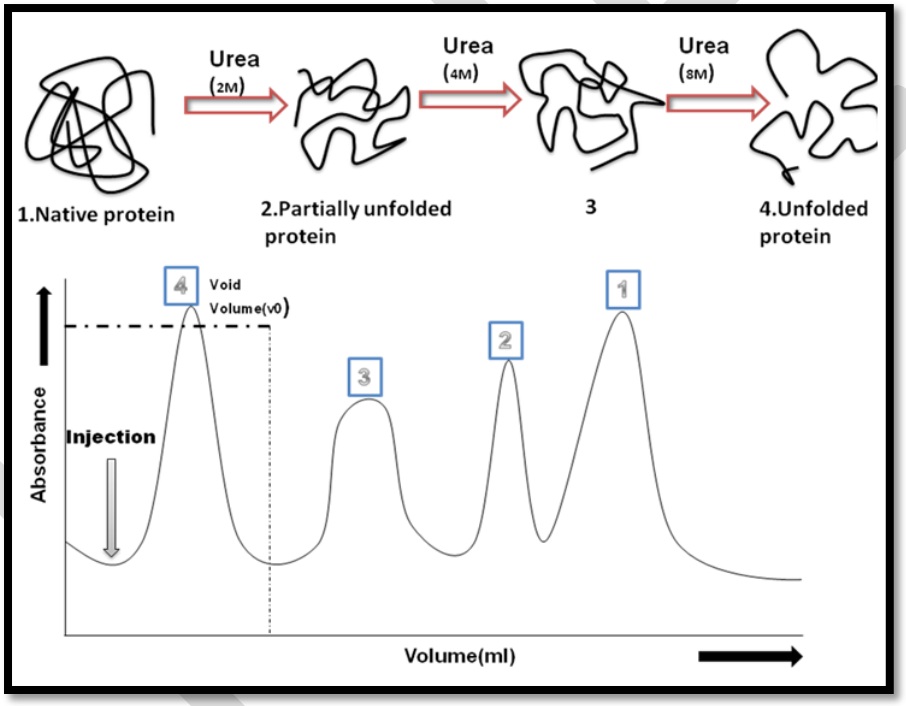


Figure 33.1: Studying Protein folding stages by gel filtration chromatography.

increase with a fixed amount of the protein, free ligand will appear in the chromatogram. The protein amount and the concentration at which free ligand appeared, and the elution data can be use to calculate the stoichiometric ratio of ligand/protein and the equilibrium constant.

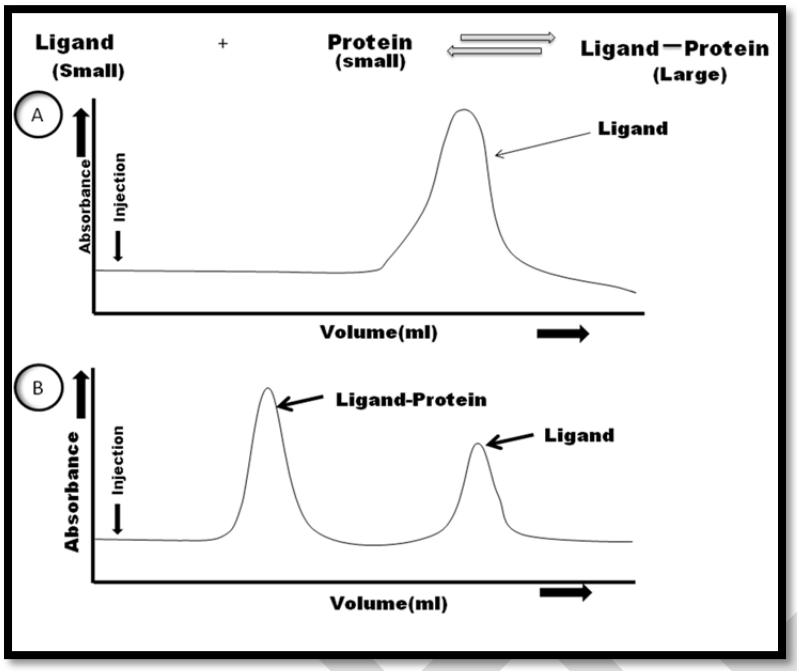


Figure 33.2: Studying Protein-ligand interaction by gel filtration chromatography.

Desalting- Desalting or removal of the small molecule from the protein is important for activity assay and other down stream processes. A gel filtration column is equilibrated with the buffer or water and then the sample for desalting is loaded. After the run the protein and salt are eluted separaetely as peak (Figure 33.3).

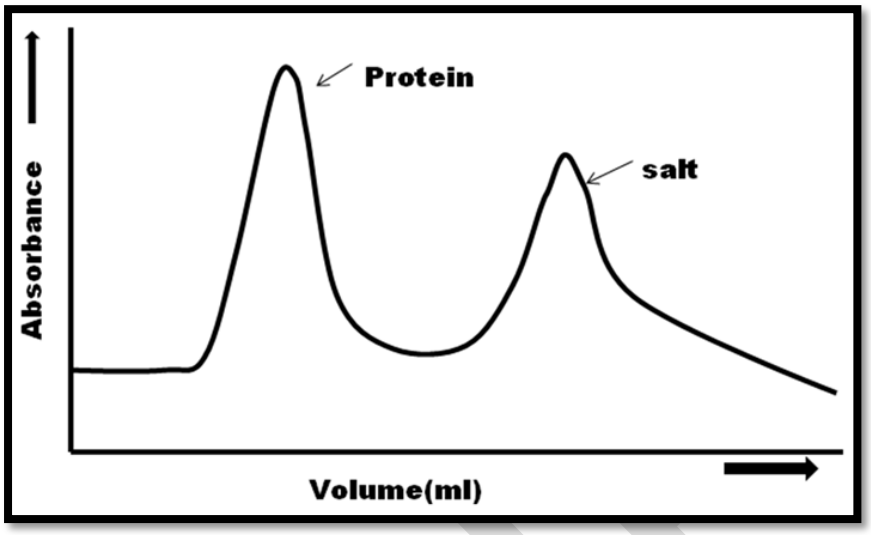


Figure 33.3: Desalting of a sample by gel filtration chromatography.

High Performance Liquid Chromatography (HPLC)

High Performance Liquid Chromatography (HPLC) : Principle, Types, Instrumentation and Applications

Chromatography is a technique to separate mixtures of substances into their components on the basis of their molecular structure and molecular composition. This involves a stationary phase (a solid, or a liquid supported on a solid) and a mobile phase (a liquid or a gas). The mobile phase flows through the stationary phase and carries the components of the mixture with it. Sample components that display stronger interactions with the stationary phase will move more slowly through the column than components with weaker interactions. This difference in rates cause the separation of variuos components. Chromatographic separations can be carried out using a variety of stationary phases, including immobilized silica on glass plates (thin-layer chromatography),

volatile gases (gas chromatography), paper (paper chromatography) and liquids (liquid chromatography).

High perfomance Liquid Chromatography

High performance liquid chromatography (HPLC) is basically a highly improved form of column liquid chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres. That makes it much faster. All chromatographic separations, including HPLC operate under the same basic principle; separation of a sample into its constituent parts because of the difference in the relative affinities of different molecules for the mobile phase and the stationary phase used in the separation.

Types of HPLC

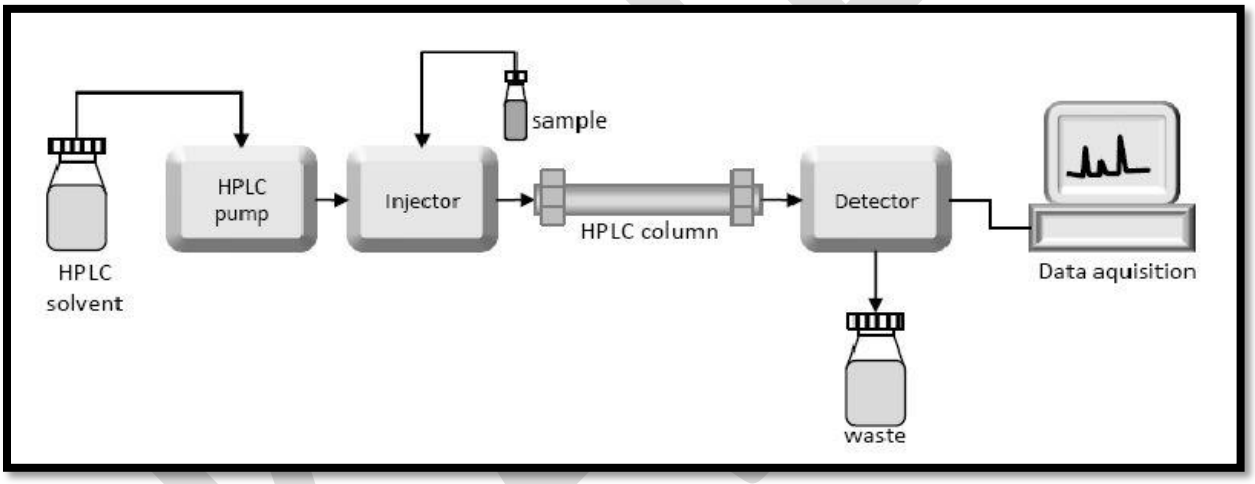
There are following variants of HPLC, depending upon the phase system (stationary) in the process :

1. **Normal Phase HPLC**:
   This method separates analytes on the basis of polarity. NP-HPLC uses polar stationary phase and non-polar mobile phase. Therefore, the stationary phase is usually silica and typical mobile phases are hexane, methylene chloride, chloroform, diethyl ether, and mixtures of these. Polar samples are thus retained on the polar surface of the column packing longer than less polar materials.
2. **Reverse Phase HPLC:**
   The stationary phase is nonpolar (hydrophobic) in nature, while the mobile phase is a polar liquid, such as mixtures of water and methanol or acetonitrile. It works on the principle of hydrophobic interactions hence the more nonpolar the material is, the longer it will be retained.
3. **Size-exclusion HPLC:**
   The column is filled with material having precisely controlled pore sizes, and the particles are separated according to its their molecular size. Larger molecules are rapidly washed through the column; smaller molecules penetrate inside the porous of the packing particles and elute later.

4. **Ion-Exchange HPLC:**

The stationary phase has an ionically charged surface of opposite charge to the sample ions. This technique is used almost exclusively with ionic or ionizable samples. The stronger the charge on the sample, the stronger it will be attracted to the ionic surface and thus, the longer it will take to elute. The mobile phase is an aqueous buffer, where both pH and ionic strength are used to control elution time.

Instrumentation of HPLC



As shown in the schematic diagram in Figure above, HPLC instrumentation includes a pump, injector, column, detector and integrator or acquisition and display system. The heart of the system is the column where separation occurs.

1. **Solvent Resorvoir** : Mobile phase contents are contained in a glass resorvoir. The mobile phase, or solvent, in HPLC is usually a mixture of polar and non-polar liquid components whose respective concentrations are varied depending on the composition of the sample.
2. **Pump** : A pump aspirates the mobile phase from the solvent resorvoir and forces it through the system's column and detecter. Depending on a number of factors including column dimensions,

particle size of the stationary phase, the flow rate and composition of the mobile phase, operating pressures of up to 42000 kPa (about 6000 psi) can be generated.

3. **Sample Injector** : The injector can be a single injection or an automated injection system. An injector for an HPLC system should provide injection of the liquid sample within the range of 0.1-100 mL of volume with high reproducibility and under high pressure (up to 4000 psi).
4. **Columns** : Columns are usually made of polished stainless steel, are between 50 and 300 mm long and have an internal diameter of between 2 and 5 mm. They are commonly filled with a stationary phase with a particle size of 3–10 µm. Columns with internal diameters of less than 2 mm are often referred to as microbore columns. Ideally the temperature of the mobile phase and the column should be kept constant during an analysis.
5. **Detector** : The HPLC detector, located at the end of the column detect the analytes as they elute from the chromatographic column. Commonly used detectors are UV-spectroscopy, fluorescence, mass-spectrometric and electrochemical detectors.
6. **Data Collection Devices** : Signals from the detector may be collected on chart recorders or electronic integrators that vary in complexity and in their ability to process, store and reprocess chromatographic data. The computer integrates the response of the detector to each component and places it into a chromatograph that is easy to read and interpret.

Applications of HPLC

The information that can be obtained by HPLC includes resolution, identification and quantification of a compound. It also aids in chemical separation and purification. The other applications of HPLC include :

- **PharmaceuticalApplications**

  1.To control drug stability.

  2. Tablet dissolution study of pharmaceutical dosages form.

  3. Pharmaceutical quality control.

- **Environmental Applications**

  1. Detection of phenolic compounds in drinking water.
  2. Bio-monitoring of pollutants.

- **Applications in Forensics**

  1. Quantification of drugs in biological samples.
  2. Identification of steroids in blood, urine etc.
  3. Forensic analysis of textile dyes.
  4. Determination of cocaine and other drugs of abuse in blood, urine etc.

- **Food and Flavour**

  1. Measurement of Quality of soft drinks and water.
  2. Sugar analysis in fruit juices.
  3. Analysis of polycyclic compounds in vegetables.
  4. Preservative analysis.

- **Applications in Clinical Tests**

  1. Urine analysis, antibiotics analysis in blood.
  2. Analysis of bilirubin, biliverdin in hepatic disorders.
  3. Detection of endogenous Neuropeptides in extracellular fluid of brain etc.

Gas Chromatography & principle (principle of GC):

The principle in gas chromatography principle involves separation of components of the sample under test due to partition in between gaseous mobile phase and stationary liquid phase. The elements partitioned into gas come out first while other come later.

Gas chromatography runs on the **principle of partition chromatography** for separation of components. Based on the stationary and mobile phases it is categorized under *the gas-liquid type of chromatography,* i.e., the stationary phase is a liquid layer supported over a stationary phase

while the mobile phase is an inert and stable gas. Hence the perfect name as Gas-Liquid chromatography (GLC).

How gas chromatography works:

The gas is set to flow at a constant rate from the cylinder on to the liquid layer impregnated on a solid support in a column. The sample is injected into the injection point and is carried by the mobile gas into the column. Inside the column, the components get separated by the differential partition in between the mobile phase gas and stationary phase liquid. The component that partitioned into gascomes out of the column first and is detected by the detector. The one partitioned into liquid phase comes out later and is also detected. The recordings are displayed onto a computer software. From these peaks, one can identify the components and also their concentration.

Must read article Gas Chromatography Theory for details on other important aspects of GC.

Gas chromatography method: Below is the video of the instrumentation and method simultaneously.

Gas chromatography instrumentation

The gas chromatography apparatus can be listed as

1. The mobile phase gas in a cylinder: The mobile phase is an **inert gas (monoatomic elementgases or non-reactive gases** like **nitrogen, helium & hydrogen**. The carrier gas is kept in a metallic cylinder and outflow is controlled by a regulator. From gas carrier cylinder, the gas is passed under fixed rate through a pressure gauge which indicates the speed of flow of gas into the column. Most **commonly used gas is helium**.

2. The injection system: This is present before column yet inside the thermal chamber to load sample under analysis into the system.

3. The column for gas chromatography. The gas chromatography column is a usually long (few meters like 3 to 6 meters) and coiled for accommodation into a small thermal chamber. The column is mostly made of steel or glass.

The GC columns are of three types viz.

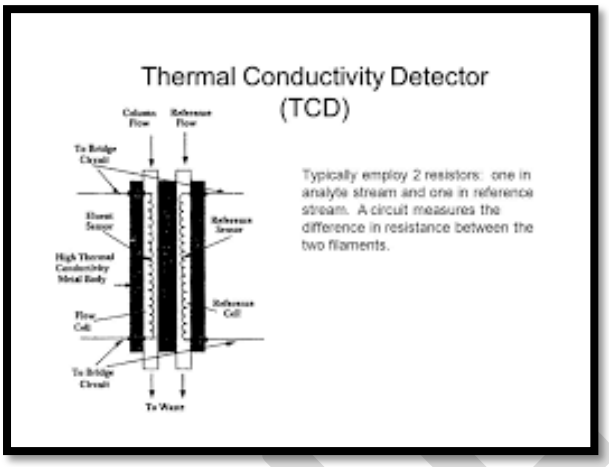
♠ Packed column. This is a column into which solid beads are packed. This column has advantages like efficient separation and precise readings.

♠ Tubular column. Here into a stainless steel hollow tube a thin layer of liquid is coated to act as a stationary phase. This column offers least resistance to flow of gas.

♠ Support coated tubular column**.** Here into stainless steel column a thin solid layer is coated on to which a thin layer of liquid stationary phase is present.

4. The Detector: is another vital component of the gas chromatography apparatus. GC detectors detect the isolated components and helps in identification and quantification of the sample. They are of 4 types of GC detectors like

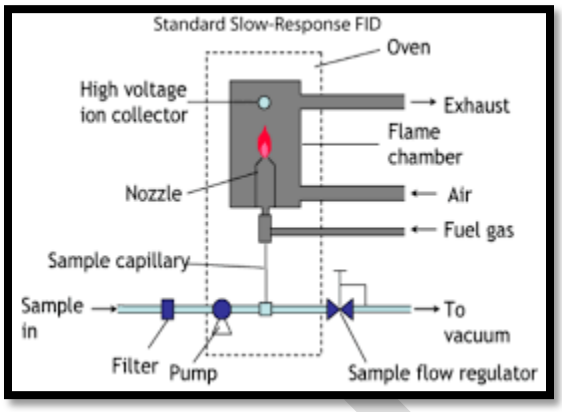
♦ Thermal conductivity detector: Here there are two columns which have a conducting wire in between. The gas is allowed to pass through the two columns of detectors i.e to one the effluent from gas chromatography column and to other gas from the gas cylinder directly. Since the temperature of both gases is same, the thermal conduction is constant.



Thermal conductivity detector

When the sample is injected into gas chromatography column. The effluent gas carries the sample components into the detector column. Since effluent gas is mixed with sample components there results in difference in thermal conductivity from prior one recording. This difference in conductivity is specific for the component analyzed. This is recorded for further comparison and identification of the components and their quantity.

♦ Flame ionization detectors: Here the sample components from effluent are ionized by subjecting to flame in a chamber. These ions raise upwards and are attracted towards anode or cathode based on charge on them. When they impinge on the electrodes, current is passed which is recorded. The strength and intensity of current depends on the sample and is specific.



flame-ionisation-detector

♦ Argon ionization detector; These detectors are similar to flame ionization detectors with only difference that argon ion gas is used to ionize the sample molecules. The argon ions are obtained by reacting argon gas with radioactive elements. Once argon ionizes they try to get back to stable state by either taking or giving electron from the sample components thus making sample molecules to ions for detection.

♦ Electron capture detector, etc.

5. The **computer to record** the analysed readings. This is connected with the detector and hence records the detector changes in reference to the flow of separated components from the exit of the column. The record is called gas chromatograph.

5. The **thermal chamber** to fix or maintain fixed temperature.

* Precolumn and post column treatment of sample (if necessary). This is done to modify the sample. The sample should be stable on heating and also be separated properly. For this precolumn derivatization is used and for the sample to be detected properly, post-column derivatization is done by making a suitable chemical change.

As a further improvement in GC, the gas chromatography apparatus is fixed with Mass spectroscopy system (GC-ms) for better analysis of components regarding their mass.

Reference Books :

Text Books:

T1: Sharma.B.K.(2012) Instrumental methods of chemical analysis (28th Edition). Meerut: Krishna Prakashan Media (p) Ltd

POSSIBLE QUESTIONS:

Part-A (20 x 1= 20 marks) Online Examinations

(Each Question Carry One Mark)

1. Chromatography is a technique used for --------- compounds
a.separation
b.identification
c.measure
d. analysis
Answer:a

2. Column chromatography is a type of
a. partition
b. adsorption
c. Absorption

d. thin layer

Answer:b

3. Thin layer chromatography is

a. ionization

b. partition chromatography

c. electrical mobility of ionic species

d. adsorption chromatography

Answer: d

4. In gas chromatography, the basis for separation of the components of the volatile material is the difference in

a. partition coefficients

b. conductivity

c. molecular weight

d. elements percentage

Answer: a

5. In reverse phase chromatography, the stationary phase is made

a. non-polar

b. either non-polar or polar

c. polar

d. low polar

Answer: a

6.Ion exchange chromatography is based on the

a. electrostatic attraction

b. electrical mobility of ionic species

c. adsorption chromatography

d. partition chromatography

Answer: a

7. The general expression for the appearance of a solute in an effluent is (where V is the elution volume of a substance , V_0 void volume, k_D distribution constant and V_i internal water volume)

a. $V = V_0 + k_D V_i$

b. $V = V_0/V_i$

c. $V = V_0 - k_D V_i$

d. $V/V_0 = k_D V_i$

Answer:a

8. A combination of paper chromatography and electrophoresis involves

a. Absorption

b. electrical mobility of the ionic species

c. ionization

d. adsorption chromatography

Answer:b

9. Which of the following is not used for detection in GC?

a. Infrared spectroscopy

b. NMR

c. Flame ionisation

d. Electrical conductivity

Answer: b

10. Which of these effects result from slow injection of a large sample volume?

a. Increased resolution

b. Decreased resolution

c. Non-linear detector response

d. Constant resolution

Answer: b

11. The GC trace obtained after an experiment is called a

a. Chromatograph

b. chromatogram

c. chromatophore

d. graph

Answer: b

12. Which of the following detectors give concentration-dependent signals?

a. Electron-capture detector

b. non thermal conductivity

c. UV detector

d. Scintillation counter

Answer: a

13. What useful information can be found from a Van Deemter plot?

a. The selectivity factor

b. Optimum mobile phase flow rate

c. Optimum column temperature

d. Optimum column length

Answer: b

14. What is the typical internal diameter of fused silica capillary columns?

a. 0.2-0.3 mm

b. 0.3-0.5mm

c. 0.5-1.0 mm

d. 1.0-2.0 mm

Answer: a

15. Resolution is proportional to the

a. number of theoretical plates in a column

b. square root of the number of theoretical plates in a column

c. square of the number of theoretical plates in a column

d. cube root of the number of theoretical plates in a column

Answer: b

16. Derivatisation of a sample is carried out to

a. reduce polarity of the analytes

b. decrease the detector response

c. decrease volatility of the analytes

d. irreducible polarity

Answer: a

17. Which of the statements is correct?

a. Gas chromatography is notused to analyse gases

b. Gas chromatography is used to analyse solids

c. Gas chromatography is not used to analyse gases, solutions

d. Gas chromatography is not used to analyse solid , gases

Answer: b

18. In column switching chromatography

a. compounds trapped on one column are eluted to another column

b. One column is removed and replaced by another

c. the flow to the column is switched on and off repeatedly

d. compounds does not move

Answer:a

19. A retention gap is placed between the injector and the front of the column to

a. retain contaminants and prevent them from reaching the column

b. retain the sample and release it gradually to the column

c. prevent back flush of the injected solution

d. release random to the column

Answer: a

20. Which of the following detectors give mass flow-dependent signals?

a. Electron capture detector

b. Field ionisation detector

c. Thermal conductivity detector

d. Proton capture

Answer: b

Part-B (5 x 6 =30 marks)

(Each Question Carry Six Marks)

1. Write notes on electrophoresis and its applications?

2. Write notes on GLC.

3. Discuss in detail about coloumn chromatography touching upon adsorbents, solvents, column preparation, elution and analysis.

4. Discuss the Principle, procedure and applications of paper chromatography

5. Explain with example ion-exchange chromatography. What are its advantages?

6. Describe a method how one can separate a mixture of amino acids using paper

Part-C (1 x10= 10 marks) Compulsory Questions

1. Paper chromatography and column chromatography can be used as examples to explain the difference between adsorption and partition chromatographic techniques, identify

i. The stationary and mobile phase

ii. How the mobile phase moves

Identify with a reason, which of the technique is more suitable for collecting samples of pure compounds for further analysis.

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DEPARTMENT OF CHEMISTRY

Class: II M.Sc Chemistry

Subject Title: Physical Methods in chemistry

Subject code: 17CHP303

UNIT-I

Multiple choice questions (Each question carry one mark)

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c.measure
d. analysis
Answer:a

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c. Absorption
d. thin layer
Answer:b

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Answer: d

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b. $V = V_0/V_i$
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c. Gas chromatography is not used to analyse gases, solutions
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d. release random to the column
Answer: a

20. Which of the following detectors give mass flow-dependent signals?
a. Electron capture detector
b. Field ionisation detector
c. Thermal conductivity detector
d. Proton capture
Answer: b

21. Headspace analysis is carried out in order to
a. analyse volatile compounds from solid or liquid samples
b. determine the psychological state of the tutor
c. analyse the column contents ahead of the sample
d. determine non-volatiles
Answer: a

22. Split injection is carried out by
a. splitting the sample into smaller portions to inject sequentially
b. splitting the sample into smaller portions to inject at the same time through parallel ports
c. splitting off some of the sample so that it does not enter the column
d. It does not splitting the sample portions
Answer: c

23. What does the retention factor, k', describe?
a. The distribution of an analyte between the stationary and the mobile phase
b. The migration rate of an analyte through a column
c. The velocity of the mobile phase
d. The velocity of the stationary phase
Answer: b

24. Theoretical plates are used to
a. estimate the efficiency of a column

b. determine the thickness of the stationary phase
c. measure the distribution of the analyte between mobile and stationary phases
d. Estimate the compounds
Answer: a

25. What does the selectivity factor describe?
a. The proportional difference in widths of two chromatographic peaks
b. The maximum number of different species which a column can separate simultaneously
c. The relative separation achieved between two species
d. does not separate the species
Answer: c

26. Helium is generally preferred as carrier gas over nitrogen and hydrogen because
a. it is inert
b. it has a high viscosity
c. it not doubles up as a party gas for balloons and funny voices
d. it is reactive
Answer: a

27. The column is heated to
a. prevent analyte condensation within the column
b. control elution of the same analytes
c. irreduce band broadening to get sharper peaks
d. control elution of the different analytes
Answer: d

28. What are the benefits of decreasing the column internal diameter?
a. Increased sample capacity
b. Increased resolution
c. Reduced risk of column overloading
d. non-Reduced risk of column overloading
Answer: b

29. Sample retention in the column is measured by
a. retention time
b. factor
c. index
d. co-efficient
Answer: a

30. Column bleeding occurs when
a. elution of the analyte is extended over time
b. the column is cracked and stationary phase leaks out
c. traces of the stationary phase are eluted
d. the column breaks during installation and causes personal injury
Answer: c

31. Which of the following are not used as stationary phases in a GC column?
a. Polysiloxanes
b. Silica
c. Cyclodextrins
d. None are used as stationary phases
Answer: b

32. Doubling the column's length increases resolution by a factor of
$(2)^{0.5}$
a. 3
b. 2
c. 4
d. 5
Answer: a

33. Sample injection is considered successful if
a. all of the sample in the injector has been added to the column
b. the sample is concentrated at the start of the column
c. the sample is spread evenly along the column
d. the sample is homogenously spread along the column
Answer: b

34. Which of the following gases is unsuitable for use as a GC carrier gas?
a. Nitrogen
b. Helium
c. Oxygen
d. Hydrogen
Answer: c

35. characteristic feature of any form of chromatography is the ...
a. use of molecules that are soluble in water.
b. use of an inert carrier gas.
c. calculation of an R_f value for the molecules separated.
d. use of a mobile and a stationary phase.
Answer: d

36. A student sets up a paper chromatogram and places a spot of green food dye on the origin. After six minutes the solvent has moved 12 cm and a blue spot has advanced 9 cm. After fourteen minutes the solvent has advanced a further 8 cm. How many cm from the origin is the blue spot likely to be?
a. 26
b. 8
c. 18
d. 15
Answer: d

37. Thin layer chromatography can be used to distinguish between different amino acids. If a particular amino acid has low solubility in the mobile phase used, then the amino acid ...
a. will have a low R_f value.
b. will spend more time dissolved in the mobile phase than attached to the stationary phase.
c. must have a high molecular mass.
d. will move at a speed close to that of the solvent.
Answer: a

38. Which of the following is the most suitable gas to use as a carrier gas in a gas chromatogram?
a. Helium
b. Oxygen
c. Methane
d. Carbon dioxide
Answer: a

39. A new youth drink contains sugar, salt, alcohol and vitamin C. A gas chromatogram could be used to determine the
a. alcohol content only.
b. alcohol, sugar and vitamin C content only.
c. concentration of all ingredients in the drink.
d. alcohol and sugar content only.
Answer: a

40. The basis of the technique of chromatography for separating components of a mixture is ...
a. the absorption of infrared radiation by the components.
b. the interaction of the components with both stationary and mobile phases.
c. the differing movement of particles of different mass in an electric field.
d. the deflection of charged particles in a magnetic field.
Answer: b

41. Acetone is an organic molecule with a semi-structural formula of CH_3COCH_3. A student runs a sample of acetone through a gas chromatogram at 50°C. The acetone produces a peak after 4.2 minutes. The student then injects a mixture of unknown organic substances into the same column at the same temperature. There are peaks after 3.1, 4.2 and 7.4 minutes. From this information, it can be concluded that ...
a. the mixture has three components, one of which must be acetone.
b. the mixture has at least three components, one of which might be acetone.
c. the mixture has at least three components, one of which must be acetone.
d. the mixture has three components, but acetone is not one of them.
Answer: b

42. A mixture of ethanol (C_2H_6O) and butanol ($C_4H_{10}O$) is approximately 90% ethanol and 10% butanol. The mixture is passed through a gas liquid chromatogram. The printout obtained is likely to show that, compared to butanol, the ethanol has ...
a. a shorter retention time and a smaller area under the peak.

b. a shorter retention time and a larger area under the peak.
c. a longer retention time and a larger area under the peak.
d. a smaller retardation factor and a larger area under the peak.
Answer: b

43. High performance liquid chromatography (HPLC) cannot be used to ...
a. separate types of organic pesticides.
b. determine the mercury content of a fish sample.
c. identify the various pigments from a leaf extract.
d. determine the caffeine content of coffee samples.
Answer: b

44. Which of the following statements about paper and gas chromatography is correct?
a. The R_f and R_t values of a substance are determined solely by the interaction of the substance with the stationary phase.
b. A substance with a long retention time in gas chromatography is likely to have a high R_f value in paper chromatography.
c. A high R_f value is indicative of a substance that adsorbs strongly onto the stationary phase.
d. A long retention time in gas chromatography is indicative of a substance with a strong adsorption on to the stationary phase.
Answer: d

45. The example of bulk property detector used in HPLC is
a. Refractive index detector
b. UV detector
c. fluorescence detector
d. UV-visible detector
Answer: a

46. In-vitro hydrolysis studies of drugs & kinetic studies of reaction can be performed by
a. Polarimetry
b. Refractometry
c. Potentiometry
d. Conductometry
Answer: d

47. The alkenes and aromatic compounds can be suitably detected using
a. Refractive index detector
b. conductivity detector
c. Spectrophotometric detector
d. Potentiometric detector
Answer: c

48. Formic acid is an example of
a. protogenic solvent
b. protophillic solvent

c. amphiprotic solvent
d. Aprotic solvent
Answer: a

49. The relationship between concentration, temperature & potential of a solution is given by
a. Ilkovic equation
b. Henderson equation
c. Nernst equation
d. Hassalbach equation
Answer: c

50. In refractometric analysis, if temperature is increased by 1 °C then refractive index decreases by
a. 0.001 to 0.002
b. 0.002 to 0.003
c. 0.003 to 0.004
d. 0.004 to 0.005
Answer: d

51. Sucrose can be determined after silylation using which chromatographic technique
a. HPLC
b. Gel chromatography
c. Gas liquid chromatography
d. Paper chromatography
Answer: c

52. R_f is refered as
a. retention time
b. retard factor
c. resistant value
d. reduced value
Answer: a

53. The composition of Silica gel G is
a. silica gel without binder
b. silica gel + $CaSO_4$
c. Silica gel + alumina
d. silica gel + $MaSO_4$
Answer: c

54. The formula for resolution (R) between peaks in gas chromatography is (where d = distance between peak 1 and 2; W1 and W2 are width of peak 1 and 2, respectively)
a. 2d / (W1+W2)
b. d / (W1+W2)
c. 2d / (W1-W2)
d. d / (W1-W2)

Answer: a

55. Oxygen --------be used as carrier gas in gas chromatography
a. can
b. cannot
c. often
d. always
Answer: a

56. Snells law is related to
a. Refractometry
b. Potentiometry
c. Non-aqueous titrations
d. Chromatography
Answer: a
57. Relative flow (Rf) value ranges from
a. 0 to 1
b. 0 to 2.0
c. +2 to -2
d. +1 to -1
Answer: a

58. Electrostatic attraction is a function present in ------ chromatography
a. column
b. paper
c. ion exchange
d. gas
Answer: c

59. TLC means
a. thinlayer chromatography
b. thicklayer chromatography
c. thermolinear chromatography
d. therotical layer chromatography
Answer: a

60. The mechanism present in the ion exchange chromatography
a. reversible
b. irreversible
c. ionisation
d. bromination
Answer: a

Lecture Notes

UNIT-II

SYLLABUS

Thermal analysis: *Differential thermal analysis DTA and differential scanning calorimetry DSC - basic principles - thermo gravimetric analysis.*

Electron spectroscopy: *ESCA XPS: Principle, chemical shifts - description of ESCA spectrometer, X-ray sources, samples, analysis, detectors and recording devices-applications.*

Auger electron spectroscopy AES and Ultra-Violet photo electron spectroscopy UPS/PES principles and applications.

Differential Thermal Analysis (DTA)

DTA consists of heating a sample and reference material at the same rate and monitoring the temperature difference between the sample and reference. In this method, the sample is heated along with a reference standard under identical thermal conditions in the same oven. The temperature difference between the sample and reference substance is monitored during the period of heating. As the samples undergo any changes in state, the latent heat of transition will be absorbed/ evolved and the temperature of the sample will differ from that of the reference material. This difference in temperature is recorded. Hence, any change in state can be detected along with the temperature at which it occurs.

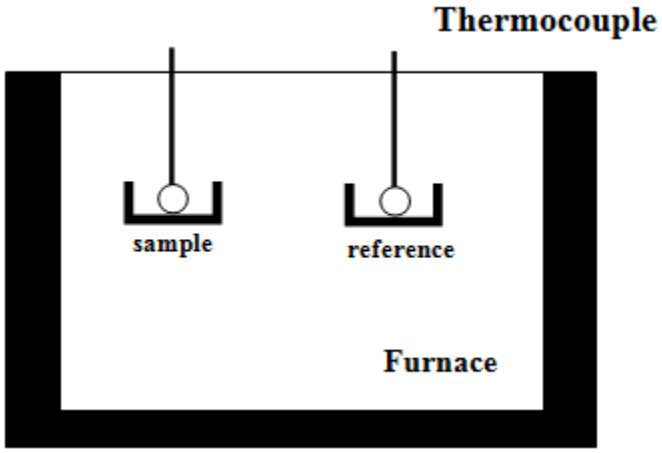

Fig. 1. Schematic diagram for differential thermal analysis technique.

When an endothermic process occurs (ΔH positive) in the sample, the temperature of sample (T_s)lags behind the temperature of reference (T_r). The temperature difference $\Delta T = (T_s - T_r)$ is recorded against reference temperature T_r and the corresponding plot is shown in Fig 12. In DTA, by convention, endothermic response is represented as negative that is by downward peaks. When an exothermic process (ΔH negative) occurs in the sample, the response will be in the reverse direction and the peaks are upward. Since the definition of $\Delta T = T_s - T_r$ is rather arbitrary, the DTA curves are usually marked with endo or exo direction.

It is essential that reference sample must not undergo any change in state over the temperature range used and both the thermal conductivity and heat capacity of reference must be similar to those of samples. Both sample and reference materials should be also inert towards sample holder or thermocouples. Alumina or silicon carbide are most commonly used standard reference samples. DTA profiles are affected by heating rate, sample size and thermocouple position within the sample.

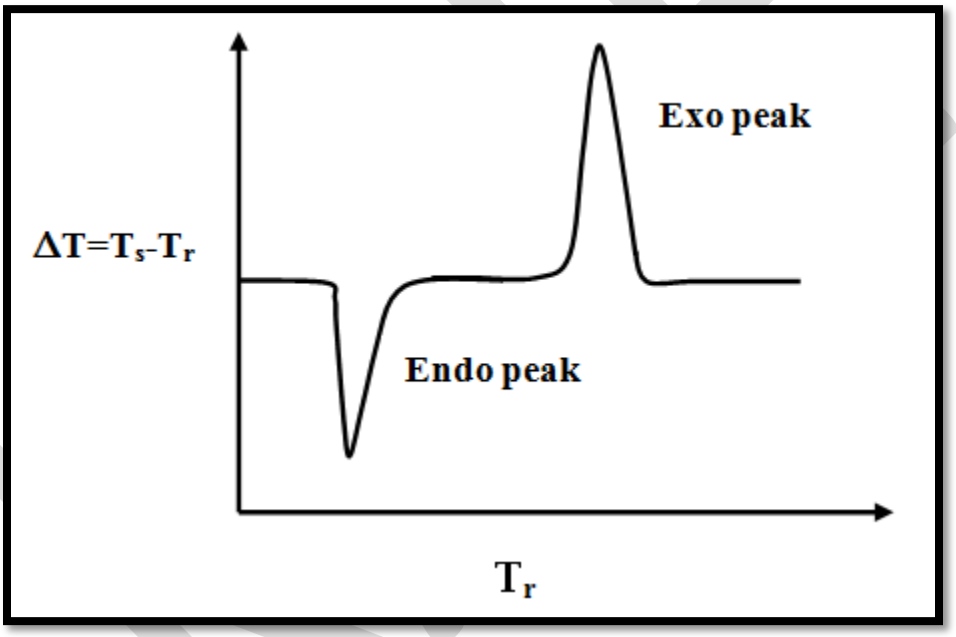

Fig. 2. Typical exo and endo peak in a DTA profile.

Application:

Any change associated with enthalpy change can be studied by DTA. In general DTA curves are used to get informations about temperature and enthalpy changes for decomposition, crystallization, melting, glass transition etc. In solid catalysis it is particularly useful to detect phase changes associated with calcination process. For example change of aluminum hydroxide to alumina can be easily detected by DTA.

Differential scanning calorimetry:

• Differential scanning calorimetry (DSC) technique was developed by E.S. Watson and M. J. O'Neill in 1962 and commercial introduction was done at 1963 in Pittsburgh conference.

• DSC is a thermo-analytical technique in which the differences in the amount of heat required to increase the temperature of a sample and reference are measured as a function of temperature.

• Both the sample and reference are maintained at nearly the same temperature throughout the experiment. The reference sample should have a well defined heat capacity over the range of temperatures to be scanned and analyzed.

• In general, the temperature program of the DSC is designed to increase the sample holder temperature linearly as a function of time.

• The main application of DSC is in studying phase transitions such as melting point, glass transitions, or exothermic decompositions. These transitions involve energy changes or heat capacity changes that can be detected by DSC with great sensitivity.

Description of DSC:

There are two types of DSC commercially available: Heat Flux (HF) Type and Power Compensation (PC) Type. Figure 22.01 shows the block diagram of HF and PC types.

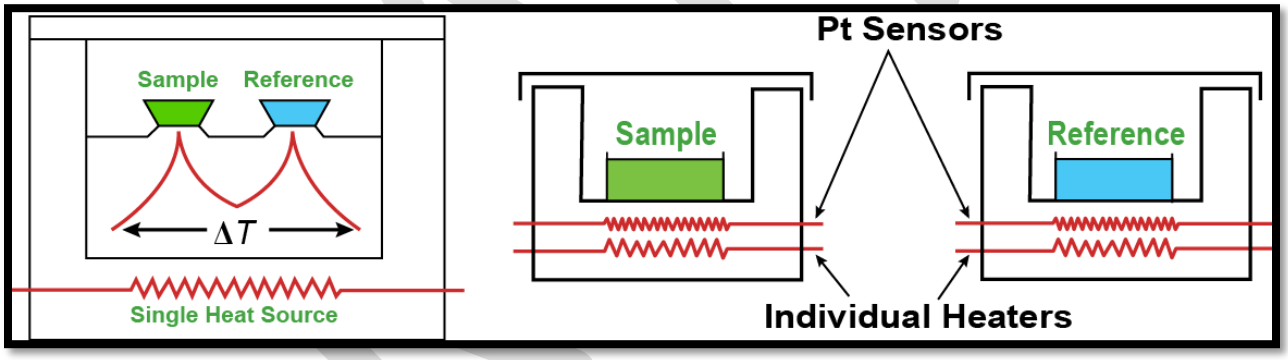

Figure 3: Schematic diagram of HF and PC types DSC.

In HF type DSC:

• Both sample and reference pans are heated by a single furnace through heat sink and heat resistor. Heat flow is proportional to the heat difference of heat sink and holders. The temperature versus time profile through a phase transition in a heat flux instrument is not linear.
• At a phase transition, there is a large change in the heat capacity of the sample, which leads to a difference in temperatures between the sample and reference pan.
• A set of mathematical equations convert the signal into heat flow information. By calibrating the standard material, the unknown sample quantitative measurement is achievable.

In PC type DSC:

Both sample and reference pans are heated by a different furnaces. When an event occurs in the sample, sensitive Platinum Resistance Thermometer (PRT) detects the changes in the sample, and power (energy) is applied to or removed from the sample furnace to compensate for the change in heat flow to or from the sample. As a result, the system is maintained at a "thermal null" state at all times. The amount of power required to maintain system equilibrium is directly proportional to the energy changes occurring in the sample. No complex heat flux equations are necessary with a power compensation DSC because the system directly measures energy flow to and from the sample.

In addition, PC type DSC has enhanced modulated temperature DSC (StepScan) technique and fast scan DSC (HyperDSC) for dramatic improvements in productivity, as well as greater sensitivity.

Furthermore, the heating and cooling rate of PC types DSC can be as high as 500°C/min.

Detection of phase transitions:

The underlying principle is that when the sample undergoes a physical transformation (phase transitions, etc), more or less heat will be needed to flow to it as compared to the reference to maintain both of them at the same temperature. This certainly depends on whether the process is exothermic or endothermic.

For example:

When a solid sample melts into a liquid, then it requires more heat flowing to the sample to increase its temperature at the same rate as the reference. This is due to the absorption of heat by the sample as it undergoes the endothermic phase transition from solid to liquid. Similarly, when the sample undergoes exothermic processes (such as crystallization) less heat is required to raise the sample temperature.

By observing the difference in heat flows between the sample and reference, DSC is able to measure the amount of heat absorbed or released during such transitions. DSC may also be used to observe more subtle phase changes, such as glass transitions.

Information about the DSC curves:

In general, the result of a DSC experiment is a curve of heat flux versus temperature or versus time. This curve can be used to calculate enthalpies of transitions, i.e., $\Delta H = kA$ (where, H is the enthalpy of transition, k is the calorimetric constant, and A is the area under the curve) , which is done by integrating the peak corresponding to a given transition.

The value of k is typically given by the manufacturer for an instrument or can generally be determined by analyzing a well-characterized sample with known enthalpies of transition.

Applications of DSC:

DSC technique can be used to obtain glass transition, melting points, crystallization times and temperatures, heats of melting and crystallization, percentage of crystallinity, oxidative stabilities, heat capacity, completeness of cure, purities, thermal stabilities, polymorphism, recyclates or regrinds

Evaluation and interpretation of DSC curves:

Figure 22.02 shows the typical DSC curve for a sample exhibiting endotherm of melting at a particular heating rate.

The onset of melting (122.8°C) and peak temperature of melting (123.66°C) can be determined by extrapolation technique and peak values, respectively.

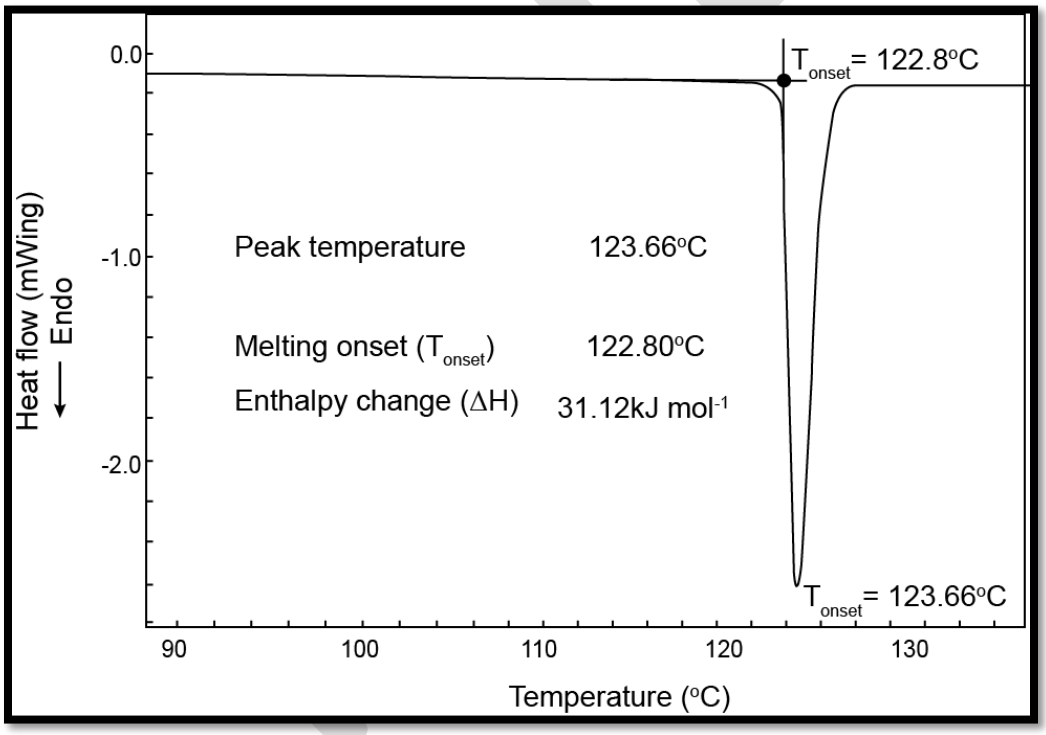

Figure 4: Typical DSC curve of a sample.

The enthalpy change can be calculated by integrating the area under the curve. The unit can be either J/g or J/mole depending on the nature of the sample.

Effect of heating rate:

Heating rate affects the melting point and enthalpy of melting. Figure 22.03 shows the typical DSC curves taken at different heating rate.

With increasing heating rate, the onset of the melting does not change significantly, but the peak point of melting shifts slowly to higher temperature.

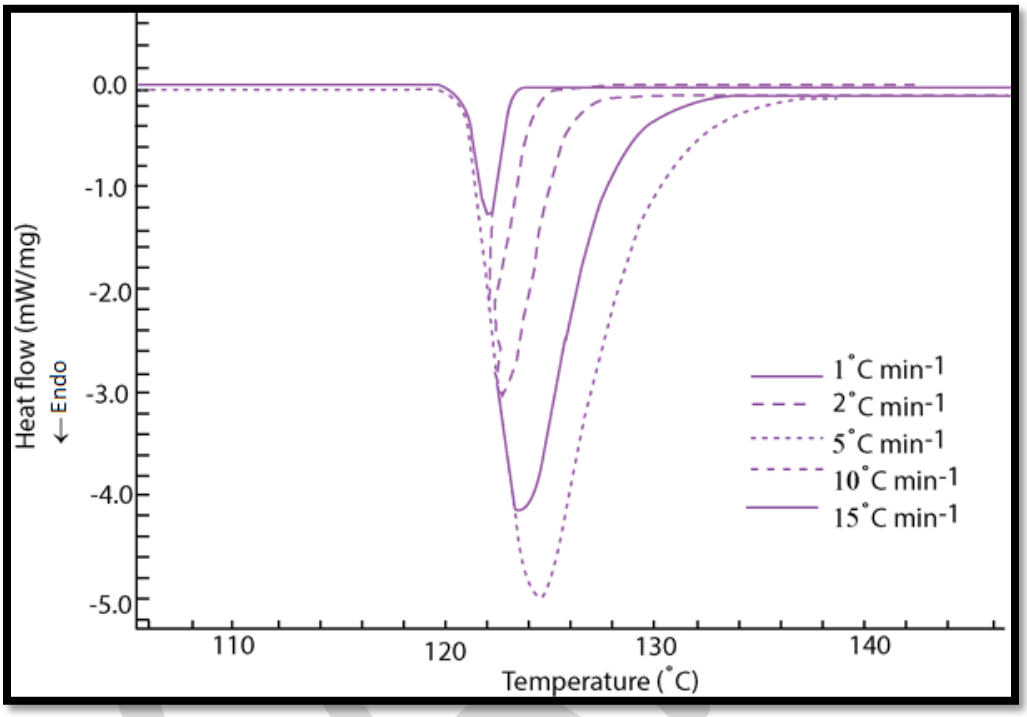

Figure 5: Typical DSC curves taken at different heating rates.

Effect of sample weight:

The sample weight also affects the thermal properties significantly. Figure 22.04 shows the typical DSC curves taken at a constant heating rate for different mass of the samples.

It could be clearly seen that the onset of melting, peak point of melting and enthalpy undergo small variations when the sample mass is changed.

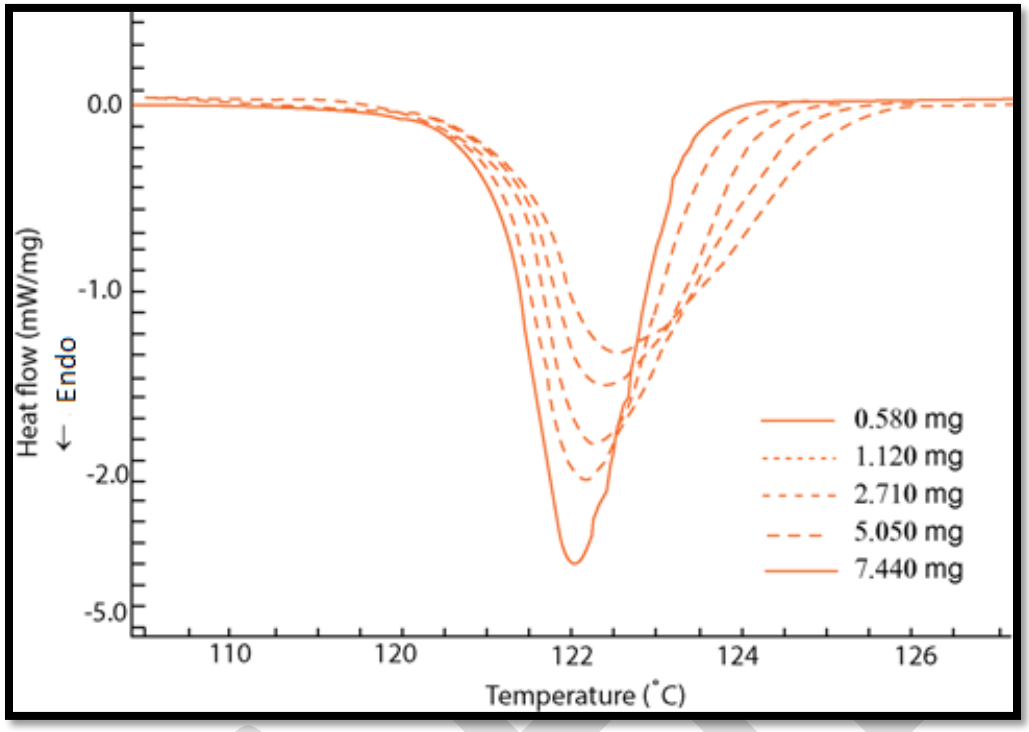

Figure 6: Typical DSC curves taken for different weighed samples.

Thermogravimetric analysis:

• Thermogravimetric (TG) is a branch of thermal analysis examining the mass changes of a sample as a function off temperature (in the scanning mode) or as a function of time (in the isothermal mode).

• Thermal gravimetric analysis or thermogravimetric analysis (TGA) is a method of thermal analysis in which changes in physical and chemical properties of materials are measured as a function of increasing temperature (with constant heating rate), or as a function of time (with constant temperature and/or constant mass loss).

• Changes in the mass of a sample due to various thermal events (desorption, absorption, sublimation, vaporization, oxidation, reduction and decomposition) are studied while the sample is subjected to a program of change in temperature. Therefore, it is used in the analysis of volatile products, gaseous products lost during the reaction in thermoplastics, thermosets, elastomers, composites, films, fibers, coatings, paints, etc.

• There are different types of TGA available:

i. Isothermal or Static TGA: In this case, sample is maintained at a constant temperature for a period of time during which change in weight is recorded.

ii. Quasi-static TGA: In this technique, the sample is heated to a constant weight at each of a series of increasing temperature.

iii. Dynamic TGA: In this type of analysis, the sample is subjected to condition of a continuous increase in temperature at a constant heating rate, i.e., usually linear with time.

Thermogravimetric analysis:

• The instrument used for TGA analysis is a programmed precision balance for a rise in temperature (called as Thermobalance, see Figure 23.01). Thermobalance consists of an electronic microbalance (important component), a furnace, a temperature programmer and a recorder.

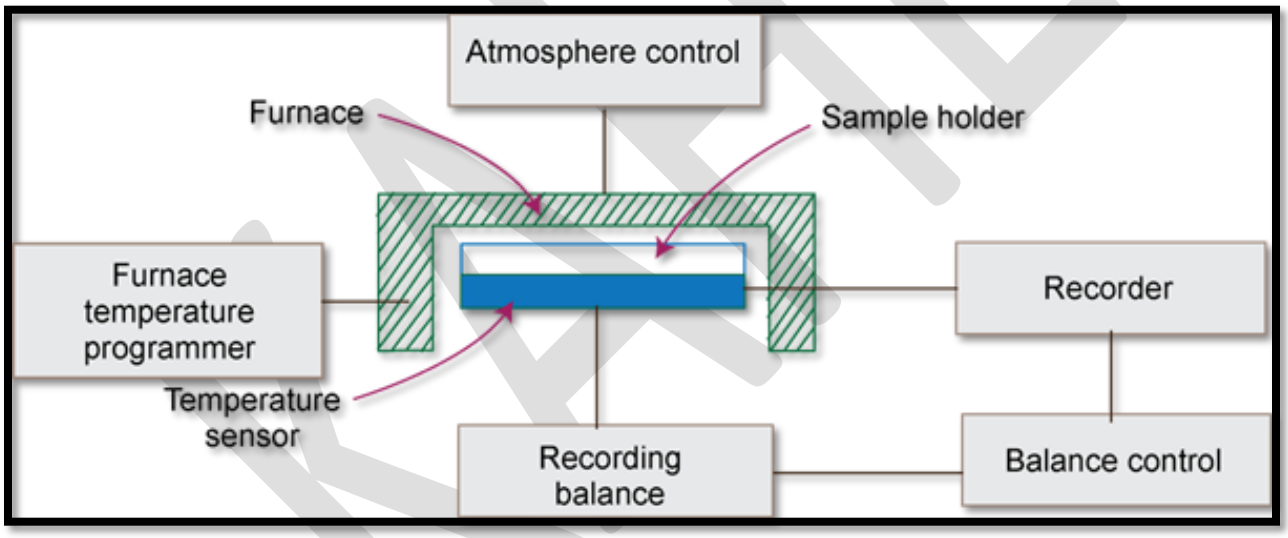

Figure 7: Block Diagram of a Thermobalance.

• The plot of mass change in percentage versus temperature or time (known as TGA curves) is the typical result of TGA analysis as shown in Figure 23.02.

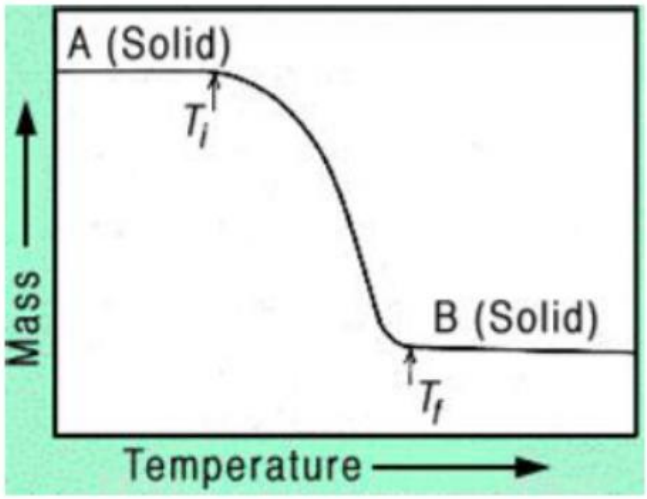

Figure 8: The plot of mass change with temperature.

• There are two temperatures in the reaction: T_i (starting of decomposition temperature) and T_f(final temperature) representing the lowest temperature at which the onset of a mass change is seen and the lowest temperature at which the process has been completed, respectively. The reaction temperature and interval (T_f - T_i) strongly depend on the conditions of the experiments. Hence, they can not have any fixed values.

Interpretation of TGA Curves:

TGA curves are typically classified into seven types according to their shapes. Figure 23.03 shows schematic of various types of TGA curves.

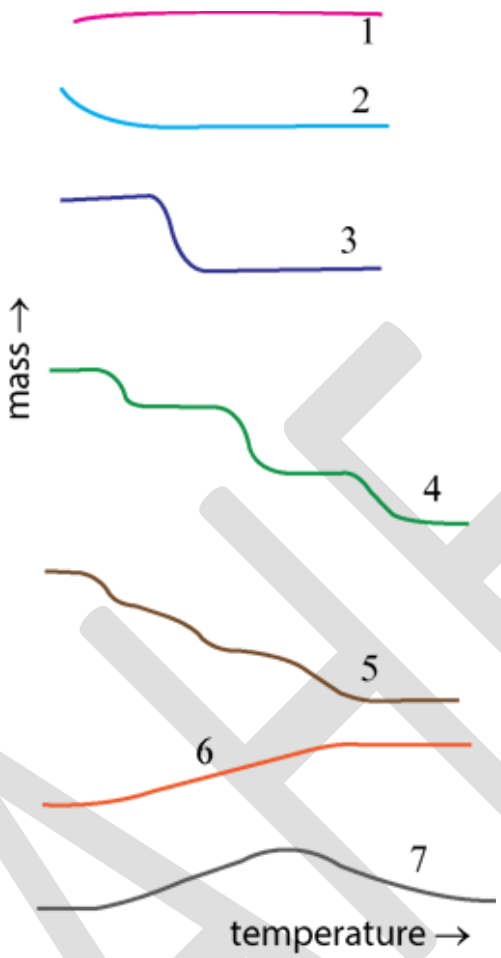

Figure 9: Schematic of various types of TGA curves.

• Curve 1: No change: This curve depicts no mass change over the entire range of temperature, indicating that the decomposition temperature is greater than the temperature range of the instrument.

• Curve 2: Desorption / drying: This curve shows that the mass loss is large followed by mass plateau. This is formed when evaporation of volatile product(s) during desorption, drying or polymerization takes place. If a non-interacting atmosphere is present in the chamber, then curve 2 becomes curve 1.

• Curve 3: Single stage decomposition: This curve is typical of single-stage decomposition temperatures having T_i and T_f.

• Curve 4: Multistage decomposition: This curve reveals the multi-stage decomposition processes as a result various reactions.

• Curve 5: Similar to 4, but either due to fast heating rate or due to no intermediates.

• Curve 6: Atmospheric reaction: This curve shows the increase in mass. This may be due to the reactions such as surface oxidation reactions in the presence of an interacting atmosphere.

• Curve 7: Similar to curve 6, but product decomposes at high temperatures. For example, the reaction of surface oxidation followed by decomposition of reaction product(s).

Processes that leads to Weight Gain and loss in TGA experiment:

Process	Weight gain	Weight loss
Adsorption or absorption	✓	
Desorption, drying		✓
Dehydration, desolvation		✓
Sublimation		✓
Vaporization		✓
Solid-state reactions (some cases)		✓
Solid-gas reactions	✓	✓
Magnetic transitions	✓	✓

Applications of TGA:

a) Thermal stability of the related materials can be compared at elevated temperatures under the required atmosphere. TGA curve helps to explicate decomposition mechanisms.

b) Materials Characterization: TGA curves can be used to fingerprint materials for identification or quality control.

c) Compositional analysis: By a careful choice of temperature programming and gaseous environment, many complex materials/ mixtures can be analyzed by decomposing or removing their components. For example: filler content in polymers; carbon black in oils; ash and carbon in coals, and the moisture content of many substances.

d) Kinetic studies: A variety of methods can be used to analyze the kinetic features of weight loss or gain through controlling the chemistry or predictive studies.

e) Corrosion studies: TGA provides a means of studying oxidation or some reactions with other reactive gases or vapors.

Examples of TGA curves:

Figure 23.04 shows the heat decomposition mass curve of Whewellite (calcium oxalate monohydrate) [1]

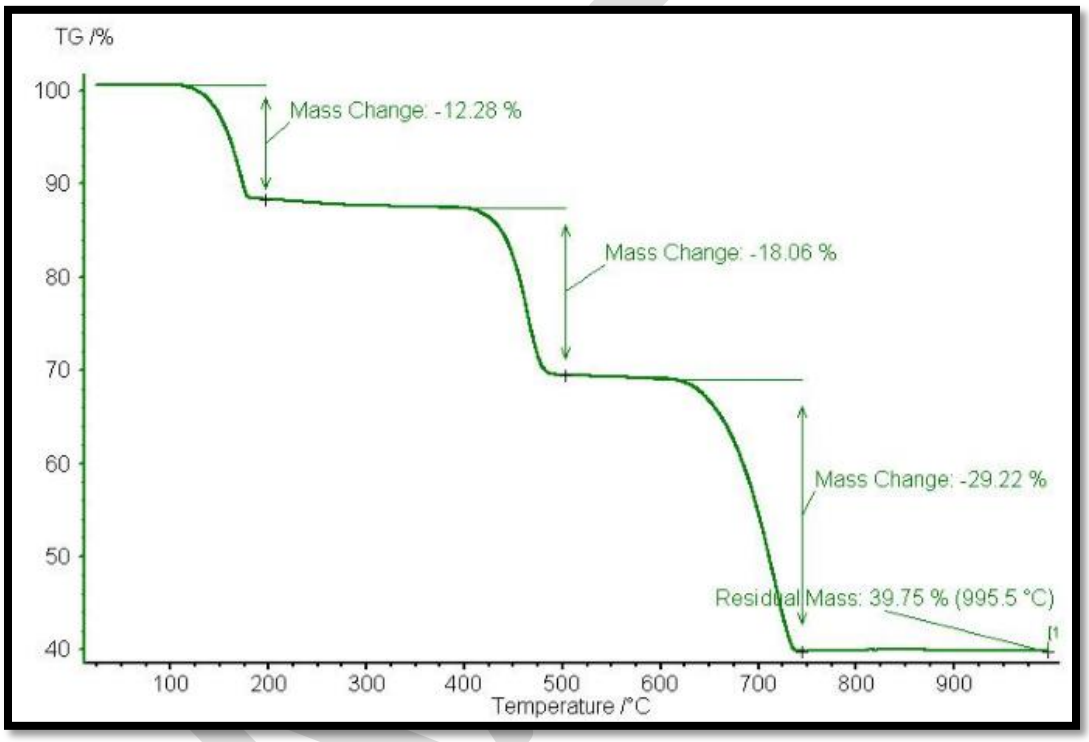

Figure 10: TGA curve of Whewellite.

Examples of TGA curves:

Thermal decomposition of calcium oxalate monohydrate studied by TGA

(a) $Ca(COO)_2\ H_2O \rightarrow [200°C] \rightarrow Ca(COO)_2 + H_2O$ (g);

(b) $Ca(COO)_2 \rightarrow [500°C] \rightarrow CaCO_3 + CO$ (g); and,

(c) $CaCO_3 \rightarrow [800°C] \rightarrow CaO + CO_2$ (g).

Figure 23.05 depicts the mass change corresponding to each reactions of calcium oxalate monohydrate.

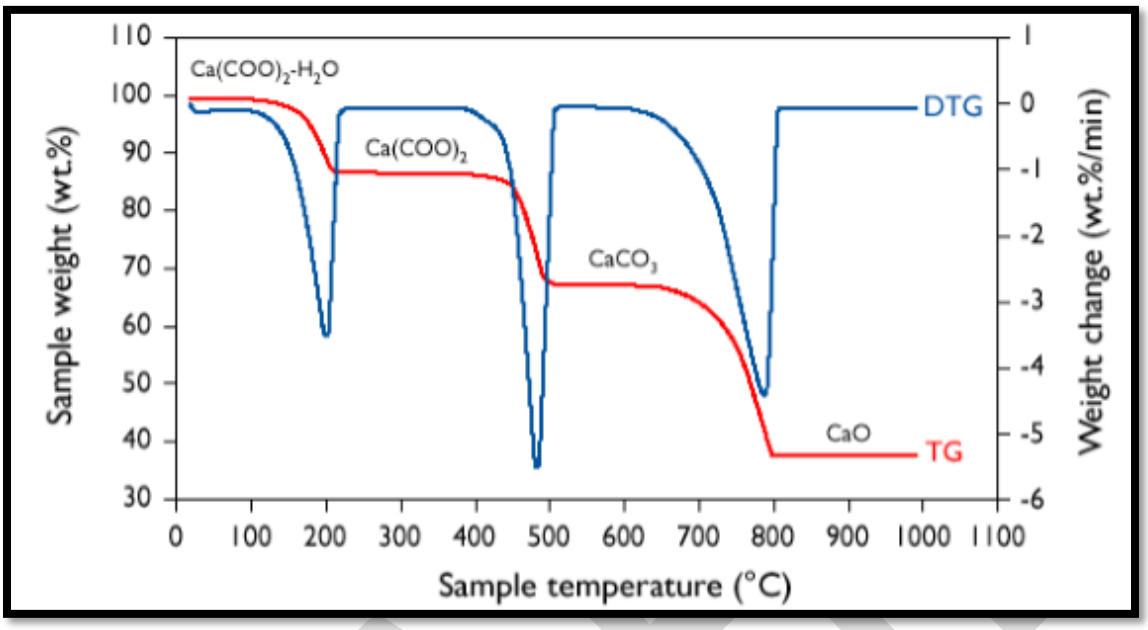

Figure 11: TGA curve of calcium oxalate monohydrate [2].

Spectroscopic methods:

These methods make use of the principle that when a beam of electrons or high-energy radiation (usually X-rays) are bombarded at the surface, the surface electrons from the upper layer of are ejected. These electrons are called secondary electrons. Since the energy of these electrons is very low, these are assumed to be produced from only a few layers of the surface. Electrons of this energy (10-30 eV) cannot reach the surface from underlying layers. In spectroscopic methods, surfaces are analyzed by capturing secondary electrons of different energies, which are characteristic of their environment. Based on spectroscopic theory some of the techniques are discussed below.

(a) X-Ray Photoelectron Spectroscopy (XPS):

X-ray photoelectron spectroscopy (XPS) was developed in mid 1960 by K. Siegbohn and his colleagues. Later on, K. Siegbohn was awarded Nobel Prize for physics in 1981 for the work on XPS. This technique provides a wide range of information regarding atomic composition, oxidation states, and chemical structures. Because of its versatility it is also called Electron Spectroscopy for Chemical Analysis (ESCA). Basic principle of this technique is based on phenomenon called photoelectric effect, rationalized by Albert Einstein in 1905. When photons of known energy (usually X-rays) knock the surface, an electron from K-shell is knocked out; kinetic energy of this electron is measured in the spectroscopy. The spectrum is given as the binding energy as function of electron counting rate. Binding is one unique character of different elements. Binding energy of

an atom can be calculated from the following equation.

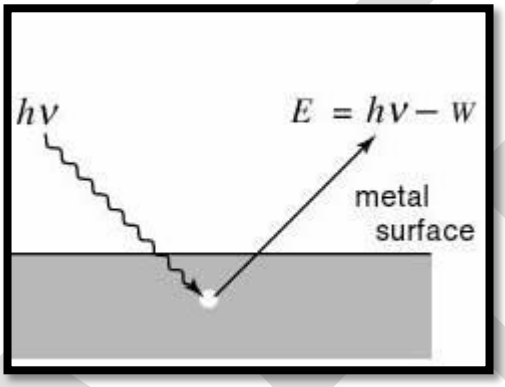

Figure 13: Photoelectric Effect

Binding Energy (eV), B.E. = h υ – K.E – W (37.4)

where, h υ = Incident energy, K.E = Kinetic Energy of the ejected electron and W = Work function.

In the XPS spectrum, the innermost orbital appears a at higher binding energy than the outer orbital. Binding energies of 1s orbitals increase with atomic number.

Instrumentation and working

Basic components of XPS instrumentation are:

1. Source, 2. Sample Holder, 3. Analyzer, 4.Detector and 5. Signal Processor

Source: A common source is an X-ray tube with both Al or Mg target and a suitable filter. For the analysis of heavy elements Cu, Mn and Ag targets with suitable filters are used.

Sample holder: Samples like a piece of metal are mounted close to source, which is evacuated upto 10^{-9} torr to avoid contamination by oxygen and water vapors.

Analyzer: Hemispheric analyzers are used to distinguish electrons of different energies by applying electrostatic and magnetic fields.

Detector and signal Processor: Usually Channeltrons or solid-state detectors are used to attain required sensitivities.

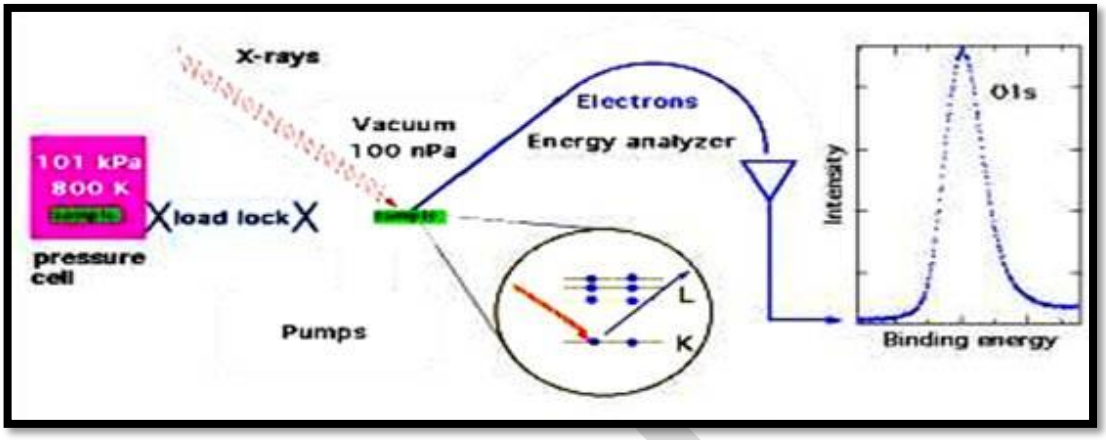

Figure 14: Block diagram of X-ray Photoelectron Spectroscopy

Applications

1. Surface analysis for elemental composition.
2. To determine different oxidation states of the elements.
3. For structural analysis of molecules.
4. It is also useful for qualitative analysis of the surfaces.

Auger Electron Spectroscopy (AES)

Unlike ESCA Auger (pronounced as OJ) Electron Spectroscopy is based on a two step process. In AES, the source is electron beam unlike x-rays in XPS. Electrons of energy 3-20 keV are incident upon a conducting sample. These electrons cause core electrons from the atoms contained in the sample to be knocked out, giving photoelectrons and atoms with a core hole. The atom then relaxes when electrons from higher level drop into the core hole, some energy is released in this transition, this released energy ejects another electron from the next higher level, which is called auger electron. The kinetic energy of auger electron is the difference between the energy released in relaxation of excited ion and energy required to remove the second electron from its orbit. The energy of auger electron is characteristic of the element that emits it and can be used to identify the element.

Auger electron spectroscopy is a popular technique for determining the composition of the top few layers of the surface. Except Hydrogen and Helium it is sensitive for all other elements. But it is more sensitive to lighter elements than heavy elements.

Auger electron emission is described as KLL, LMM, and MNN etc. For example KLL process involves initial removal of a K electron followed by a transition of an electron from L or higher levels to K. The energy released in this process ejects second electron from L Shell, which is called auger electron. This process occurs almost simultaneously. Auger electron emission is purely a surface phenomenon. Since auger electrons are very

weak, only electrons from the surface of a few atomic layers can reach the detector.

Instrumentation:

It is somewhat similar to XPS except for the electron source. A common electron gun is the source of electrons. Its basic mechanism involves the application of a high voltage of about 50keV applied to "V" Wehnelt cylinder which produces electrons. These produced electrons are converged to a spot by positive potential. This spot is used to scan the surface.

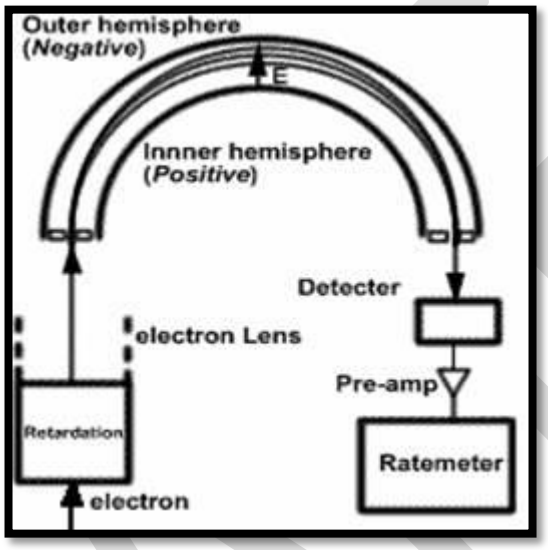

Figure 15: Auger Electron Spectroscopy

Applications of AES

1. Qualitative analysis of solid surfaces
2. Depth profiling of solids
3. Line scanning (Characterizing surface composition of solid as function of distance along the line)

UV Photoelectron Spectroscopy

Ultraviolet Photoelectron Spectroscopy (UPS) operates on the same principles as XPS, the only difference being that ionising radiation at energies of 10s of eV are used to induce the photoelectric effect, as opposed to photons of greater than 1keV that are used in XPS. In the laboratory setting ultraviolet photons are produced using a gas discharge lamp, typically filled with helium, although other gases such as argon and neon can also be used. The photons emitted by helium gas have energies of 21.2eV (He I) and 40.8eV (He II)

As lower energy photons are used, most core level photoemissions are not accessible using UPS, so spectral acquisition is limited to the valence band region. There are two types of experiment performed using UPS: Valence band acquisition and electronic workfunction measurement.

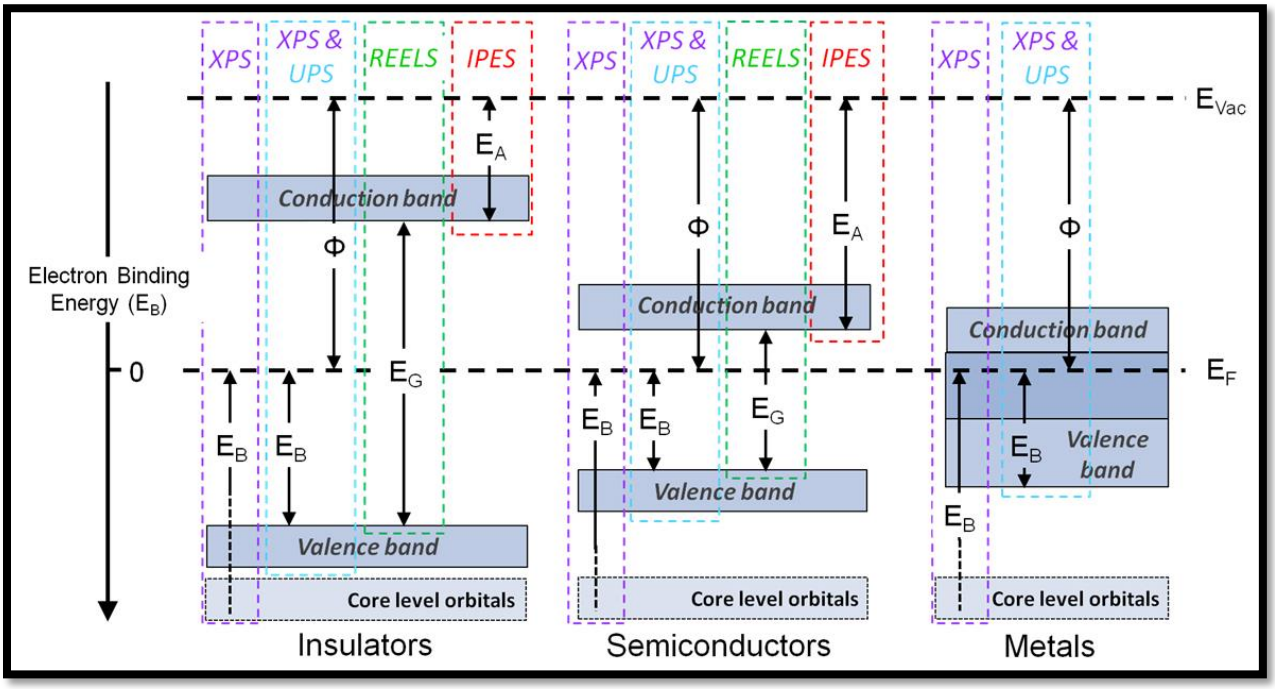

Figure16

Valence band

Many of the molecular orbitals from which valence band photoelectron signal originates posses a high degree of hybridisation, therefore the shifts in peak binding energy are far more varied and subtle than those observed for core level photoemission peaks. For this reason valence band spectra are predominantly used for material characterisation through spectral fingerprinting, and individual peak assignment is either performed on surfaces with well known electronic structure, or in conjunction with computational studies. Due to this ambiguity in the assignment of valence band peaks, these spectra are not used for quantification.

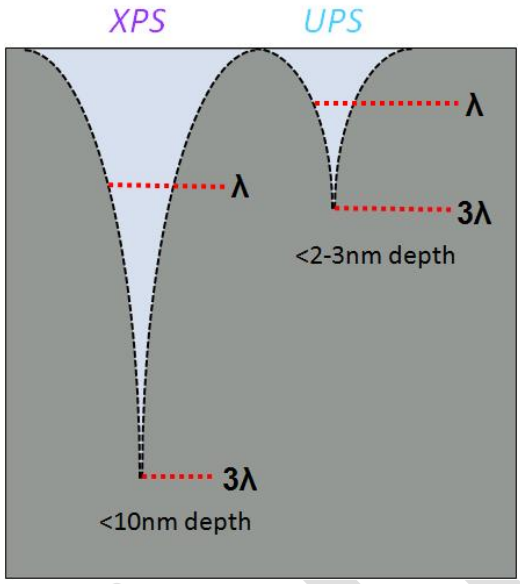

Figure17: Difference in information depth for XPS and UPS

XPS is also widely used to collect valence band spectra, the combination of both XPS and UPS to investigate the valence band can be extremely powerful as the ionisation cross section of an orbital is dependent on the incident photon energy, therefore different electronic transitions and states can be probed by using different photon energies.

UPS also exhibits greater surface sensitivity than XPS, the inherent surface sensitivity of XPS is due to the short inelastic mean free path (IMFP, or λ) of free electrons within a solid, with the so-called 'information depth' from which > 99% of a photoemission signal originates conventionally being defined at 3 mean free path lengths from the surface, which in XPS is often quoted as 10 nm. This is an approximation as the IMFP of an electron is determined by the material properties of the solid media through which it is travelling and its kinetic energy, with electrons of lower kinetic energy having shorter path lengths. The lower incident photon energies used in UPS give emit photoelectrons of much lower kinetic energies than those measured in XPS, therefore giving UPS an approximate information depth of 2-3nm.

Workfunction

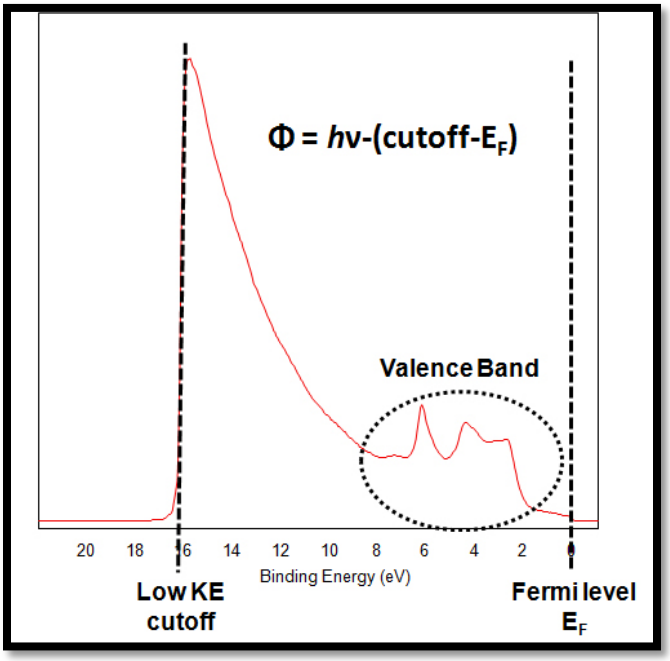

Figure-18:Measuring a material's work function using a UP spectrum

The difference between the Fermi level and Vacuum level is referred to as the electronic workfunction, a material property applied in the development of electronics devices, for example where matching of valence and conduction bands in multilayered devices is required. As a surface property, the workfunction is strongly influenced by variation in composition or structure at the surface, such as atmospheric contamination.

The electronic workfunction is acquired spectroscopically by measuring the difference between the Fermi Level and the cutoff of the 'tail' at the low kinetic energy end of the spectrum (a.k.a. spectrum width) and subtracting this value from the incident photon energy. This value can of course be measured using X-ray incident radiation, however UPS allows workfunction calculation from a single spectrum.

When measuring the electronic workfunction using photoelectron spectroscopy it is necessary to apply a small bias (typically 5-10 V) to the sample surface, so as to deconvolute the true workfunction of the surface from the internal workfunction of the spectrometer.

Reference Books :

Text Books:

T1: Sharma.B.K.(2012) Instrumental methods of chemical analysis (28th Edition). Meerut: Krishna Prakashan Media (p) Ltd

POSSIBLE QUESTIONS:

Part-A (20 x 1= 20 marks) Online Examinations

(Each Question Carry One Mark)

1. Which of the abbreviation is refered to thermogravimetry?
a.TG
b.DTA
c. DTG
d. Th
Answer: a

2. According to thermal methods, when the matter is heated then it undergo -------- changes
a. physical changes only
b. chemical changes only
c. biological change
d. physical and chemical changes
Answer: d

3.What is the instrument used in the DTG
a. DTA apparatus
b. calorimeter
c. thermobalance
d. photometer
Answer: c

4. What is the abbreviation for DTA
a. Differential thermal analysis
b. Differentiate thermal analysis
c. Different temperature analysis
d. Differential thermo analysis
Answer: a

5. The parameter reflectance is measured by which instrument

a. DSC
b.DTG
c. DRS
d. DTA
Answer: c

6. The dynamic reflectance spectroscopy used ------ instruments
a. calorimeter
b. spectrophotometer
c. thermobalance
d. detector
Answer: b

7. The radioactivity of the sample is detected by using
a. ETA
b. EC
c. EGD
d. DTG
Answer: a

8. The meaning of EC is
a. Electrodeconductance
b. Electrical conductivity
c. Energy concentration
d. Electroniccurrent
Answer: b

9. The horizontal lines present in the TG curve is called as
a. Plates
b. Plateaus
c. Curve
d. Peak
Answer: b

10. In which technique,the sample is heated to constant weight at each of a serious of increasing temperature
a. Quasistatic Thermogravimetry
b. Dynamic Thermogravimetry
c. Isothermal Thermogravimetry
d. Static Thermogravimetry
Answer: a

11. In the dynamic Thermogravimetry, the sample is heated in environment with ---------- manner

a. slow rate
b. continous rate
c. linear rate
d. fast rate
Answer: c

12. The meaning of TMA is
a.Thermo mechanical analysis
b. Thermo magnetic analysis
c. thermal continous analysis
d. thermo gravimetric analysis
Answer: a

13. The thermal conductivity cell instrument used in ------ analysis
a. Evolved gas detection
b. Energy gas detection
c. Electrical gas detection
d. Equipped gas detection
Answer: a
14. The instrument of Electrical conductivity is
a. photometer
b. spectrophotometer
c. Electrometer
d. Thermometer
Answer: c

15. The main reaction occurred in thermal analysis is
a. oxidation
b. reduction
c.decomposition
d. dehydrogenation
Answer: c

16. Thermogravimetery is used to analyse ------ of the samples
a. size
b. mass
c. nature
d.elements percentage
Answer: b

17. Thermomechanical analysis is used to measure the ----- of the samples
a. nature
b. refractive index

c. magnitude
d. Volume
Answer: d

18. The change in dH of the sample according change in ------- of the sample
a. temperature
b. volume
c. size
d. index
Answer: a

19. The instrument dilatometer is used to measure the ------ of the sample
a. length
b. height
c. density
d. mass
Answer: a

20. The brief of DSC is
a. Differential thermal analysis
b.Differential scanning calorimetry
c. Differentioalscanning calorimeter
d. Difficultscannedconductometer
Answer: b

Part-B (5 x 6 =30 marks)

(Each Question Carry Six Marks)

1. What is meant by TGA?
2. Explain Compton scattering.
3. Explain the basic theory of Ultraviolet photoelectron spectroscopy
4. Give the merits of TGA.
5. Explain the medical uses of X-ray.
6. What is the principle involved in DTA.
7. What is the principle involved in thermogravimetric analysis?
8. What factors that affect the DTA curves
9. Discuss the instrumentation of Auger electron spectroscopy.

10. Explain the characteristics of TGA curves for $CaC_2O_4.H_2O$ and $CuSO_4.5H_2O$

11. Write notes on (i) Photoelectric absorption (ii) Compton scattering (iii) Rayleigh scattering

12. Describe the instrumentation of thermogravimetry?

. 13. Explain the uses of X-ray.

Part-C (1 x10= 10 marks) Compulsory Questions

1. (i) What is Differential thermal analysis? In typical DTA curve what does sharp endothermic peak and broad endothermic peak indicate?

(ii). How a purity of a compound be checked by Differential scanning calorimetry (DSC)? (Unit-II)

2. Describe the following detectors (i) Scintillation counters, (ii)Gas ionization detectors

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DEPARTMENT OF CHEMISTRY

Class: II M.Sc Chemistry

Subject Title: Physical Methods in chemistry

Subject code: 17CHP303

UNIT-II

Multiple choice questions (Each question carry one mark)

1. Which of the abbreviation is refered to thermogravimetry?
a.TG
b.DTA
c. DTG
d. Th
Answer: a

2. According to thermal methods, when the matter is heated then it undergo -------- changes
a. physical changes only
b. chemical changes only
c. biological change
d. physical and chemical changes
Answer: d

3.What is the instrument used in the DTG
a. DTA apparatus
b. calorimeter
c. thermobalance
d. photometer
Answer: c

4. What is the abbreviation for DTA
a. Differential thermal analysis
b. Differentiate thermal analysis
c. Different temperature analysis

d. Differential thermo analysis
Answer: a

5. The parameter reflectance is measured by which instrument
a. DSC
b.DTG
c. DRS
d. DTA
Answer: c

6. The dynamic reflectance spectroscopy used ------ instruments
a. calorimeter
b. spectrophotometer
c. thermobalance
d. detector
Answer: b

7. The radioactivity of the sample is detected by using
a. ETA
b. EC
c. EGD
d. DTG
Answer: a

8. The meaning of EC is
a. Electrodeconductance
b. Electrical conductivity
c. Energy concentration
d. Electroniccurrent
Answer: b

9. The horizontal lines present in the TG curve is called as
a. Plates
b. Plateaus
c. Curve
d. Peak
Answer: b

10. In which technique,the sample is heated to constant weight at each of a serious of increasing temperature
a. Quasistatic Thermogravimetry
b. Dynamic Thermogravimetry
c. Isothermal Thermogravimetry
d. Static Thermogravimetry
Answer: a

11. In the dynamic Thermogravimetry, the sample is heated in environment with ---------- manner
a. slow rate
b. continous rate
c. linear rate
d. fast rate
Answer: c

12. The meaning of TMA is
a.Thermo mechanical analysis
b. Thermo magnetic analysis
c. thermal continous analysis
d. thermo gravimetric analysis
Answer: a

13. The thermal conductivity cell instrument used in ------ analysis
a. Evolved gas detection
b. Energy gas detection
c. Electrical gas detection
d. Equipped gas detection
Answer: a
14. The instrument of Electrical conductivity is
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d. mass
Answer: a

20. The brief of DSC is
a. Differential thermal analysis
b.Differential scanning calorimetry
c. Differentioalscanning calorimeter
d. Difficultscannedconductometer
Answer: b

21. The parameter temperature of the sample is measured by
a. TG
b. DTA
c. thermoprocess
d. thermo titrimetry
Answer: d

22. The electrode conductance measured by using ---- apparatus
a. EG
b. EC
c. DC
d. AC
Answer: b

23. The change in temperature (ΔT) is analysed by using----
a. DTG
b. DSC
c. DTA
d. TG
Answer: c

24. The isothermal thermogravimetry also called as

a. Static thermogravimetry
b. Dynamic thermogravimetry
c. Differential thermogravimetry
d. Thermogravimetry
Answer: a

25. The thermogravimetry is concerned the change in ----- of samples
a. volume
b. weight
c. temperature
d. density
Answer: b

26. The 10% of polystyrene decomposed at ------ °C
a. 250
b. 375
c. 500
d. 625
Answer: b

27. The decomposition of polymerisation of the complex is
a. 198
b.123
c. 100
d. 151
Answer: d

28. The atmospheric air is allowed flow the furnace is called ----- air
a. Dynamic
b. static
c. cool
d. hot
Answer: b

29. In the dynamic air flow, -------- air is passed by using cylinder
a. free
b. cool
c. ionzed
d. compressed
Answer: d

30.-Which one of the following nitrogen gas is used for the inert environment?
a. oxygen free
b. sulphur free
c. acidic free
d. carbon free

Answer: a

31. In the thermogravimetry, the balance must be ------ stability
a. mechanical
b. electrical
c. mannual
d. mechanical & electrical
Answer: d

32. In the thermobalance ------- response to change in weight of the sample
a. slow
b. modrate
c. linear
d. Rapid
Answer: d

33. The furnace is approximatly ---- mm in diameter
a. 23
b. 34
c. 12
d. 10
Answer: c

34.The furnace is ----- mm long
a. 30
b. 15
c. 10
d. 20
Answer: d

35. Electronic microbalance is sesitive from ---- to --- mg full scale deflection
a. 1 to 243
b. 45 to 157
c. 1 to 250
d. 30 to 180
Answer: c

36. The thermobalance placed ----- to the sample crucible
a. above
b. below
c. adjacent
d. near
Answer: b

37. The ----- of the balance should be commensurate with the sample size
a. position

b. shape
c. sensitivity
d. output
Answer: c

38. L-glutamic acids has howmany stages of decomposition in the air
a.2
b.5
c. 3
d. 4
Answer: c

39. The information of the sample is determined by the ----- of the peak
a. Shape only
b. size only
c. shape and size
d. not the shape
Answer: c

40. The physical changes of the samples measured by ------- curve
a. exothermic
b. endothermic
c. long
d. short
Answer: b

41. The exothermic peaks give about the ------- information of the compound
a. chemical reaction
b. physical change
c. biological
d. valuble
Answer: a

42. DTA did not measure the ------ change in the samples
a. temperature
b. weight
c. valuble
d. metals
Answer: b

43. In DTA instruments ----- not change within the temperature range
a. heat of reaction
b. size only
c. nature
d. mass
Answer: a

44. In the gaseous environment, which technique is more sensitive
a. DTA
b.TG
c. DSC
d. DTG
Answer: a

45. In DSC, energy necessary to establish a -------- temperature difference between sample and reference
a. zero
b. optimum
c. high
d. low
Answer: a

46. The sample size which is under DSC analysis
a. 1-7mg
b. 2-3mg
c. 8-9 mg
d. 2-10mg
Answer: d

47. The sensitivity of heat transition in DTA is
a. 1kJ/mole
b. 3kJ/mol
c. 0.5 kJ/mol
d. 4 kJ/mol
Answer: c

48. The specific heat measurement in the DSC is ------ value
a. accurate
b. not accurate
c. low
d. high
Answer: a

49. LVDT is refered as
a. linear variabledifferentiate transform
b. linear variable differential transformer
c. linear differential volume transformer
d. linear differential volume transistor
Answer: b

50. In the Dilatometry, the displacement range is
a. ± 0.0025cm

b. ± 0.25cm
c. ± 1.0025cm
d. ± 2.0025cm
Answer: a

51. In TG, the accuracy of weight loss in the range
a. ±100
b. ±1
c. ±1000
d. ±10
Answer: b

52. How many % of reduction of polystyrene at 375°C
a. 20
b. 10
c.50
d. 40
Answer: b

53. TG 750 operates from ambient room temperatuer to 1000 °C
a. Room temperature to 100
b. 100 to 1000
c. room temperature to 1000
d. 1 to 10
Answer: c

54. Automatic thermogravimetric analysis----minutes required for entire operation carried out
a. 1 to 5
b. 5
c. 10
d.12
Answer: d

55. The curves for the mixture of ($MgO + CaCo_3$)
a. 1000° C
b. 3000 °C
c. 5000 °C
d. 7000 °C
Answer: C

56. TG curves for MgO + CaO) mixture occurred at -----
a. 2000
b. 900
c. 2500
d. 4000
Answer: b

57. In 500 °C dry magnesium oxalate reduced from 1g to 0.73g
a. 1g to 0.93 g
b. 1g to 0.50g
c. 1g to 0.25g
d. 1g to 0.73g
Answer: d

58. In DTA analysis, We can measure the peak corresponds to-------- of the sample
a. decomposition
b. combination
c. reduction
d. oxidation
Answer: a

59. 12 minutes for recording the results occur in ----- thermogravimetry
a. dynamic
b. static
c. automatic
d. mannual
Answer: c
60. In which thermogravimetry used in the temperature range from room temperature to 1000°C
a. TG759
b. TG321
c. TG750
d. TG43
Answer: c

61. Give the equation for X-ray monochromators?
a. $m\lambda = d \sin \Theta$
b. $m\lambda = 2d \sin \Theta$
c. $m\lambda = d \cos e \Theta$
d. $m\lambda = d \cos \Theta$
Answer: a

Lecture Notes

UNIT-III

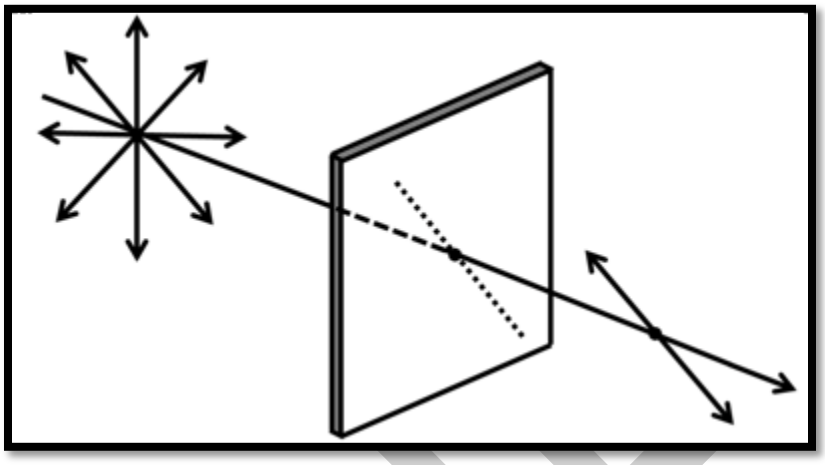
SYLLABUS

Circular Dichroism and Optical Rotatory Dispersion: *Basic principles -cotton effects-octants rule –axial halo ketone rule-application of ORD and CD. Tyndal Scattering-turbidimetry and nephelometry-applications. Atomic absorption spectroscopy.*

Circular Dichroism and Optical Rotatory Dispersion

Introduction

Before going ahead to see what circular dichroism (abbreviated as CD) means, let us have a quick revisit on the polarized light. Light, as we have discussed in lecture 3 is electromagnetic radiation where electric field and the magnetic field are always perpendicular to each other. From now on, we shall mention only electric field; it is implicit that at all points in time and space, the magnetic field vector is perpendicular to both the electric field vector and the direction of the propagation of light. Unpolarized light is comprised of several electromagnetic waves with their electric field vectors (and therefore magnetic field vectors also) pointing in all possible directions, but perpendicular to the direction of light propagation. If the vectors in all, but one, directions are cut off, the resulting radiation is a plane polarized light as the electric field vector is confined to one plane (Figure 1). Looking towards the light source will exhibit electric field fluctuations in one line; the plane polarized light is therefore also referred to as the linearly polarized light.



Plane polarized light produced by a Linear Polarizer

Superposition of polarized waves

Two electromagnetic waves can be superposed through vector addition of their electric field vectors. The properties of the resultant waves depend on the wavelength, polarization, and the phase of the superposing waves. In-phase superposition of two waves of same wavelength that are linearly polarized in two perpendicular planes results in a linearly polarized light with its electric field vector oscillating in a plane that is inclined at an angle of 45° to the polarization planes of both the waves (Figure 2).

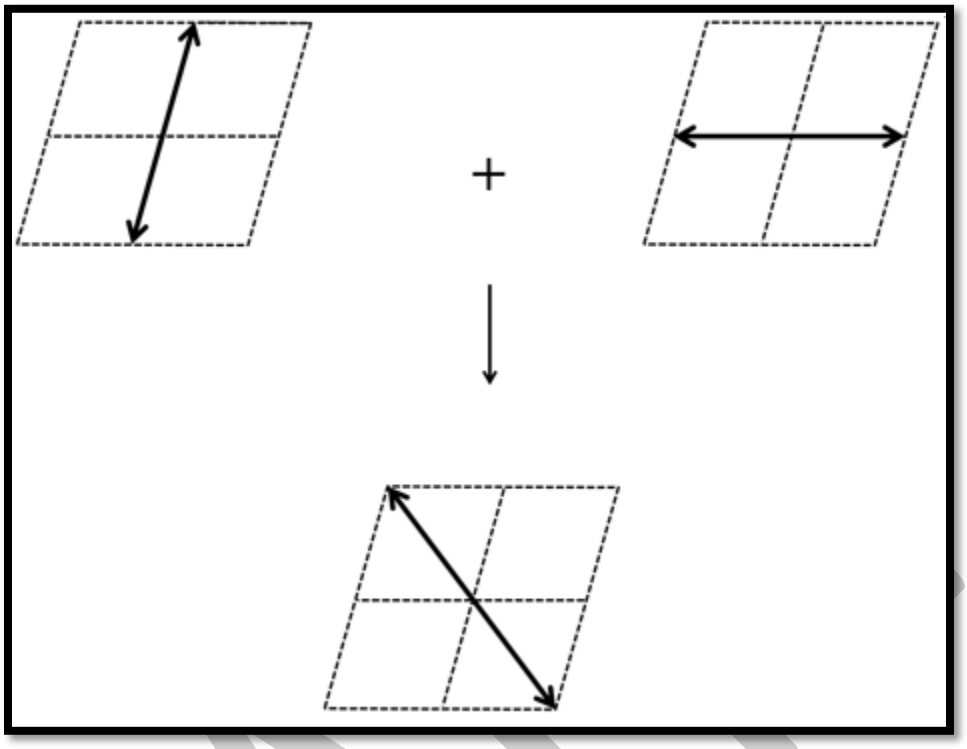


Figure : Superposition of linearly polarized waves

Let us see what happens when the two plane polarized waves, polarized in two perpendicular planes meet each other out of phase. Suppose the two waves have a phase difference of 90°. As the two waves have same wavelength, a 90° phase difference implies that when one of the wave is at maximum amplitude, the amplitude of the other one is minimum and vice versa. If the amplitudes of the two waves are equal, their superposition with a 90° phase difference results in a wave wherein electric field vector traverses a circular path (Figure 8.3). The electric field of the resultant wave is never zero but a vector of constant length. When looked at the travelling wave from the direction of propagation, the electric field appears to be rotating in a circle. The resulting light is therefore termed as circularly polarized light (Figure 8.3).

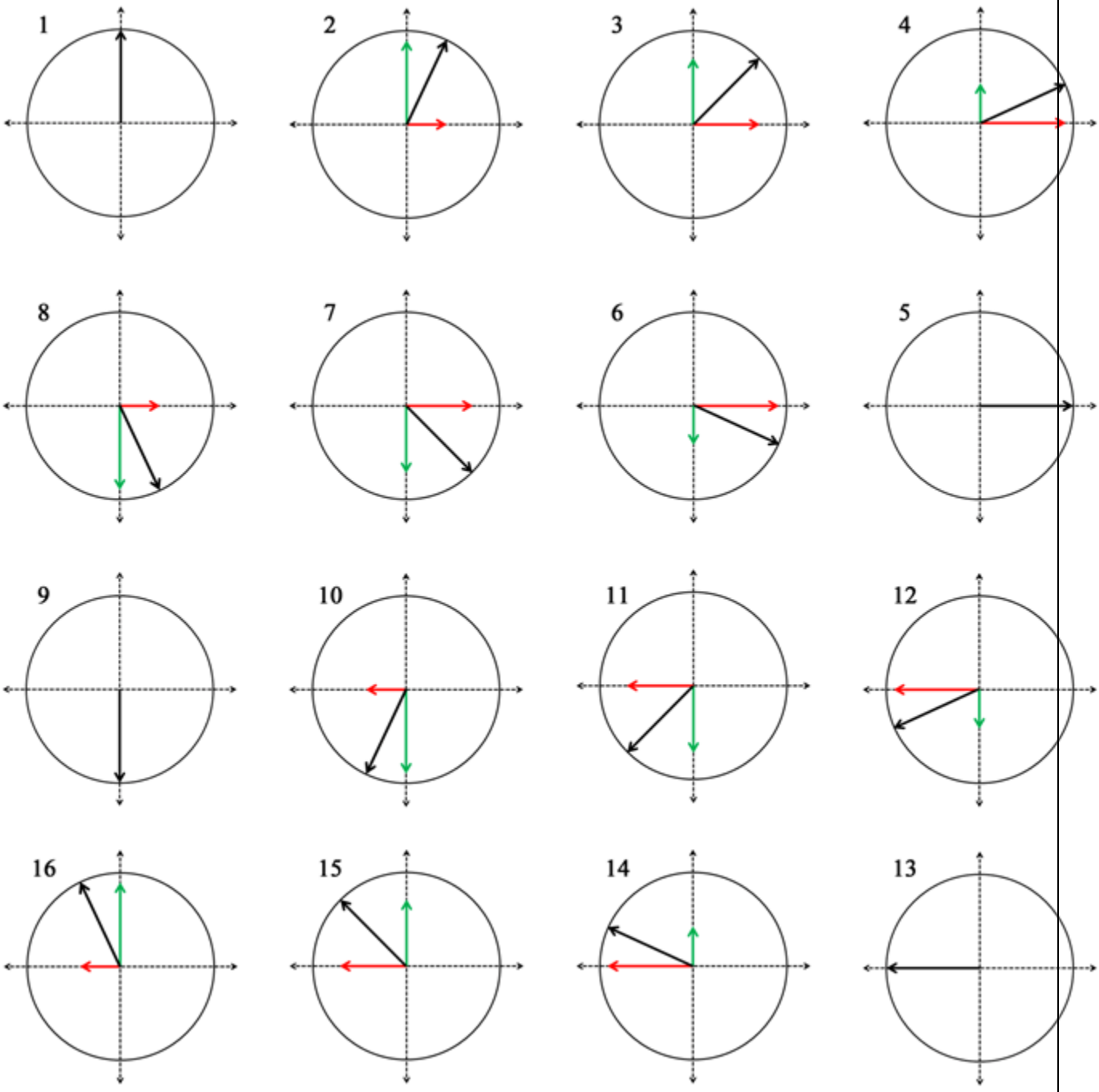

1
2
3
4
8
7
6
5
9
10
11
12
16
15
14
13

Figure : Superposition of waves linearly polarized in mutually perpendicular plain and that meet together 90° out of phase.

The direction of rotation depends on phase difference; a -90° phase difference would result in a circularly polarized light where the electric field rotates in opposite direction. When looked towards the light source, the electric field vector of a right circularly polarized wave appears to rotate counterclockwise in space while that of a left circularly polarized wave rotates clockwise. What happens when the right circularly polarized light (RCPL) and the left circularly polarized light (LCPL) superpose? The resultant wave is a linearly polarized wave (Figure 8.4). A linearly polarized light can therefore be considered as being composed of a right circularly polarized light and a left circularly polarized light.

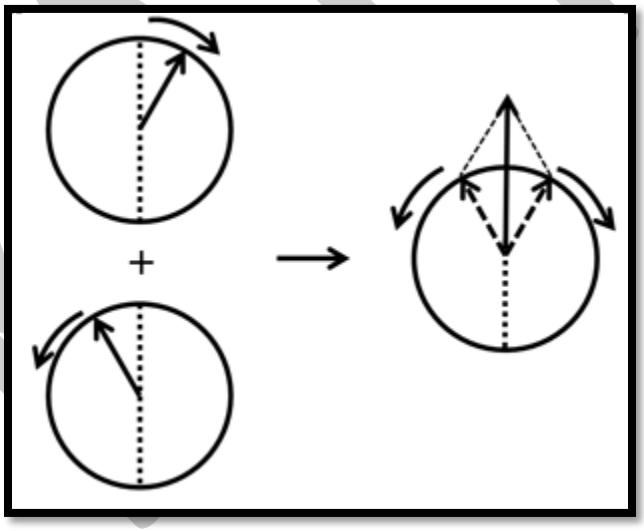


Figure : Superposition of left and right circularly polarized light resulting in plane polarized light.

Circular Dichroism

Circular dichroism, abbreviated as CD, is a chiroptical spectroscopic method. A chiral molecule or an achiral molecule in asymmetric environment interacts differently with the LCPL and the RCPL. The literal meaning of dichroism is 'two colors'. In chiroptical spectroscopy, dichroism means differential absorption of the lights with different polarizations. Circular dichroism, therefore, refers to the differential absorption of the left and right circularly polarized light and is defined as:

$$CD = \Delta A = A_l - A_r \quad (6.1)$$

where, A_l and A_r are the absorbances for the left and right circularly polarized lights, respectively.

We can therefore say that the molar absorption coefficients for the two lights are different and can write the equation 6.1 can be written as:

$$CD = (\varepsilon_l - \varepsilon_r)cl \quad (6.2)$$

$$CD = \Delta\varepsilon cl \quad (6.3)$$

The preferential absorption of LCPL over RCPL (or vice versa) results in elliptical polarized light (Figure).

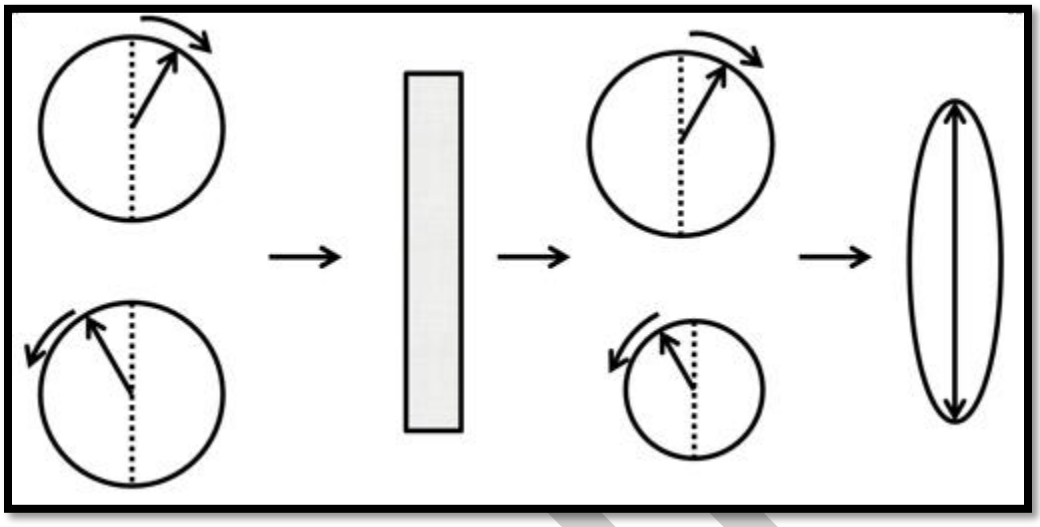


Figure : Differential absorption of the left and right circularly polarized light resulting in elliptically polarized light. Notice that if one component is completely absorbed, the resultant wave will be circularly polarized.

CD is historically represented in terms of ellipticity (θ) which is the tangent of ratio of minor to major axis of the ellipse. The relationship between CD and θ is give by:

$$\theta\ (\text{radians}) = \frac{2.303}{4} \times CD \qquad (6.4)$$

$$\theta\ (\text{degrees}) = \frac{2.303}{4} \times CD \times \frac{180}{\pi} \qquad (6.5)$$

$$\theta\ (\text{degrees}) \approx 33.0 \times CD \qquad (6.6)$$

A plot between ΔA or $\Delta\varepsilon$ or θ against the wavelength of light represents a CD spectrum. In this lecture, we shall be discussing only electronic CD. That means that we shall be looking at the electromagnetic region that causes electronic transition, which of course is UV/Visible region.

Circular birefringence

If a sample reduces the velocity of the LCPL and RCPL to different extents, the sample is said to be circularly birefringent and the phenomenon circular birefringence. Let us see what happens when the linearly polarized light (having two components, LCPL and RCPL) traverses a circular birefringent medium: the velocities of the two components are reduced to different extents *i.e.* they have different wavelengths in the sample. After emerging from the samples, the wavelength is restored but two components can be out of phase. This results in the rotation of the polarization axis. If the material is not circularly dichroic, the plane of the linearly polarized light is rotated (Figure 8.6A). If the material is both circularly dichroic and birefringent, the plane polarized light will become elliptically polarized light with the major axis of the ellipse tilted with respect to the polarization axis of the incident polarized light (Figure 8.6B).

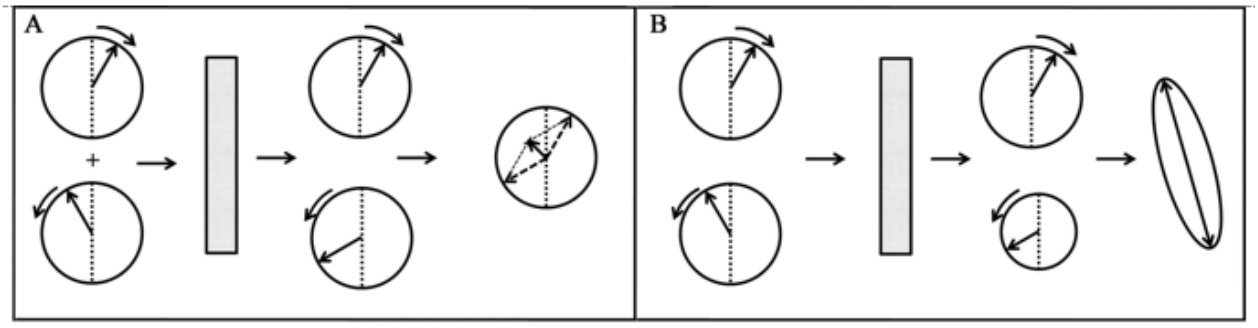


Figure : A linearly polarized light passing through a circular birefringent but not circular dichroic material (A) and through a material that is both circular birefringent and circular dichroic (B). Circular dichroism results in elliptically polarized light while circular birefringence causes change in the polarization axis.

Instrumentation

Photoelastic modulator: A photoelastic material is the one that exhibits birefringenece under mechanical stress. The photoelastic modulator in a CD instrument comprises of a quartz crystal fused to a piezoelectric material. Oscillations in the piezoelectric material drive the quartz crystal to oscillate at the same frequency. The crystal optical axis is at 45° to the linearly polarized light. The crystal retards one component of the light more than the other when compressed. When expanded the velocity of the two components gets reversed. A PEM, therefore gives alternating LCPL and RCPL.

As CD is simply the difference in the absorbance of the LCPL and RCPL lights, a CD spectrometer, also known as a CD spectropolarimeter, is basically an absorption spectrophotometer (Figure 8.7). The instrument has a light source, usually a Xenon lamp. The polychromatic light from the source is converted to monochromatic radiation which is further converted to linearly polarized light by a polarizer. The linearly polarized light passes through a photoelastic modulator that alternately converts the linearly polarized light into LCPL and RCPL. The LCPL and the RCPL, therefore pass through the sample alternately and their absorbance gets recorded. Absorbance is recorded at various wavelengths to obtain a CD spectrum. Single wavelength CD values are also important in studying the fast reactionssuch as protein folding/unfolding (discussed in the next lecture).

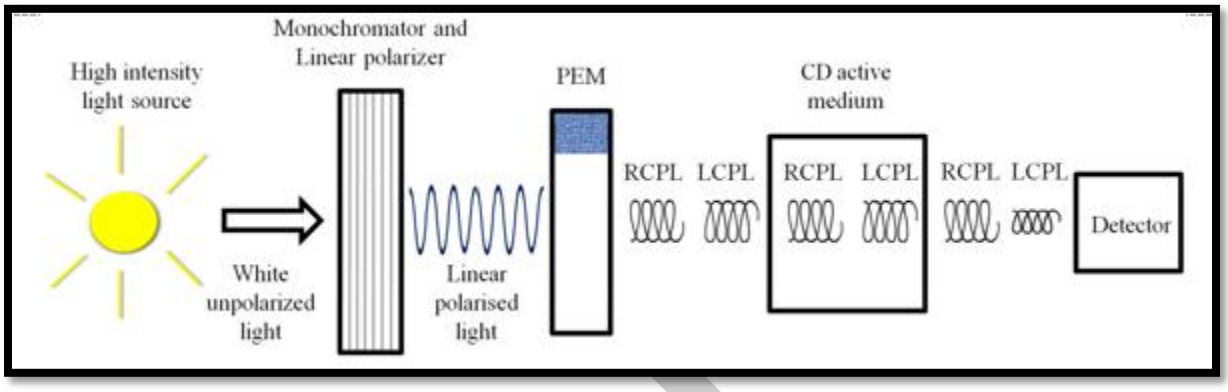


Figure : Schematic diagram of a CD spectropolarimeter.

CD of biomolecules

Most biomolecules are chiral and the biomacromolecules are composed of chiral components. Folding of biomacromolecules into higher order structures further imparts them the asymmetry. CD has not been used as much to study other biomolecules probably, as it has been used to study proteins.

CD of proteins

Proteins are usually composed of 20 amino acids, 19 of which (except glycine) are chiral. This chirality also reflects in the higher order structures that the polypeptides adopt; α-helix, for example, is a right handed helix. If a polypeptide adopting α-helical structure is synthesized using D-amino acids, it folds into the left-handed α-helix under identical conditions. The other structural features of a polypeptide backbone include β-sheets, that are comprised of extended polypeptide chains ; β-turns, that usually, but not essentially, link the β-strands in an antiparallel β-sheet; and unordered conformation. CD spectra of the proteins contain information about the asymmetric features of the polypeptide backbone. Furthermore, it can provide information about the orientation of the side chains. CD,

therefore, is capable of providing information about the structure of proteins which in turn helps understanding their function. The chromophore that provides information about the conformation of the peptide backbone is the peptide bond (Figure); the spectra are therefore recorded in the far UV region, the region where peptide bond absorbs.

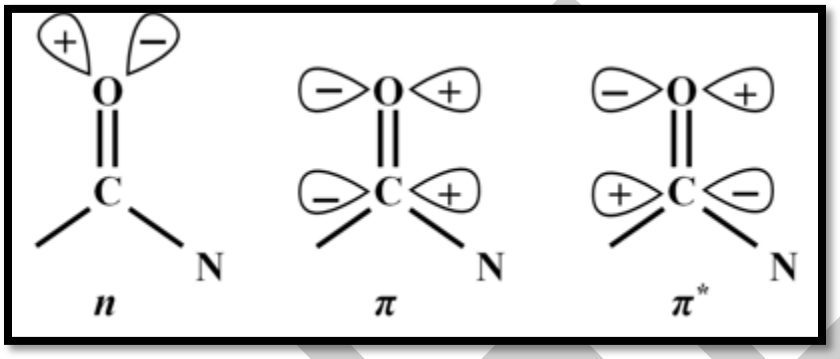


Figure : The peptide bond showing molecular orbitals involved in electronic transitions

Let us have a look at the CD spectra characteristic of the different structural components of the proteins (Figure 9.2).

- *α-helix*: The right handed α-helix displays two negative absorption bands centered around 222 nm ($n \rightarrow \pi^*$ transition) and 208 nm (a part of the $\pi \rightarrow \pi^*$ transition) and a strong positive band around 192 nm (a part of the $\pi \rightarrow \pi^*$ transition).

- *β-sheet:* β-sheets are characterized by the presence of a negative band centered around 216-218 nm ($n \rightarrow \pi^*$ transition) and a positive band of comparable intensity at around 195 nm ($\pi \rightarrow \pi^*$ transition).

- *β-turn:* A β-turn comprises of a four residue protein motif that causes the polypeptide backbone to take an approximately 180° turn. The CD spectrum for a β-turn is not well defined. A typical β-turn, however, shows a weak negative band around 225 nm ($n \rightarrow$

π*transition), a strong positive band between 200 – 205 nm ($\pi \rightarrow \pi^*$ transition), and a strong negative band ($\pi \rightarrow \pi^*$ transition) between 180 – 190 nm.

- *Random coil:* Random coil or unordered conformation shows a weak positive band around 218 nm ($n \rightarrow \pi^*$ transition) and a strong negative band ($\pi \rightarrow \pi^*$ transition) below 200 nm.

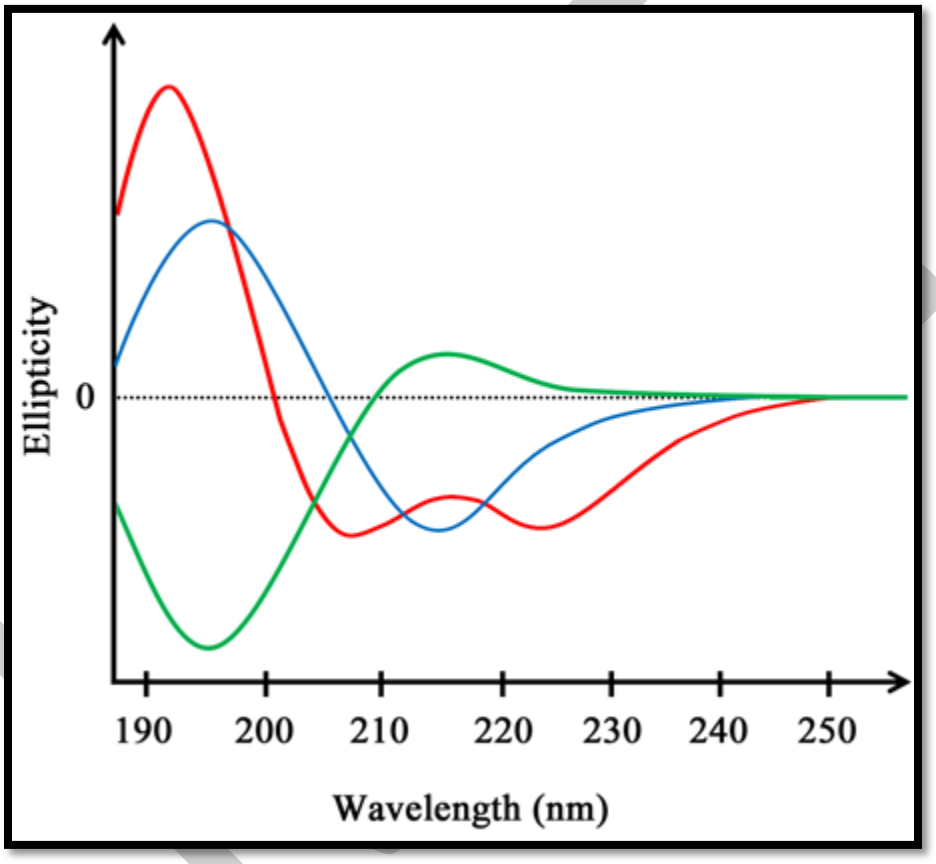


Figure : Far UV circular dichroism spectra of α-helix (red), β-sheet (blue), and unordered conformation (green)

The CD spectrum of a protein can be written as a linear combination of the spectra of all the structural components:

CD (protein) = a CD(α-helix) + b CD(β-sheet) + c CD (Random coil)

As the CD spectra of different structural components are quite distinct, it is possible to estimate the fraction of different structural components in a protein from its CD spectrum. As discussed in lecture 5, proteins also have chromophores that absorb in the near UV region. These include the aromatic amino acids and disulfide linkages. The CD of aromatic amino acids is highly dependent on their environment and therefore near UV CD of proteins can provide the information about the environments these residues reside in as well as their orientations in the structure. As it provides information about the tertiary region, near UV CD is also referred to as tertiary CD in the context of the proteins.

CD of nucleic acids

As mentioned in lecture 5, nitrogenous bases constitute the chromophores of nucleic acids in the near and far UV region. The CD of the stacked bases is larger in magnitude as compared to that of the isolated bases. As the double helical nucleic acids have stacked bases, what we measure essentially is the CD that arises due to coupling of the chromophores. As the stacking geometries are different for different forms of nucleic acids such as B-DNA, Z-DNA, and A DNA; CD can help in determining which DNA form is present in a given sample.

Applications in biomolecular analysis

i. Determination of protein/peptide structure: As has already been discussed earlier, far UV CD spectroscopy provides information about the secondary structural elements in a protein. A mixture of structures can be deconvoluted to obtain the fraction of different structural elements. Furthermore, near UV CD provides information about the tertiary structure of the protein.

ii. Comparison of structures: Mutants of proteins are often required for understanding the functions of the proteins. It, however, needs to be ascertained that the mutation does not cause any significant change in the overall structure of the protein. CD spectroscopy happens to be a fast and extremely reliable tool to compare the conformations of the wild type proteins with their mutants.

iii. Stability of proteins: Stability of the proteins to denaturants or heat can be studied using CD spectroscopy. In such studies CD is usually monitored at a single wavelength, typically around 220 nm. Plotting the change in ellipticity against increasing denaturant concentration/temperature provides the denaturation curve. Figure shows the denaturation curves for three related proteins. The denaturation curves suggest that the protein indicated with the blue trace is most stable while the one indicated with red trace the least.

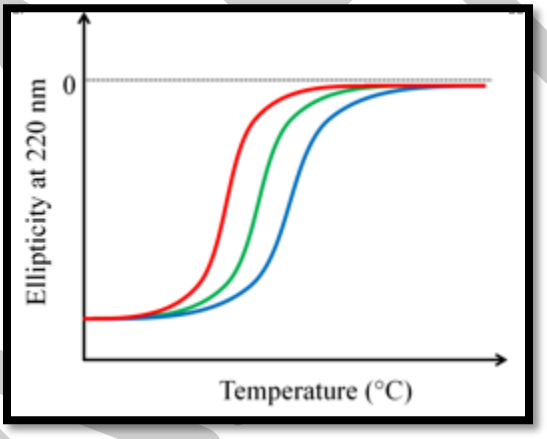


Figure : Comparison of thermostability of three related proteins. The blue trace represents the most stable protein.

iv. Binding of ligands to proteins: Binding of a ligand to a protein usually does not affect the secondary structural elements significantly. However, such a binding can cause changes in the local tertiary structure. Binding of ligands accompanying such conformational changes

can be studied using tertiary CD if the binding region happens to have one or more aromatic residues . Short peptides, on the other hand, can undergo large scale structural changes sometime involving completely switching from one secondary structure to another. Such changes can easily be observed using far UV CD.

v. DNA structure: CD in the 200 – 300 nm region can be used to identify which structural isoform of DNA is present in the given sample. The left-handed helical DNA form, the Z-DNA was indeed identified using CD spectroscopy. The typical CD signatures of the B, Z, and A form of DNA are:

B-DNA: In its most common form *i.e.* B-DNA with ~10.4 bases per turn, a positive band ~275 nm, a crossover ~258 nm, and a negative band at ~240 nm are observed.

Z-DNA: A negative band ~290 nm and a positive band ~260 nm; a crossover between 180-185 nm.

A-DNA: A positive band ~260 nm, a negative band ~210 nm.

vi. Protein folding/unfolding: CD is used for studying the folding and unfolding of proteins. For monitoring the fast reactions such as protein folding, a single wavelength CD is recorded in a stopped flow experiment wherein the protein solution is mixed with a denaturant and CD is recorded as a function of time. Modern instruments take ~1 millisecond time between mixing and recording data allowing the understanding of the folding/unfolding events that occur on milliseconds to seconds timescale. A diagrammatic unfolding experiment is shown in figure 9.4

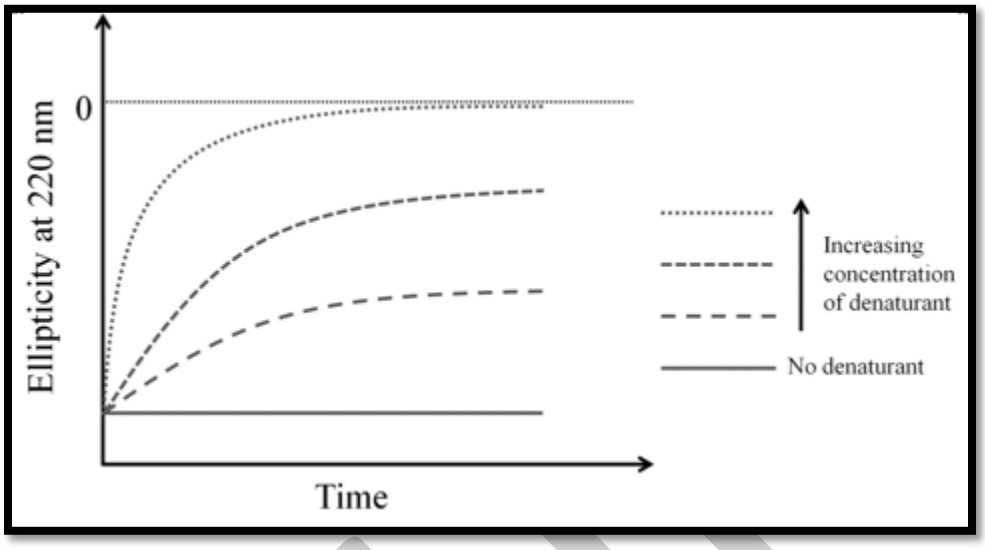


Figure : A diagram showing the kinetics of unfolding of a hypothetical protein. The protein is unfolded with different concentrations of a denaturant. Protein and denaturant are mixed in a stopped flow apparatus (mixing time typically ~1 ms) and changes in ellipticity are monitored over time.

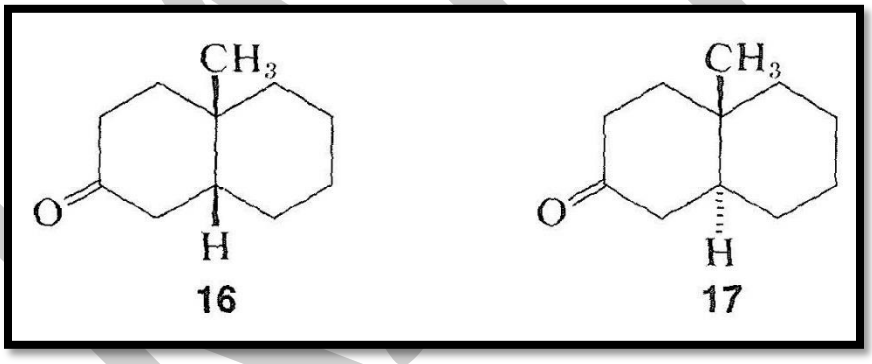
viii. Molecular self-assembly: Self-assembly into structural and functional superstructures is integral to biomolecules and therefore to living systems. Inspired by the naturally occurring superstructures, short peptides have attracted considerable attention as the monomers for designing superstructures with novel properties and applications in biomedicine. Circular dichroism has been central in elucidating the conformations of the peptides in superstructures as well as the interactions that drive this assembly.

Circular dichroism, therefore, is a powerful tool in studying the conformations of biomolecules as well as the processes these molecules are involved in.

Optical Rotatory Dispersion and Circular Dichroism

Optical rotations usually are measured at just one wavelength, namely 589.3nm589.3nm, simply because sodium-vapor lamps provide an especially convenient source of monochromatic light. Measurements at other wavelengths are less easily made without specialized instruments, with which relatively few laboratories are currently equipped. Nevertheless, much information has been obtained about structure, conformation, and configuration of organic compounds from measurements of optical rotation as a function of wavelength (i.e., **optical rotatory dispersion**).

Like other phenomena involving interactions between electromagnetic radiation and organic molecules, as in infrared, ultraviolet, and nmr spectroscopy, optical rotatory dispersion curves often are quite sensitive to small changes in structure. As an example, the rotatory dispersion curves for enantiomers of *cis*- and *trans*-10-methyl-2-decalones, 1616 and 1717, are reproduced in Figure 19-7:



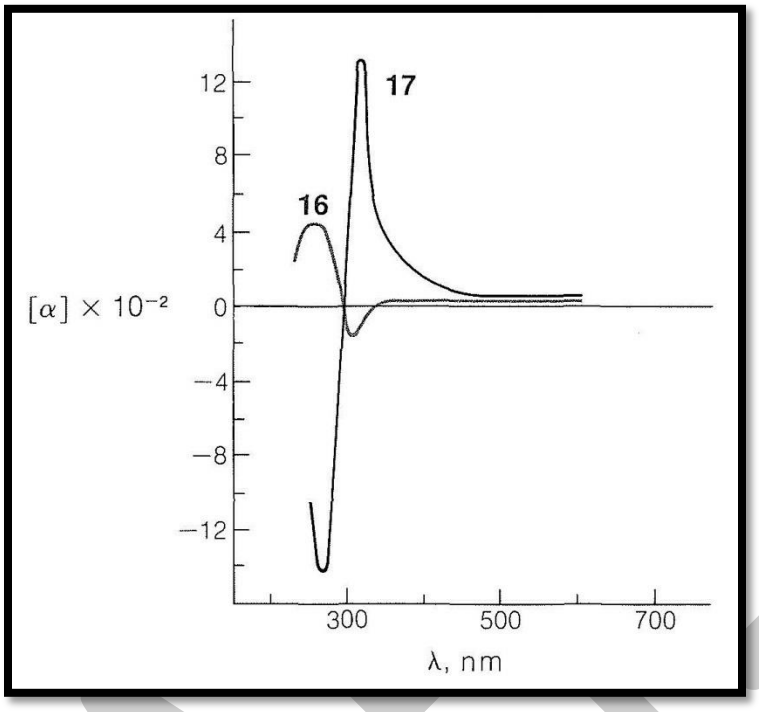


Figure : *Rotatory dispersion curves for* cis-*10-methyl-2-decalone,* 1616, *and* trans-*10-methyl-2-decalone* 1717. *(By permission from C. Djerassi, O*ptical Rotatory Dispersion, *McGraw-Hill Book Co., New York, 1960.)*

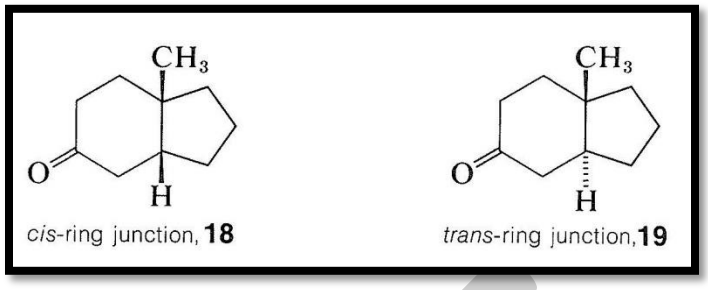
Only a small positive rotation is observed for the particular enantiomers at the wavelength of the sodium line (589.3nm)(589.3nm) compared to the large, both positive and negative, rotations found at wavelengths between 270nm270nm and 400nm400nm. If we measure the rotations as a function of wavelength and if, as we approach shorter wavelengths, the rotation rises to a *maximum*before changing sign, as it does with the trans isomer, 1717, then the compound is said to exhibit a **positive Cotton effect**. The opposite behavior, as with the cis isomer, 1616, is called a **negative Cotton effect**. The wavelength at the center point for the very rapid change in rotation for 1717 is 300nm300nm and corresponds to the $n\rightarrow\pi*$ absorption maximum of the carbonyl group in the ultraviolet absorption curve of the same compound. Thus excitation of the carbonyl group by absorption of

ultraviolet light and strong rotatory dispersion of polarized light are associated phenomena. In fact, when a substance exhibits a Cotton effect, not only does it transmit clockwise and counterclockwise circularly polarized light with unequal velocities , it also absorbs the two forms of light unequally.

This means that the molar extinction coefficients of the two enantiomers (єlєl and єrєr) are unequal in circularly polarized light. These differences in absorption (єlєl and єrєr) can be measured as a function of wavelength, and the curves obtained are called **circular dichroism** curves. They have positive or negative signs (Cotton effect) just as for optical rotatory dispersion curves.

Most of the research on optical rotatory dispersion to date has been with optically active ketones because the carbonyl chromophore conveniently has a weak absorption band in the 300nm300nm region. Compounds with chromophores that absorb light *strongly* in the ultraviolet usually are unsatisfactory for rotatory dispersion measurements because insufficient incident light is transmitted to permit measurement of optical rotation. Weak absorption bands below about 210nm210nm have not been exploited because of experimental difficulties in making the necessary measurements.

Many rotatory dispersion curves have been obtained for optically active ketones derived from steroids and triterpenes, which are monocyclic, bicyclic, and open-chain compounds. Enough data have been accumulated so that the various shapes and magnitudes of the curves are recognized as characteristic of particular structural features. A good illustration is provided by the rotatory dispersion curves for the *cis*- and *trans*-8-methylhydrindan-5-ones, 1818 and 1919, which are shown in Figure 19-8:



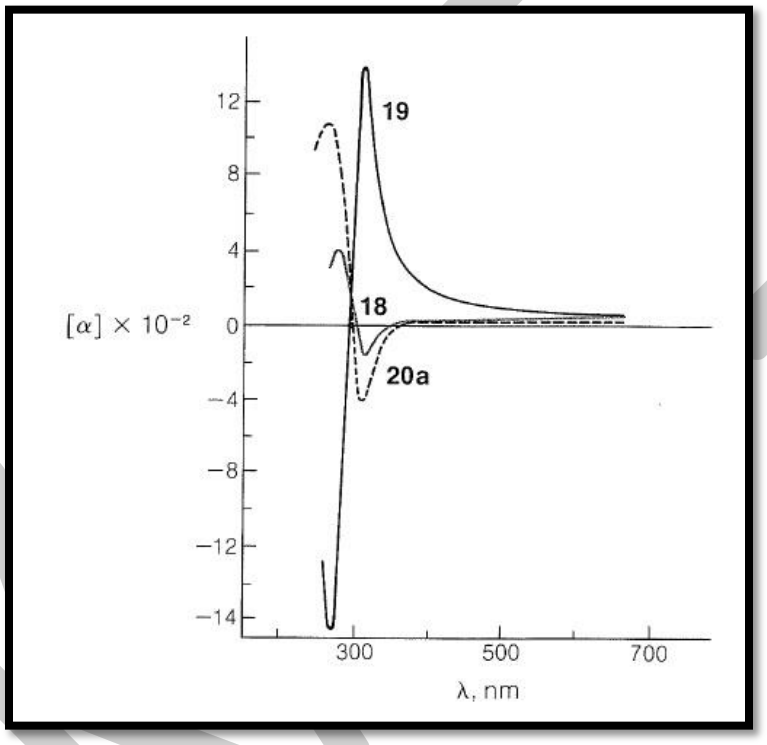
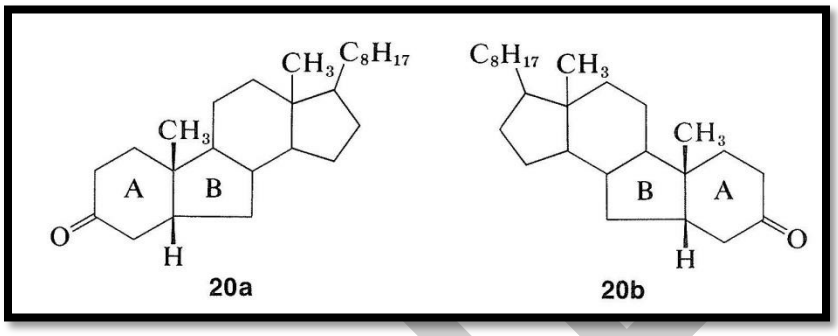


Figure ***:*** *Rotatory dispersion curves for* trans-*8-methylhydrindan-5-one*, 1919, cis-*8-methylhydrindan-5-one*, 1818, *and* BB-*norcoprostan-3-one*, 20a20a. *(By permission from C. Djerassi*, Optical Rotatory Dispersion, *McGraw-Hill Book Co., New York, 1960.)*

The remarkable differences in these curves are due to changes in the environment of the carbonyl groups arising from the different configurations of the hydrogens at the ring junctions. Because the rotatory dispersion curve of the closely related structure 20a20a is

very similar to that of the *cis*-hydrindanone, 1818, the rings labeled AA and BB in 20a20a can be inferred also to be cis oriented (see Figure 19-8):



Rotatory dispersion curves often are helpful in establishing configurations; thus the relative configurations of compounds 1818 and 20a20a must be the same because, if they were not, the two curves would resemble mirror images of one another. Therefore, if the absolute configuration of 1818 corresponds to the formula shown, then compound 20a20a has the configuration shown and not 20b20b.

Cotton effect curves and octant rule, How would you relate stereo chemical features of a compound with them?

Cotton Effect Curve

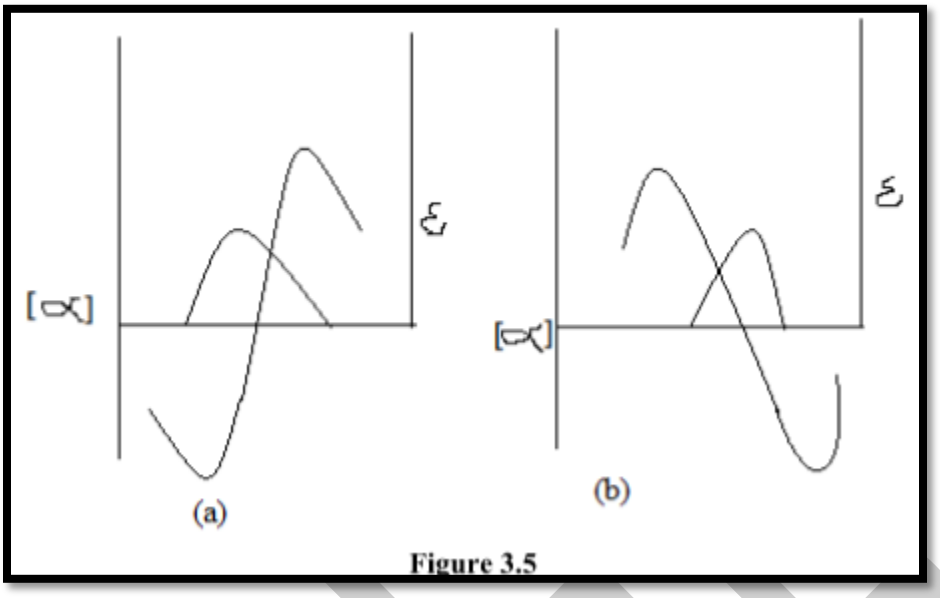


Figure 3.5

Any medium which is exhibiting circular birefringence may also exhibit circular dichroism. The combination of these two effects in the region in which the optically active absorption bands are observed gives rise to the phenomenon called the cotton effect. And obtain curve known as cotton effect curve. It gives two types of absorption curve that is positive or negative cotton effect curve.

Optically active bands are absorption bands of the chromophores which are either intrinsically asymmetric or which become asymmetric because of the interaction with asymmetric environment.

As an example of the former we may discuss the hexahelicene molecule. In this case the entire molecule acts as one big chromophore.

As an example of latter we consider the carbonyl group which is symmetric but becomes optically active in an asymmetric environment. Hence the carbonyl group in acetone is optically inactive because it is a symmetric molecule environment. The same carbonyl group

in 3-methylcyclohexanone, however, becomes optically active because there is an asymmetric carbon atom present. It is expected that such induced optical activity would be appreciably smaller than the case in which the whole molecule acts as chromophore. This is indeed true in general.

Both the absorption and circular dichroism phenomenon are having their origin in the charge displacements which are caused by the interaction with the electromagnetic radiation. As a result there will be induced electric and magnetic dipoles. We define the rotational strength Rk of a chromophore for the k electronic transition as follows:

$$Rk=\rho\mu\cos\theta$$

Where ρ and μ refer to the electric and magnetic transition moments and θ refer to the angle between the directions of these two moments. For a molecule having either a centre of inversion or a reflation plane of symmetry, we can have any of the following three situations:
$\rho \neq 0$ but $\mu = 0$ electric dipole allowed but magnetic dipole forbidden transition
$\rho \neq 0$ but $\mu = 0$ electric dipole allowed but electric dipole forbidden transition
$\rho \perp \mu$ $\cos\theta = 0$ in all cases, Rk = 0 and the molecule is optically inactive.

Octant Rule

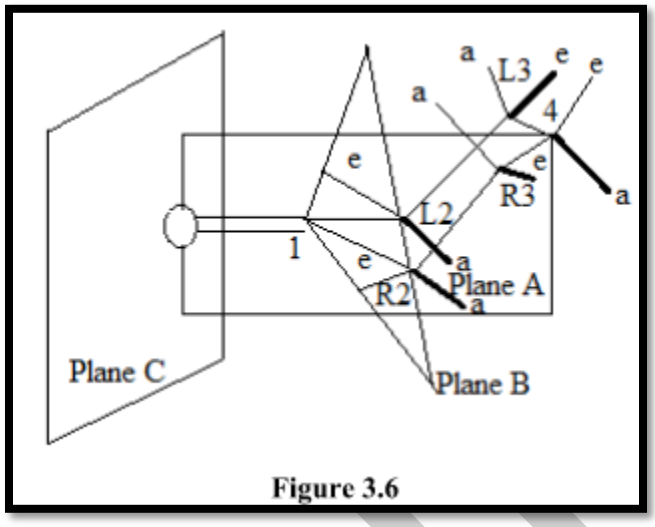


Figure 3.6

This is very useful empirical rule in predicting the sign and magnitude of the cotton effect. This rule applies only to the substitute cyclohexanones. The cyclohexanone molecule is divided into eight octants by three planes A, Band C shown in figure1.4. Plane A passes carbon atoms 1 and 4; the substituents attached to carbon atoms 4 thus lie in this plane.

Plane B encompasses carbon atoms 1, L2 and R2 where L and R denotes left and right from the observer's point of view. The substituents in the equatorial positions at L2 and R2 are practically in this plane.

Plane C bisects the carbonyl group and is perpendicular to plane A and B. thus plane A and B produce four octants and plane C produce four more. The midpoint of the C = O bond is chosen to be the origin of the coordinate system.

The octant rule may be stated that substituents in the lower left and far upper right octants make a negative contribution to the cotton effect, substituents in the far lower right and far

upper left make a positive contribution; substituents in any of the three planes do not make contribution.

As rarely do substituents bend over the carbonyl group towards the oxygen atom beyond, the four octants in the front of plane C are usually vacant and will not concern us. Thus we shall consider only the four octants defined by planes A and B. as a simple illustration of this rule let us consider 3-methylcyclohexanone molecule.

Relation of Stereochemical features of compounds with them

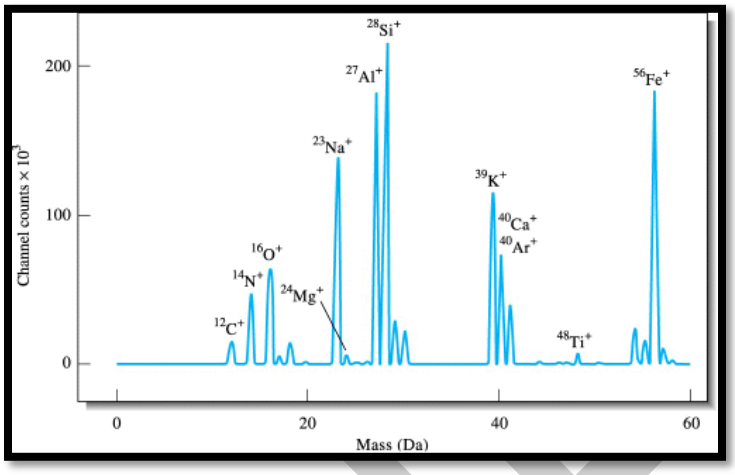
The sign of Cotton effect gives information about the stereochemistry in the nearby environment of the chromophore, i.e., the carbonyl group ($n \rightarrow \pi^*$ absorption of the carbonyl group around 280 nm) acts as a probe of the chirality of its environment. Consider the following points

(i) When two compounds display curves of the same sign and shape, the stereochemical features near the chromophore are same.

(ii) When two compounds show cotton effects of opposite sign then the stereochemical features near the chromophore are mirror image type.

(iii) The compound (II and III,) are not enantiomers (these are different compounds), however, their ORD curves have an almost mirror image relationship. Thus the compounds have an object mirror image i.e., enantiomeric stereochemistry of the groups in the immediate vicinity of the carbonyl group and this is A/B ring junction.

Atomic Absorption Spectroscopy: Instrumentation



Atomic identification and quantification

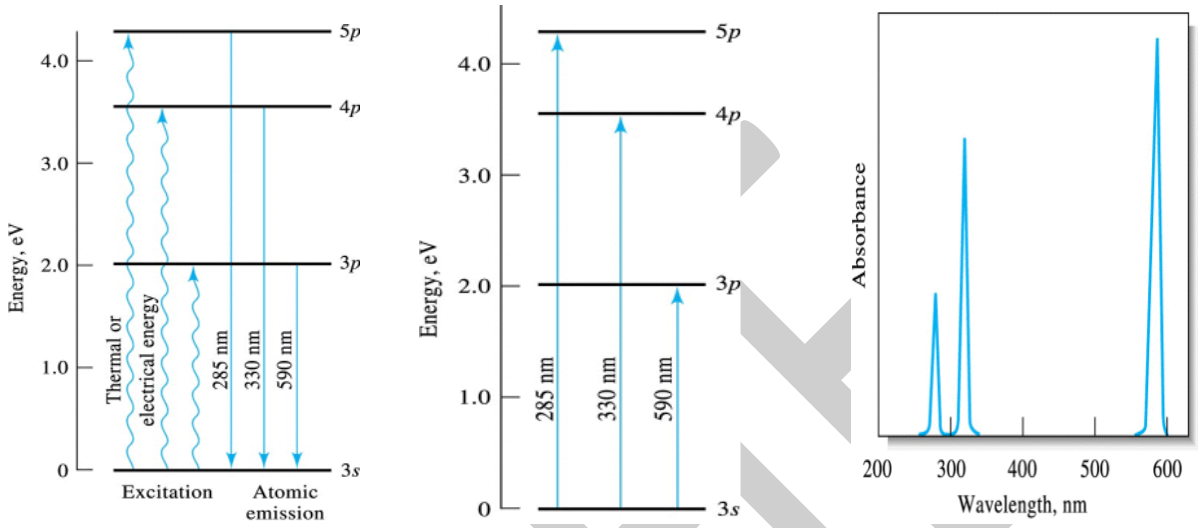
Atomic spectroscopy is the determination of elemental composition by its electromagnetic or mass spectrum. Atomic spectroscopy is closely related to other forms of spectroscopy. It can be divided by atomization source or by the type of spectroscopy used. The basic principle is that light is passed through a collection of atoms. If the wavelength of the light has energy corresponding to the energy difference between two energy levels in the atoms, a portion of the light will be absorbed. The relationship between the concentrations of atoms, the distance the light travels through the collection of atoms, and the portion of the light absorbed is given by the Beer-Lambert law.

Origins of Atomic Spectroscopy

Spectroscopy of atoms or ions does not involve vibrations or rotation transitions. Transition involves promoting an electron from a ground state to a higher empty atomic state orbital, this state is referred to as the excited state.

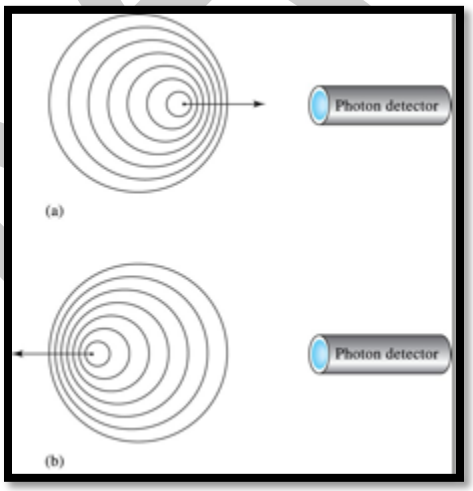
Shown to the right are the three sodium absorption and emission process and the emission lines. Atomic p-orbitals are in fact split into two energy levels for the multiple spins of the

electron. The energy level is so small however that a single line observed. A high resolution would show the line as a doublet.



Resolution (Width) Lines Spectra

Atomic spectral lines have finite widths with factors to line broadening due to:



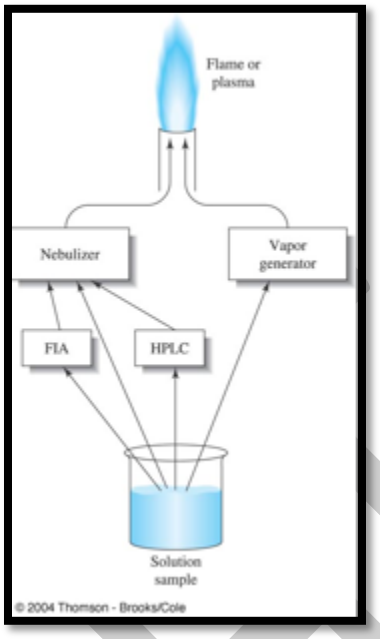
• Natural Broadening - The lifetime of the excited states lead to uncertainty leading to broadening due to shorter excited state lifetimes. Lifetimes of 10-8 s lead to width of 10-5 nm.

• Collisional Broadening -Also referred to as **Pressure Broadening** is the result of collision of the excited state leads to shorter lifetimes and broadening of the spectral lines.

• Doppler Broadening - When molecules are moving towards a detector or away from a detector the frequency will be offset by the net speed the radiation hits the detector. This is also known as the Doppler effect and the true frequency will either be red shifted (if the chemical is moving away from the detector) or blue shifted (if the chemical is moving towards the detector)

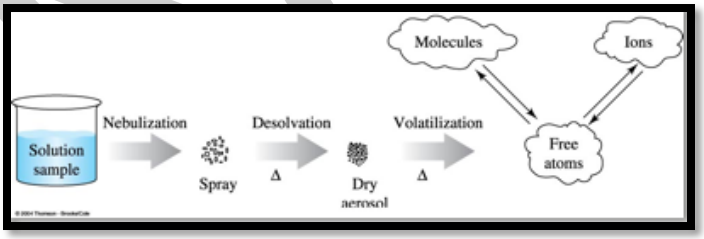
Instrumentation

Atomic spectroscopy begins with atomizing the sample.

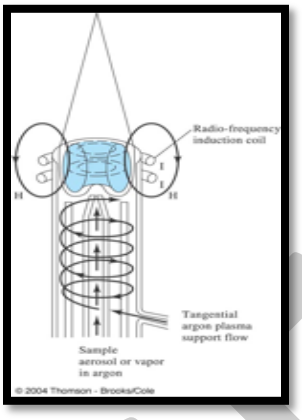


• Sample introduction - Atomizer devices are either continuous or discrete. Continuous are in the form of plasmas and flame. Discrete are in the form of electrothermal. Nebulizers are the method to introduce samples into the atomizer. Direct nebulizer creates fine droplets by aerosol.

• Shown is the continuous sample method. Samples are frequently introduced into plasmas or flame by means of nebulizer which takes the sample and convert it to a spray or mist.



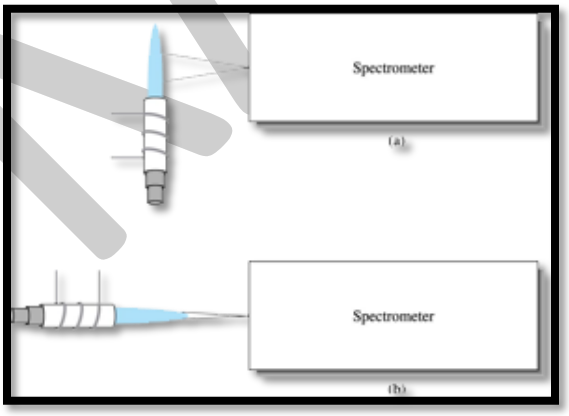
Plasma Source



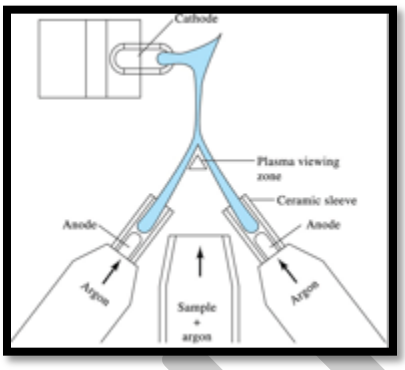
• Plasma is the phase of matter with its electrons stripped. In argon plasma, argon ions and electrons act as the conducting species. Three power sources are dc-electric, radio and microwave frequency generators. The most advantageous is the radio or inductively coupled plasma (ICP) because of sensitivity and minimal interference. DC plasma source (DCP) are also advantageous and is also simple and less expensive

•Inductive Coupled Plasma consists of three concentric quartz tubes in which streams of argon flow. Ionization of the argon is initiated by a spark from a Tesla coil.

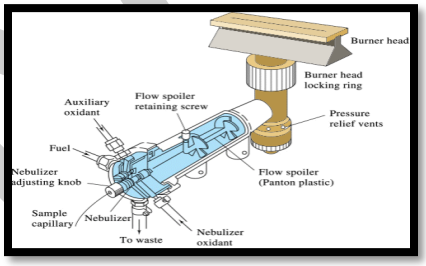
The geometries of CP source, in radial geometry or axial geometry.

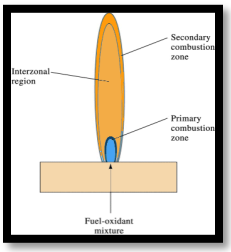


Flame Atomizer



• Flame atomizers contain a pneumatic nebulizer, which converts the sample solution into a mists or aerosol. Shown is a diagram of a three electrode dc plasma jet. Two separate dc plasmas have a single common cathode. The overall plasma burns n the form of an inverted Y. Samples are introduced as aerosol from the area between the two graphite anodes. Observation of emission in the region beneath the strongly emitting plasma core avoids much of the plasma background emission. When a nebulized sample is carried into a flame, desolvation of the droplets occurs in the primary combustion zone, located in the tip of the burner. The fine solid particles are carried to a region in the center of the the flame called the inner core.





Flames Used in Atomic Spectroscopy

Fuel and Oxidant	Temperature, °C
*Gas/Air	1700–1900
*Gas/O_2	2700–2800
H_2/air	2000–2100
H_2/O_2	2500–2700
†C_2H_2/air	2100–2400
†C_2H_2/O_2	3050–3150
†C_2H_2/N_2O	2600–2800

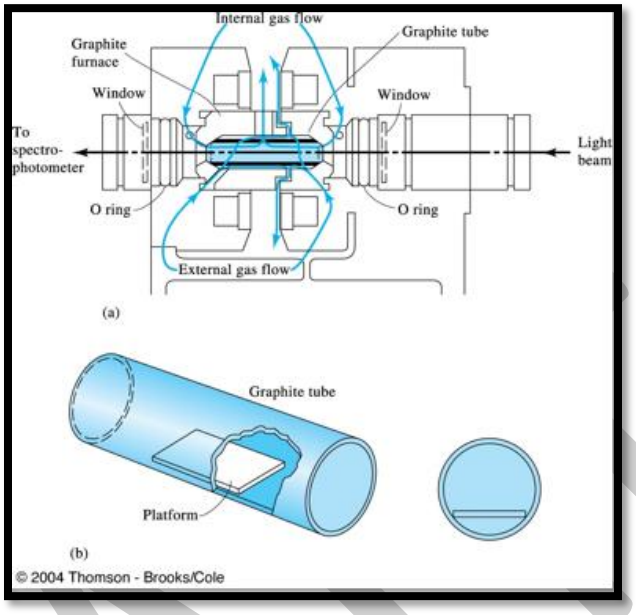
*Propane or natural gas
†Acetylene

Electrothermal Atomizer

Electrothermal atomizer deposits a few microliters of sample in the furnace with a syringe or an autosampler. This is followed by drying ashing and atomization steps that is carried out by instrument programming. .

There are other types of atomizers devices. Examples are the gas discharge which results in glow discharge. Early atomizers include dc and ac arcs which have been replaced almost entirely by ICP.

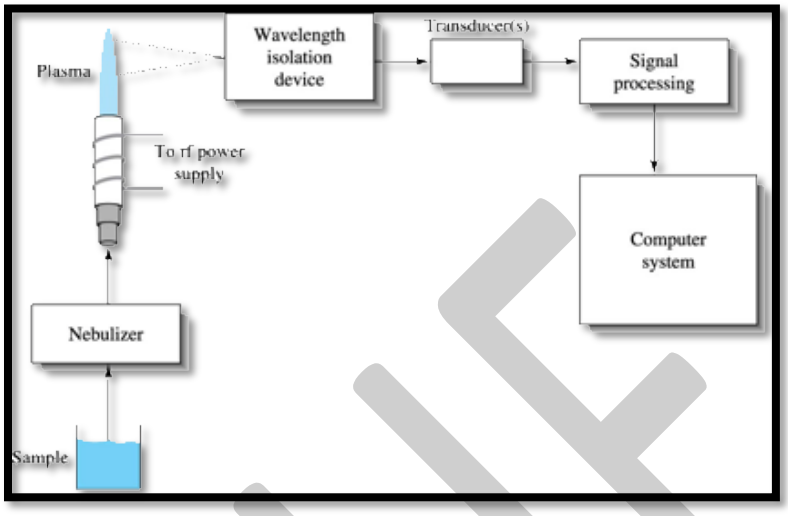
Shown is the cross-sectional view of a graphite furnace atomizer. The L'vov platform and its position in the graphite furnace.y ICP.



Atomic Emission Spectroscopy

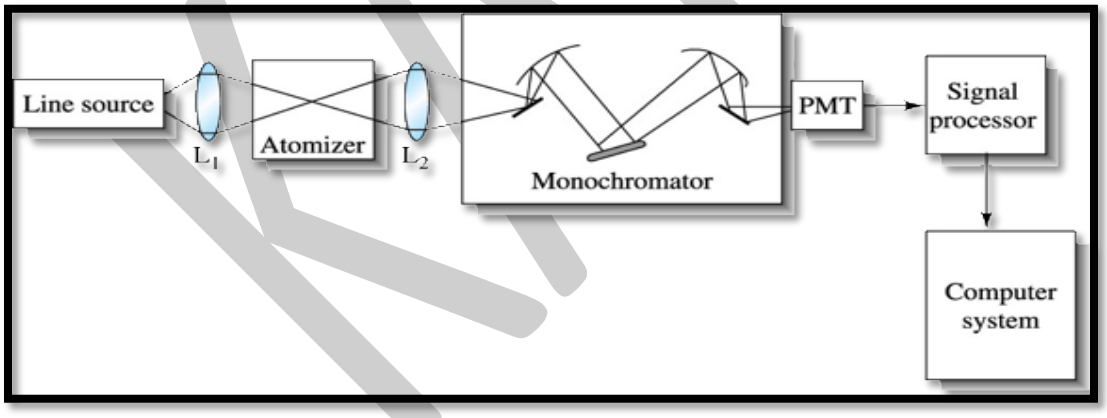
Atomic emission spectroscopy is widely used in elemental analysis.

Shown is the block diagram of a typical ICP atomic emission spectrometer



Atomic Absorption Spectroscopy

Flame atomic absorption spectroscopy (AAS) is the most used of atomic methods.



Block diagram of a single-beam atomic absorption spectrometer. Radiation from a line source is focused on the atomic vapor in aflame or an electrothermal atomizer. The attenuated source radiation then enters a monochromator, which isolates the line of interest. Next the radiant power from the source, attenuated by absorption, is measured by the

photomultiplier tube (PMT). The signal is then processed and directed to a computer system for output.

References:

Text Books:

1. Sharma, B. K. (2005). *Instrumental Methods of Chemical Analysis* (24th Edition). Meerut: Krishna Prakashan Media (P) Ltd.
2. Jag mohan (2007). Organic Spectroscopy: Principles and Applications (II Edition). New delhi: Narose publishing house

POSSIBLE QUESTIONS:

Part-A (20 x 1= 20 marks) Online Examinations

(Each Question Carry One Mark)

1. Electric field and magnetic field oscillates only in one plane ----- occur
a. linearly polarized light
b. circularly polarized light
c. square polarized light
d. randomly polarized light
Answer: b

2. Electric field and magnetic field oscillates perpendicular to the plane ----- occur
a. linearly polarized light
b. circularly polarized light
c. square polarized light
d. randomly polarized light
Answer: a

3. The effect of CE associates with ----- transition
n-π* transition
n-n transition
n-σ transition
σ-σ transition

Answer: a

4. The CE means
a. cotton effect
b. coefficient
c. conductance
d. coherence
Answer: d

5. In the octant rule consider which sector
a. front sector
b. side plane
c. side sector
d. rear sector
Answer: a

6. In CE optical rotation increases with wavelength decrease is occur
a. +ve CE
b. -ve CE
c. ±ve CE
d. no CE
Answer: b

7. In CE optical rotation decreases with wavelength increases is occur
a. +ve CE
b. -ve CE
c. ±ve CE
d. no CE
Answer: b

8. The cotton effect was discovered by
a. compton
b. amine cotton
c. roberg
d. john dolton
Answer: b

9. The octant rule s applied for steroids because of its ----
a. structure
b. chemical charge

c. rigid property
d. reactivity
Answer: c

10. Which rule applied on ORD MEASUREMENTS?
a. Axial halo ketone rule
b. octant rule
c. cotton rule
d. maxwell rule
Answer: a

11. Ketones substituted with a halogen atom at the------
a. α-carbon
b. β-carbon
c. ϒ-carbon
d. 4-th carbon
Answer: a

12. Tyndall effect also known as-------
a. collidal scatterin
b. Tyndall scattering
c. α-emission
d. α-radiation
Answer: b

13. The light is -------- while the shorter wavelength
a. transmissed
b. scattered
c. emitted
d. reflected
Answer: b

14. In the rayleigh scattering , the indensity of light depends on the ----th power of freguency
a.3
b. 2
c. 4
d. 5

15. The wavelength dependence of --------
a. optical activity

b. optical rotation
c. rotation
d. optical dispersion
Answer: a

16. The indices of refraction of an optical medium for the left and right hand circularly polarized components may not be the same, and will be designated
a. n_L
b. n_R
c. n_L,n_R
d. n_L - n_R
Answer: c

17. The corresponding absorptivities denotes as
a. a_L
b. a_R
c. aL + aR
d. a_L,a_R
Answer: d

18. Which of the following represents the two radius vector in CD
a. E_L,Er
b. E_R
c. E_L, E_R
d. E_L - E_R
Answer: c

19. What is the elipticity equation in radius scale?
a. $\Theta = 1/4 (K_L - K_R)d$
b. $\Theta = 1/4 (K_R - K_L)d$
c. $\Theta = 1/4 (KL + KR)d$
d. $\Theta = 1/4 (KL + KR)d$
Answer: a

20. The combined phenomena of nonzero circular birefringence and dichroism is known as the
a. Inductive effect
b. None inductive effect
c. Cotton effect
d. electrostatic effect

Answer: c

Part-B (5 x 6 =30 marks)

(Each Question Carry Six Marks)

1. Explain –Circular dichromism.
2. Explain optical rotator dispersion.
3. Give the expression for molar circular dichromism.
4. Explain the principle of atomic absorption spectroscopy.
5. Explain molar ellipticity.
6. Explain Octant rule and give one example.
7. Discuss the application of the axial halo ketone rule in structure determination.
8. Write notes on (i) single beam atomic absorption spectrometer (ii) Double beam atomic

Part-C (1 x10= 10 marks) Compulsory Questions

1. Write notes on circular polarization of light.
2. Write notes on light scattering.
3. Write a note on effect of circular dichroism
4. Describe the following (i) ORD Photometer, (ii) CD Apparatus

KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University)

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Coimbatore – 641 021.

DEPARTMENT OF CHEMISTRY

Class: II M.Sc Chemistry

Subject Title: Physical Methods in chemistry

Subject code: 17CHP303

UNIT-III

Multiple choice questions (Each question carry one mark)

1. Electric field and magnetic field oscillates only in one plane ----- occur
a. linearly polarized light
b. circularly polarized light
c. square polarized light
d. randomly polarized light
Answer: b

2. Electric field and magnetic field oscillates perpendicular to the plane ----- occur
a. linearly polarized light
b. circularly polarized light
c. square polarized light
d. randomly polarized light
Answer: a

3. The effect of CE associates with ----- transition
n-π* transition
n-n transition
n-σ transition
σ-σ transition
a

4. The CE means
a. cotton effect
b. coefficient
c. conductance
d. coherence

Answer: d

5. In the octant rule consider which sector
a. front sector
b. side plane
c. side sector
d. rear sector
Answer: a

6. In CE optical rotation increases with wavelength decrease is occur
a. +ve CE
b. -ve CE
c. ±ve CE
d. no CE
Answer: b

7. In CE optical rotation decreases with wavelength increases is occur
a. +ve CE
b. -ve CE
c. ±ve CE
d. no CE
Answer: b

8. The cotton effect was discovered by
a. compton
b. amine cotton
c. roberg
d. john dolton
Answer: b

9. The octant rule s applied for steroids because of its ----
a. structure
b. chemical charge
c. rigid property
d. reactivity
Answer: c

10. Which rule applied on ORD measurements
a. Axial halo ketone rule
b. octant rule
c. cotton rule
d. maxwell rule
Answer: a

11. Ketones substituted with a halogen atom at the------
a. α-carbon

b. β-carbon
c. ϒ-carbon
d. 4-th carbon
Answer: a

12. Tyndall effect also known as-------
a. collidal scatterin
b. Tyndall scattering
c. α-emission
d. α-radiation
Answer: b

13. The light is -------- while the shorter wavelength
a. transmissed
b. scattered
c. emitted
d. reflected
Answer: b

14. In the rayleigh scattering , the indensity of light depends on the ----th power of freguency
a.3
b. 2
c. 4
d. 5

15. The wavelength dependence of --------
a. optical activity
b. optical rotation
c. rotation
d. optical dispersion
Answer: a

16. The indices of refraction of an optical medium for the left and right hand circularly polarized components may not be the same ,and will be designated
a. n_L
b. n_R
c. n_L,n_R
d. n_L - n_R
Answer: c

17. The corresponding absorptivities denotes as
a. a_L
b. a_R
c. aL + aR
d. a_L,a_R
Answer: d

18. which of the following represents the two radius vector in CD
a. $E_{L,}$Er
b. E_R
c. E_L, E_R
d. E_L - E_R
Answer: c

19. What is the elipticity equation in radius scale?
a. $\Theta = 1/4 (K_L - K_R)d$
b. $\Theta = 1/4 (K_R - K_L)d$
c. $\Theta = 1/4 (KL + KR)d$
d. $\Theta = 1/4 (KL + KR)d$
Answer: a

20. The combined phenomena of nonzero circular birefringence and dichroism is known as the
a. Inductive effect
b. None inductive effect
c. Cotton effect
d. electrostatic effect
Answer: c

21. The servoamplifier responds only to the ------- frequency
a. 12-H_Z
b. 14-H_Z
c. 16-H_Z
d. 17-H_Z
Answer: a

22. The Faraday cell,which consists of a glass or silica rod surrounded by a coil carrying ----- alternating current
a. 12-H_Z
b. 14-H_Z
c. 16-H_Z
c. 60-H_Z
Answer: d

23. ORD curves are affected by
a. chromophoric bands
b. chromophoric absorption
c. chromophoric adsorption
d. chromophoric reflection
Answer: a

24. Conventional spectrophotometers are designed to determine the difference between ---------- of a sample

a. adsorbances of a sample
b. size of sample
c. reflection of sample
Answer: a

25. What are the second steps of circular polarisation
a.The beam of radiation must not plane polarized
b. The polarized beam must be passed through a device
c. The beam of radiation should not be plane polarized
d. The polarized beam may not be passed through a device
Answer: b

26. How many types are in the circular resolvers?
a. 1
b. 2
c. 3
d. 4
Answer: c

27. The pockels modulator , a high potential is applied across a plate of crystalline--------?
a. KH_3PO_4
b. KH_2PO_4
c. $KHPO_4$
d. KH_2PO_3
Answer: b

28. The majority of CD measurements are for the purpose of what?
a. Structure determination
b. identify plane of symmetry
c. identify the phase effect
d. identify sample density
Answer: a

29. What is the value for 1 nm in Â unit?
a. 5
b. 10
c. 20
d. 50
Answer: b

30. In the 4th position of the cyclohexanonen has ------ CE effect
a. less
b. no
c. high
c. very high
Answer: b

31. In the 2,6th position of the cyclohexanonen has ------ to the CE effect
a. small contribution
b. high contribution
c. medium contribution
d. optimum contribution
Answer:a

32.In the octant rule consideration the carbonyl group of the cyclohexanone is ----- of the chair position
a. tail
b.axial
c. equtorial
d. head
Answer: d

33. The compound (R)-(+)-3-methyl cyclo hexanone exhibits ------ CE
a. negative
b. positive
c. ±
d. null
Answer: b

34. CE estimates the ------- of ketosteroids
a. dipole effect
b. magnitute
c. interaction
d. position
Answer: b

35. Why Axial substitution often occurred in the compound because
a. dipole-dipole intraction
b. dipole-dipole repulsion
c. dipole-dipole combination
d. steric effect
Answer: b

36. In axial haloketone rule, when the +ve cotton effect occurred, the α-halogen present in the ---- side of the view
a. right
b. left
c. axial
d. equtorial
Answer: a

37. In axial haloketone rule, when the -ve cotton effect occurred, the α-halogen present in the ---- side of the view
a. right
b. axial
c. left
d. equtorial
Answer: c

38. According to rayleigh formula, the particle need ----- size
a. 40nm
b. above 40 nm
c. below 40nm
d. near to 40 nm
Answer: c

39. A blue iris in an eye because of
a. Rayleigh scattering
b.Compton effect
c. Rayleigh absorption
d. Tyndall scattering
Answer: d
40. In shorter wavelengths scattering has ----- extent
a. less
b. greater
c. better
d. high
Answer: b

41. The size and density of the particles determined by -------
a. Rayleigh effect
b. Compton effect
c. cotton effect
d. Tyndall effect
Answer: d

42. Tyndall scattering is mathematically analysable in terms of----- theory
a. langmuir
b. rayleigh
c. mie
d. binomial
Answer: c

43. In the -------- combination causes the red rays appear in sunrises and sunset
a. Rayleigh ,cotton effect
b. Tyndall effect, cotton effect
c. Rayleigh, Tyndall effect

d. Tyndall,+ cotton effect
Answer: c

44. The degree of red in a sunset varies depending on ------
a. light
b. weather
c. scattering particle
d. cotton effect
Answer: b

45. Tyndall effect applied to light scattering for macroscopic particles -------- takes place
a. scattering
b. reflection
c. transmission
d. absorption
Answer: b

46. Tyndall effect in ------ glass,appears the blue colour with orange shining
a. transparent
b. opaque
c. quartz
d. opalescent
Answer: d

47. The french physicst discover the ------- Effect
a. compton
b. tyndall
c. cotton
d. rayleigh
Answer: c

48. ORD and CD are known as ------- properties
a. chiral
b. achiral
c. optical
d. chiroptical
Answer: d

49. Magnetic optical rotation is known as
a. Tyndall effect
b. Compton effect
c. cotton effect
d. Faraday effect
Answer: d

50. Both nephelometry and turbidimetry based on the scattering light by -------- particles in the solution
a. transparent
b. non-transparent
c. opaque
d. solubled
Answer: b

51. Nephelometry and turbidimetry only differ in the ----- of scattered radiation
a. measuring
b. solublity of particles
c. Absorption
d. reflection
Answer: a

52. Turbidimetry is similar to the
a. conductometry
b. colorimetry
c. polarimetry
d. flurometry
Answer: b

53. Nephelometry is similar to the
a. conductometry
b. colorimetry
c. polarimetry
d. flurometry
Answer: d

54. Turbidimetry method applied for ----- concentration of collidal particles suspention
a. low
b. high
c. very low
d. minute
Answer: b

55. Nephlometry method applied for ----- concentration of collidal particles suspention
low
high
very low
minute
a

56. Which source is used for occuring monochromatic radiation
a. white light
b. UV light

c. IR light
d. gaseous
Answer: a

57. 1 Part of the ammonia concentration in 160 millian parts of ammonia detected by adding indicator
a. Nessler's reagent
b. buffer solution
c. acidic solution
Answer:b

58. AAS refered as a
a. Atomic absorption spectroscopy
b. Atomic adsorption spectroscopy
c. Analytical adsorption spectroscopy
d. Analytical absorption spectroscopy
Answer: a

59. ------ type of molecules have the CD and ORD
a. Chiral only
b. optical only
c. symmetrical
d. chiral,optical
Answer: d

60. Atomic absorption spectroscopy used to quantitative determination of ------ metals
a. trans
b. trace
c. alkali
d. non-metals
Answer: b

Lecture Notes

UNIT-IV

SYLLABUS

ESR spectroscopy: *Theory - derivative curves - g shift - hyperfine splitting-isotropic and anisotropic systems-zero field splitting and Kramer degeneracy. Identification of free radicals – applications to copper complexes.*

Electron paramagnetic resonance

Electron paramagnetic resonance (EPR) or **electron spin resonance** (ESR) spectroscopy is a technique for studying materials with unpaired electrons. The basic concepts of EPR are analogous to those of nuclear magnetic resonance (NMR), but it is electron spins that are excited instead of the spins of atomic nuclei. EPR spectroscopy is particularly useful for studying metal complexes or organic radicals. EPR was first observed in Kazan State University by Soviet physicist Yevgeny Zavoisky in 1944, and was developed independently at the same time by Brebis Bleaney at the University of Oxford.

EPR spectrometer

Theory

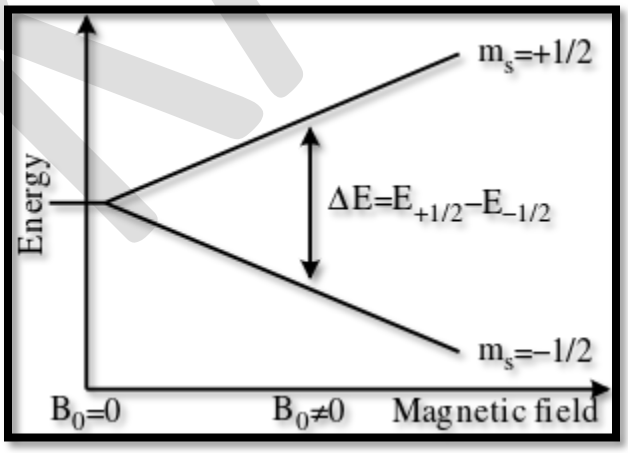
Origin of an EPR signal

Every electron has a magnetic moment and spin quantum number $s = \frac{1}{2}$, with magnetic components $m_s = +\frac{1}{2}$ and $m_s = -\frac{1}{2}$. In the presence of an external magnetic field with strength B_0, the electron's magnetic moment aligns itself either parallel ($m_s = -\frac{1}{2}$) or antiparallel ($m_s = +\frac{1}{2}$) to the field, each alignment having a specific energy due to the Zeeman effect :

$E = m_s g_e \mu_B B_0$ where

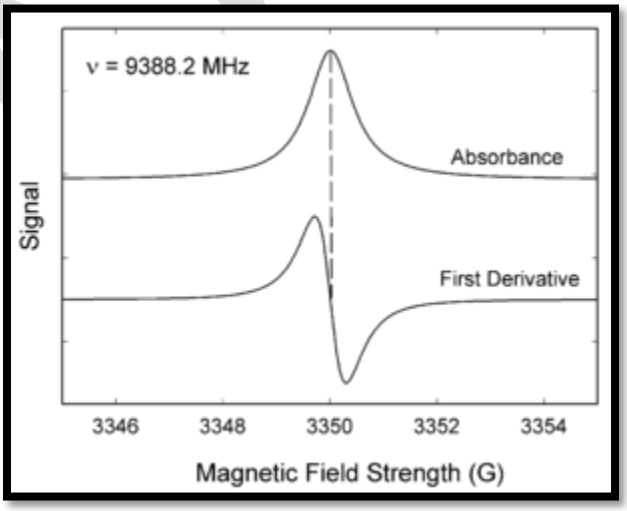
- g_e is the electron's so-called g-factor (see also the Landé *g*-factor). $g_e = 2.0023$ for the free electron.
- μ_B is the Bohr magneton.

Therefore, the separation between the lower and the upper state is $\Delta E = g_e \mu_B B_0$ for unpaired free electrons. This equation implies that the splitting of the energy levels is directly proportional to the magnetic field's strength, as shown in the diagram below.



An unpaired electron can move between the two energy levels by either absorbing or emitting a photon of energy $h\nu$ such that the resonance condition, $h\nu = \Delta E$, is obeyed. This leads to the fundamental equation of EPR spectroscopy: $h\nu = g_e \mu_B B_0$.

Experimentally, this equation permits a large combination of frequency and magnetic field values, but the great majority of EPR measurements are made with microwaves in the 9000–10000 MHz (9–10 GHz) region, with fields corresponding to about 3500 G (0.35 T). Furthermore, EPR spectra can be generated by either varying the photon frequency incident on a sample while holding the magnetic field constant or doing the reverse. In practice, it is usually the frequency that is kept fixed. A collection of paramagnetic centers, such as free radicals, is exposed to microwaves at a fixed frequency. By increasing an external magnetic field, the gap between the $m_s = +\frac{1}{2}$ and $m_s = -\frac{1}{2}$ energy states is widened until it matches the energy of the microwaves, as represented by the double-arrow in the diagram above. At this point the unpaired electrons can move between their two spin states. Since there typically are more electrons in the lower state, due to the Maxwell–Boltzmann distribution (see below), there is a net absorption of energy, and it is this absorption that is monitored and converted into a spectrum. The upper spectrum below is the simulated absorption for a system of free electrons in a varying magnetic field. The lower spectrum is the first derivative of the absorption spectrum. The latter is the most common way to record and publish EPR spectra.



For the microwave frequency of 9388.2 MHz, the predicted resonance position is a magnetic field of about $B_0 = h\nu / g_e \mu_B$ = 0.3350 tesla = 3350 gauss.

Because of electron-nuclear mass differences, the magnetic moment of an electron is substantially larger than the corresponding quantity for any nucleus, so that a much higher electromagnetic frequency is needed to bring about a spin resonance with an electron than with a nucleus, at identical magnetic field strengths. For example, for the field of 3350 G shown at the right, spin resonance occurs near 9388.2 MHz for an electron compared to only about 14.3 MHz for ^{1}H nuclei. (For NMR spectroscopy, the corresponding resonance equation is $h\nu = g_N \mu_N B_0$ where g_N and μ_N depend on the nucleus under study.)

Maxwell–Boltzmann distribution

In practice, EPR samples consist of collections of many paramagnetic species, and not single isolated paramagnetic centers. If the population of radicals is in thermodynamic equilibrium, its statistical distribution is described by the Maxwell–Boltzmann equation

$$\frac{n_{\text{upper}}}{n_{\text{lower}}} = \exp\left(-\frac{E_{\text{upper}} - E_{\text{lower}}}{kT}\right) = \exp\left(-\frac{\Delta E}{kT}\right) = \exp\left(-\frac{\epsilon}{kT}\right) = \exp\left(-\frac{h\nu}{kT}\right) (Eq.1)$$

where n_{upper} is the number of paramagnetic centers occupying the upper energy state, k is the Boltzmann constant, and T is the temperature in kelvins. At 298 K, X-band microwave frequencies ($\nu \approx 9.75$ GHz) give $n_{\text{upper}}/n_{\text{lower}} \approx 0.998$, meaning that the upper energy level has a smaller population than the lower one. Therefore, transitions from the lower to the higher level are more probable than the reverse, which is why there is a net absorption of energy.

The sensitivity of the EPR method (i.e., the minimum number of detectable spins N_{min}) depends on the photon frequency ν according to

$$N_{\text{min}} = \frac{k_1 V}{Q_0 k_f \nu^2 P^{1/2}} (Eq.2)$$

where k_1 is a constant, V is the sample's volume, Q_0 is the unloaded quality factor of the microwave cavity (sample chamber), k_f is the cavity filling coefficient, and P is the microwave power in the spectrometer cavity. With k_f and P being constants, $N_{\min} \propto (Q_0\nu^2)^{-1}$, i.e., $N_{\min} \propto \nu^{-\alpha}$, where $\alpha \approx 1.5$. In practice, α can change varying from 0.5 to 4.5 depending on spectrometer characteristics, resonance conditions, and sample size.

A great sensitivity is therefore obtained with a low detection limit $N_{\min}$ and a large number of spins. Therefore, the required parameters are:

- A high spectrometer frequency to maximize the eq.2. Common frequencies are discussed below
- A low temperature to decrease the number of spin at the high level of energy as shown in eq.1. This condition explain why spectra are often recorded on sample at the boiling point of liquid nitrogen or liquid helium.

Spectral parameters

In real systems, electrons are normally not solitary, but are associated with one or more atoms. There are several important consequences of this:

1. An unpaired electron can gain or lose angular momentum, which can change the value of its *g*-factor, causing it to differ from g_e. This is especially significant for chemical systems with transition-metal ions.
2. The magnetic moment of a nucleus with a non-zero nuclear spin will affect any unpaired electrons associated with that atom. This leads to the phenomenon of hyperfine coupling, analogous to J-coupling in NMR, splitting the EPR resonance signal into doublets, triplets and so forth.
3. Interactions of an unpaired electron with its environment influence the shape of an EPR spectral line. Line shapes can yield information about, for example, rates of chemical reactions.[ref needed]

4. The *g*-factor and hyperfine coupling in an atom or molecule may not be the same for all orientations of an unpaired electron in an external magnetic field. This anisotropy depends upon the electronic structure of the atom or molecule (e.g., free radical) in question, and so can provide information about the atomic or molecular orbital containing the unpaired electron.

The *g* factor

Knowledge of the *g*-factor can give information about a paramagnetic center's electronic structure. An unpaired electron responds not only to a spectrometer's applied magnetic field B_0 but also to any local magnetic fields of atoms or molecules. The effective field B_{eff}experienced by an electron is thus written

$$B_{\text{eff}} = B_0(1 - \sigma)$$

where σincludes the effects of local fields (σ can be positive or negative). Therefore, the $h\nu = g_{\text{e}}\mu_{\text{B}}B_{\text{eff}}$resonance condition (above) is rewritten as follows:

$$h\nu = g_{\text{e}}\mu_B B_{\text{eff}} = g_{\text{e}}\mu_B B_0(1 - \sigma)$$

The quantity $g_{\text{e}}(1 - \sigma)$is denoted $g$and called simply the g-factor, so that the final resonance equation becomes

$$h\nu = g\mu_{\text{B}}B_0$$

This last equation is used to determine $g$in an EPR experiment by measuring the field and the frequency at which resonance occurs. If $g$does not equal $g_{\text{e}}$the implication is that the ratio of the unpaired electron's spin magnetic moment to its angular momentum differs from the free electron value. Since an electron's spin magnetic moment is constant (approximately the Bohr magneton), then the electron must have gained or lost angular momentum through spin-orbit coupling. Because the mechanisms of spin-orbit coupling are well understood, the

magnitude of the change gives information about the nature of the atomic or molecular orbital containing the unpaired electron.

In general, the g factor is not a number but a second-rank tensor represented by nine numbers arranged in a 3×3 matrix. The principal axes of this tensor are determined by the local fields, for example, by the local atomic arrangement around the unpaired spin in a solid or in a molecule. Choosing an appropriate coordinate system (say, x,y,z) allows to "diagonalize" this tensor thereby reducing the maximum number of its components from nine to three, g_{xx}, g_{yy} and g_{zz}. For a single spin experiencing only Zeeman interaction with an external magnetic field, the position of the EPR resonance is given by the expression $g_{xx}B_x + g_{yy}B_y + g_{zz}B_z$. Here B_x, B_y and B_z are the components of the magnetic field vector in the coordinate system (x,y,z); their magnitudes change as the field is rotated, so as the frequency of the resonance. For a large ensemble of randomly oriented spins, the EPR spectrum consists of three peaks of characteristic shape at frequencies $g_{xx}B_0$, $g_{yy}B_0$ and $g_{zz}B_0$: the low-frequency peak is positive in first-derivative spectra, the high-frequency peak is negative, and the central peak is bipolar. Such situation is commonly observed in powders and the spectra are therefore called "powder-pattern spectra". In crystals, the number of EPR lines is determined by the number of crystallographically equivalent orientations of the EPR spin (called "EPR center").

Hyperfine coupling

Since the source of an EPR spectrum is a change in an electron's spin state, it might be thought that all EPR spectra for a single electron spin would consist of one line. However, the interaction of an unpaired electron, by way of its magnetic moment, with nearby nuclear spins, results in additional allowed energy states and, in turn, multi-lined spectra. In such cases, the spacing between the EPR spectral lines indicates the degree of interaction between the unpaired electron and the perturbing nuclei. The hyperfine coupling constant of a nucleus is directly related to the spectral line spacing and, in the simplest cases, is essentially the spacing itself.

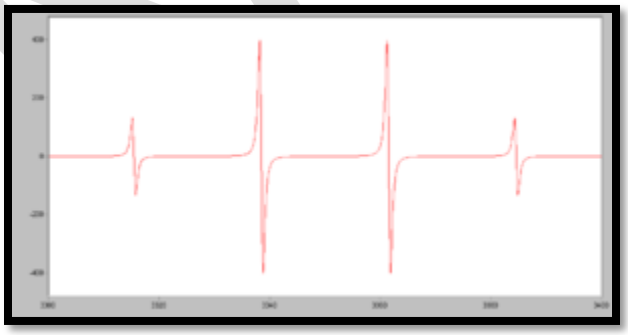
Two common mechanisms by which electrons and nuclei interact are the Fermi contact interaction and by dipolar interaction. The former applies largely to the case of isotropic

interactions (independent of sample orientation in a magnetic field) and the latter to the case of anisotropic interactions (spectra dependent on sample orientation in a magnetic field). Spin polarization is a third mechanism for interactions between an unpaired electron and a nuclear spin, being especially important for π-electron organic radicals, such as the benzene radical anion. The symbols "*a*" or "*A*" are used for isotropic hyperfine coupling constants while "*B*" is usually employed for anisotropic hyperfine coupling constants.

In many cases, the isotropic hyperfine splitting pattern for a radical freely tumbling in a solution (isotropic system) can be predicted.

- For a radical having M equivalent nuclei, each with a spin of I, the number of EPR lines expected is $2MI + 1$. As an example, the methyl radical, CH_3, has three ^1H nuclei each with $I = 1/2$, and so the number of lines expected is $2MI + 1 = 2(3)(1/2) + 1 = 4$, which is as observed.

- For a radical having M_1 equivalent nuclei, each with a spin of I_1, and a group of M_2 equivalent nuclei, each with a spin of I_2, the number of lines expected is $(2M_1I_1 + 1)(2M_2I_2 + 1)$. As an example, the methoxymethyl radical, $H_2C(OCH_3)$, has two equivalent ^1H nuclei each with $I = 1/2$ and three equivalent ^1H nuclei each with $I = 1/2$, and so the number of lines expected is $(2M_1I_1 + 1)(2M_2I_2 + 1) = [2(2)(1/2) + 1][2(3)(1/2) + 1] = [3][4] = 12$, again as observed.

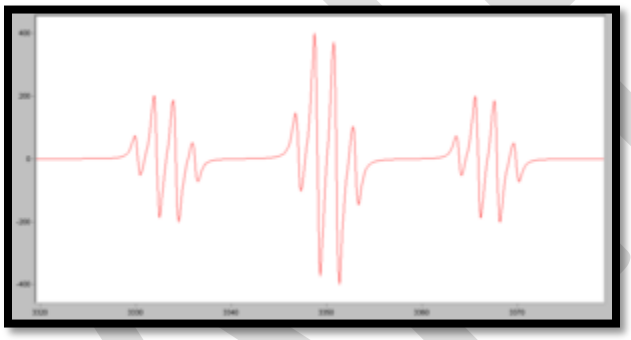


Simulated EPR spectrum of the CH_3 radical

- The above can be extended to predict the number of lines for any number of nuclei.

While it is easy to predict the number of lines a radical's EPR spectrum should show, the reverse problem, unraveling a complex multi-line EPR spectrum and assigning the various spacings to specific nuclei, is more difficult.

In the oft-encountered case of $I = 1/2$ nuclei (e.g., ^{1}H, ^{19}F, ^{31}P), the line intensities produced by a population of radicals, each possessing M equivalent nuclei, will follow Pascal's triangle. For example, the spectrum at the right shows that the three ^{1}H nuclei of the CH_3 radical give rise to $2MI + 1 = 2(3)(1/2) + 1 = 4$ lines with a 1:3:3:1 ratio. The line spacing gives a hyperfine coupling constant of $a_H = 23$ G for each of the three ^{1}H nuclei. Note again that the lines in this spectrum are *first derivatives* of absorptions.



Simulated EPR spectrum of the $H_2C(OCH_3)$ radical

As a second example, consider the methoxymethyl radical, $H_2C(OCH_3)$. The two equivalent methyl hydrogens will give an overall 1:2:1 EPR pattern, each component of which is further split by the three methoxy hydrogens into a 1:3:3:1 pattern to give a total of 3×4 = 12 lines, a triplet of quartets. A simulation of the observed EPR spectrum is shown at the right, and agrees with the 12-line prediction and the expected line intensities. Note that the smaller coupling constant (smaller line spacing) is due to the three methoxy hydrogens, while the larger coupling constant (line spacing) is from the two hydrogens bonded directly to the carbon atom bearing the unpaired electron. It is often the case that coupling constants decrease in size with distance from a radical's unpaired electron, but there are some notable exceptions, such as the ethyl radical (CH_2CH_3).

Resonance linewidth definition

Resonance linewidths are defined in terms of the magnetic induction *B*, and its corresponding units, and are measured along the *x* axis of an EPR spectrum, from a line's center to a chosen reference point of the line. These defined widths are called halfwidths and possess some advantages: for asymmetric lines values of left and right halfwidth can be given. The halfwidth $\Delta B_h$is the distance measured from the line's center to the point in which absorption value has half of maximal absorption value in the center of resonance line. First inclination width $\Delta B_{1/2}$is a distance from center of the line to the point of maximal absorption curve inclination. In practice, a full definition of linewidth is used. For symmetric lines, halfwidth $\Delta B_{1/2} = 2\Delta B_h$, and full inclination width $\Delta B_{max} = 2\Delta B_{1s}$

Pulsed EPR

For more details on this topic, see Pulsed EPR.

The dynamics of electron spins are best studied with pulsed measurements. Microwave pulses typically 10–100 ns long are used to control the spins in the Bloch sphere. The spin-lattice relaxation time can be measured with an inversion recovery experiment.

As with pulsed NMR, the Hahn echo is central to many pulsed EPR experiments. A Hahn echo decay experiment can be used to measure the dephasing time, as shown in the animation below. The size of the echo is recorded for different spacings of the two pulses. This reveals the decoherence, which is not refocused by the πpulse. In simple cases, an exponential decay is measured, which is described by the T_2time.

Applications

EPR/ESR spectroscopy is used in various branches of science, such as biology, chemistry and physics, for the detection and identification of free radicals and paramagnetic centers such as F centers. EPR is a sensitive, specific method for studying both radicals formed in chemical reactions and the reactions themselves. For example, when ice (solid H_2O) is decomposed by exposure to high-energy radiation, radicals such as H, OH, and HO_2 are produced. Such radicals

can be identified and studied by EPR. Organic and inorganic radicals can be detected in electrochemical systems and in materials exposed to UV light. In many cases, the reactions to make the radicals and the subsequent reactions of the radicals are of interest, while in other cases EPR is used to provide information on a radical's geometry and the orbital of the unpaired electron.

Miniature Electron Spin Resonance Spectroscopy with Micro-ESR

Miniaturisation of military radar technologies allowed the development of miniature microwave electronics as a spin-off by the California Institute of Technology. Since 2007 these sensors have been employed in miniaturized electron spin resonance spectrometers called Micro-ESR.

The high cost, large size, and difficult maintenance of electron spin resonance spectrometers has limited their use to specialized research centers with highly trained personnel. Micro-ESR makes ESR feasible for nonspecialists to determine oxidation by directly measuring of free radicals.

Applications include real-time monitoring of free radical containing Asphaltenes in (crude) oils; Biomedical R&D to measure oxidative stress; Evaluation of the shelf-life of food products;

Medical and biological applications of EPR also exist. Although radicals are very reactive and so do not normally occur in high concentrations in biology, special reagents have been developed to spin-label molecules of interest. These reagents are particularly useful in biological systems. Specially-designed nonreactive radical molecules can attach to specific sites in a biological cell, and EPR spectra can then give information on the environment of these so-called spin-label or spin-probes. *Spin-labeled fatty acids* have been extensively used to study dynamic organisation of lipids in *biological membranes*, lipid-protein interactions and temperature of transition of gel to liquid crystalline phases.

A type of dosimetry system has been designed for reference standards and routine use in medicine, based on EPR signals of radicals from irradiated polycrystalline α-alanine(the alanine deamination radical, the hydrogen abstraction radical, and the $(CO^{-}(OH))=C(CH_3)NH_2^{+}$ radical) . This method is suitable for measuring gamma and x-rays, electrons, protons, and high-linear energy transfer (LET) radiation of doses in the 1 Gy to 100 kGy range.

EPR/ESR spectroscopy can be applied only to systems in which the balance between radical decay and radical formation keeps the free-radicals concentration above the detection limit of the spectrometer used. This can be a particularly severe problem in studying reactions in liquids. An alternative approach is to slow down reactions by studying samples held at cryogenic temperatures, such as 77 K (liquid nitrogen) or 4.2 K (liquid helium). An example of this work is the study of radical reactions in single crystals of amino acids exposed to x-rays, work that sometimes leads to activation energies and rate constants for radical reactions.

The study of radiation-induced free radicals in biological substances (for cancer research) poses the additional problem that tissue contains water, and water (due to its electric dipole moment) has a strong absorption band in the microwave region used in EPR spectrometers.

EPR/ESR also has been used by archaeologists for the dating of teeth. Radiation damage over long periods of time creates free radicals in tooth enamel, which can then be examined by EPR and, after proper calibration, dated. Alternatively, material extracted from the teeth of people during dental procedures can be used to quantify their cumulative exposure to ionizing radiation. People exposed to radiation from the Chernobyl disaster have been examined by this method.

Radiation-sterilized foods have been examined with EPR spectroscopy, the aim being to develop methods to determine if a particular food sample has been irradiated and to what dose.

Because of its high sensitivity, EPR was used recently to measure the quantity of energy used locally during a mechanochemical milling process.

EPR/ESR spectroscopy has been used to measure properties of crude oil, in particular asphaltene and vanadium content. EPR measurement of asphaltene content is a function of spin density and solvent polarity. Prior work dating to the 1960s has demonstrated the ability to measure vanadium content to sub-ppm levels.

In the field of quantum computing, pulsed EPR is used to control the state of electron spin qubits in materials such as diamond, silicon and gallium arsenide.

High-field high-frequency measurements

High-field high-frequency EPR measurements are sometimes needed to detect subtle spectroscopic details. However, for many years the use of electromagnets to produce the needed fields above 1.5 T was impossible, due principally to limitations of traditional magnet materials. The first multifunctional millimeter EPR spectrometer with a superconducting solenoid was described in the early 1970s by Prof. Y. S. Lebedev's group (Russian Institute of Chemical Physics, Moscow) in collaboration with L. G. Oranski's group (Ukrainian Physics and Technics Institute, Donetsk), which began working in the Institute of Problems of Chemical Physics, Chernogolovka around 1975. Two decades later, a W-band EPR spectrometer was produced as a small commercial line by the German Bruker Company, initiating the expansion of W-band EPR techniques into medium-sized academic laboratories.

Waveband	L	S	C	X	P	K	Q	U	V	E	W	F	D	—	J	—
λ/mm	300	100	75	30	20	12.5	8.5	6	4.6	4	3.2	2.7	2.1	1.6	1.1	0.83
ν/GHz	1	3	4	10	15	24	35	50	65	75	95	111	140	190	285	360
B_0/T	0.03	0.11	0.14	0.33	0.54	0.86	1.25	1.8	2.3	2.7	3.5	3.9	4.9	6.8	10.2	12.8

The EPR waveband is stipulated by the frequency or wavelength of a spectrometer's microwave source (see Table).

EPR experiments often are conducted at X and, less commonly, Q bands, mainly due to the ready availability of the necessary microwave components (which originally were developed for radar applications). A second reason for widespread X and Q band measurements is that electromagnets can reliably generate fields up to about 1 tesla. However, the low spectral resolution over *g*-factor at these wavebands limits the study of paramagnetic centers with comparatively low anisotropic magnetic parameters. Measurements at $\nu >$ 40 GHz, in the millimeter wavelength region, offer the following advantages:

EPR spectra of TEMPO, a nitroxide radical, as a function of frequency. Note the improvement in resolution from left to right. EPR spectra are simplified due to the reduction of second-order effects at high fields.

1. Increase in orientation selectivity and sensitivity in the investigation of disordered systems.
2. The informativity and precision of pulse methods, e.g., ENDOR also increase at high magnetic fields.
3. Accessibility of spin systems with larger zero-field splitting due to the larger microwave quantum energy $h\nu$.
4. The higher spectral resolution over *g*-factor, which increases with irradiation frequency ν and external magnetic field B_0. This is used to investigate the structure, polarity, and dynamics of radical microenvironments in spin-modified organic and biological systems through the spin label and probe method. The figure shows how spectral resolution improves with increasing frequency.
5. Saturation of paramagnetic centers occurs at a comparatively low microwave polarizing field B_1, due to the exponential dependence of the number of excited spins on the radiation frequency ν. This effect can be successfully used to study the relaxation and dynamics of paramagnetic centers as well as of superslow motion in the systems under study.

6. The cross-relaxation of paramagnetic centers decreases dramatically at high magnetic fields, making it easier to obtain more-precise and more-complete information about the system under study. This was demonstrated experimentally in the study of various biological, polymeric and model systems at D-band EPR.

Reference Books :

Text Books:

T1: Sharma.B.K.(2012) Instrumental methods of chemical analysis (28th Edition). Meerut: Krishna Prakashan Media (p) Ltd

POSSIBLE QUESTIONS:

Part-A (20 x 1= 20 marks) Online Examinations

(Each Question Carry One Mark)

1. Electron resonance is a branch of spectroscopy?
a. Absorption
b. Adsorption
c. Chemisorptions
d. Physisorption
Answer: a

2. What is the abbreviation for EPR?
a. Electron paramagnetic resonance
b. Energy paramagnetic resonance
c. Electron non paramagnetic resonance
d. Electron proton resonance
Answer: a

3. Write the energy, transition equation?
a. $E = h\vartheta = g\beta H_o$
b. $E = hv = g\beta H_o$
c. $E = h\vartheta = G\beta Ho$
d. $E = h\vartheta = g\beta H$
Answer: b

4. Choose the system with axial symmetry equation?
$g^2 = g_1^2 \cos^2 \theta + g_12 \sin2 \theta$
$g^2 = g_1^2 \cos^2 \theta + g_1^2 \sin^2 \theta$
$g^2 = g_1^2 \sin^2 \theta + g_1^2 \cos^2 \theta$
$g^2 = g_12 \cos^2 \theta + g_12 \sin^2 \theta$
a

5. The magnetic moment of an electron is about ----?
a. -9270×10^{24}
b. -9720×10^{24}
c. -9230×10^{24}
d. -9250×10^{24}
Answer: c

6. How many gauss are required in EPR transition?
a. 12,000
b. 6,500
c. 10,000
c. 15,000
Answer: a

7. In which microwave region, we are obtained EPR transition?
a. 9,000 mc sec^{-1}
b. 9,000 m sec^{-1}
c. 9,200 mc sec^{-1}
d. 9,600 mc sec^{-1}
Answer: a

8. The shape of the spectrum and the separation of the peaks will depend upon?
a. rseonant field
b. Magnetic field
c. electromagnetic field
d. electric field
Answer: b

9. Choose the actual value for a free electron is?
a. 2.203
b. 2.0023
c. 2.0067
d. 2.0987
Answer: c

10. The effect of spin orbit coupling is much larger in-----field?

a. Magnetic field
b. Non magnetic field
c. Crystal field
d. electric field
Answer: a

11. The effect of crystal field is
a. weak
b. strong
c. highly
d. partially
Answer: a

12. When a metal ion is placed in a crystalline field, the degeneracy of the d-orbital resolved by which interaction?
a. electrostatic
b. steric
c. Electromagnetic field
d. dipole to dipole
Answer: c

13. Given the following, which field applied for the spin degeneracy will remain?
a. Magnetic field
b. electric field
c. crystal field
d. no field effect
Answer: d

15. The spin levels may be split even in the absence of a magnetic field spliting is also called?
a. Zero-field splitting
b. crystal field splitting
c. low field splitting
d. high field splitting
Answer: a

16. The spin degeneracy of every level remains _____?
a. doubly degenerates
b. degenerates
c. tribly degenerates
d. no degenerates
Answer: a

17. The effect of quadrupole interaction is usually complicated because it is accompanied by a much larger magnetic ------ interaction?

a. hyperfine
b. electrostatic
c. dipole to dipole
d. electro magnetic
Answer: a

18. The values of g depends on ----- electron?
a. metal ion
b. free electron
c. outermost electron
d. sigma bond electron
Answer: a

19. The g value of the ------- electron is close to that of a free electron?
a. Even electron
b. Odd electron
c. paired electron
d. unpaired electron
Answer: b

20. The magnitude of g depends on ------- electron?
a. unpaired electron
b. paired electron
c. outershell electron
d. innershell electron
Answer: a

Part-B (5 x 6 =30 marks)

(Each Question Carry Six Marks)

1. Write a note on g value?

2. Explain with suitable example hyperfine splitting?

3. Write the note on basic principles of ESR spectroscopy?

4. Discuss about zero field splitting and Kramer degeneracy.

5. Explain isotropic and anisotropic systems.

Part-C (1 x10= 10 marks) Compulsory Questions

1. Write a note on Identification of free radicals – applications to copper complexes.

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DEPARTMENT OF CHEMISTRY

Class: II M.Sc Chemistry

Subject Title: Physical Methods in chemistry

Subject code: 17CHP303

UNIT-IV

Multiple choice questions (Each question carry one mark)

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a. Absorption
b. Adsorption
c. Chemisorptions
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$g^2 = g_1 2 \cos^2 \theta + g_1 2 \sin^2 \theta$
a

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b. -9720×10^{24}
c. -9230×10^{24}
d. -9250×10^{24}
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b. strong
c. highly
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b. degenerates
c. tribly degenerates
d. no degenerates
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b. electrostatic
c. dipole to dipole
d. electro magnetic
Answer: a

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b. free electron

c. outermost electron
d. sigma bond electron
Answer: a

19. The g value of the ------- electron is close to that of a free electron?
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b. Odd electron
c. paired electron
d. unpaired electron
Answer: b

20. The magnitude of g depends on ------- electron?
a. unpaired electron
b. paired electron
c. outershell electron
d. innershell electron
Answer: a

21. If the paramagnetic radical is located in a perfectly cubic
a. crystal
b. non-crystal
c. complex
d. metal
Answer: a

22. In a crystal site of lower symmetry the g value depends upon the orientation of the crystal and is said to be an--------?
a. Anisotropic
b. Isotropic
c. isomeric
d. tatumeric
Answer: a

23. Choose the value of 1 gauss?
a. 2.80 mc sec^{-1}
b. 2.70 mc sec^{-1}
c. 3.80 mc sec^{-1}
d. 3.50 mc sec^{-1}
Answer: a

24. The EPR spectrum of a hydrogen atom in a solid matrix consists of ------- peak ?
a. Three peaks
b. Four peaks
c. Two peaks
d. Five peaks
Answer: c

25. The spectrum of a free electron would consist of a ------- peak?
a. double
b. trible
c. single
d. no peak
Answer: c

26. Choose the energy level equation ?
a. $E = g\beta H m_s + A\ m_s m_1$
b. $E = g\beta H m_s$
c. $E = g\beta H m_l$
d. $E = g\beta H m_n$
Answer: a

27. A unpaired electron interacting with ------- spin ?
a. Nuclear
b. electron
c. equal
d. non equal
Answer: a
28. The radical containing-------type of proton?
a. non-equivalent
b. equivalent
c. metal
d. nonmetal
Answer: a

29. The hyperfine energy levels interaction with ------?
a. paired electron
b. unpaired electron
c. outershell electron
d. innershell electron
Answer: b

30. In the naphthalene negative ion, the two sets of four equivalent protons should give a total of -----lines in the ESR spectrum?
a. Twenty
b. Five
c. Twenty five
d. Ten
Answer: c

31. If the electron is delocalized on nuclei with spin greater than ------?
a. 1/2
b. - 1/2

c. 0
d. 1
Answer: a

32. The metal ion present in the molecule affects the ----- value
a. d
b. g
c. s
d. a
Answer: b

33. Which of the following have three peaks in the EPR SYSTEM?
a. Hydrogen
b. carbon
c. helium
d. sodium
Answer: a

34. 280 mc sec^{-1} is equal to
a. 1cm
b. 1mm
c. 1 gauss
d. 1newton
Answer: c

35. The weak field obtained in ------ field
a. metal ion
b. crystal
c. non-metal
d. noble gas
Answer: b

36. Which one of the following is example for an absorption
a. EPR
b. FES
c. AES
d. ES
Answer: a

37. $E = hv = g\beta H_o$, equation belongs to ------ energy equation
a. higher
b. lower
c. emission
d. transitition
Answer: d

38. $g^2 = g_1^2 \cos^2 \theta + g_1 2 \sin 2 \theta$, the equation belongs to the system which have -------- symmetry
a. equtorial
b. axial
c. plane
d. non-plane
Answer: b

39. -9230×10^{24} the equation is wriiten for the electron -------
a. energy
b. position
c. momentum
d. magnetic momentum
Answer: d

40. 12000 is a number of gauss present in the -----
a. NMR
b. ESR
c. IR
d. mass
Answer: b

41. Which of the following is affected in EPR spectrum at the presence of magnetic field?
a. size
b. moment
c. shape
d. numbers
Answer: c

42. Whose separation is interpreted in the EPR spectrum with the presence of magnetic field?
a. substance
b. peak
c. curve
d. metals
Answer: b

43. 2.0067 the value is belongs to ----- electron
a. normal
b. acctual
c. spin paired
d. unspin
Answer: b

44. 9,000 mc sec^{-1} the value belongs to
a. UV range
b. Radio wave
c. microwave

d. visible
Answer: c

45. The non-equivalent type of proton present in -----
a. positive ion
b. negative ion
c. radical
d. substance
Answer: c

46. The effect of quadrupole interaction is usually complicated because it is accompanied by a much larger magnetic ------ inetraction ?
a.hyperfine
b. electrostatic
c. dipole to dipole
d. electro magnetic
Answer: a

47. The values of g depends on ----- electron ?
a. metal ion
b. free electron
c. outermost elctron
d. sigma bond electron
Answer: a

48. The g value of the Cu+ metal present in ------- is 0.3467?
a. oxalate
b. citrate
c. histidine
d. dipyridyl
Answer: b

49. The magnitude of g depends on ------- electron ?
a. unpaired electron
b. paired electron
c. outershell electron
d. innershell electron
Answer: a

50. If the paramagnetic radical is located in a perfectly cubic
a. crystal
b. non-crystal
c. complex
d. metal
Answer:a

51. In a crystal site of lower symmetry the g value depends upon the orientation of the crystal and is said to be an--------?
a. Anisotropic
b. Isotropic
c. isomeric
d. tatumeric
Answer: a

52. The g-value of Cu+ present in the histidine
a. 0.19
b. 0.2277
c. 0.3134
d. 0.3447
Answer: a

53. Anisotropic system then spin depends on the ------ of the crystal field
a. number
b. energy
c. orientation
d. axes
Answer: c

54. The g value for the Cu+ in the oxalate system is
a. 0.985
b. 0.3137
c. 0.3347
d. 0.3467
Answer: b

55. The frequency range of 9000-10,000 MHz lies in the region
a. Visible
b. Microwave
c. Ultraviolet
d. Infrared
Answer: b

56. The value of gyromagnetic ratio g is equal to 2.0023 for
a.Free electrons
b. Free radicals
c. Transition metal ions
d. All species containing unpaired electrons
Answer: a

57. Free radicals can be produced by
a. Pyrolysis
b.Adsorption of alumina or zeolites

c. reduction or oxidation by chemical or electrolytic means
d. All are correct
Answer: d

58. The most widely used standard reference substance in ESR is
a. 1,1-diphenyl-2-picryl hydrazyl free radical
b. TMS
c. 1,1-diphenyl-2-picryl hydroxyl free radical
d.DMSO
Answer: a

59. Mn^{2+} has a nuclear spin of
a.1/2
b. 3/2
c. 5/2
d. 1
Answer: c

60. The Klystron tube source produces radiation at a constant frequency of about
a.5000 MHz
b. 2000 MHz
c. 9500 MHz
d. 1000 MHz
Answer: c

Lecture Notes

UNIT-5

SYLLABUS

Flame Emission Spectroscopy: *Introduction, flames and flame spectra, flames temperature, chemical reaction in flame and flame background. Flame photometers, Flame spectrophotometers, photosensitive detectors, single beam and double beam instruments, calibration curve, errors in flame photometers, applications.*

Flame

A **flame** (from Latin *flamma*) is the visible, gaseous part of a fire. It is caused by a highly exothermic reaction taking place in a thin zone. Some flames, such as the flame of a burning candle, are hot enough to have ionized gaseous components and can be considered plasma. This subject is, however, hotly debated.

Mechanism

Color and temperature of a flame are dependent on the type of fuel involved in the combustion, as, for example, when a lighter is held to a candle. The applied heat causes the fuel molecules in the candle wax to vaporize. In this state they can then readily react with oxygen in the air, which gives off enough heat in the subsequent exothermic reaction to vaporize yet more fuel, thus sustaining a consistent flame. The high temperature of the flame causes the vaporized fuel molecules to decompose, forming various incomplete combustion products and free radicals, and these products then react with each other and with the oxidizer involved in the reaction. Sufficient energy in the flame will excite the electrons in some of the

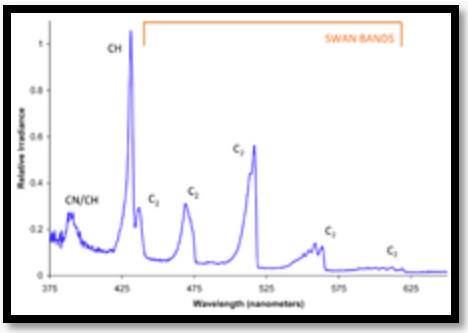
transient reaction intermediates such as CH and C_2, which results in the emission of visible light as these substances release their excess energy (see spectrum below for an explanation of which specific radical species produce which specific colors). As the combustion temperature of a flame increases (if the flame contains small particles of unburnt carbon or other material), so does the average energy of the electromagnetic radiation given off by the flame (see Black body).

Other oxidizers besides oxygen can be used to produce a flame. Hydrogen burning in chlorine produces a flame and in the process emits gaseous hydrogen chloride (HCl) as the combustion product. Another of many possible chemical combinations is hydrazine and nitrogen tetroxide which is hypergolic and commonly used in rocket engines. Fluoropolymers can be used to supply fluorine as an oxidizer of metallic fuels, e.g. in the magnesium/teflon/vitoncomposition.

The chemical kinetics occurring in the flame is very complex and involves typically a large number of chemical reactions and intermediate species, most of them radicals. For instance, a well-known chemical kinetics scheme, GRI-Mech, uses 53 species and 325 elementary reactions to describe combustion ofbiogas.

There are different methods of distributing the required components of combustion to a flame. In a diffusion flame, oxygen and fuel diffuse into each other; where they meet the flame occurs. In apremixed flame, the oxygen and fuel are premixed beforehand, which results in a different type of flame. Candle flames (a diffusion flame) operate through evaporation of the fuel which rises in alaminar flow of hot gas which then mixes with surrounding oxygen and combusts.

Flame Color



Spectrum of the blue (premixed, i.e., complete combustion) flame from a butanetorch showing molecular radical band emission and Swan bands. Note that virtually all the light produced is in the blue to green region of the spectrum below about 565 nanometers, accounting for the bluish color of sootless hydrocarbon flames.

Different flame types of a Bunsen burnerdepend on oxygen supply. On the left a rich fuel with no premixed oxygen produces a yellow sooty diffusion flame; on the right a lean fully oxygen premixed flame produces no soot and the flame color is produced by molecular radicals, especially CH and C2band emission. The purple color is an artifact of the photographic process.

Flame color depends on several factors, the most important typically being black-body radiation and spectral band emission, with both spectral lineemission and spectral line absorption

playing smaller roles. In the most common type of flame, hydrocarbon flames, the most important factor determining color is oxygen supply and the extent of fuel-oxygen pre-mixing, which determines the rate of combustion and thus the temperature and reaction paths, thereby producing different color hues.

In a laboratory under normal gravity conditions and with a closed oxygen valve, a Bunsen burner burns with yellow flame (also called a safety flame) at around 1,000 °C (1,800 °F). This is due to incandescence of very fine soot particles that are produced in the flame. With increasing oxygen supply, less black body-radiating soot is produced due to a more complete combustion and the reaction creates enough energy to excite and ionize gas molecules in the flame, leading to a blue appearance. The spectrum of a premixed (complete combustion) butane flame on the right shows that the blue color arises specifically due to emission of excited molecular radicals in the flame, which emit most of their light well below ~565 nanometers in the blue and green regions of the visible spectrum.

The colder part of a diffusion (incomplete combustion) flame will be red, transitioning to orange, yellow, and white as the temperature increases as evidenced by changes in the black-body radiation spectrum. For a given flame's region, the closer to white on this scale, the hotter that section of the flame is. The transitions are often apparent in fires, in which the color emitted closest to the fuel is white, with an orange section above it, and reddish flames the highest of all. A blue-colored flame only emerges when the amount of soot decreases and the blue emissions from excited molecular radicals become dominant, though the blue can often be seen near the base of candles where airborne soot is less concentrated.

Specific colors can be imparted to the flame by introduction of excitable species with bright emission spectrum lines. In analytical chemistry, this effect is used in flame tests to determine presence of some metal ions. In pyrotechnics, the pyrotechnic colorants are used to produce brightly coloredfireworks.

Flame Temperature

A flame test for sodium. Note that the yellow color in this gas flame does not arise from the blackbody emission of sootparticles (as the flame is clearly a blue premixed complete combustion flame) but instead comes from the spectral lineemission of sodium atoms, specifically the very intense sodium D lines.

When looking at a flame's temperature there are many factors which can change or apply. An important one is that a flame's color does not necessarily determine a temperature comparison because black-body radiation is not the only thing that produces or determines the color seen; therefore it is only an estimation of temperature. Here are other factors that determine its temperature:

- Adiabatic flame; i.e., no loss of heat to the atmosphere (may differ in certain parts).
- Atmospheric pressure
- Percentage oxygen content of the atmosphere.
- The fuel being burned (i.e., depends on how quickly the process occurs; how violent the combustion is.)
- Any oxidation of the fuel.
- Temperature of atmosphere links to adiabatic flame temperature (i.e., heat will transfer to a cooler atmosphere more quickly).
- How stoichiometric the combustion process is (a 1:1 stoichiometricity) assuming no dissociation will have the highest flame temperature... excess air/oxygen will lower it and likewise not enough air/oxygen.

In fires (particularly house fires), the cooler flames are often red and produce the most smoke. Here the red color compared to typical yellow color of the flames suggests that the temperature is lower. This is because there is a lack of oxygen in the room and therefore there is incomplete combustion and the flame temperature is low, often just 600–850 °C (1,112–1,562 °F). This means that a lot of carbon monoxide is formed (which is a flammable gas if hot

enough) which is when in fire and arson investigation there is greatest risk of backdraft. When this occurs flames get oxygen, carbon monoxide combusts and temporary temperatures of up to 2,000 °C (3,632 °F) occur.

Flame temperatures of common items include a candle at 1,400 °C (2,600 °F), a blow torch – at around 1,600 °C (2,900 °F) a propane torch at 1,995 °C(3,620 °F), or a much hotter oxyacetylene combustion at 3,000 °C (5,400 °F).

Common flame temperatures

This is a rough guide to flame temperatures for various common substances (in 20 °C air at 1 atm. pressure):

Material burned	**Flame temperature (°C)**
Charcoal fire	750–1,200
Methane (natural gas)	900–1,500
Bunsen burner flame	900–1,600 (depending on the air valve)
Propane blowtorch	1,200–1,700
Candle flame	~1,100 (majority), hot spots may be 1300–1400
Backdraft flame peak	1,700–1,950

Material burned	**Max. flame temperature (°C, in air, diffusion flame)**
Animal fat	800–900
Kerosene	990
Wood	1027

Charcoal(forced draft)	1390

Hottest flame temperature

Temperature of over 4,525 °C (8,180 °F) when it burns in oxygen. Dicyanoacetylene, a compound of carbon and nitrogen with chemical formula C_4N_2burns in oxygen with a bright blue-white flame at a temperature of 5260 K (4990 °C, 9010 °F), and at up to 6000 K in ozone.[This high flame temperature is also the result of the absence of hydrogen, and, therefore, water as a combustion product. Because of its high specific heat, water vapor as a combustion product tends to lower the flame temperature of hydrogen containing compounds. The endothermic dissociation of water at high temperatures above 2000 °C also prevents flame temperatures to rise above 3000 to 4000 °C.

Cool flames

At temperatures as low as 120 °C, fuel-air mixtures can react chemically and produce very weak flames called cool flames. The phenomenon was discovered by Humphry Davy in 1817. The process depends on a fine balance of temperature and concentration of the reacting mixture, and if conditions are right it can initiate without any external ignition source. Cyclical variations in the balance of chemicals, particularly of intermediate products in the reaction, give oscillations in the flame, with a typical temperature variation of about 100 K, or between "cool" and full ignition. Sometimes the variation can lead to explosion.

Flames in microgravity

In zero gravity, convection does not carry the hot combustion products away from the fuel source, resulting in a spherical flame front.

In 2000, experiments by NASA confirmed that gravity plays an indirect role in flame formation and composition. The common distribution of a flame under normal gravity conditions depends on convection, as soot tends to rise to the top of a flame (such as in a candle in normal gravity conditions), making it yellow. In microgravity or zero gravity environment, such as in orbit, natural convection no longer occurs and the flame becomes spherical, with a tendency to become bluer and more efficient. There are several possible explanations for this difference, of which the most likely is the hypothesis that the temperature is sufficiently evenly distributed that soot is not formed and complete combustion occurs. Experiments by NASAreveal that diffusion flames in microgravity allow more soot to be completely oxidized after they are produced than do diffusion flames on Earth, because of a series of mechanisms that behave differently in microgravity when compared to normal gravity conditions. These discoveries have potential applications in applied science and industry, especially concerning fuel efficiency.

FLAME PHOTOMETRY

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Objective:

To determine the concentration of alkali and alkaline earth metals in various samples.

Introduction

Atomic spectroscopy is thought to be the oldest instrumental method for the determination of elements. These techniques are introduced in the mid of 19th Century during which Bunsen and Kirchhoff showed that the radiation emitted from the flames depends on the characteristic element present in the flame. The potential of atomic spectroscopy in both the qualitative as well as quantitative analysis were then well established. The developments in the instrumentation area led to the widespread application of atomic spectroscopy. Atomic spectroscopy is an unavoidable tool in the field of analytical chemistry. It is divided into three types which are absorption, emission, and luminescence spectroscopy. The different branches of atomic absorption spectroscopy are (1) Flame photometry or flame atomic emission spectrometry in which the species is examined in the form of atoms (2) Atomic absorption spectrophotometry, (AAS), (3) Inductively coupled plasma-atomic emission spectrometry (ICP-AES).

Theory:

Photoelectric flame photometry, a branch of atomic spectroscopy is used for inorganic chemical analysis for determining the concentration of certain metal ions such as sodium, potassium, lithium, calcium, Cesium, etc. In flame photometry the species (metal ions) used in the spectrum are in the form of atoms. The International Union of Pure and Applied Chemistry (IUPAC) Committee on Spectroscopic Nomenclature has recommended it as flame

atomic emission spectrometry (FAES). The basis of flame photometric working is that, the species of alkali metals (Group 1) and alkaline earth metals (Group II) metals are dissociated due to the thermal energy provided by the flame source. Due to this thermal excitation, some of the atoms are excited to a higher energy level where they are not stable. The absorbance of light due to the electrons excitation can be measured by using the direct absorption techniques. The subsequent loss of energy will result in the movement of excited atoms to the low energy ground state with emission of some radiations, which can be visualized in the visible region of the spectrum. The absorbance of light due to the electrons excitation can be measured by using the direct absorption techniques while the emitting radiation intensity is measured using the emission techniques. The wavelength of emitted light is specific for specific elements.

Flame Photometer

Parts of a flame photometer

1. Source of flame:

A burner that provides flame and can be maintained in a constant form and at a constant

temperature.

2. Nebuliser and mixing chamber:

Helps to transport the homogeneous solution of the substance into the flame at a steady rate.

3. Optical system (optical filter):

The optical system comprises three parts: convex mirror, lens and filter. The convex mirror helps to transmit light emitted from the atoms and focus the emissions to the lens. The convex lens help to focus the light on a point called slit. The reflections from the mirror pass through the slit and reach the filters. This will isolate the wavelength to be measured from that of any other extraneous emissions. Hence it acts as interference type color filters.

4. Photo detector:

Detect the emitted light and measure the intensity of radiation emitted by the flame. That is, the emitted radiation is converted to an electrical signal with the help of photo detector. The produced electrical signals are directly proportional to the intensity of light.

A schematic representation of flame photometer is shown in figure 1,

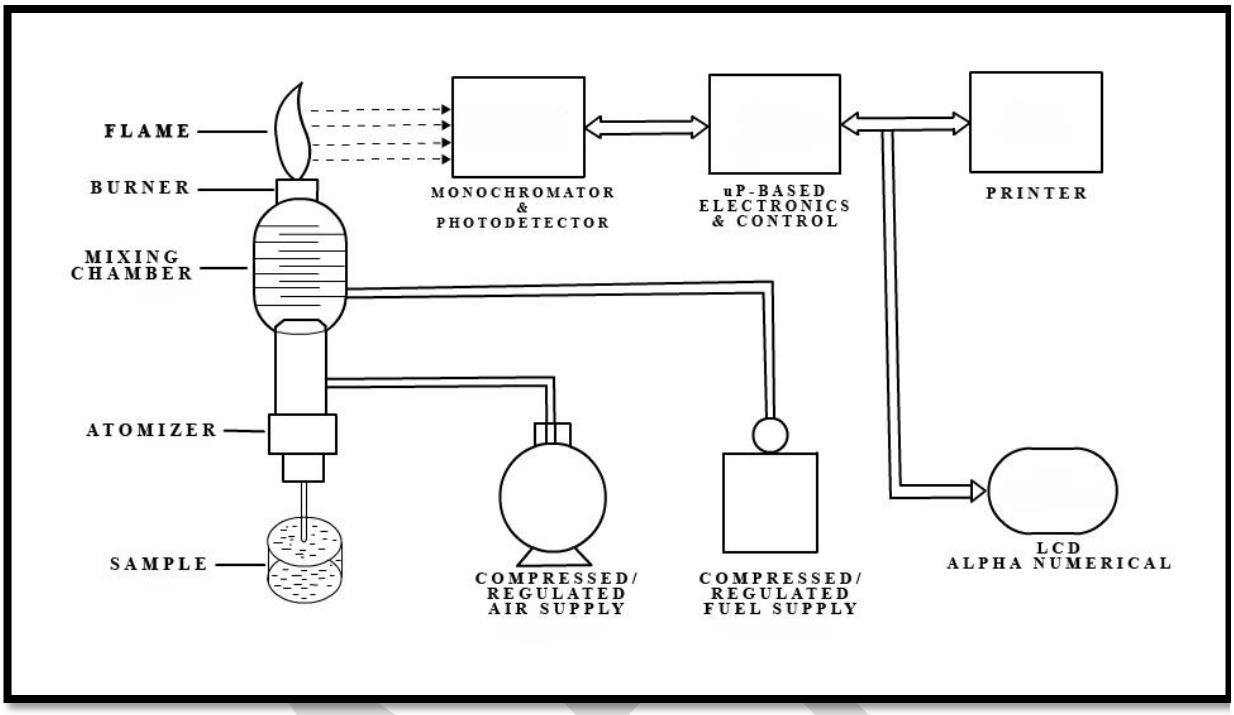


Fig 1: A schematic representation of flame photometer

Mechanism of working:

The working of the flame photometer involves a series of steps which is discussed in the following sections.

Nebulisation:

The solution of the substance to be analyzed is first aspirated into the burner, which is then dispersed into the flame as fine spray particles.

A brief overview of the process:

1. The solvent is first evaporated leaving fine divided solid particles.
2. This solid particles move towards the flame, where the gaseous atoms and ions are produced.
3. The ions absorb the energy from the flame and excited to high energy levels.
4. When the atoms return to the ground state radiation of the characteristic element is emitted.
5. The intensity of emitted light is related to the concentration of the element.

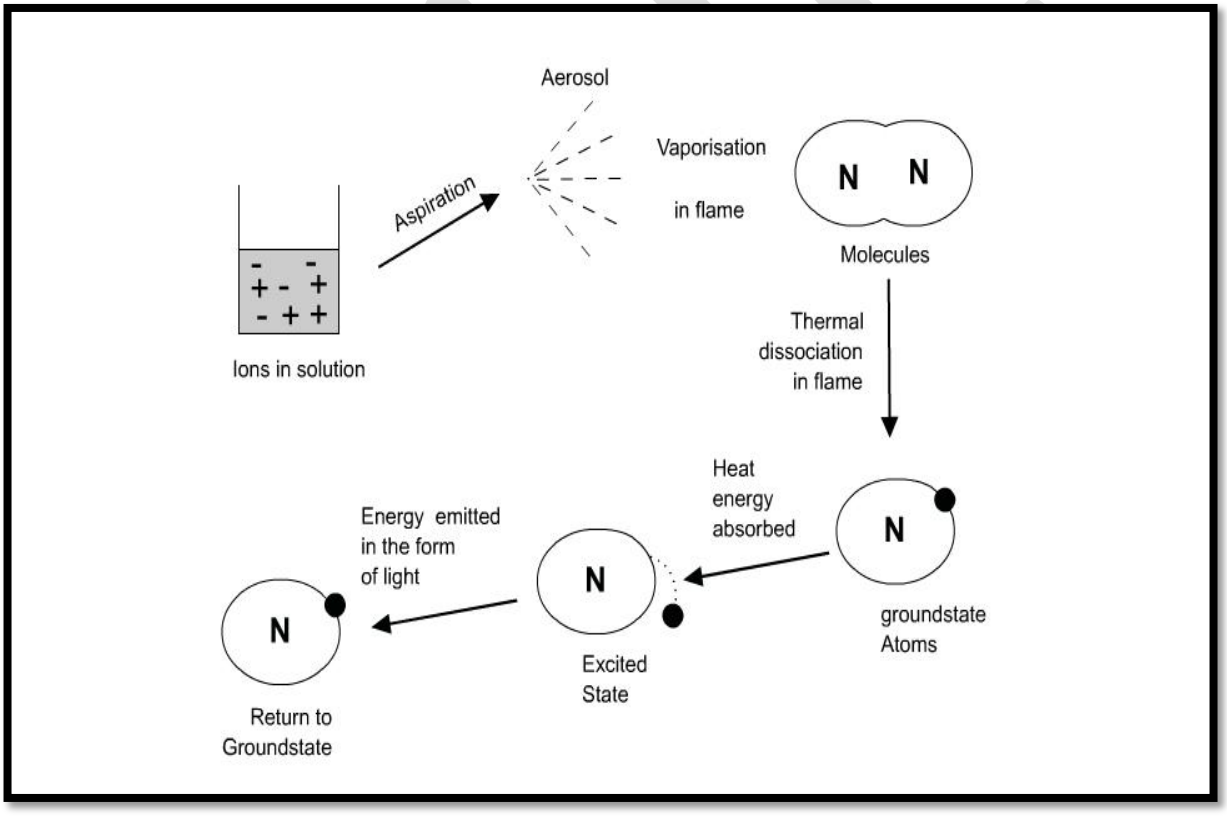


Fig 2: Brief overview of the process

Events occurring in the flame:

Flame photometry employs a variety of fuels mainly air, oxygen or nitrous oxide (N_2O) as oxidant. The temperature of the flame depends on fuel-oxidant ratio.

The various processes in the flame are discussed below:

Desolvation: The metal particles in the flame are dehydrated by the flame and hence the solvent is evaporated.

Vapourisation: The metal particles in the sample are dehydrated. This also led to the evaporation of the solvent.

Atomization: Reduction of metal ions in the solvent to metal atoms by the flame heat.

Excitation: The electrostatic force of attraction between the electrons and nucleus of the atom helps them to absorb a particular amount of energy. The atoms then jump to the exited energy state.

Emission process: Since the higher energy state is unstable the atoms jump back to the stable low energy state with the emission of energy in the form of radiation of characteristic wavelength, which is measured by the photo detector.

The energy level diagram of the sodium atom is shown in figure 3

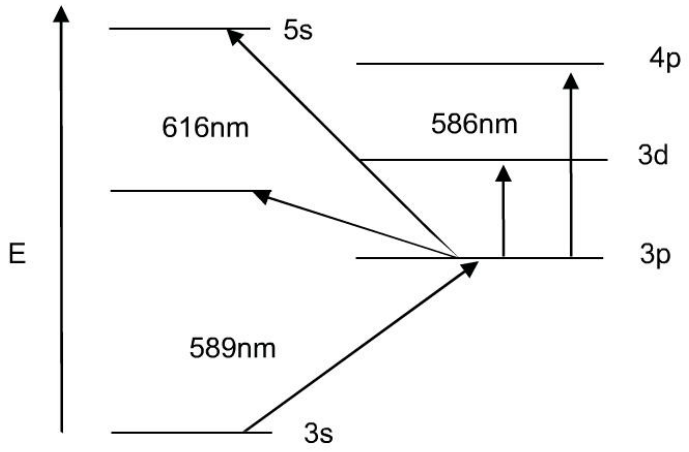


Fig 3: Energy level diagram for atomic sodium

The intensity of the light emitted could be described by the Scheibe-Lomakin equation:

$$I = k \times c^n$$

Where:

I = Intensity of emitted light

c = the concentration of the element

k = constant of proportionality

$n \sim 1$ (at the linear part of the calibration curve)

Then,

$$I = k \times c$$

That is the intensity of emitted light is directly related to the concentration of the sample.

The comparison of emission intensities of unknown samples to either that of standard solutions (plotting calibration curve), or to those of an internal standard (standard addition method), helps in the quantitative analysis of the analyte metal in the sample solution.

The flame emissions of the alkali and alkaline earth metals in terms of the emission wavelength and the characteristic color produced by each element is shown in table 1

Flame photometer has both quantitative and qualitative applications. Flame photometer with monochromators emits radiations of characteristic wavelengths which help to detect the presence of a particular metal in the sample. This help to determine the availability of alkali and alkaline earth metals which are critical for soil cultivation. In agriculture, the fertilizer requirement of the soil is analyzed by flame test analysis of the soil. In clinical field, Na^+ and K^+ ions in body fluids, muscles and heart can be determined by diluting the blood serum and aspiration into the flame. Analysis of soft drinks, fruit juices and alcoholic beverages can also be analyzed by using flame photometry.

Advantages:

- Simple quantitative analytical test based on the flame analysis.
- Inexpensive.
- The determination of elements such as alkali and alkaline earth metals is performed easily with most reliable and convenient methods.
- Quite quick, convenient, and selective and sensitive to even parts per million (ppm) to parts per billion (ppb) range.

Disadvantages:

Moreover the flame photometer has a wide range of applications in the analytical chemistry, it possess many disadvantages which are explained below:

- The concentration of the metal ion in the solution cannot be measured accurately.
- A standard solution with known molarities is required for determining the concentration of the ions which will corresponds to the emission spectra.
- It is difficult to obtain the accurate results of ions with higher concentration.
- The information about the molecular structure of the compound present in the sample solution cannot be determined.
- The elements such as carbon, hydrogen and halides cannot be detected due to its non radiating nature.

Spectrophotometry

In chemistry, **spectrophotometry** is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength. It is more specific than the general term electromagnetic spectroscopy in that spectrophotometry deals with visible light, near-ultraviolet, and near-infrared, but does not cover time-resolved spectroscopic techniques.

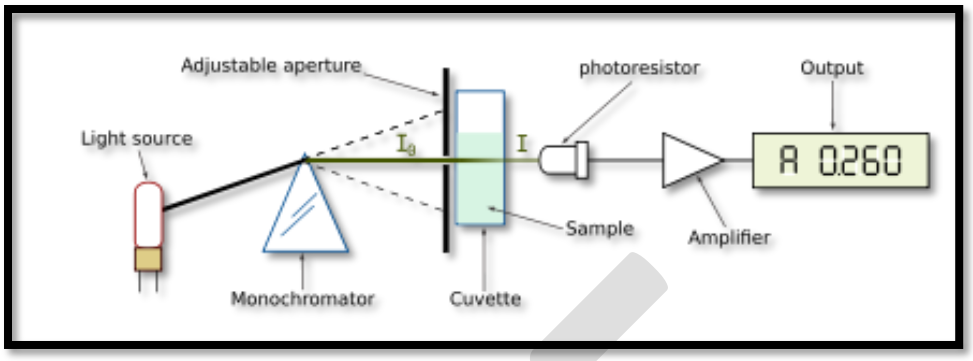
Spectrophotometry uses photometers that can measure a light beam's intensity as a function of its color (wavelength) known as spectrophotometers. Important features of spectrophotometers are spectral bandwidth, (the range of colors it can transmit through the test sample), and the percentage of sample-transmission, and the logarithmic range of sample-absorption and sometimes a percentage of reflectance measurement.

A spectrophotometer is commonly used for the measurement of transmittance or reflectance of solutions, transparent or opaque solids, such as polished glass, or gases. However they can also be designed to measure the diffusivity on any of the listed light ranges that usually cover around 200 nm - 2500 nm using different controls and calibrations. Within these ranges of light, calibrations are needed on the machine using standards that vary in type depending on the wavelength of the *photometric determination*.

An example of an experiment in which spectrophotometry is used is the determination of the equilibrium constant of a solution. A certain chemical reaction within a solution may occur in a forward and reverse direction where reactants form products and products break down into reactants. At some point, this chemical reaction will reach a point of balance called an equilibrium point. In order to determine the respective concentrations of reactants and products at this point, the light transmittance of the solution can be tested using spectrophotometry. The amount of light that passes through the solution is indicative of the concentration of certain chemicals that do not allow light to pass through.

The use of spectrophotometers spans various scientific fields, such as physics, materials science, chemistry, biochemistry, and molecular biology. They are widely used in many industries including semiconductors, laser and optical manufacturing, printing and forensic examination, as well in laboratories for the study of chemical substances. Ultimately, a spectrophotometer is able to determine, depending on the control or calibration, what substances are present in a target and exactly how much through calculations of observed wavelengths.

Design



Single beam spectrophotometer

There are two major classes of devices: single beam and double beam. A double beam spectrophotometer compares the light intensity between two light paths, one path containing a reference sample and the other the test sample. A single-beam spectrophotometer measures the relative light intensity of the beam before and after a test sample is inserted. Although comparison measurements from double-beam instruments are easier and more stable, single-beam instruments can have a larger dynamic range and are optically simpler and more compact. Additionally, some specialized instruments, such as spectrophotometers built onto microscopes or telescopes, are single-beam instruments due to practicality.

Historically, spectrophotometers use a monochromator containing a diffraction grating to produce the analytical spectrum. The grating can either be movable or fixed. If a single detector, such as a photomultiplier tube or photodiode is used, the grating can be scanned stepwise so that the detector can measure the light intensity at each wavelength (which will correspond to each "step"). Arrays of detectors, such as charge coupled devices (CCD) or photodiode arrays (PDA) can also be used. In such systems, the grating is fixed and the intensity of each wavelength of light is measured by a different detector in the array. Additionally, most modern mid-infrared spectrophotometers use a Fourier transform technique to acquire the spectral information. The technique is called Fourier transform infrared spectroscopy.

When making transmission measurements, the spectrophotometer quantitatively compares the fraction of light that passes through a reference solution and a test solution. For reflectance measurements, the spectrophotometer quantitatively compares the fraction of light that reflects from the reference and test samples. Light from the source lamp is passed through a monochromator, which diffracts the light into a "rainbow" of wavelengths and outputs narrow bandwidths of this diffracted spectrum. Discrete frequencies are transmitted through the test sample. Then the photon flux density (watts per metre squared usually) of the transmitted or reflected light is measured with a photodiode, charge coupled device or other light sensor. The transmittance or reflectance value for each wavelength of the test sample is then compared with the transmission or reflectance values from the reference sample.

In short, the sequence of events in a modern spectrophotometer is as follows:

1. The light source is imaged upon the sample.
2. A fraction of the light is transmitted or reflected from the sample.
3. The light from the sample is imaged upon the entrance slit of the monochromator.
4. The monochromator separates the wavelengths of light and focuses each of them onto the photodetector sequentially.

Many older spectrophotometers must be calibrated by a procedure known as "zeroing." The absorbency of a reference substance is set as a baseline value, so the absorbencies of all other substances are recorded relative to the initial "zeroed" substance. The spectrophotometer then displays % absorbency (the amount of light absorbed relative to the initial substance).

UV-visible spectrophotometry

The most common spectrophotometers are used in the UV and visible regions of the spectrum, and some of these instruments also operate into the near-infrared region as well.

Visible region 400–700 nm spectrophotometry is used extensively in colorimetry science. It is a known fact that it operates best at the range of 0.2-0.8 O.D. Ink manufacturers, printing companies, textiles vendors, and many more, need the data provided through colorimetry. They take readings in the region of every 5–20 nanometers along the visible region, and produce a spectral reflectance curve or a data stream for alternative presentations. These curves can be used to test a new batch of colorant to check if it makes a match to specifications, e.g., ISO printing standards.

Traditional visible region spectrophotometers cannot detect if a colorant or the base material has fluorescence. This can make it difficult to manage color issues if for example one or more of the printing inks is fluorescent. Where a colorant contains fluorescence, a bi-spectral fluorescent spectrophotometer is used. There are two major setups for visual spectrum spectrophotometers, d/8 (spherical) and 0/45. The names are due to the geometry of the light source, observer and interior of the measurement chamber. Scientists use this instrument to measure the amount of compounds in a sample. If the compound is more concentrated more light will be absorbed by the sample; within small ranges, the Beer-Lambert law holds and the absorbance between samples vary with concentration linearly. In the case of printing measurements two alternative settings are commonly used- without/with uv filter to control better the effect of uv brighteners within the paper stock.

Samples are usually prepared in cuvettes; depending on the region of interest, they may be constructed of glass, plastic (visible spectrum region of interest), or quartz (Far UV spectrum region of interest).

Applications

- Estimating dissolved organic carbon concentration
- Specific Ultraviolet Absorption for metric of aromaticity

- Bial's Test for concentration of pentoses

IR spectrophotometry

Spectrophotometers designed for the infrared region are quite different because of the technical requirements of measurement in that region. One major factor is the type of photosensors that are available for different spectral regions, but infrared measurement is also challenging because virtually everything emits IR light as thermal radiation, especially at wavelengths beyond about 5 μm.

Another complication is that quite a few materials such as glass and plastic absorb infrared light, making it incompatible as an optical medium. Ideal optical materials are salts, which do not absorb strongly. Samples for IR spectrophotometry may be smeared between two discs of potassium bromide or ground with potassium bromide and pressed into a pellet. Where aqueous solutions are to be measured, insoluble silver chloride is used to construct the cell.

Spectroradiometers

Spectroradiometers, which operate almost like the visible region spectrophotometers, are designed to measure the spectral density of illuminants. Applications may include evaluation and categorization of lighting for sales by the manufacturer, or for the customers to confirm the lamp they decided to purchase is within their specifications. Components:

1. The light source shines onto or through the sample.
2. The sample transmits or reflects light.
3. The detector detects how much light was reflected from or transmitted through the sample.
4. The detector then converts how much light the sample transmitted or reflected into a number.

A Double Beam Absorption Spectrometer

This describes a double beam UV-visible absorption spectrometer.

The overall design

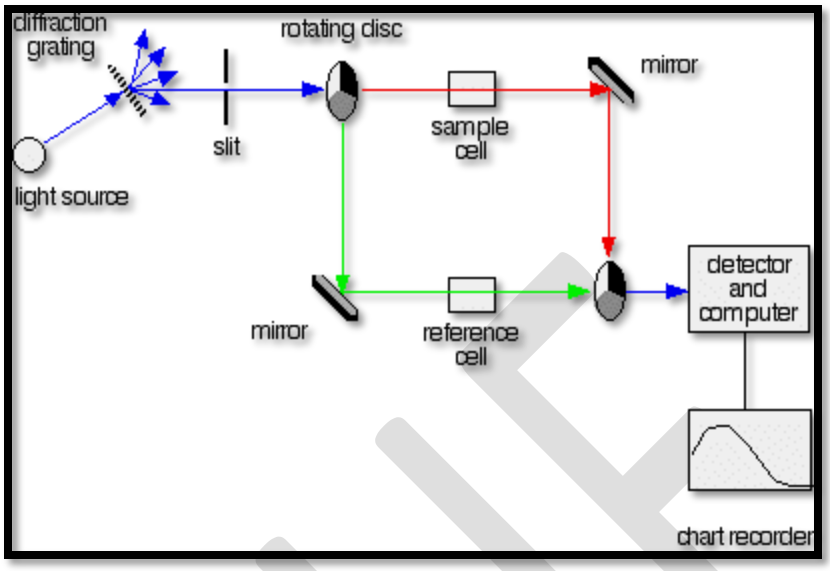
If you pass white light through a colored substance, some of the light gets absorbed. A solution containing hydrated copper(II) ions, for example, looks pale blue because the solution absorbs light from the red end of the spectrum. The remaining wavelengths in the light combine in the eye and brain to give the appearance of cyan (pale blue).

Some colorless substances also absorb light - but in the ultra-violet region. Since we can't see UV light, we don't notice this absorption. Different substances absorb different wavelengths of light, and this can be used to help to identify the substance - the presence of particular metal ions, for example, or of particular functional groups in organic compounds.

The amount of absorption is also dependent on the concentration of the substance if it is in solution. Measurement of the amount of absorption can be used to find concentrations of very dilute solutions. An absorption spectrometer measures the way that the light absorbed by a compound varies across the UV and visible spectrum.

A simple double beam spectrometer

We'll start with the full diagram, and then explain exactly what is going on at each stage.



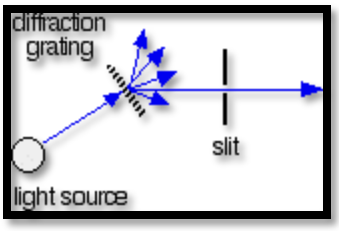
The light source

You need a light source which gives the entire visible spectrum plus the near ultra-violet so that you are covering the range from about 200 nm to about 800 nm. (This extends slightly into the near infra-red as well.)

You can't get this range of wavelengths from a single lamp, and so a combination of two is used - a deuterium lamp for the UV part of the spectrum, and a tungsten / halogen lamp for the visible part. The combined output of these two bulbs is focused on to a diffraction grating.

The diffraction grating and the slit

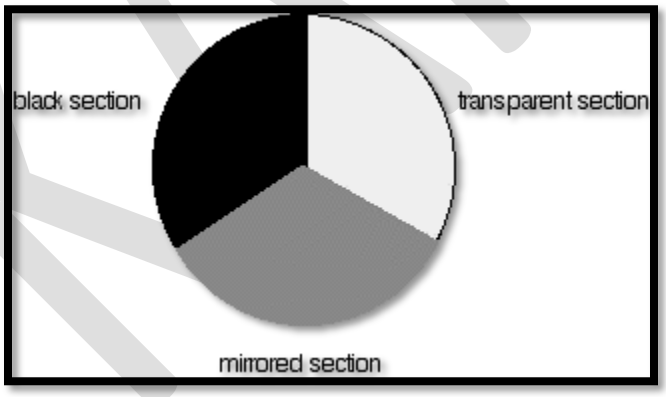
You are probably familiar with the way that a prism splits light into its component colors. A diffraction grating does the same job, but more efficiently.



The blue arrows show the way the various wavelengths of the light are sent off in different directions. The slit only allows light of a very narrow range of wavelengths through into the rest of the spectrometer. By gradually rotating the diffraction grating, you can allow light from the whole spectrum (a tiny part of the range at a time) through into the rest of the instrument.

The rotating disks

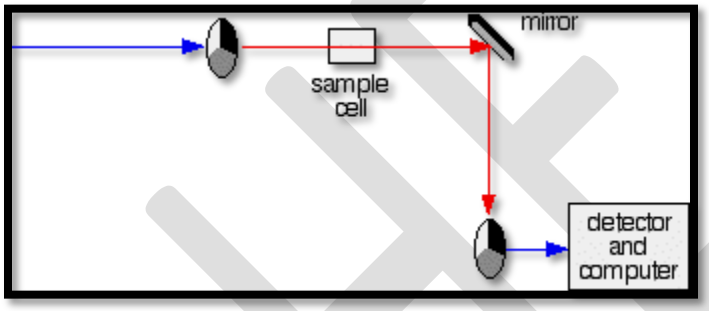
This is the clever bit! Each disk is made up of a number of different segments. Those in the machine we are describing have three different sections - other designs may have a different number.



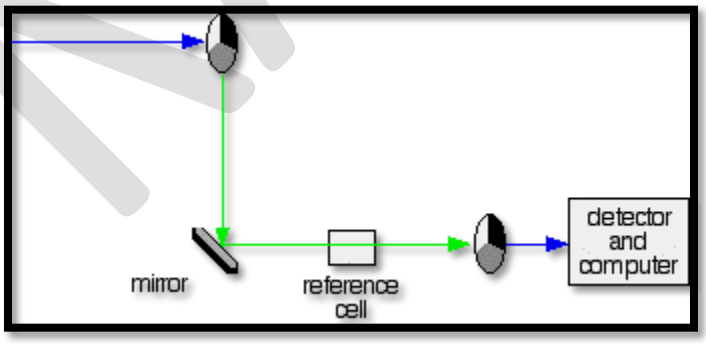
The light coming from the diffraction grating and slit will hit the rotating disk and one of three things can happen.

1. If it hits the transparent section, it will go straight through and pass through the cell containing the sample. It is then bounced by a mirror onto a second rotating disk. This disk is rotating such that when the light arrives from the first disk, it meets the mirrored section of the second disk. That bounces it onto the detector.

   It is following the red path in the diagram:



2. If the original beam of light from the slit hits the mirrored section of the first rotating disk, it is bounced down along the green path. After the mirror, it passes through a reference cell (more about that later). Finally the light gets to the second disk which is rotating in such a way that it meets the transparent section. It goes straight through to the detector.



3. If the light meets the first disk at the black section, it is blocked - and for a very short while no light passes through the spectrometer. This just allows the computer to make allowance for any current generated by the detector in the absence of any light.

The sample and reference cells

These are small rectangular glass or quartz containers. They are often designed so that the light beam travels a distance of 1 cm through the contents. The sample cell contains a solution of the substance you are testing - usually very dilute. The solvent is chosen so that it doesn't absorb any significant amount of light in the wavelength range we are interested in (200 - 800 nm). The reference cell just contains the pure solvent.

The detector and computer

The detector converts the incoming light into a current. Higher the current, greater the intensity of the light. For each wavelength of light passing through the spectrometer, the intensity of the light passing through the reference cell is measured. This is usually referred to as I_o - that's I for Intensity.

The intensity of the light passing through the sample cell is also measured for that wavelength - given the symbol, I.

If I is less than I_o, then obviously the sample has absorbed some of the light. A simple bit of math is then done in the computer to convert this into something called the *absorbance* of the sample - given the symbol, A.

For reasons which will become clearer when we do a bit of theory on another page, the relationship between A and the two intensities is given by:

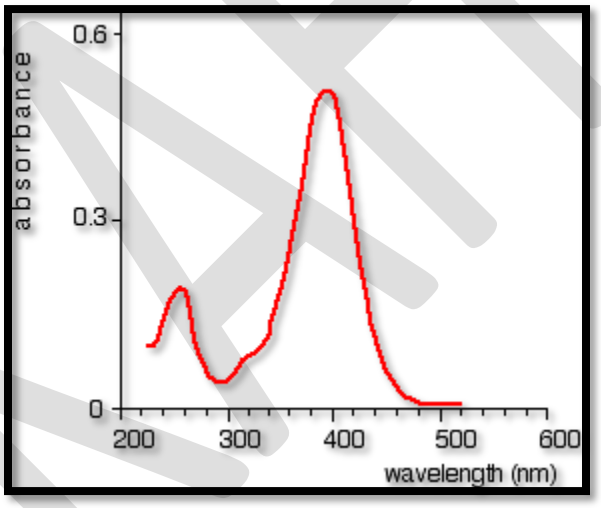
$$A=\log(II_o)$$

On most of the diagrams you will come across, the absorbance ranges from 0 to 1, but it can go higher than that. An absorbance of 0 at some wavelength means that no light of that particular wavelength has been absorbed. The intensities of the sample and reference beam are both the same, so the ratio I_o/I is 1. Log_{10} of 1 is zero.

An absorbance of 1 happens when 90% of the light at that wavelength has been absorbed - which means that the intensity is 10% of what it would otherwise be.

In that case, I_o/I is 100/I0 (=10) and log_{10} of 10 is 1.

The absoprtion spectrum is a plot of absorbance against wavelength. The output might look like this:



This particular substance has what are known as *absorbance peaks* at 255 and 395 nm. How these arise and how they are interpreted are diskussed on another page.

Atomic Emission Spectroscopy

The focus of this section is on the emission of ultraviolet and visible radiation following the thermal excitation of atoms. Atomic emission spectroscopy has a long history. Qualitative applications based on the color of flames were used in the smelting of ores as early as 1550 and were more fully developed around 1830 with the observation of atomic spectra generated by flame emission and spark emission.[18] Quantitative applications based on the atomic emission from electric sparks were developed by Lockyer in the early 1870 and quantitative applications based on flame emission were pioneered by Lundegardh in 1930. Atomic emission based on emission from a plasma was introduced in 1964.

Atomic Emission Spectra

Atomic emission occurs when a valence electron in a higher energy atomic orbital returns to a lower energy atomic orbital. Figure shows a portion of the energy level diagram for sodium, which consists of a series of discrete lines at wavelengths corresponding to the difference in energy between two atomic orbitals.

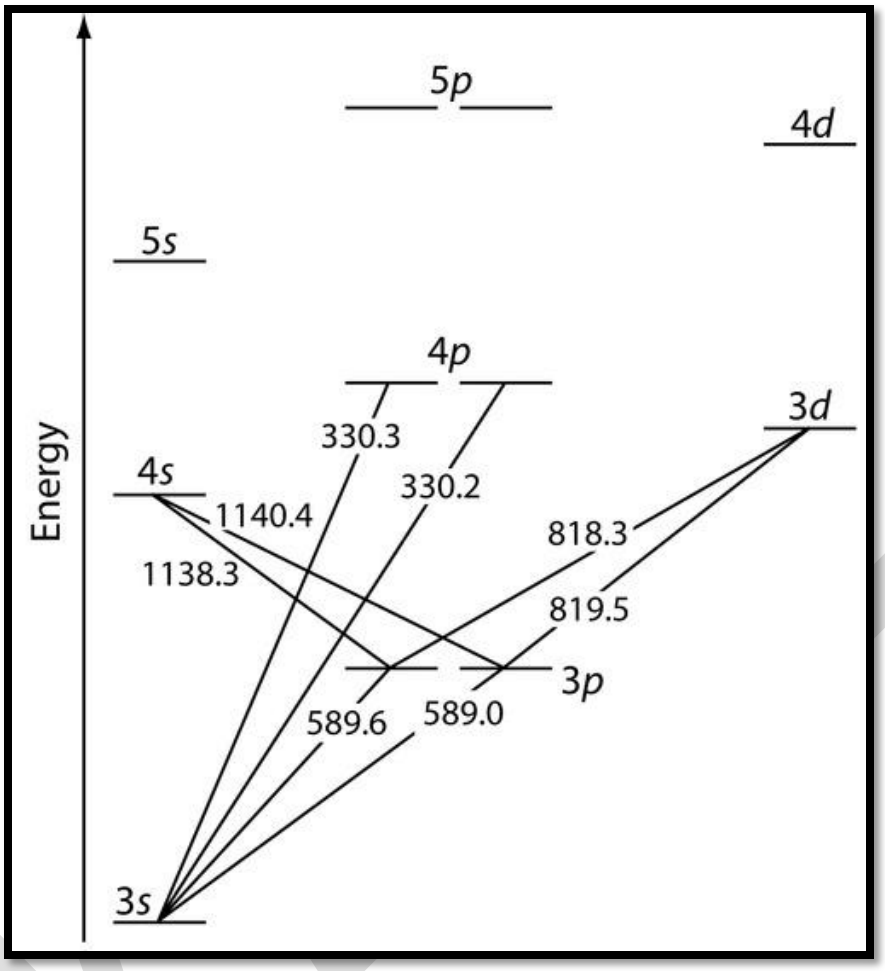


Figure Valence shell energy level diagram for sodium. The wavelengths corresponding to several transitions are shown. Note that this is the same energy level diagram as Figure .

The intensity of an atomic emission line, I_e, is proportional to the number of atoms, N^*, populating the excited state,

$$I_e = kN^* \qquad 5.30$$

where k is a constant accounting for the efficiency of the transition. If a system of atoms is in thermal equilibrium, the population of excited state i is related to the total concentration of atoms, N, by the Boltzmann distribution. For many elements at temperatures of less than 5000 K the Boltzmann distribution is approximated as

$$N^* = N(g_i / g_0)e^{-E_i / kT} \qquad 5.31$$

where g_i and g_0 are statistical factors that account for the number of equivalent energy levels for the excited state and the ground state, E_i is the energy of the excited state relative to a ground state energy, E_0, of 0, k is Boltzmann's constant (1.3807×10^{-23} J/K), and T is the temperature in kelvin. From equation 5.31 we expect that excited states with lower energies have larger populations and more intense emission lines. We also expect emission intensity to increase with temperature.

Equipment

An atomic emission spectrometer is similar in design to the instrumentation for atomic absorption. In fact, it is easy to adapt most flame atomic absorption spectrometers for atomic emission by turning off the hollow cathode lamp and monitoring the difference in the emission intensity when aspirating the sample and when aspirating a blank. Many atomic emission spectrometers, however, are dedicated instruments designed to take advantage of features unique to atomic emission, including the use of plasmas, arcs, sparks, and lasers as atomization and excitation sources, and an enhanced capability for multielemental analysis.

Atomization and Excitation

Atomic emission requires a means for converting a solid, liquid, or solution analyte into a free gaseous atom. The same source of thermal energy usually serves as the excitation source.

The most common methods are flames and plasmas, both of which are useful for liquid or solution samples. Solid samples may be analyzed by dissolving in a solvent and using a flame or plasma atomizer.

Flame Sources

Atomization and excitation in flame atomic emission is accomplished using the same nebulization and spray chamber assembly used in atomic absorption (Figure). The burner head consists of single or multiple slots, or a Meker style burner. Older atomic emission instruments often used a total consumption burner in which the sample is drawn through a capillary tube and injected directly into the flame.

Plasma Sources

Plasma is a hot, partially ionized gas that contains an abundant concentration of cations and electrons. The plasmas used in atomic emission are formed by ionizing a flowing stream of argon gas, producing argon ions and electrons. A plasma's high temperature results from resistive heating as the electrons and argon ions move through the gas. Because plasmas operate at much higher temperatures than flames, they provide better atomization and a higher population of excited states.

A schematic diagram of the inductively coupled plasma source (ICP) is shown in Figure . The ICP torch consists of three concentric quartz tubes, surrounded at the top by a radio-frequency induction coil. The sample is mixed with a stream of Ar using a nebulizer, and is carried to the plasma through the torch's central capillary tube. Plasma formation is initiated by a spark from a Tesla coil. An alternating radio-frequency current in the induction coils creates a fluctuating magnetic field that induces the argon ions and the electrons to move in a circular path. The resulting collisions with the abundant unionized gas give rise to resistive heating, providing temperatures as high as 10 000 K at the base of the plasma, and between 6000 and

8000 K at a height of 15–20 mm above the coil, where emission is usually measured. At these high temperatures the outer quartz tube must be thermally isolated from the plasma. This is accomplished by the tangential flow of argon shown in the schematic diagram.

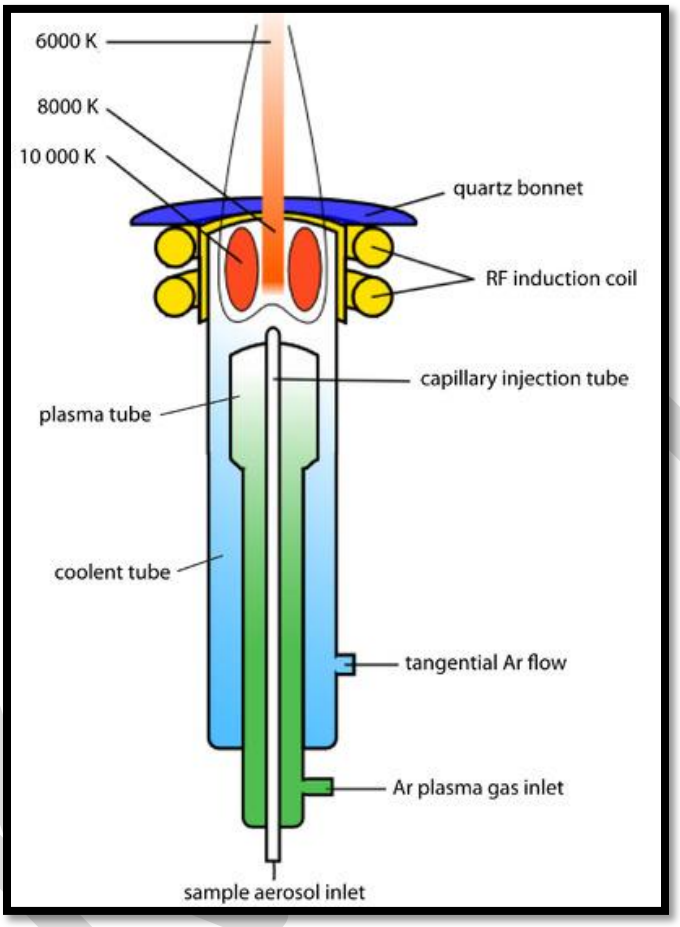


Figure Schematic diagram of an inductively coupled plasma torch.

Multielemental Analysis

Atomic emission spectroscopy is ideally suited for multielemental analysis because all analytes in a sample are excited simultaneously. If the instrument includes a scanning monochromator,

we can program it to move rapidly to an analyte's desired wavelength, pause to record its emission intensity, and then move to the next analyte's wavelength. This sequential analysis allows for a sampling rate of 3–4 analytes per minute.

Another approach to a multielemental analysis is to use a multichannel instrument that allows us to simultaneously monitor many analytes. A simple design for a multichannel spectrometer couples a monochromator with multiple detectors that can be positioned in a semicircular array around the monochromator at positions corresponding to the wavelengths for the analytes (Figure 10.59).

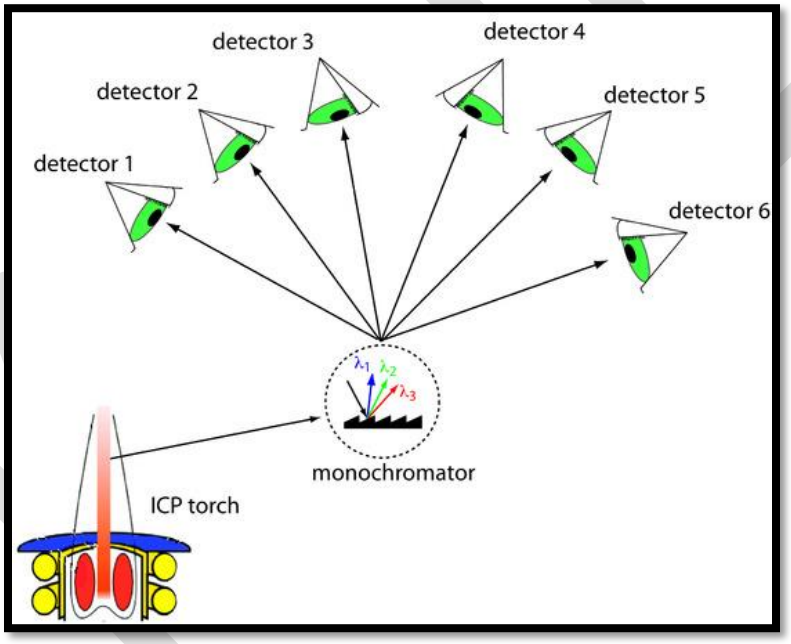


Figure 10.59 Schematic diagram of a multichannel atomic emission spectrometer for the simultaneous analysis of several elements. The ICP torch is modified from Xvlun. Instruments may contain as many as 48–60 detectors.

Quantitative Applications

Atomic emission is widely used for the analysis of trace metals in a variety of sample matrices. The development of a quantitative atomic emission method requires several considerations, including choosing a source for atomization and excitation, selecting a wavelength and slit width, preparing the sample for analysis, minimizing spectral and chemical interferences, and selecting a method of standardization.

Choice of Atomization and Excitation Source

Except for the alkali metals, detection limits when using an ICP are significantly better than those obtained with flame emission (Table 10.14). Plasmas also are subject to less spectral and chemical interference. For these reasons a plasma emission source is usually the better choice.

Table 10.14 Detection Limits for Atomic Emission[a]

	detection limit in µg/mL	
element	**flame emission**	**ICP**
Ag	2	0.2
Al	3	0.2
As	2000	2
Ca	0.1	0.0001
Cd	300	0.07
Co	5	0.1
Cr	1	0.08
Fe	10	0.09

Table 10.14 Detection Limits for Atomic Emission[a]		
	detection limit in µg/mL	
Hg	150	1
K	0.01	30
Li	0.001	0.02
Mg	1	0.003
Mn	1	0.01
Na	0.01	0.1
Ni	10	0.2
Pb	0.2	1
Pt	2000	0.9
Sn	100	3
Zn	1000	0.1

[a] Source: Parsons, M. L.; Major, S.; Forster, A. R.; App. Spectrosc. 1983, 37, 411–418.

Selecting the Wavelength and Slit Width

The choice of wavelength is dictated by the need for sensitivity and the need to avoid interferences from the emission lines of other constituents in the sample. Because an analyte's atomic emission spectrum has an abundance of emission lines—particularly when using a high temperature plasma source—it is inevitable that there will be some overlap between emission lines. For example, an analysis for Ni using the atomic emission line at 349.30 nm is complicated by the atomic emission line for Fe at 349.06 nm. Narrower slit widths provide better resolution, but at the cost of less radiation reaching the detector. The easiest approach to selecting a

wavelength is to record the sample's emission spectrum and look for an emission line that provides an intense signal and is resolved from other emission lines.

Preparing the Sample

Flame and plasma sources are best suited for samples in solution and liquid form. Although a solid sample can be analyzed by directly inserting it into the flame or plasma, they usually are first brought into solution by digestion or extraction.

Minimizing Spectral Interferences

The most important spectral interference is broad, background emission from the flame or plasma and emission bands from molecular species. This background emission is particularly severe for flames because the temperature is insufficient to break down refractory compounds, such as oxides and hydroxides. Background corrections for flame emission are made by scanning over the emission line and drawing a baseline (Figure). Because plasma's temperature is much higher, background interference due to molecular emission is less of a problem. Although emission from the plasma's core is strong, it is insignificant at a height of 10–30 mm above the core where measurements normally are made.

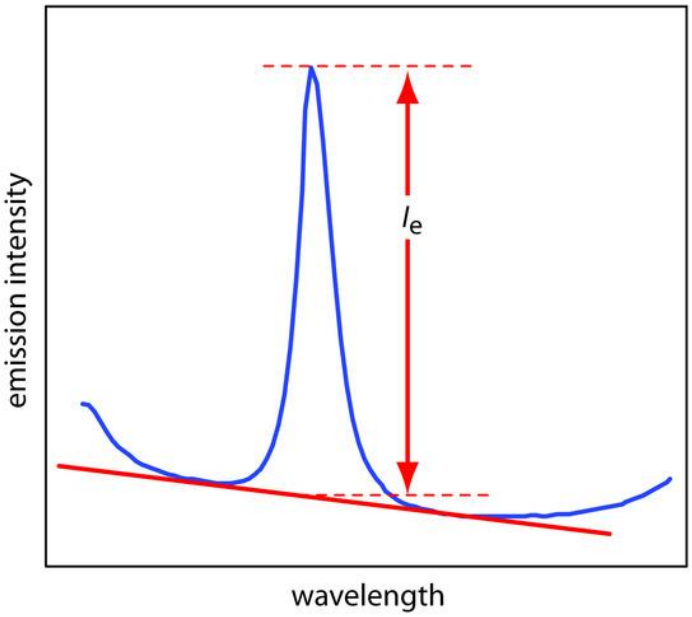


Figure : Method for correcting an analyte's emission for the flame's background emission.

Minimizing Chemical Interferences

Flame emission is subject to the same types of chemical interferences as atomic absorption. These interferences are minimized by adjusting the flame's composition and adding protecting agents, releasing agents, or ionization suppressors. An additional chemical interference results from **self-absorption**. Because the flame's temperature is greatest at its center, the concentration of analyte atoms in an excited state is greater at the flame's center than at its outer edges. If an excited state atom in the flame's center emits a photon while returning to its ground state, then a ground state atom in the cooler, outer regions of the flame may absorb the photon, decreasing the emission intensity. For higher concentrations of analyte self-absorption may invert the center of the emission band (Figure 10.61).

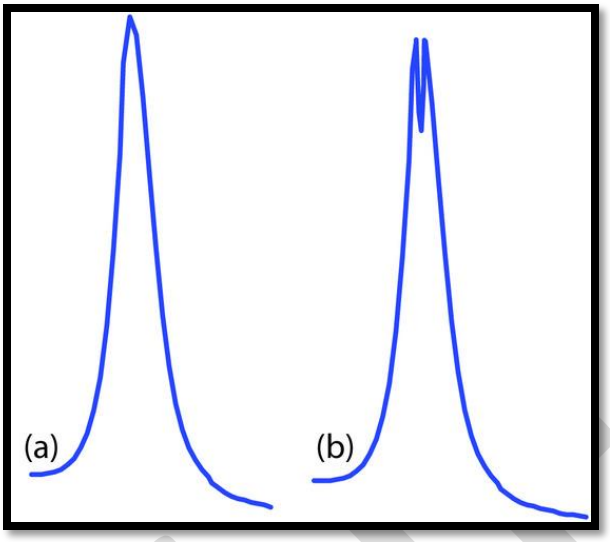


Figure: Atomic emission lines for (a) a low concentration of analyte, and (b) a high concentration of analyte showing the effect of self-absorption.

Chemical interferences with plasma sources generally are not significant because the plasma's higher temperature limits the formation of nonvolatile species. For example, PO_4^{3-} is a significant interferent when analyzing samples for Ca^{2+} by flame emission, but has a negligible effect when using a plasma source. In addition, the high concentration of electrons from the ionization of argon minimizes ionization interferences.

Standardizing the Method

From equation 10.30 we know that emission intensity is proportional to the population of the analyte's excited state, N^*. If the flame or plasma is in thermal equilibrium, then the excited state population is proportional to the analyte's total population, N, through the Boltzmann distribution (equation 10.31).

A calibration curve for flame emission is usually linear over two to three orders of magnitude, with ionization limiting linearity when the analyte's concentrations is small and self-absorption limiting linearity for higher concentrations of analyte. When using a plasma, which suffers from fewer chemical interferences, the calibration curve often is linear over four to five orders of magnitude and is not affected significantly by changes in the matrix of the standards.

Emission intensity may be affected significantly by many parameters, including the temperature of the excitation source and the efficiency of atomization. An increase in temperature of 10 K, for example, produces a 4% increase in the fraction of Na atoms occupying the 3p excited state. This is potentially significant uncertainty that may limit the use of external standards. The method of internal standards can be used when variations in source parameters are difficult to control. To compensate for changes in the temperature of the excitation source, the internal standard is selected so that its emission line is close to the analyte's emission line. In addition, the internal standard should be subject to the same chemical interferences to compensate for changes in atomization efficiency. To accurately compensate for these errors the analyte and internal standard emission lines must be monitored simultaneously.

The best way to appreciate the theoretical and practical details discussed in this section is to carefully examine a typical analytical method. Although each method is unique, the following description of the determination of sodium in salt substitutes provides an instructive example of a typical procedure. The description here is based on Goodney, D. E. *J. Chem. Educ.* **1982**, *59*, 875–876.

Representative Method

Determination of Sodium in a Salt Substitute

Description of Method

Salt substitutes, which are used in place of table salt for individuals on low–sodium diets, replaces NaCl with KCl. Depending on the brand, fumaric acid, calcium hydrogen phosphate, or potassium tartrate also may be present. Although intended to be sodium-free, salt substitutes contain small amounts of NaCl as an impurity. Typically, the concentration of sodium in a salt substitute is about 100 μg/g The exact concentration of sodium is easily determined by flame atomic emission. Because it is difficult to match the matrix of the standards to that of the sample, the analysis is accomplished by the method of standard additions.

Procedure

A sample is prepared by placing an approximately 10-g portion of the salt substitute in 10 mL of 3 M HCl and 100 mL of distilled water. After the sample has dissolved, it is transferred to a 250-mL volumetric flask and diluted to volume with distilled water. A series of standard additions is prepared by placing 25-mL portions of the diluted sample into separate 50-mL volumetric flasks, spiking each with a known amount of an approximately 10 mg/L standard solution of Na^+, and diluting to volume. After zeroing the instrument with an appropriate blank, the instrument is optimized at a wavelength of 589.0 nm while aspirating a standard solution of Na^+. The emission intensity is measured for each of the standard addition samples and the concentration of sodium in the salt substitute is reported in μg/g.

Questions

1. Potassium ionizes more easily than sodium. What problem might this present if you use external standards prepared from a stock solution of 10 mg Na/L instead of using a set of

standard additions?

Because potassium is present at a much higher concentration than sodium, its ionization suppresses the ionization of sodium. Normally suppressing ionization is a good thing because it increases emission intensity. In this case, however, the difference between the matrix of the standards and the sample's matrix means that the sodium in a standard experiences more ionization than an equivalent amount of sodium in a sample. The result is a determinate error.

2. One way to avoid a determinate error when using external standards is to match the matrix of the standards to that of the sample. We could, for example, prepare external standards using reagent grade KCl to match the matrix to that of the sample. Why is this not a good idea for this analysis?

Sodium is a common contaminant, which is found in many chemicals. Reagent grade KCl, for example, may contain 40–50 μg Na/g. This is a significant source of sodium, given that the salt substitute contains approximately 100 μg Na/g.

3. Suppose you decide to use an external standardization. Given the answer to the previous questions, is the result of your analysis likely to underestimate or overestimate the amount of sodium in the salt substitute?

The solid black line in Figure 10.62 shows the ideal calibration curve assuming that we match the matrix of the standards to the sample's matrix, and that we do so without adding an additional sodium. If we prepare the external standards without adding KCl, the emission for each standard decreases due to increased ionization. This is shown by the lower of the two dashed red lines. Preparing the standards by adding reagent grade KCl increases the concentration of sodium due to its contamination. Because we underestimate the actual concentration of sodium in the standards, the resulting calibration curve is shown by the other

dashed red line. In both cases, the sample's emission results in our overestimating the concentration of sodium in the sample.

4. One problem with analyzing salt samples is their tendency to clog the aspirator and burner assembly. What effect does this have on the analysis?

Clogging the aspirator and burner assembly decreases the rate of aspiration, which decreases the analyte's concentration in the flame. The result is a decrease in the emission intensity and a negative determinate error.

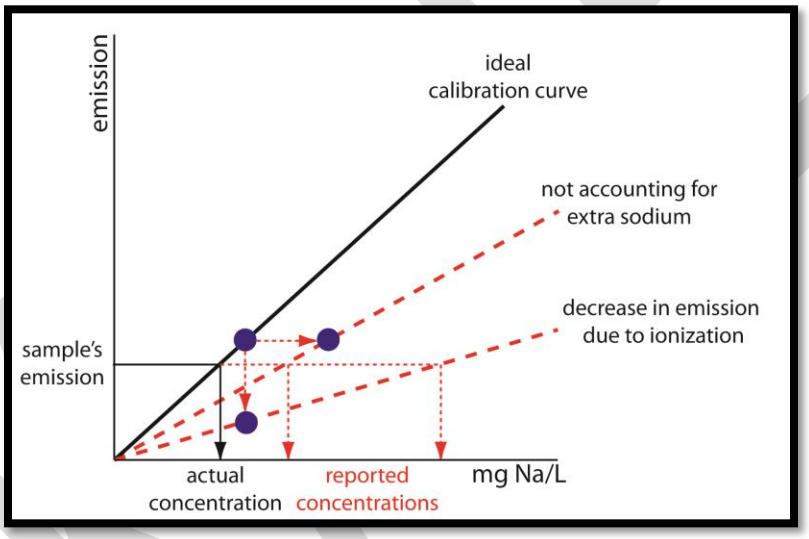


Figure : External standards calibration curves for the flame atomic emission analysis of Na in a salt substitute. The solid black line shows the ideal calibration curve assuming matrix matching of samples and standards with pure KCl. The lower of the two dashed red lines shows the effect of failing to add KCl to the external standards, which decreases emission. The other dashed red line shows the effect of using KCl that is contaminated with NaCl, which causes us to underestimate the concentration of Na in the standards. In both cases, the result is a positive determinate error in the analysis of samples.

Example

To evaluate the method described in Representative Method , a series of standard additions is prepared using a 10.0077-g sample of a salt substitute. The results of a flame atomic emission analysis of the standards is shown here.

added Na (μg/mL)	I_e (arb. units)
0.000	1.79
0.420	2.63
1.051	3.54
2.102	4.94
3.153	6.18

What is the concentration of sodium, in μg/g, in the salt substitute.

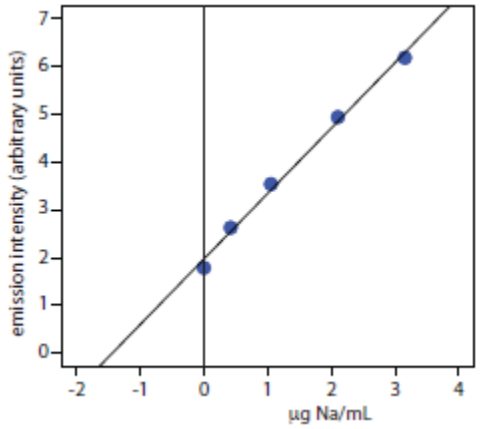
See Section 5C.3 in Chapter 5 to review the method of standard additions.

Solution

Linear regression of emission intensity versus the concentration of added Na gives a standard

additions calibration curve with the following equation.

I_e= 1.97 + 1.37 × g Na / mL



The concentration of sodium in the sample is equal to the absolute value of the calibration curve's *x*-intercept. Substituting zero for the emission intensity and solving for sodium's concentration gives a result of 1.44 µg Na/mL. The concentration of sodium in the salt substitute is

((1.44 g Na / mL) × (50.00 mL / 25.00 mL) × 250.0 mL) / 10.0077 g sample = 71.9 g Na/g

Evaluation of Atomic Emission Spectroscopy

Scale of Operation

The scale of operations for atomic emission is ideal for the direct analysis of trace and ultratrace analytes in macro and meso samples. With appropriate dilutions, atomic emission also can be applied to major and minor analytes.

See Figure 3.5 to review the meaning of macro and meso for describing samples, and the

meaning of major, minor, and ultratrace for describing analytes.

Accuracy

When spectral and chemical interferences are insignificant, atomic emission is capable of producing quantitative results with accuracies of between 1–5%. Accuracy frequently is limited by chemical interferences. Because the higher temperature of a plasma source gives rise to more emission lines, the accuracy of using plasma emission often is limited by stray radiation from overlapping emission lines.

Precision

For samples and standards in which the analyte's concentration exceeds the detection limit by at least a factor of 50, the relative standard deviation for both flame and plasma emission is about 1–5%. Perhaps the most important factor affecting precision is the stability of the flame's or the plasma's temperature. For example, in a 2500 K flame a temperature fluctuation of ±2.5 K gives a relative standard deviation of 1% in emission intensity. Significant improvements in precision may be realized when using internal standards.

Sensitivity

Sensitivity is strongly influenced by the temperature of the excitation source and the composition of the sample matrix. Sensitivity is optimized by aspirating a standard solution of analyte and maximizing the emission by adjusting the flame's composition and the height from which we monitor the emission. Chemical interferences, when present, decrease the sensitivity of the analysis. The sensitivity of plasma emission is less affected by the sample matrix. In some cases a calibration curve prepared using standards in a matrix of distilled water can be used for samples with more complex matrices.

Selectivity

The selectivity of atomic emission is similar to that of atomic absorption. Atomic emission has the further advantage of rapid sequential or simultaneous analysis.

Time, Cost, and Equipment

Sample throughput with atomic emission is very rapid when using automated systems capable of multielemental analysis. For example, sampling rates of 3000 determinations per hour have been achieved using a multichannel ICP, and 300 determinations per hour with a sequential ICP. Flame emission is often accomplished using an atomic absorption spectrometer, which typically costs between $10,000–$50,000. Sequential ICP's range in price from $55,000–$150,000, while an ICP capable of simultaneous multielemental analysis costs between $80,000–$200,000. Combination ICP's that are capable of both sequential and simultaneous analysis range in price from $150,000–$300,000. The cost of Ar, which is consumed in significant quantities, can not be overlooked when considering the expense of operating an ICP.

Reference Books :

Text Books:

T1: Sharma.B.K.(2012) Instrumental methods of chemical analysis (28th Edition). Meerut: Krishna Prakashan Media (p) Ltd

POSSIBLE QUESTIONS:

Part-A (20 x 1= 20 marks) Online Examinations

(Each Question Carry One Mark)

1. What is the visible,gaseous part of a fire.
a. Flame
b. Non-flame
c. oxygen
d. fuel
Answer: c

2. Which of a flame are dependent on the type of fuel involved in the combustion?
a. color
b. temperature
c. color and temperature
d. pressure
Answer: c

3. What are the transient reaction intermediates?
a. CH
b. C_2
c. CH and C_2
d. CH_2
Answer: b

4. What is produced in the burning of Hydrogen, chlorine?
a. Cl
b. HCl
c. H fumes
d. NaCl
Answer: a

5. What is said in the chemical combination of hydrazine and nitrogen tetroxide?
a. hypergolic
b. hyperpolic
c. hydrophilic
d. hydrophobic
Answer: a

6. The light produced is in the blue to green region of the spectrum below-------
a. 565 nm
b. 589 nm
c. 645 nm

d. 343 nm
Answer: b

7. The bunsen burner burns with yellow flame is also called ?
a. non-safety flame
b. safety flame
c. dangerous flame
d. luminous flame
Answer: a

8. The bunsen burner burns with yellow flame at around -----°C ?
a. 1,000°C
b. 1,800°C
c. 1,200°C
d. 1,400°C
Answer: c

9. In which radiating soot is produced due to a more complete combustion?
a. high black body
b. medium block body
c. less block body
d. normal level black body
Answer: c

10. The colder part of a diffusion (incomplete combustion) flame will be------?
a. Orange
b. Yellow
c. Red
d. Blue
Answer: a

11. In which flame, it no loss of heat to the atmosphere?
a. adiabatic
b. non-adiabatic
c. excothermic
d. endothermic
Answer: a

12. In which color compared to typical yellow color of the flames suggests that the temperature is lower?

a. Red
b. Green
c. Orange
d. Blue
Answer: a

13. What is the flame temperature of candle?
a. 1,400 °C
b. 1,450 °C
c. 1,560 °C
d. 1,230 °C
Answer: a

14. What is the flame temperature for charcole fire ?
a. 750 - 1,200 °C
b. 1,000 - 1,350 °C
c. 1,300 °C
d. 500°C
Answer: d

15. What is the maximum flame temperature for wood?
a. 1,400 °C
b. 1,450 °C
c. 1,560 °C
d. 1,027 °C
Answer: b

16. Given in the following, In which ----burns in oxygen with a bright blue-white flame at the temperature of 5,260 k and at up to 6,000 k in ozone.
a. C_3N_2
b. C_4N_2
c. C_2N_4
d. CN_2
Answer: b

17. The very weak flames are also called?
a. Hot flames
b. Cool flames
c. a and b
d. None of these

Answer: a

18. In which temperature, the fuel- air mixture can react chemically ?
a. low as 120 °C
b. low as 130 °C
c. low as 125 °C
d. high as 140 °C
Answer: c

19. In flame photometry the concentration of ----- metals are determined?
a. alkali only
b. alkaline only
c. alkali as well as alkaline
d. metals
Answer: a

20. The radiation emitted from the flames depends on the characteristic ----- present in the flame?
a. Element
b. Metal
c. Alloys
d.Non metal
Answer: a

Part-B (5 x 6 =30 marks)

(Each Question Carry Six Marks)

1. Explain the disadvantages of calibration curve

2 . What are the clinical applications of flame photometry?

3. Explain Scheibe-Lomakin equation.

4. What is a flame photometer?

5. What are the different branches of atomic absorption spectroscopy?

6. Give the mathematical expression of calibration curve.

7. Write notes on (i) Nebulization (ii) Sample delivery

8. Write notes on flame photometry?

9. Discuss the parts of flame photometer?

10. Write notes on flame temperature

Part-C (1 x10= 10 marks) Compulsory Questions

1. Write notes on flame atomizes?

2. Write notes on flame color?

3. Write notes on burner?

4. Describe the premix chamber burner and the total consumption burner. Compare them with respect to efficiency and sensitivity?

5. Describe the mechanism of operation of hallow-cathode lamp.

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DEPARTMENT OF CHEMISTRY

Class: II M.Sc Chemistry

Subject Title: Physical Methods in chemistry

Subject code: 17CHP303

UNIT-V

Multiple choice questions (Each question carry one mark)

1. What is the visible,gaseous part of a fire.
a. Flame
b. Non-flame
c. oxygen
d. fuel
Answer: c

2. Which of a flame are dependent on the type of fuel involved in the combustion?
a. color
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d. endothermic
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c. low as 125 ℃
d. high as 140 ℃
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b. alkaline only
c. alkali as well as alkaline
d. metals
Answer: a

20. The radiation emitted from the flames depends on the characteristic ----- present in the flame?
a. Element
b. Metal
c. Alloys
d.Non metal
Answer: a

21. Given the following spectroscopy, in which spectroscopy is well established in the qualitative and quantitative analysis?
a. Atomic spectroscopy
b. UV spectroscopy
c. IR Spectra
d. flame spectra
Answer: c

22. What are the types of branches of atomic absorption spectroscopy?
a.1
b. 2
c. 3
d. 4
Answer: a

23. What is the abbreviation of ICP-AES?
a. Inductively coupled plasma atomic emission spectrometry
b. Inductively coupled plasma atomic energy spectrometry
c. Inductively coupled plasma atomic emission spectroscopy
d. Induced coupled plasma atomic energy spectrometry
Answer: a

24. What is the abbreviation of IUPAC?
a. International union of pure and applied chemistry
b. International union of pure and analytical chemistry
c. International union of pure and industrial chemistry
d. International un-union of pure and analytical chemistry
Answer: a

25. In which metal ion are used in flame photometry?
a. Sodium
b. Carbon
c. Magnesium
d. sulphur
Answer: c

26. In which helps to transport the homogeneous solution of the substance into flame ?
a. Optical system
b. Photo detector
c. Nebuliser and mixing chamber
d. source of flame
Answer: b

27. The optical system comprises ---- parts?
a. 5
b. 3
c. 4
d.2
Answer: a

28. In the photo detector, the electrical signals are directly proportional to the ---- of light ?
a. Intensity
b. velocity
c. pressure
d. volume
Answer: b

29. The intensity of emitted light is related to the concentration of the----?
a. Metal
b. Element
c. Non-metal
d. metal ion
Answer: c

30. The temperature of the flame depends?
a. fuel
b. oxidant

c. fuel-oxidant ratio
d. intermediate
Answer: a

31. In which ions in the solvent to metal atoms by the flame heat?
a. Reduction of metal
b. oxidation of metal
c. bromination
d. hydrogenatiom
Answer: b

32. What is the Scheibe-Lomakin equation?
a. $I = K X C^{m}$
b. $I = K X C^{n}$
c. $I = K X C^{n-m}$
d. $I = K X C^{n+m}$
Answer: a

33. In the flame photometry, in which element cannot be detected?
a. Carbon
b. Nitrogen
c. Magnesium
d. Helium
Answer: a

34. What is the abbreviation for FES?
a. Flame emission spectroscopy
b. Flame energy spectroscopy
c. Flame emission spectrometer
d. None of these
Answer: b

35. How many methods are present in the instrumentation for flame spectrometric?
a. 3
b. 5
c. 4
d. 2
Answer: a

36. Droplets larger than about ----- µm are trapped in the spray chamber and flow to waste
a. 20
b. 30
c. 16
d. 15
Answer: b

37. The components of the flame gases limit the usable range to wavelengths longer than ----nm
a. 200
b. 210
c. 220
d. 260
Answer: a

38. How many types of components are present in the flame photometer?
a. 5
b. 4
c. 2
d. 1
Answer: a

39. How many requirements are used in the flame?
a. 3
b. 2
c. 4
d. 5
Answer: a
40. How many types in the atomizers?
a. 2
b. 3
c. 1
d. 4
Answer: a

41. Why is a monochromator used in flame photometry?
a. To eliminate spectral interference
b. To joined spectral interference
c. spectral peak
d. spectral orientation
Answer: b

42. What type of detectors can be used in flame photometers?
a. Phototube
b. PMT
c. ionization
d. photometer
Answer: a

43. What substances are used as internal standard?
a. Lithium
b. Carbon
c. Magnesium
d. Nitrogen

Answer: a

44. How many kinds of molecular interference can occur?
a. 5
b. 3
c. 2
d. 6
Answer: a

45. Which technique is most used in the atomic spectroscopy determination?
a. paeumatic nebulization
b. trubitic nebulization
c. active nebulization
d. bulk nebulization
Answer: a

46. What is used for flame?
a. propane
b. methane
c. ethane
d. pentane
Answer: c

47. The atomic spectroscopy is used for--------- analysis ?
a. Organic chemical
b. Physical chemical
c. Inorganic chemical
d. Analytical chemical
Answer: b

48. The metal particle in the flame are dehydrated and solvent is evaporated
a. vapourisation
b. desolvation
c. atomization
d. excitation
Answer: c

49. In the reduction of metal ion takes place in ------ process
a. desolvation
b. vapourisation
c. atomization
d.emission
Answer: c

50. Ions in solution converted into aerosol by ------ process
a. vapourisation

b. desolvation
c. aspiration
d. spray
Answer: a

51. Array of detectors is known as
a. charge coupled device
b. current unit
c. energy circuit
d. energy emission
Answer: c

52. Charge coupled devices also known as
a. Phototube
b. Photo detector
c. photodiode arrays
d. photometer
Answer: c

53. The remaining wavelength of lights in the absorption combines with eye to give a ----- appearance
a. green
b. pale yellow
c. pale blue
d. violet
Answer: d

54. The visible part in the spectral absorption source is -------
a. duterium lamb
b. sodium lamb
c. hydrogen lamb
d. tungsten lamb
Answer: a

55. The burner consists of single and multiple holes are called as
a. Meker style burner
b. punsen burner
c. gas purner
d. other types
Answer: a

56. Ni shows the atomic emission line at nm
a. 349.3
b. 240
c. 321
d. 412

Answer: b

57. Fe shows the spectra line at ---- nm
a. 321
b. 349.06
c. 435
d. 534
Answer: a

58. What is the meaning of incandescence?
a. emission of radiation from hot body
b. emission of radiation from cool body
c. emission of radiation from black body
d. emission of radiation from flame
Answer: a

59. The flame in bunsen burner depend upon the
a. oxygen supply
b. nitrogen supply
c. helium supply
d. hydrogen supply
Answer: d

60. Spectro radiometers are design for measure the ------
a. spectral line
b. spectral length
c. spectral peak
d. spectral density
Answer: a