



NATIONAL OPEN UNIVERSITY OF NIGERIA

SCHOOL OF SCIENCE AND TECHNOLOGY

**COURSE CODE: CHM312**

**COURSE TITLE: Instrumental Methods of Analysis**

# Module 1: Spectroscopic Analyses

## Unit 1: Spectroscopic Techniques

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## 1.0 Introduction

Spectroscopic method of analysis involves the measurements of the intensity and wavelength of radiation that is either absorbed or transmitted. This provides the basis for sensitive methods of detection and quantitation. Absorption spectroscopy is most frequently used in the quantitation or estimation of molecules and some atoms. Emission spectroscopy covers several techniques that involve the emission of radiation by either atoms or molecules, but varies in the manner in which the emission is induced. This method of analysis involves the use of equipment, which may be simple and inexpensive, or extremely complex, with design features involving the latest technological development.

## 2.0 Objectives

At the end of this unit, student should be able to:

- Explain the meaning of spectroscopy;
- Describe the nature of electromagnetic radiation;
- Describe the interaction of radiation with matter;
- Distinguish between absorption and emission spectroscopy; and
- State the different types of spectroscopy.

## 3.0 Definition of spectroscopy

Spectroscopy is a technique which concerned with the study of the frequencies involved when electromagnetic radiation interacts with matter.

### 3.1 Interaction of radiation and matter

Radiation is a form of energy which could either be absorbed or transmitted. The interaction of radiation in spectroscopy involves transition between the different energy levels of atoms or molecules. The other types of interactions such as reflection, refraction and diffraction, are often related to the bulky properties of materials rather than to energy levels of specific atoms or molecules. Generally, the absorption or emission of radiation by matter involves the exchange of energy. Thus, to understand the principle of this exchange, it is necessary to know the distribution of energy within an atom or molecule. The internal energy of a molecule is due to the energy associated with:

- ❖ The electrons
- ❖ Vibrations between atoms
- ❖ The rotation of various groups of atoms within a molecule

The energy levels can be altered by the absorption or emission of radiant energy. This is because atoms exist only within a limited number of energy levels. A study of the wavelength

or frequency of radiation absorbed or emitted by an atom or molecule will give information about its identity. This technique is known as qualitative spectroscopy.

### 3.2 Electromagnetic radiation

Radiation is a form of energy which has both magnetic and electrical properties, hence called electromagnetic radiation. The electromagnetic radiation covered a long range of radiations which are described or characterised by either wavelength or frequency.

- Wavelength ( $\lambda$ ) is defined as the distance between the successive peaks which is measured in the unit nanometers (nm).
- Frequency ( $\nu$ ) of radiation is defined as the number of successive peaks passing a given point in 1 second.

The relationship between the two parameters is:

- $\nu \propto \frac{1}{\lambda}$
- But, the energy (E) is directly proportional to the frequency and inversely proportional to wavelength.

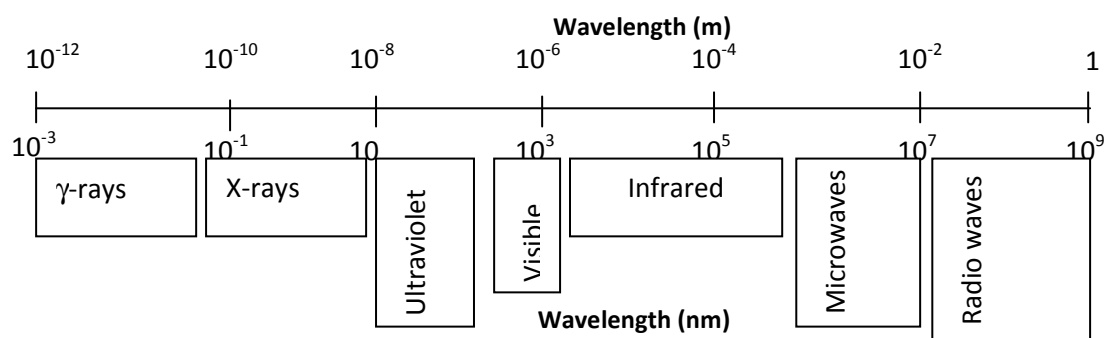
$$i.e. E = h\nu = \frac{hc}{\lambda}$$

- Where,  $h$  is a Plank's constant and  $c$  is the speed of light

The electromagnetic radiation consists of:

- Gamma rays ( $\gamma$ ) in the range of  $10^{-3}$  to  $10^{-1} nm$
- X-rays in the range of 1 to  $10^3 \text{ \AA}$
- Ultraviolet radiation in the range of 180 to 380 nm
- Visible region in the range of 380 to 780 nm
- Infra red region in the range of  $0.78 \mu m$  to  $50 \mu m$
- Microwave in the range of  $10^{-2}$  to 10 cm
- Radiowave in the range of  $10^7$  to  $10^9 nm$

This can also be represented as bellow:



Source: Holme, D.J. and Hazel, P. 1998.

Fig. 1.1: Range of electromagnetic radiation

### 3.3 Absorption of radiation

Every molecular specie is capable of absorbing its own characteristic frequencies or wavelength of electromagnetic radiation. This process transfers energy to the molecule or atom and results in a decrease in the intensity of the incident radiation. Thus, absorption of radiation could be:

- Atomic absorption which involves the absorption of electromagnetic radiation by free atomic species. The wavelength that is most strongly absorbed usually corresponds to an electronic transition from the ground state to the lowest excited state known as the resonance line.
- Molecular absorption which involves the absorption of radiation by molecules in solution. This type of absorption usually takes place in ultraviolet and visible region of the electromagnetic spectrum. Absorption of radiation in this region causes transition of electrons from molecular bonding orbital to the higher energy molecular antibonding orbital.

### 3.4 Emission of radiation

When an atom or molecule absorbs electromagnetic radiation, it will result in either electronic or vibrational transition from lower to higher energy level. Such atom or molecule is said to be in an excited or unstable state. If the atom or molecule loses all or part of this energy as radiation, photons of energy will be emitted which correspond to the difference between the energy levels involved. The radiation emitted is of specific frequencies and will show up as a bright line if dispersed as a spectrum. Just like absorption, emission could also be atomic or molecular.

Atomic emission is displayed by many elements; particularly the metals which after being excited either thermally or electrically emit a discontinuous lines spectrum due to transition ending in the ground state.

Molecular emissions are due to electronic transition within the molecule but are modified or influenced by bond length. Molecular emissions are more complex than atomic emissions. The radiation emitted consists of broad bands of radiation rather than the narrow lines associated with atomic emission. Also the resulting spectrum is approximately the mirror image of the absorption spectrum of the compound.

### 3.5 Types of spectroscopy

Based on the nature of the radiation that is being absorbed or emitted, there are different types of spectroscopy. These could be:

- ❖ Ultraviolet/visible spectroscopy
- ❖ Infra-red (I R) spectroscopy

- ❖ Nuclear Magnetic Resonance (NMR) spectroscopy
- ❖ Mass spectroscopy
- ❖ X- ray spectroscopy

Apart from the above classification, spectroscopy can also be broadly classified into three categories:

- ❖ Electronic spectroscopy
- ❖ Vibrational spectroscopy
- ❖ Rotational spectroscopy

The electronic spectroscopy involves the movement of electrons between their various energy levels, as a result of absorption or emission of electromagnetic radiation. The energy required for electronic transition comes from ultraviolet or visible region.

Vibrational spectroscopy is concerned with the vibrations of molecules when photons of radiation are absorbed. Molecules can vibrate with particular amounts of energy whose values are determined by a quantum number. The energy needed for vibration of molecule comes from the infra red region.

Rotational spectroscopy is concerned with the energy that causes the rotation of a molecule. The energy of the rotational level depends on the rotational quantum numbers. The energy required for the molecular rotation is provided by the micro wave region of the spectrum.

#### **4.0 Conclusion**

Spectroscopic method of analysis, is a technique which involves the measurements of the intensity of radiation that is either absorbed or transmitted.

#### **5.0 Summary**

In this unit we have learnt that:

- Spectroscopy is a technique which involves the study of the absorption and emission of radiation by matter.
- Electromagnetic radiation or spectrum covers a long range of radiations measured in the unit of frequency or wavelength.
- Absorption spectroscopy measures electromagnetic radiation that is taken in by atoms or molecules.
- Emission spectroscopy measures the radiation that is given out by atoms or molecules
- Spectroscopy could be electronic, infra-red (or Vibrational) and microwave (or rotational).

#### **6.0 Tutor Marked Assignment (T M A)**

1. What is meant by spectroscopy?
2. Distinguish clearly between absorption and emission spectroscopy
3. Calculate the corresponding energy of a UV-radiation having a wavelength of 250nm

## 7.0 Further reading/ References

- 1.0 Robert, M.S., Clayton, G.B. and Terence, C.M., (1974). *Spectrometric Identification of Organic Compounds*, 3<sup>rd</sup> Edition, Wiley International
- 2.0 David, J.H. and Hazel, P., (1998). *Analytical Biochemistry*, Third edition, Longman
- 3.0 Douglas, A.S. , Donald, M.W., James, F.H. and Stanley, R.C., (2004). *Fundamentals of Analytical Chemistry*, Eight Edition.

## Unit 2 : Spectrochemical optical methods

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## **Unit 2: Spectrochemical optical methods**

### **1.0 Introduction**

Optical method of analysis involves the measurement of light intensity. To relate optical measurement to the amount of a particular substance in a sample, it is necessary to exploit the ability of atoms or molecules to absorb or emit light radiation when exposed to a particular set of conditions. Thus this method of analysis involves the use of instrument with varying degrees of sophistication.

### **2.0 Objectives**

At the end of this unit the student should be able to:

- Explain the different types of optical method of analysis;
- Explain the operational principle of molecular absorption method of analysis;
- State the basic laws of light absorption;
- Differentiate between colorimetry and spectrophotometry; and
- Name the basic components of colorimeter and spectrophotometer and state their functions.

### **3.0 Definition**

Optical methods are spectroscopic techniques that are based on the absorption or emission of ultraviolet, visible and infrared radiation.

### **3.1 Types of optical methods of analysis**

- Colorimetry which involves the measurement of the absorption of light radiation by a coloured solution.
- Spectrophotometry which measures the absorption of light of a narrow wavelength band by molecules in solution.
- Atomic absorption analysis which measures the absorption light radiation by a free atomic specie.
- Fluorimetry which involves the estimation of the amount of fluorescent substance in a given sample.
- Atomic emission analysis which based on the emission light atoms in excited electronic state.

### **3.2 Molecular absorption analysis**

The absorption of light by a compound in solution increases with the concentration of the compound and this effect is fully exploited in colorimetry and spectrophotometry. Coloured compounds can be estimated directly while other compounds which give coloured derivatives with particular chemical reagents can also be analysed.

The absorption of light radiation by solutions can be elucidated by a combination of the laws of Beer and Lambert. These two laws relate the absorption to concentration and to the thickness of the absorbing layer respectively.

### 3.2.1 Beer's law

This law states that the absorption of light is directly proportional to the number of the absorbing molecules. That is, the transmittance decreases exponentially with the number or concentration of the absorbing molecules.

Mathematically, Beer's law is represented as:

$$\text{Log}_{10} \frac{I_0}{I} \propto C \quad \text{or} \quad \text{Log}_{10} \frac{I_0}{I} = \epsilon C$$

Where,  $\text{Log}_{10} \frac{I_0}{I}$  is the absorbance (A), C is the concentration and  $\epsilon$  is a constant

$I_0 =$  incident light,  $I =$  transmitted light

This law can also be represented as:  $A \propto C$  or  $A = \epsilon C$

### 3.2.2 Lambert's law

This law states that same proportion of incident light is absorbed per unit thickness irrespective of its intensity, and that each successive unit layer absorbs the same proportion of light falling on it. For example, if the incident light is 100% and 50% of it is absorbed per unit layer, the intensity of light will decrease exponentially as follows: 50%, 25%, 12.5%, 6.25%, etc .

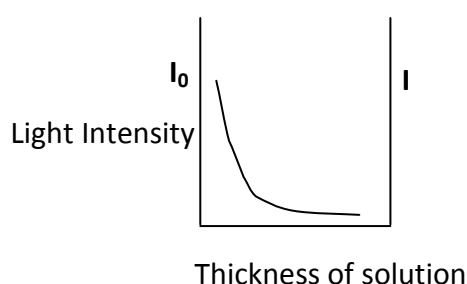


Fig 2.1: Light Intensity decreasing while passing through a solution

Thus, according to this law,  $\text{Log}_{10} \frac{I_0}{I} \propto l$  or  $\text{log}_{10} \frac{I_0}{I} = kl$

Note that: absorbance (A) =  $\text{log}_{10} \frac{I_0}{I} = kl$

Where,  $k$  is a constant and  $l$  is the path length.

The two laws are combined together and called Beer-Lambert's law:

$$A = \log_{10} \frac{I_0}{I} \propto Cl \quad \text{or} \quad \log_{10} \frac{I_0}{I} = \epsilon cl$$

where,  $\epsilon$  is a constant called molar extinction coefficient (or molar absorptivity) which is numerically equal to the absorbance of a molar solution in a cell of 1cm path length.

Note: While Lambert's law holds for all cases, Beer's law is only obeyed by dilute solutions. This is because at certain concentrations association of absorbing molecules occurs which causes a tailing off in the absorption of light. Hence, estimation of the unknown is carried out in the concentration range which Beer's law is obeyed. It is usual to plot a standard curve or graph of absorbance against concentration to determine the concentration range in which Beer's law is obeyed.

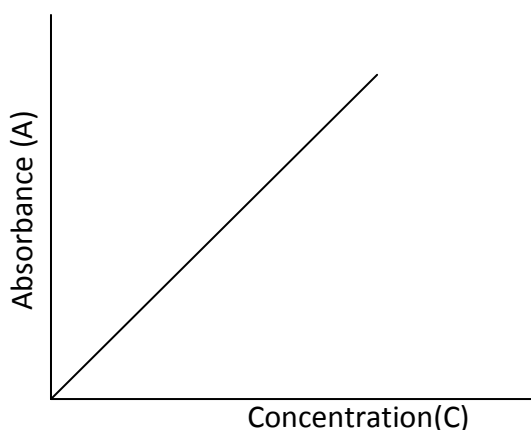


Fig. 2.2: A graph of Absorbance against Concentration

### 3.2.3 Limitations of Beer's Law

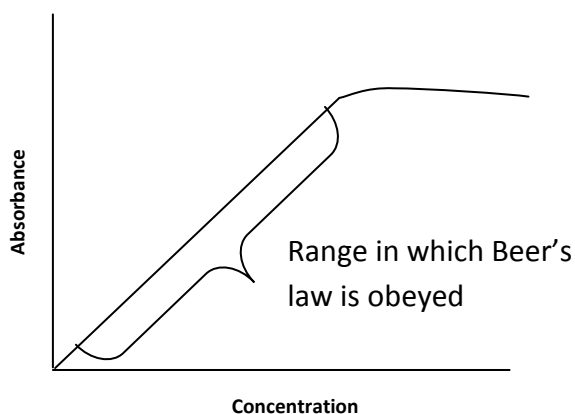


Fig. 2. 3: Graph showing the limitations of Beer's Law

Beer's law describes the absorption behaviour only for dilute solutions, hence it is limited. At concentrations exceeding 0.01M, the average distances between the absorbing ions or molecules are reduced to a point where each particle affects the charge distribution, and the extent of absorption of its neighbours. Since the extent of interaction depends on concentration, the occurrence of this phenomenon causes deviations from the linear relationship between absorbance and concentration. On the other hand, at certain concentrations, association of absorbing molecules is thought to occur, which causes a tailing off in the absorption of light. Hence, Beer's law is only obeyed by dilute solutions, but Lambert's law holds for all cases.

### 3.2.4 Absorbance and transmittance

The absorbance (A) is the measure of the fraction of light radiation that is absorbed by a given sample solution, while transmittance (T) is the fraction of incident light that is not absorbed (i.e. transmitted by the solution). Transmittance is often expressed as a percentage called percent transmittance.

$$\text{Percentage transmittance (T)} = \frac{I}{I_0} \times 100$$

But, absorbance is related to transmittance as follows:

$$A = \log_{10} \frac{100}{T}$$

Note that as the absorbance of a solution increases, the transmittance decreases.

### 3.3 Colorimetry

This method of analysis involves the measurement of the absorption of visible radiation of light by a coloured solution. This type of measurement is carried out by the use of an instrument called colorimeter. The colorimeter consists of:

- Light source (tungsten lamp)
- Monochromator (filter)
- Slit
- Optical cell or cuvette
- Photo electric cell
- Galvanometer

The instrumental set up is as bellow:

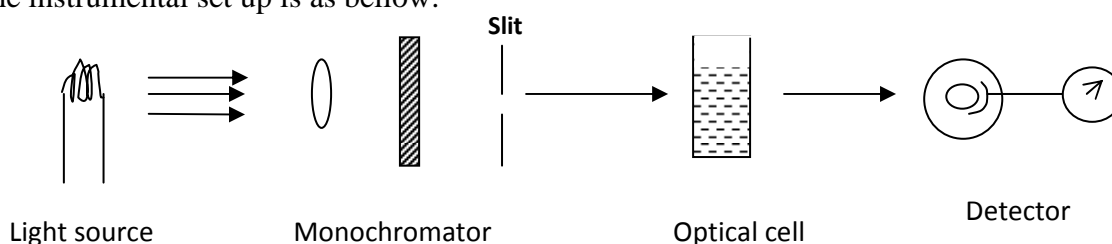


Fig. 2.4: Schematic diagram of a simple colorimeter

#### 3.3.1 Mode of operation

White light from a tungsten lamp passes through a condenser lens to give a parallel beam which falls on the filter that is positioned to select radiation of specific wavelength to impinge on a glass cuvette containing the solution. As the light is passing through the solution, some

part of it is absorbed by the sample component, while the part that is not absorbed is transmitted, and detected by a photo electric cell (detector). In order to measure the absorbance of a solution, the meter reading is first adjusted to 100% transmittance (zero absorbance) with a blank solution. The sample is then inserted in place of the blank and the absorbance is read directly. The concentration corresponding to the absorbance of the sample is then obtained from the standard or calibration graph.

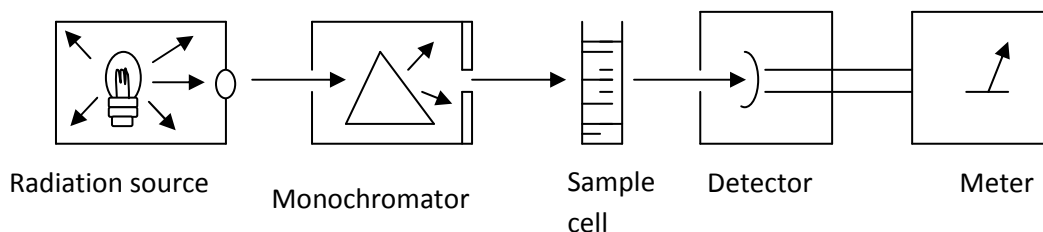
### 3.4 Spectrophotometry

This method of analysis involves the measurement of the absorption of a narrow wavelength band of radiation by molecules in solution. The instruments that are used to study or to measure the absorption or emission of electromagnetic radiation as a function of wave length are called spectrometers or spectrophotometers. The optical and electronic principles employed in these instruments are basically the same for all the regions of the spectrum.

The essential components of a spectrophotometer include:

- ❖ stable source of radiant energy
- ❖ system of lenses, mirrors and slits which define collimate(make parallel) and focus the beam of light
- ❖ monochromator to resolve the radiation into its individual wavelength
- ❖ cuvette or cell for holding the sample
- ❖ radiation detector with an associated read out system(meter or recorder)

Below is a schematic diagram of a typical spectrophotometer:



**Fig. 2.5: Components of a Spectrophotometer**

**Source: Analytical biochemistry by David J. Holme and Hazel Peck, 1998**

#### 3.4.1 Radiation source

The source of ultraviolet radiation is provided by hydrogen and deuterium lamps. These lamps consist of a pair of electrodes which are enclosed in a glass tube provided with a quartz window and filled with hydrogen or deuterium gas at low pressure. These lamps provide radiation in the range of 180 – 350nm.

A tungsten filament lamp is the most satisfactory source of visible and near infrared radiation. This lamp provides radiation in the region between 350 and 2500nm. Other sources of radiation include: xenon lamp , Globar and Nernst glower.

### 3.4.2 Monochromator

Monochromator is a device which is used to resolve polychromatic radiation into its individual wavelength and isolates these wavelengths into a very narrow band. This device produces light radiation of only particular (single) wavelength. Thus, light of single or only one wavelength is called monochromatic radiation. A monochromator consists of:

- ✓ an entrance slit which admits polychromatic radiation from the source;
- ✓ a collimating device which is either a lens or a mirror;
- ✓ a dispersion device, prism or grating which resolves the radiation into its individual components;
- ✓ a focusing lens or mirror; and
- ✓ an exit slit.

### 3.4.3 Detectors

A detector is a device which identifies and translates the transmitted radiation from the sample into an electrical signal that activates a meter or recorder. Example, a photographic film can be used as a detector. A detector usually indicates the existence of some physical phenomenon. It is capable of absorbing the energy of photons and converts it into a measurable quantity. Photoelectric detectors are used for the detection of ultraviolet and visible radiations. These detectors are classified as phototubes and photoelectric cells.

Another detector is photoconductive cell which is commonly used for the detection of near infrared radiation. But for middle and far infrared radiation, thermocouple detectors are used.

Generally, the detectors used in spectrophotometric instruments could be:

- photoelectric or barrier cell.
- photomultiplier tube.
- thermocouple.
- photoconductivity cell.

### 3.5 Differences between colorimeter and spectrophotometer

Unlike colorimeter, spectrophotometer can discriminate effectively between compounds with overlapping absorption spectra. The filter system found in colorimeter is replaced by prism or grating in spectrophotometer. The light passing through the sample solution is detected by a photocell in colorimeter, while it is detected by photomultiplier tube in most spectrophotometers. With most spectrophotometers, measurements can be done in both ultraviolet and visible region of the spectrum, which is not possible with colorimeters. Thus, the advantages displayed by spectrophotometer in comparison with colorimeter are improved resolution and sensitivity together with greater versatility. The more elaborate spectrophotometers have a scanning device which allows for the automatic determination of

the absorbance of a solution as a continuous function of wavelength. Spectrophotometers with direct reading device are also available.

#### **4.0 Conclusion**

Spectrochemical optical method of analysis is concerned with the measurement of the light radiation that is either absorbed or emitted by molecules in solution. This is achieved by the use of different types of equipment which may be simple or complex.

#### **5.0 Summary**

In this unit we have learnt that:

- Optical methods are spectroscopic techniques that are based on the absorption or emission of ultraviolet, visible and infrared radiation.
- There are different types of optical methods of analysis such as colorimetry, spectrophotometry, etc.
- The absorption of light radiation increases with the corresponding increase in concentration of the molecules in solution.
- Colorimetry involves the measurement of light absorption by coloured solution
- Spectrophotometry is the method which involves the measurement of the absorption of a narrow wavelength band by molecules in solution.

#### **6.0 Tutor Marked Assignment**

1. a. State Beer's and Lambert's laws.  
b. What is the relationship between absorbance and transmittance?
2. a. Distinguish clearly between a colorimeter and a spectrophotometer  
b. Several spectrophotometers have scales that are read either in absorbance or in % transmittance. What would be the absorbance reading at 20% T and 80% T respectively?

#### **7.0 Further reading/References**

1. David, J. H. And Hazel, P. (1998) . *Analytical biochemistry, third edition*, Longman.
2. Robert, M.S., Clayton, G.B. and Terence, C.M. (1974). *Spectrometric Identification of Organic Compounds*, 3<sup>rd</sup> Edition, Wiley International.
3. Daniel, C. H., (1982). *Qualitative Chemical Analysis*, 2<sup>nd</sup> Edition.

## Unit: 3 Infrared spectroscopy

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## Unit 3: Infrared spectroscopy

### 1.0 Introduction

Infrared spectrophotometry is a branch of spectroscopy which is concerned with the measurement of absorption of electromagnetic radiation, by molecules due to their vibrational ability. It is very important here to note that atoms in a molecule usually vibrate within the bonds that link them together. The vibrational energy in a molecule is obtained within infrared region of the electromagnetic spectrum. Thus, when ever an infrared radiation of a specific frequency interacts with a molecule, the energy is absorbed leading to an increased vibrational energy of that bond. This can even lead to change in the dipole moment of the bond concerned. Thus, the infrared instrument (spectrophotometer) is designed to measure the energy absorbed by the bond in the molecule at different wavelengths (nm) or wave number ( $\text{cm}^{-1}$ ) to produce a chart called infrared spectrum.

### 2.0 Objectives

By the end of this unit, student should be able to:

- explain the basic principles of infrared spectrometry;
- describe the different types of molecular vibrations;
- describe the instrumental arrangement of infrared spectrophotometer; and
- interpret the IR spectrum.

### 3.0 Definition

Infrared spectroscopy is a method which involves the study of the absorption of electromagnetic radiation in the range of 0.78 to  $300\mu\text{m}$ .

### 3.1 Basic principles of infrared spectrometry

Every molecule possess kinetic energy (E) which is responsible for its vibration or rotation. The total energy ( $E_t$ ) of a molecule is due to vibrational, rotational and translational energy within the molecule. Thus, the total kinetic energy ( $E_t$ ) is given by the expression:

$$E_t = E_v + E_r + E_{tr}$$

Where,

$E_v$  is vibrational energy,  $E_r$  is rotational energy and  $E_{tr}$  is the translational energy.

A molecule containing n atoms will have 3n mode degrees of freedom of motion. These are made of 3 rotational, 3 translational and  $3n - 6$  vibrational motions. Thus, for a non linear poly atomic molecule, the fundamental mode of vibration is  $3n - 6$  while a linear molecule has  $3n - 5$ . With this, it is possible to predict theoretically the number of infrared bands that can be obtained from a given molecule.

Example: Ethylmethylketone,  $\text{CH}_3 - \text{CH}_2 - \text{CO} - \text{CH}_3$  which is made up of 13 atoms, has 33 theoretical mode of vibration. This is calculated as below:

I In  $CH_3 - CH_2 - CO - CH_3$ ,  $n = 13$

$$\text{Then, } 3n - 6 = (3 \times 13) - 6 = 33$$

But, it is not possible to detect all of these vibrations in the IR spectrum because many of these bands may overlap, others may be symmetrical vibration which may not absorb any radiation. In a complex molecule, stretching and bending of bond is possible and these depend on the bond strength and masses of the corresponding atoms that form such bond.

### 3.2 Types of molecular vibrations

In an organic molecule there are two major types of fundamental vibrations. These are:

- ❖ Stretching of bond
- ❖ Bending of bond

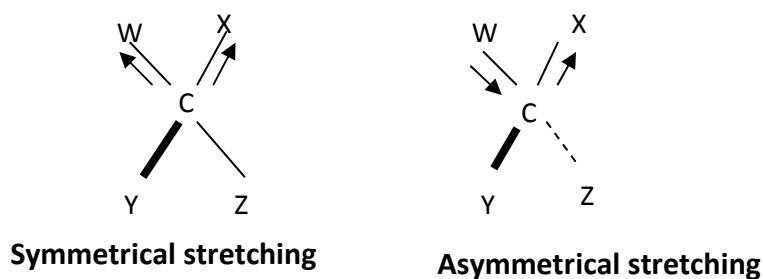


Fig. 3.1: Stretching vibrations

The stretching vibration could either be symmetrical or asymmetrical. These are represented as above

But bending vibration is of 4 different types. These are:

- ✓ Scissoring
- ✓ Rocking
- ✓ Wagging
- ✓ Twisting

These are represented as follows:

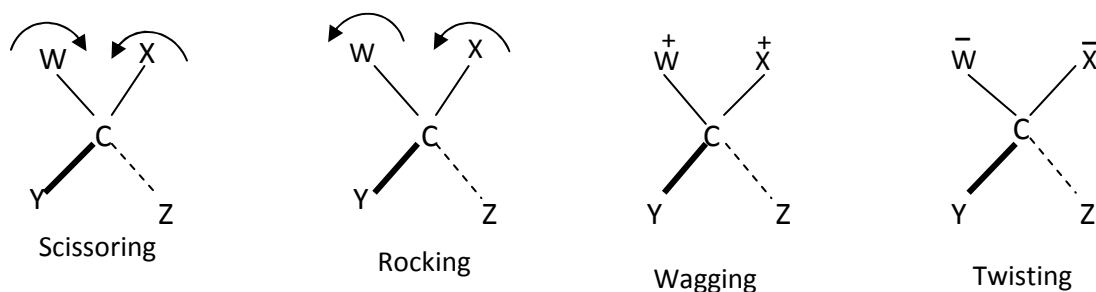


Fig. 3.2: Bending vibrations

The energy required to bend a bond is not great and falls within the range of  $400-1300\text{cm}^{-1}$ . This region is called the finger print region. Thus, this region is used to establish the identity of the chemical compounds. The energy required to stretch a bond is a little bit higher. This falls within the

region of  $1300 - 4000\text{cm}^{-1}$ . This signal is caused by groups such as OH, NH, C=O, C=C, CHO, etc. These group frequencies are independent of other parts of the molecule and are used to detect the functional groups in molecules.

### 3.3 Group frequencies

Group frequencies are the absorption bands or signals that occurs at certain frequencies due to stretching or bending vibration within a molecule. For example, the bands at  $3300\text{cm}^{-1}$  and  $1050\text{cm}^{-1}$  are characteristics of the OH group in alcohols. Group frequencies can change depending on the nature of the molecule and the solvent.

Example of some group frequencies are given below:

Vibration	type of molecule	group frequencies ( $\text{cm}^{-1}$ )
$C - H_{stretch}$	Alkanes, alcohols	2800 – 3000
$C - H_{stretch}$	Aldehydes	2700 – 2900
$C - H_{stretch}$	Alkenes	3010- 3095
$O - H_{stretch}$	Alcohols, phenols	3200 – 3600
$O - H_{stretch}$	Acids	2500 – 3000
$O - H_{bend}$	Alcohol, phenol	1260 – 1410
$N - H_{stretch}$	Amines	3300 – 3500
$C = C_{stretch}$	Alkenes	1620 – 1680
$C = O_{stretch}$	Aldehyde	1720 – 1740
$C \equiv C_{stretch}$	Alkynes	2100 – 2140
$C \equiv N_{stretch}$	Nitriles	2000 – 2500
$C = O_{stretch}$	Ketones	1705 – 1725
$C = O_{stretch}$	Carboxylic acid	1700 – 1725

### 3.4 Instrumentation

The instrument used is called infrared spectrophotometer. This instrument is basically similar to those used for the UV/ Visible measurements, but only differ from the energy or radiation sources, optical materials and the detection device.

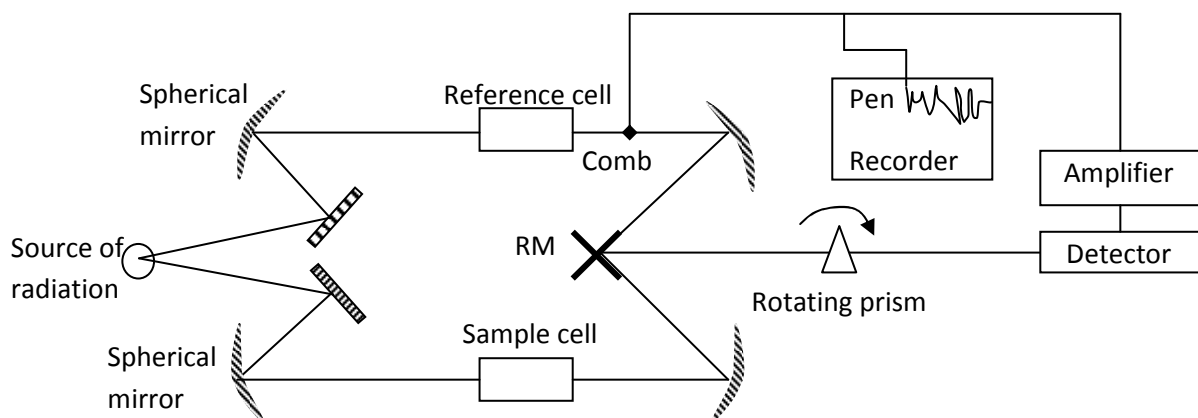


Fig. 3.3: The schematic diagram of infra-red spectrophotometer

Source: Peter R.S. Murray, 1983

#### 3.4.1 Radiation source

The radiation source may be:

- Nernst glower which is a mixture of oxides of zirconium or thorium
- Globar unit which is a small rod of silicon carbide

#### 3.4.2 Monochromator

This may be a prism or grating. The prism monochromator is usually made of sodium chloride crystals which disperse electromagnetic radiation between  $4000$  and  $650\text{cm}^{-1}$ . The grating systems monochromator have a better resolving power and disperse uniformly in all regions of electromagnetic radiation.

#### 3.4.3 Detector

The detecting device is either a thermocouple or a bolometer. A thermocouple is made of two different metals connected together to which two sensitive galvanometers are attached from the other end. The I R radiation impinges on the junction of the metals to generate a thermo electromotive force which enables the current to flow. The current generated is proportional to the quantity of the radiation impinged on the metal.

### 3.5 Application

- Used for the identification of the identity or non identity of two samples. This is done mainly in the finger print region of the spectrum.

- It is used for the detection of impurities. This is applied only when the impurities absorb strongly in the region where the main component is transparent (i.e. not absorbing any radiation)
- Identification of functional groups. I. R. Is very useful for the identification of some functional groups such as OH, CO, CHO, C = C, NH<sub>2</sub> e.t.c.

#### 4.0 Conclusion

Infrared spectroscopy is concerned with the absorption of electromagnetic radiation by molecules due to their vibrational ability. The type of spectrum obtained gives a vital information on the nature of the functional groups present in a given compound or sample.

#### 5.0 Summary

In this unit we have learnt that:

- ❖ Infrared spectrum possesses large number of absorption peaks as compared to that of UV/ Visible spectrum.
- ❖ the absorption bands provide a good information on the structural arrangement the molecule.
- ❖ almost all organic compounds absorb radiation in this region and convert it into vibrational energy.
- ❖ the absorption of I R radiation causes the bond within a molecule to either stretch or bend.

#### 6.0 Tutor Marked Assignment (T M A)

1. A sophisticated UV/ Visible near IR instrument has a wavelength range of 185 – 3000 nm. What are its wave number and frequency ranges?
2. At what range of wave numbers do the following functional groups give signal?  
(i) OH, (ii) CHO, (iii) CO and (iv) NH<sub>2</sub>

#### 7.0 Further Reading/ References

1. Robert, M.S., Clayton, G.B. and Terence, C.M. (1974). *Spectrometric Identification of Organic Compound*, 3<sup>rd</sup> Edition, Wiley International.
2. Allen, M.S., Barbara, A.G. and Melvin, L.D. (2000). *Microscale and Miniscale Organic Chemistry Laboratory Experiment*.
3. Peter R.S. Murray (1983). *Principles of Organic Chemistry*.

## Unit 4 Flame Spectroscopy

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## **Unit 4          Flame Spectroscopy**

### **1.0      Introduction:**

If a solution containing a metallic salt, e.g. sodium chloride is aspirated into a flame for example, acetylene burning in air, a vapour which contains atoms of the metal may be formed. Some of these gaseous metal atoms may be raised to an energy level which is sufficiently high to permit the emission of radiation characteristics of the metal, e.g. the characteristic yellow colour imparted to flames by compounds of sodium. This is the basis of flame photometry. However, a much larger number of the gaseous metal atoms will normally remain in an unexcited state or, in other words, in the ground state. These ground-state atoms are capable of absorbing radiant energy of their own specific resonance wavelength, which in general is the wavelength of the radiation that the atoms would emit if excited from the ground state. Hence, if light of the resonance wavelength is passed through a flame containing the atoms in question, then part of the light will be absorbed, and the extent of absorption will be proportional to the number of ground state atoms present in the flame. This is the underlying principle of atomic absorption spectroscopy (AAS).

### **2.0      Objectives:**

By the end of this unit, you should be able to:

- distinguish between flame emission and flame atomic absorption spectroscopy;
- explain the working principles of flame emission spectrometry and flame atomic absorption spectrophotometry;
- enumerate the applications of flame emission and flame atomic absorption spectroscopy; and
- mention the interferences in flame emission spectrometry and flame absorption spectrophotometer.

### **3.0      Definition of Flame Spectroscopy**

- Flame Emission Spectroscopy is a technique in which the emission of light by thermally excited atoms in a flame or furnace is used to measure the concentration of atoms, and Flame Atomic Absorption Spectroscopy is a technique in which the absorption of light by free gaseous atoms in flame or furnace is used to measure the concentration of atoms.

### 3.1 Differences between Flame Emission and Flame Atomic Absorption Spectroscopy.

Flame emission spectroscopy is basically the same as flame atomic absorption spectroscopy. The difference is that no light source is needed in flame emission. Some of the atoms in the flame are promoted to excited electronic states by collision with other atoms. The excited atoms emit their characteristic radiation as they return to their ground state. In flame emission spectroscopy, the emission intensity at a characteristic wavelength of an element is nearly proportional to the concentration of the element in the sample. For both absorption and emission, standard waves are usually used to establish the relation between signal and concentration.

### 3.2 Working Principle of Flame Emission Spectrometry (FES)

The solution is introduced into the flame as a fine spray. The solvent evaporates leaving the dehydrated salt. The salt is dissociated into free gaseous atoms in the ground state. A certain fraction of these atoms can absorb energy from the flame and be raised to an excited electronic state. The excited levels have a short lifetime and drop back to the ground state, emitting photons of characteristic wavelength. These can be detected with conventional monochromator-detector set up. The intensity of emission is directly proportional to the concentration of the analyte in solution being aspirated. A schematic diagram of a flame emission spectrometer is given below:

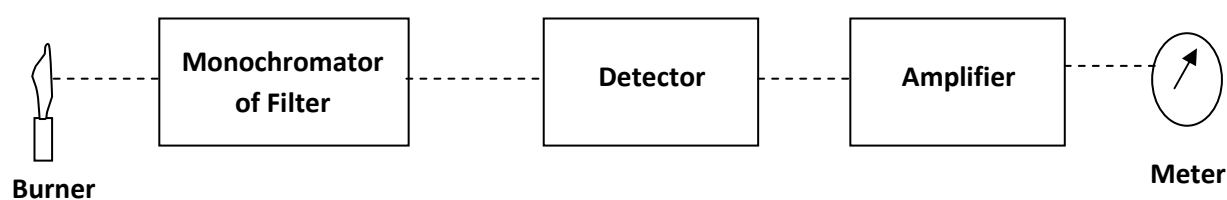


Fig 4.1: A schematic diagram of a flame emission spectrometer

Source: Modern Methods of Chemical Analysis, 2<sup>nd</sup> Edition by R, L, Pecsok et al – Page 244

### 3.3 Working Principle of Flame Atomic Absorption Spectrophotometry (FAAS)

The sample solution is aspirated into a flame as in flame emission spectrometry and the sample element is converted to atomic vapour. The flame then contains atoms of that element. Some are thermally excited by the flame, but must remain in the ground state. These ground state atoms can absorb radiation of a particular wavelength that is produced by a special source



made from that element. The wavelength of radiation given off by the sources is the same as those absorbed by the atoms in the flame. The absorption follows Beer's Law, that is, the absorbance is directly proportional to the path length in the flame and to the concentration of atomic vapour in the flame. A schematic diagram of flame atomic absorption spectrophotometer is given below:

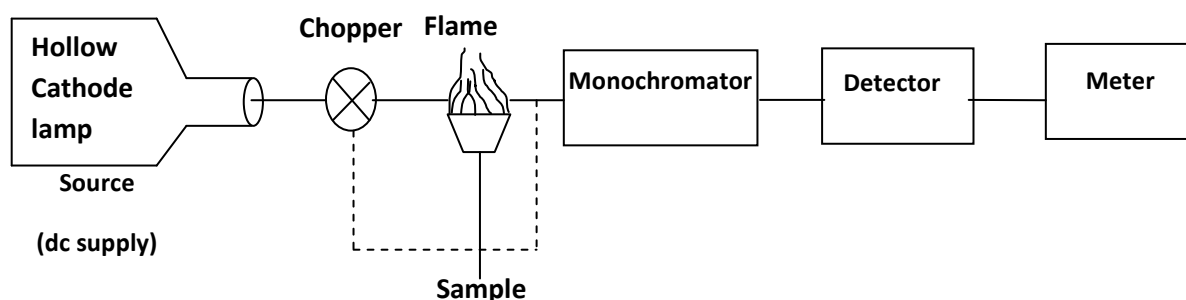


Fig 4.2: A schematic diagram of flame atomic absorption spectrophotometer

Source: *Modern Methods of Chemical Analysis*, 2<sup>nd</sup> Edition, R, L, Pecsok et al – Page 244

### 3.4 Interference:

By interference, we mean any effect that changes the signal when analyte concentration remains unchanged. In the measurement of atomic absorption or emission signals, interference is widespread and easy to overlook. If you are clever enough to discern that interference is occurring, it may be corrected by counteracting the source of interference.

#### 3.4.1 Types of interference:

- **Spectral:** refers to the overlap of analyte signal with signals due to other elements or molecules in the sample or with signals due to flame or furnace.  
The best means of dealing with overlap between lines of different elements in the sample is to choose another wavelength for analysis.
- **Chemical:** is caused by any component of the sample that decreases the extent of atomization of analyte. E.g.  $\text{SO}_4^{2-}$  and  $\text{PO}_4^{3-}$  hinder the atomization of  $\text{Ca}^{2+}$ , perhaps by forming non volatile salts. This can be solved by adding releasing agents which are chemicals to a sample to decrease chemical interference, eg EDTA and 8 – hydroxyquinoline protect  $\text{Ca}^{2+}$  from the above interference.
- **Ionization:** In this, the ionization of analyte atoms decreases the concentration of neutral analyte atoms in the flame, which results in the decrease of the desired atomic signal.

This is solved by adding an ionization suppressor to a sample so as to decrease the extent of ionization of analyte.

### **3.5 Application of the Techniques (FES and FAAS).**

- They are used in many laboratories particularly whenever trace metal analyses are required.
- Environmental samples are analyzed for heavy-metals contamination.
- Pharmaceutical samples are analyzed for metal impurities using the atomic spectrometric techniques.
- The techniques are used in the steel industry to determine minor components as well as major ones.
- The techniques are used in the measurement of sodium and potassium in serum and urine in diagnostic clinical analysis.

### **3.6 Sensitivity and Detection Limit in Atomic Absorption Analysis**

Atomic absorption spectroscopy is a sensitive technique in the analysis of metals in trace concentration.

Sensitivity is defined as that concentration of an element in aqueous solution which absorbs 1% of the incident radiation passing through a cloud of atoms being determined.

Usually, a 1% absorbance corresponds to 99% transmittance or approximately 0.004 absorbance value.

While detection limit is the concentration of an element in solution which gives a signal equal to twice the standard deviation of the series of measurements near blank level or the background signal.

Note that both the sensitivity and detection limit vary significantly with flame temperature and spectral bandwidth. For example, the sensitivity of mercury is 2.2 mg/l, while the detection limit is 0.16 mg/l. Hence, it is necessary to specify the flame type to be used in any determination.

### **4.0 Conclusion**

It is therefore clear that both flame emission and flame absorption techniques are based on the measurement of either emitted or absorbed radiation to estimate the amount of alkali metals (example sodium, potassium) and trace elements (e.g. lead, iron, copper etc) respectively.

## 5.0 Summary

In this unit, it has been learnt that:

- Flame emission is concerned with the measurement of the emitted radiation by thermally excited atoms.
- Flame absorption techniques involves the measurement of the absorbed radiation by free atomic species.
- The instrument used in flame emission is called flame photometer while that of flame absorption is called atomic absorption spectrophotometry.
- In flame emission, the emitted radiation is proportional to the concentration of the atoms in solution.
- Also, the amount of absorbed radiation is proportional to the number of the absorbing atomic species.

## 6.0 Tutor Marked Assignment (TMA)

1. (a) Compare and contrast between flame emission and flame absorption spectroscopy.  
  
(b) Explain clearly why flame emission is mainly restricted to the analysis of alkali metals.
2. (a) Outline major types of interferences encountered in atomic absorption analysis and describe how such problems can be overcome.  
  
(b) With the aid of a well-labelled schematic diagram, briefly explain the working principle of atomic absorption spectrophotometer.

## 7.0 Further Readings/References

1. Metcalfe, E. (1987) *Atomic Spectroscopy and Emission Spectroscopy*, John Wiley, UK
2. Douglas, A. S., Donald, M.W., James, F.H. and Stanley, R.C., (2004), *Fundamentals of Analytical Chemistry*, 8<sup>th</sup> edition
3. Christian, G.D. and O'Reilly, J.E., (1986). *Instrumental Analysis*, 2<sup>nd</sup> Edition.

## Unit 5 X-Ray Spectroscopy

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## Unit 5      X-Ray Spectroscopy

### 1.0      Introduction:

X-rays are electromagnetic radiation ranging in wavelength from about 0.1 – 25 Å. The shorter the wavelength of the x-ray, the greater the energy and its penetration power. Both light and x-rays are produced by electronic transition. The only difference is that light rays are produced by the transition of the outer electrons, while x-rays are produced by the transition of inner electrons.

X-ray spectroscopy is concerned with the measurement of the absorption of x-rays when it interacts with matter.

### 2.0      Objectives:

By the end of this unit, you should be able to:

- define x-ray spectroscopy;
- enumerate the different sources of x-rays;
- explain the components of x-ray spectrometers;
- describe the different types of x-ray detectors; and
- explain the working principle involved in x-ray spectroscopy.

### 3.0      Definition

X-ray spectroscopy is a technique which involves the study of the interaction between x-ray radiation with matter.

### 3.1      Sources of X- rays:

There are three common sources of X-rays for analytical purposes:

1. Electron bombardment of a metal target.
2. Irradiation of a target (sample) with primary beam of high energy x-rays to produce a secondary beam of fluorescent x-rays.
3. Exposure of a sample to a radioactive source which generates X-rays. The X-ray emission spectra produced from these sources may be continuous or discontinuous, or a combination of both.

Characteristic line spectra are produced by an electron beam. These are obtained when a fast moving electron excites an atom to a higher energy level, then x-ray emission follows when an outer electron falls onto the vacancy in the lower energy, inner shell, giving rise to a series of lines.

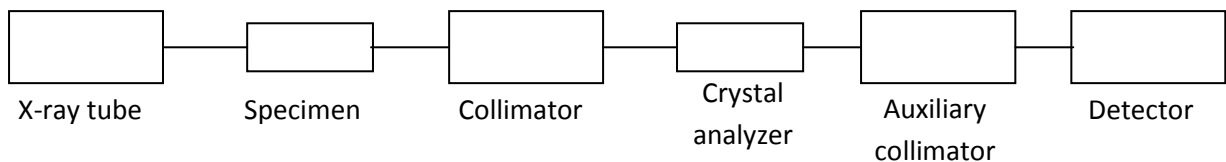
An X-ray fluorescence spectrum is produced by exciting the target atom with a beam of high energy x-ray that is sufficient enough to knock out k electrons. After a short time the excited ion returns to the ground state, producing a fluorescence spectrum similar to the emission spectrum. Unlike the line emission spectra, where the lines appear as spikes

superimposed on the continuous background, fluorescence spectra produce only the line spectrum without the continuous background. Hence fluorescence spectra show a much greater signal – background level and are preferred for analytical work.

### 3.2 X-ray Emission Spectrometers:

This is a device or instrument used to measure analytically the emission of the x-ray radiation by a sample. This instrument consists of :

1. Source of x-ray radiation called x-ray cooling tube,
2. Specimen chamber
3. Collimator made of a parallel fine tube
4. Crystal analyzer
5. Auxiliary collimator
6. Detector



**Fig. 5.1: Block diagram of X-ray emission spectrometer**

In this instrument, the fluorescence spectrum is conveniently generated with high energy x-rays from a Coolidge tube, even though the sample could be made the target in an x-ray tube. No suitable transparent materials are available for the fabrication of lenses, therefore x-rays are collimated by passage through a series of slits or a collection of long narrow tubes. Likewise no prisms are available to disperse X-rays, but fortunately crystals of many salts are able to disperse X-rays by diffraction and serves as excellent monochromators. Such crystals are: Topaz, LiF, NaCl, ammoniumdihydrogenphosphate (ADP), ethylene diamine d-tartrate, etc. The large single crystal analyzer (monochromator) is usually rotated on its axis to obtain the spectrum.

### 3.3 X-ray Detector

Three types of detectors are used to measure the intensity of an x-ray beam.

1. The simplest detector is a photographic film or plate which will darken when exposed to x-rays. The developed film is then scanned with a densitometer to record the spectrum.
2. Gas ionization detectors, such as ionization chamber, proportional counters and Geiger tubes used to measure radioactivity, are suitable for x-rays.
3. Some crystals fluoresce in the ultraviolet or visible region when exposed to x-rays.

These crystals may be incorporated in a scintillation counter that also utilizes a photomultiplier tube to measure the intensity of the fluorescence.

### 3.4 Non dispersive X – ray Spectrometers

These are compact, relatively inexpensive and give performance comparable to crystal monochromator instruments, except for somewhat poorer resolution of closely spaced lines.

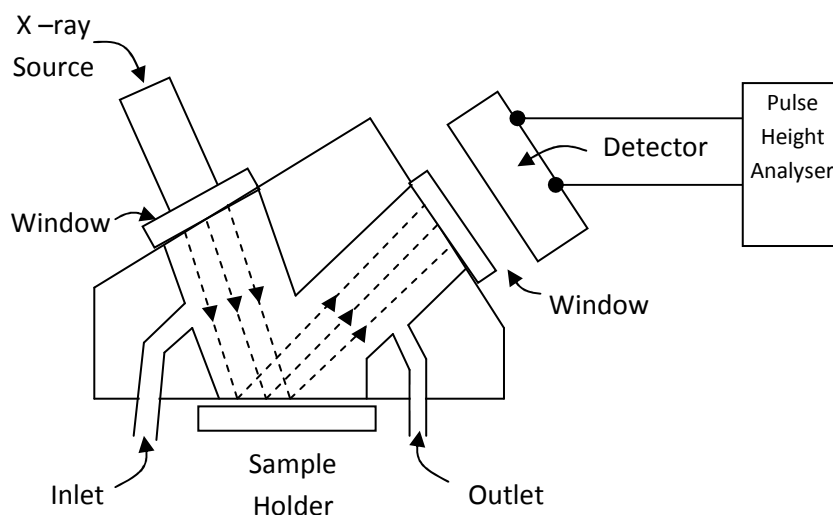


Fig. 5.2: Non-dispersive x-ray spectrometer

#### 3.4.1 Applications:

All types of solids are easily handled. If necessary, the sample may be placed in a thin walled cell or deposited as a film under cellophane. All elements above calcium ( $Z = 20$ ) are readily detected, those between sodium ( $Z = 11$ ) and calcium with difficulty, and the lighter elements not at all. X-ray emission spectroscopy is widely used for the analysis of steels and other alloys and for the determination of heavy elements in organic samples (e.g. lead and bromine in aviation fuels). It is highly specific with limits of detectability as low as a few parts per million.

### 3.5 X – Ray Absorption

The absorption of X-rays is similar to the absorption of other electromagnetic energy in the ultraviolet, visible or infrared regions. The only significant difference is the energy involved. Like the emission process, the absorption process also concerned with the innermost electron. This process identifies the element regardless of its environment. Absorption of the X-ray photon is most probable.

If the energy of the incident photon just equals the energy required to eject the electron. That is, the electron leaves with essentially zero kinetic energy. For example, the absorption spectrum of lead consists of a few broad peaks with sharp discontinuities called absorption edges. Each absorption edge corresponds to the energy required to eject a K or L electron. The wavelength of an absorption edge is slightly less than that of the

corresponding emission line, because the energy required to eject electron completely from the atom is greater than the energy associated with an outer electron (already in the atom) falling into the vacancy.

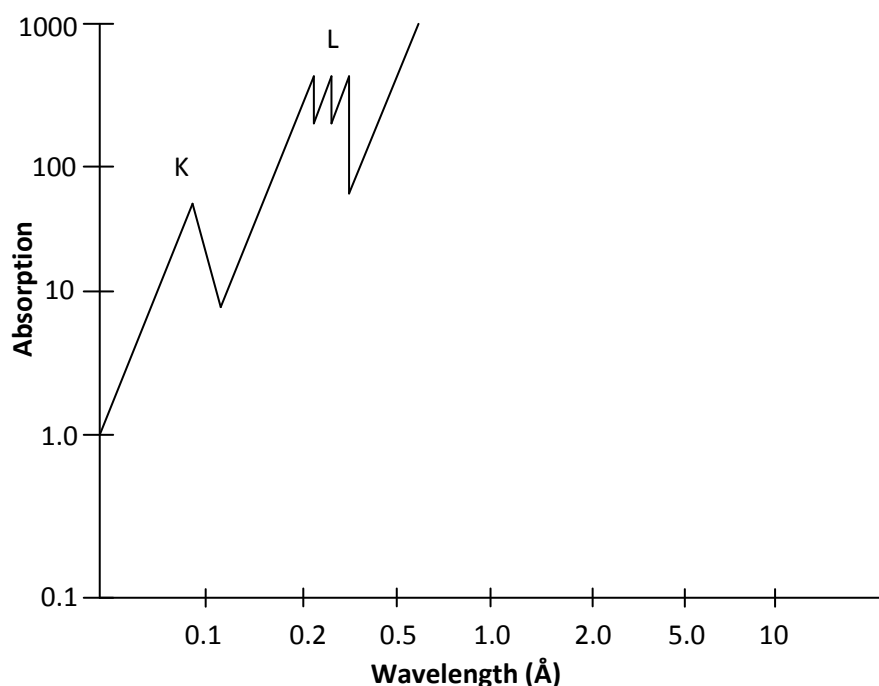


Fig. 5.3: A graph of absorption of x-ray against wavelength

Beer's law is valid for the absorption of X – rays, and is usually written as  $2.303 \log (I_0/I) = \mu x$

where  $I_0$  is the incident intensity and  $I$  is the intensity transmitted through a sample thickness of  $x$  cm. The proportionality constant,  $\mu$  is called the linear absorption coefficient.

The broad absorption bands as seen above, leads to the interference among neighbouring heavy elements. For the most part, X-ray absorption methods are limited to samples containing a single heavy element in an organic matrix (e.g. lead in gasoline or chlorine in chloro compounds).

### 3.5 Application of X – ray Fluorescence Analysis

X-ray fluorescence analysis has the advantage that it is non-destructive. It can be used for the analysis of works of art, valuable coins and forensic materials. Several elements can be determined in a few minutes, on only a tiny amount of material. The major disadvantages are

- (i) elements lighter than sodium cannot be determined readily,
- (i) lower concentrations are not so readily determined



(iii) the instruments are relatively costly and

(iv) the technique deals primarily with the surface of the sample whereas the composition of the outermost layer of a material may differ from that of the internal layer.

#### **4.0 Conclusion:**

X-ray spectroscopy is a method which deals with the study of the interaction between x-ray radiation with matter. The technique is very vital for the analysis of steel and alloys as well as for the determination of heavy metal concentration in different samples.

#### **5.0 Summary**

In this unit, we have learnt that

- X-rays are electromagnetic radiation ranging from about  $0.1 - 2.5 \text{ \AA}$ .
- There are three main sources of x-rays for analytical purposes.
- X-rays are produced by exciting the target atom with a beam of high energy that is sufficient enough for knock the inner electrons.
- Three types of detectors are used to measure the intensity of an x-ray beam namely photographic film, gas ionization detectors and some crystals which fluoresce.
- X-ray emission is used for the analysis of steel and alloys as well for determining the concentration of heavy metals

#### **6.0 Tutor Marked Assignment**

1.(a) Distinguish clearly between x-ray emission and x-ray absorption processes

(b) Calculate the frequency in hertz and energy in joules of an x-ray photon with a wavelength of  $2.35 \text{ \AA}$

2.(a) How would you differentiate between an x-ray and other types of electromagnetic radiations

(b) Briefly explain the basic application of x-ray spectroscopic analysis.

#### **7.0 Further Reading/Reference**

1.0 Christian, G.D. and O'Reilly, J.E. (1986) *Instrument Analysis*, 2<sup>nd</sup> Edition Boston, Allyn and Bacon

2.0 Douglas, A.S., Donald, M.W., Holler, F.J., and Stanley, R.C. (2004) *Fundamentals of Analytical Chemistry*

## MODULE 2: Basic Analytical Techniques

### Unit 1 - X – Ray Diffraction Method

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## **Unit 1            X – RAY DIFFRACTION METHOD**

### **1.0    Introduction**

X – rays are electromagnetic radiation with fairly high penetration power or energy. The rays have the normal properties of waves most especially, they can be diffracted.

Diffraction process usually occurs whenever a wave meets a barrier with one or more openings of about the same size as the corresponding wavelength. Usually, the atoms or ions in crystals are arranged in such a way that they are separated from one another by a particular distance. Such distances are in the right range to cause x – rays to be diffracted. Thus, the layers of atoms in a crystal act as a diffraction grating for x-rays.

### **2.0    Objectives**

By the end of this unit, students should be able to:

- explain the meaning of x-ray diffraction;
- explain the basic concept of x-ray diffraction;
- describe the different types of x-ray diffraction experiment;
- describe the arrangement of planes in crystals; and
- list the application of x-ray diffraction analysis.

### **3.0    Definition**

X-ray diffraction is a phenomenon in which waves of x-rays spread and bend as they pass through small openings or around barriers.

Note: Diffraction is more pronounced when the opening, or aperture, or the barrier is similar in size to or smaller than the wavelength of the incoming wave.

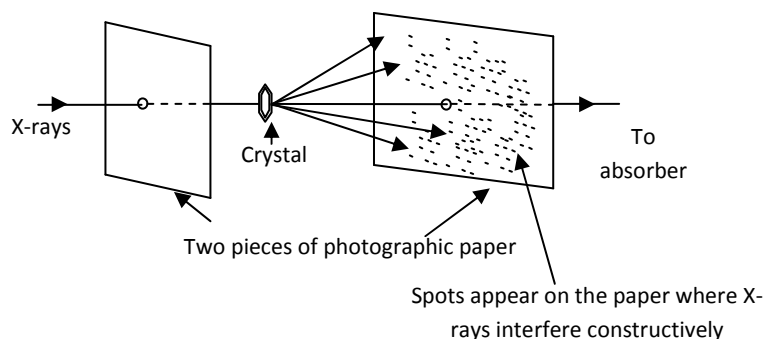
### **3.1    Basic Concept of X-ray Diffraction**

X-rays are electromagnetic waves in which the wavelengths are comparable in size to the spacing between the atoms in crystals. When these waves pass through crystals, they diffract (i.e. spread out or bend) and form interference patterns. These patterns can be analysed to gain information about geometric structure and properties of the crystals.

The main reason why atoms are able to produce diffraction patterns is due to the number of electrons they contain. The electric field of x-rays interacts with the electron cloud around the atom. Thus, the result of many of these interactions produces the diffraction pattern. Large atoms have many electrons. As a result, they produce the strongest patterns. Small atoms, such as hydrogen, have little effect on x-ray because of the few number of electrons.

### 3.2 Types of X-ray Diffraction Experiments

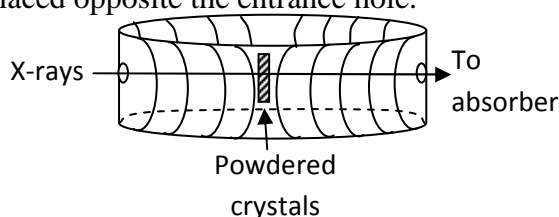
The first x-ray diffraction experiments were performed by bombarding Zinc Sulphide with high energy electrons. Then the X-rays were passed through a single crystal as shown below:



**Fig. 1.1: A simplified diagram of scattering of x-ray by a crystal**

**Source: *Advanced Chemistry: Physical and Industrial* By Philip Mathews, Pg. 170**

The second experiment involves the use of a powdered substance which is known as Powder Method. In this experiment, crystals of sample to be analysed are ground to a fine powder and then placed in a tube made of glass which has little effect on x-rays. The sample is surrounded by a cylindrical sheet of photographic paper. The x-rays are allowed to penetrate through a small hole in the cylinder and passed into the sample, where the diffraction occurs. A piece of lead which absorbs any x-rays that pass through the sample is placed opposite the entrance hole.



**Fig. 1.2: Diffraction by powdered crystals**

**Source: *Advanced Chemistry: Physical and Industrial* By Philip Mathews, Pg. 170**

As can be seen, the powdered crystals are at the centre. The reflections of the x-rays produce curved lines on the photographic paper.

Note: the above diagram represents only one set of plane in a crystal. The separation between the planes is shown as an angle to the incoming x-rays. The angle is represented as  $\theta$ . Diffractions from crystals with their sets of planes at angle  $\theta$  will be arranged in form of a cone. This is because the photographic film is in the shape of a cylinder; the reflections from the crystals produce a set of curved patterns on the film.

### 3.3 The Effect of X-ray Diffraction on the Arrangement of Atoms

Generally, the extent of diffraction depends on the nature and arrangement of the individual atoms within a crystal. Thus, the greater the number of electrons in an atom, the stronger the diffraction or reflection. If the intensities of the individual spots on X-ray photograph can be compared accurately, then it is possible to relate them with the

nature of the electron cloud around the atoms. For accurate measurement of the intensities, an instrument called diffractometer is used. Thus, the intensities of the various reflections are measured directly by electronic means rather than by photographs. When the results are computerized, electron density maps can be produced.

### 3.4 Bragg's Equation

Bragg's proposed that the formation of diffraction patterns could be explained by assuming that the X-rays were reflected from the various planes of atoms in a crystal. According to Bragg's explanation, it is possible to consider that both diffraction and reflection are equivalent to one another. Thus, when two rays are diffracted in two different planes, the difference in the distance travelled by these rays is  $2d \sin\theta$ . So, for constructive interference, we have:

$$n\lambda = 2d \sin\theta$$

where  $n\lambda$  is referring to the path difference in crystals,  $n$  is a whole number,  $\lambda$  is a wavelength. If  $n = 1$ , reflection is said to be first order. If  $n = 2$  it is a 2<sup>nd</sup> order reflection and so on.

**Example:** When X-rays of wavelength  $1.54 \times 10^{-8}$  cm passed through Sodium Chloride, an intense cone is formed at  $\theta = 15.87^\circ$ . If this is taken as first order reflection then

$$1.54 \times 10^{-8} \text{ cm} = 2 d \sin (15.87^\circ)$$

$$\therefore d = \frac{1.54 \times 10^{-8}}{2 \sin 15.87} = 2.82 \times 10^{-8} \text{ cm}$$

Note:  $d$  is referring to the spacing between the planes.

### 3.5 Applications

X-ray diffraction can be used to discover the structures of crystals. The diffraction patterns of X-ray can also be used to determine the arrangement of electrons around the atoms. This provides good evidence about the shapes of molecules.

X-ray diffraction has led to the discovery of the double helical structure of DNA.

### 4.0 Conclusion

X-ray diffraction is thus a method which involves the spreading and bending of the rays as they pass through a small opening or barriers. This method is useful in determining the arrangement of atoms in a molecule.

## 5.0 Summary

- X-ray diffraction is a method of determining atomic and molecular structures by measuring patterns of scattered X-rays after they pass through a crystalline substance.
- The diffraction pattern can be measured using single crystal or a powder.
- X-rays are diffracted from the layers of atoms or ions in a crystal according to Bragg's equation  $n\lambda = 2d \sin\theta$ .
- The intensity of the spots on an x-ray diffraction photographs depends on the electron densities of the atoms or ions.

## 6.0 Tutor marked Assignment

1. (a) What is meant by X-ray diffraction?  
(b) The X-ray powder pattern of Sodium Chloride shows a cone of  $\theta = 15.38^\circ$  using x-rays of wavelength  $1.65 \times 10^{-8}$  cm. What would be the spacing between the planes?
2. (a) Describe briefly the basic concept of x-ray diffraction.  
(b) With the aid of a diagram, explain how an x-ray is diffracted by a crystalline substance.

## 7.0 Further Reading/Reference

Philip, M. (2003) *Advanced Chemistry, Physical and Industrial*. Cambridge University Press.

## **Unit 2                      Nuclear Magnetic Resonance (NMR) Spectroscopy**

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## Unit 2      Nuclear Magnetic Resonance (NMR) Spectroscopy

### 1.0      Introduction:

Nuclear magnetic resonance (NMR) spectroscopic technique is based on the magnetic properties of certain atomic nuclei. Magnetic properties are only exhibited by molecules that have either atoms with odd mass number or an uneven number of electrons. The nucleus of atoms with odd mass number has both spin and magnetic properties. A spinning positively charged nucleus possesses a magnetic moment which is capable of interacting with an externally applied field.

### 2.0      Objectives

By the end of this unit, you should be able to:

- explain the meaning and basic concept of NMR spectroscopy;
- describe the source and pattern of NMR spectra;
- explain the operation of NMR spectrometer; and
- describe instrumental arrangement of NMR spectrometer.

### 3.0      Definition

Nuclear magnetic resonance (NMR) spectroscopy is a technique that is based on quantization of the spin angular momentum of the nucleus.

#### 3.1      Basic Principle of NMR

In order to produce an NMR spectrum, the nucleus must have a net spin. For a nucleus to have spin, it must contain an odd mass number. Thus, nuclei such as  $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^9\text{F}$  and  $^{31}\text{P}$  are suitable, whereas atoms with even mass number, like  $^{12}\text{C}$  and  $^{16}\text{O}$  are not suitable for NMR. Hydrogen is the most important element that displays NMR properties then followed by Carbon. Hence, NMR studies enable the detection of the positions of these atoms within a molecule. This is possible because the magnetic properties of various atomic nuclei differ and therefore tend to determine their energy absorption in a magnetic field, when irradiated with electromagnetic radiation in the radio frequency region.

#### 3.2      The Source of NMR Spectra

Atomic nuclei possess charge. Some isotopes such as  $^1\text{H}$  behave like a tiny spinning magnet. This is because they possess both electric charge and mechanical spin. A spinning charge generates a magnetic field. The nuclei of certain isotopes of atoms have spin quantum number (I) which is  $\pm\frac{1}{2}$ . Therefore, they can have either of two spin states:  $+\frac{1}{2}$  or  $-\frac{1}{2}$  corresponding to the magnetic moment.

In the presence of an external field, the proton can have two orientations, either aligned with the field called parallel orientation or against the field called antiparallel orientation. The parallel orientation occurs at lower energy state while antiparallel at higher energy. In addition to this, the proton will also precess around the axis of applied magnetic field. The spinning frequency of the proton doesn't change but precessional frequency is directly proportional to



the strength of the external field ( $H_0$ ). For example, a proton exposed to an external magnetic force of 14000 gauss will precess about 60 million times per second so that  $\nu = 60$  MHz

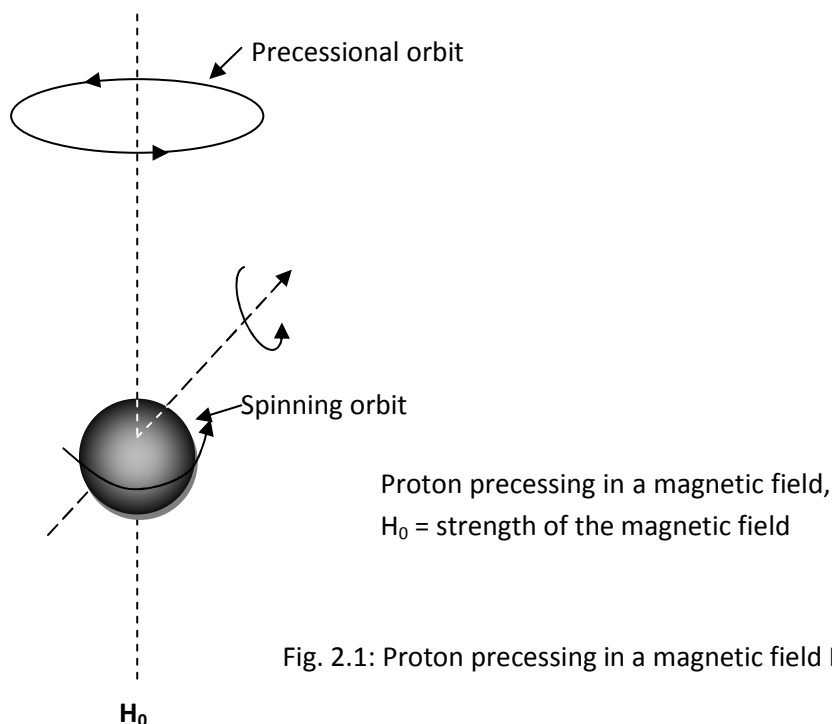


Fig. 2.1: Proton precessing in a magnetic field  $H_0$

When the precessing proton is irradiated with a beam of radio frequency, the low energy nuclei may absorb this energy and move to a higher energy state. This is possible only if the precessing frequency is equal to the frequency of the radio frequency radiation i.e. if the two are in resonance. Hence, the term is called nuclear magnetic resonance.

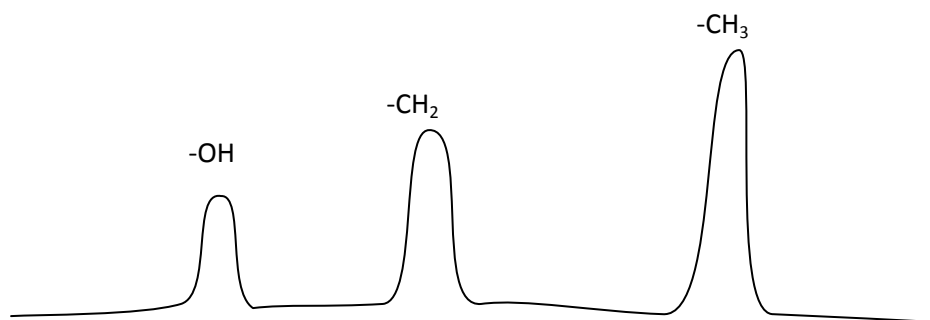
### 3.3 NMR Spectrum

In NMR experiments, a solution of substance under investigation is placed in a strong magnetic field. Then the solution is irradiated with radio frequency energy of appropriate frequencies. The energy absorbed by the protons is recorded as NMR spectrum.

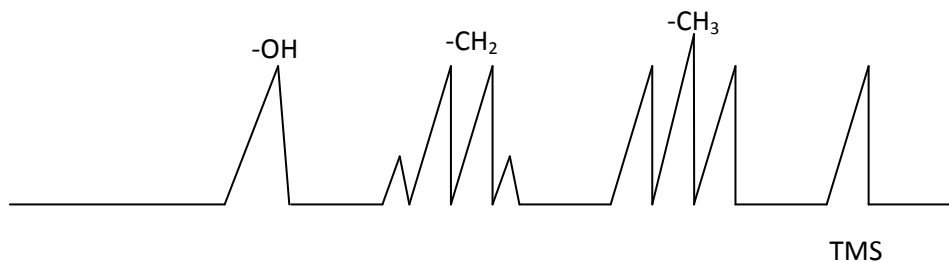
### 3.4 Pattern of the Spectrum

The environment of each nucleus in a molecule is different depending on the orbital and bonding electrons. Also the absorption of energy by the nucleus is in accordance with its environment. This enables the hydrogens in a methyl group ( $-CH_3$ ) to be differentiated from those in the methylene group ( $-CH_2$ ), and hydroxyl group ( $-OH$ ).

Example, consider the different environments of the hydrogen atoms in the ethanol molecule,  $CH_3CH_2OH$ . The frequency at which absorption occurs for the hydrogen atoms in the  $-CH_3$  groups is different from those in the  $-CH_2$  groups which in turn differs from those in the  $-OH$  group. As a result the NMR spectrum of ethanol shows three absorption peaks, caused by the hydrogen in each of these three groups.



When it is highly resolved it will be:



The spectrum is calibrated against a standard, which is usually tetramethylsilane (TMS) and then interpreted.

### 3.5 The NMR Spectrometer

The basic components of an NMR spectrometer are as follows:

- Strong magnet
- Radio frequency oscillator
- Radio frequency receiver
- Recorder with calibrator and integrator.
- Sample holder
- Temperature control

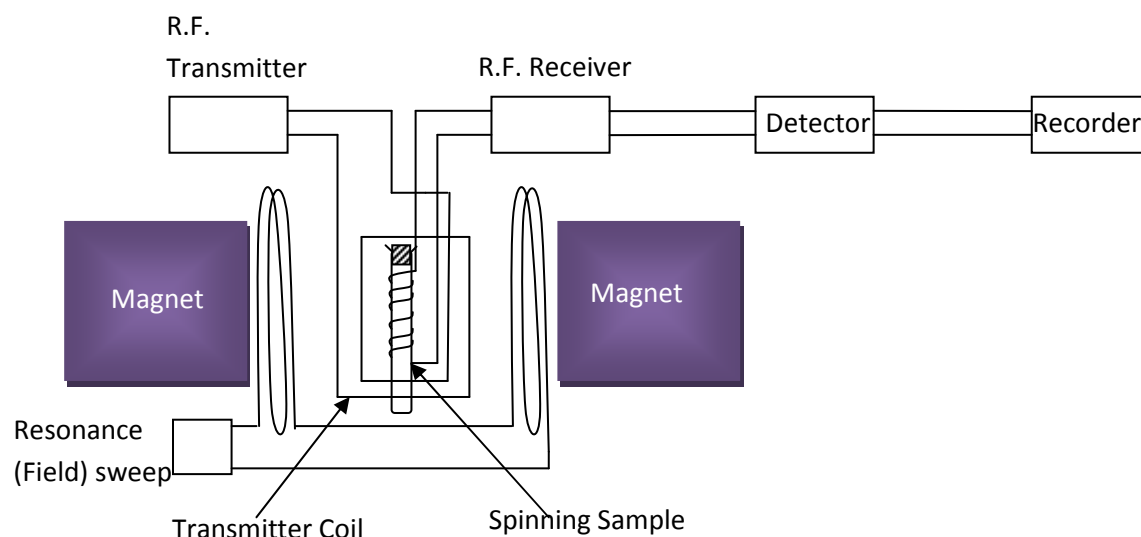


Fig. 2.3: Schematic diagram of an NMR Spectrometer

Source: Spectrometric Identification of Organic Compounds, Robert M.S., et al (1974)

### 3.6 Operational Procedure

For a non viscous liquid about 0.5 ml is used, while for solid samples 10 – 50 mg/0.5 ml solvent is used. The sample solution is filtered to remove any particles. About 0.5 ml is put into a glass sample tube with internal diameter of about 0.5cm. Few drops of the internal standard added. The sample is placed in the sample holder and left for some minutes for the temperature to equilibrate. The tube is spun at 30 – 60 rps. The spectrum is recorded, and then followed by integration of the peaks.

### 3.7 Application of NMR

The main use of this technique is in the determination of the structure of organic compounds based on the absorptions of the hydrogen nuclei. The technique is also vital in the study of reaction rates and mechanisms.

### 4.0 Conclusion

Nuclear magnetic resonance is mainly used to detect the hydrogen atoms in a molecule.

In a magnetic field, the proton in the nucleus of each hydrogen atom changes its spin state when it absorbs the required amount of energy. The frequency of the radiation absorbed depends on the chemical environment of the proton.

### 5.0 Summary

In this unit , we have learnt that:

- Nuclear magnetic resonance spectroscopic technique is based on the magnetic properties of certain atomic nuclei which are exhibited by molecules containing atoms with odd mass numbers.
- In the presence of external magnetic field, the proton can either be aligned with the field (parallel orientation) or against the field (anti-parallel orientation).
- NMR enables the detection of positions of atoms within a molecule

#### **6.0 Tutor Marked Assignment (TMA)**

1. Explain the basic principle behind NMR spectroscopy
2. With the aid of a good schematic diagram, describe the instrumental arrangement of NMR spectrometer.

#### **7.0 Further Reading/References**

1. Abraham R.J., Fischer J.P. and Loftus, P. (1992) *Introduction to NMR Spectroscopy*, John Wiley, UK
2. Robert, M.S., Clayton, G.B., and Terence, C.M., (1974). *Spectrometric Identification of Organic Compounds*. 3<sup>rd</sup> edition Wiley International.
3. Willard, H.H., Merritt, L.L., Dean, J.A. and Settle, F.A. (1988). *Instrumental Method of Analysis*, 7<sup>th</sup> Edition.

## **Unit 3                      Fluorescence Spectroscopy**

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## **Unit 3          Fluorescence Spectroscopy**

### **1.0    Introduction**

Fluorescence is a photoluminescence phenomenon in which atoms or molecules are excited by absorption of electromagnetic radiation. The excited atoms then return to the ground state, while giving up their excess energy as photons. One of the most interesting features of molecular fluorescence is its inherent sensitivity which is an advantage over the conventional absorption spectroscopy. The fluorescence method also has a larger linear concentration range than those encountered in absorption spectroscopy. Fluorescence methods are much less widely applicable than the absorption methods because of the limited number of molecules that show appreciable fluorescence.

### **2.0    Objectives**

By the end of this unit, students should be able to:-

- explain the meaning of molecular fluorescence spectroscopy;
- highlight the basic concept of molecular fluorescence;
- distinguish between excitation spectra and fluorescence spectra;
- relate fluorescence with the molecular structure;
- describe the effect of temperature and solvent on fluorescence; and
- illustrate the basic arrangement of fluorescence instrument.

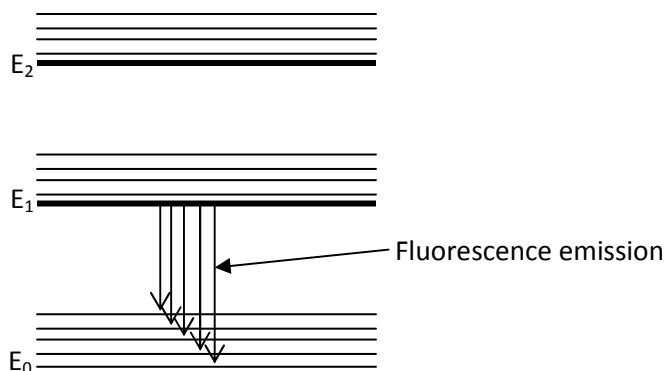
### **3.0    Definition**

Fluorescence is a radiation produced by an atom or molecule that has been excited by photons to a singlet and excited state.

#### **3.1    Basic Concept of Molecular Fluorescence**

When a fluorescent substance absorbs a photon or quantum of light, one of its orbital electrons is raised to a remote orbit and consequently elevates the molecule to a transient high energy excited state. As the elevated electron drops back to its original orbit, the photon activated molecule regains its original low energy ground state by emitting part of the absorbed energy as specific light wavelengths. Such an emission is called fluorescence if the emitted light has a longer wavelength than the absorbed light and its emission commences almost immediately on exposure to the exciting short wavelengths and stops within  $10^{-8}$  sec of termination of the source exposure.

The energy level diagram of fluorescence radiation is shown below:

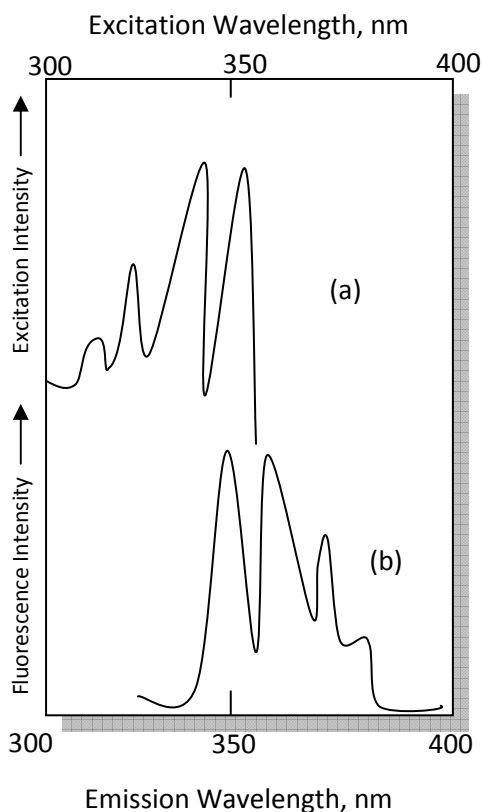


**Fig. 3.1: Energy level diagram of fluorescence emission**

Where  $E_0$  is the ground state, while  $E_1$  and  $E_2$  are the corresponding excited states.

### 3.2 Relationship between Excitation and Fluorescence Spectra

The excitation spectra or absorption spectrum approximately appear as a mirror image of fluorescence spectrum. This is because the energy differences between vibrational states are about the same for both ground and excited states. This effect is demonstrated by the spectra of anthracene, as fluorescent substance.



**Fig. 3.2: (a) Excitation spectra, (b) Emission spectra**

There are many exceptions for this mirror image rule, most especially when the excited and ground states have different molecular geometries or when different fluorescence bands originate from different parts of the body.

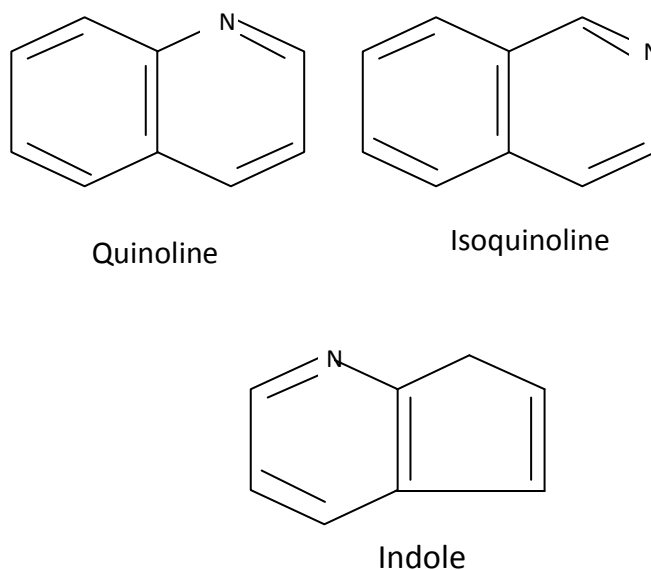
### 3.3 Fluorescence and Structure

Under normal condition, compounds containing aromatic rings of benzene give the most intense and most useful molecular fluorescence emission. Certain aliphatic and alicyclic carbonyl compounds as well as the highly conjugated double bond compounds also fluoresce, but these are very few as compared to those of aromatic ring structure.

Most of the unsubstituted aromatic hydrocarbons fluoresce in solution and the efficiency increases with the number of rings and their degree of condensation. The simplest heterocyclic molecules such as pyridine, furan, thiophene and pyrrole do not exhibit or possess molecular fluorescence properties. But fused ring compounds structures containing these rings often fluoresce. Also substitution on an aromatic ring causes the wavelength of absorption to shift as well as corresponding changes in the fluorescence peaks. Structural rigidity also increases the ability of a compound to fluoresce

Additionally, substitution affects the fluorescence efficiency.

Examples of fluorescent compounds include:



**Fig. 3.3: Typical aromatic compounds that fluoresce**

### 3.4 Effects of Temperature and Solvent

Generally, quantum efficiency of fluorescence decreases with increase in temperature. This is because the increased frequency of collision at elevated temperatures increases the probability of collisional relaxation. Also a decrease in solvent viscosity leads to similar effect.



### 3.5 Effect of Concentration

The intensity of fluorescence radiation (F) is proportional to the radiant power of the exciting beam absorbed by the system.

$$\text{ie } F = K (I_0 - I) \quad (1)$$

Where  $I_0$  is the power of the incident beam on the solution and  $I$  is the power of the transmitted radiation, while  $K$  is a constant.

Thus, to relate  $F$  to the concentration ( $C$ ) of the fluorescent substance, Beer's law is applied as follows:

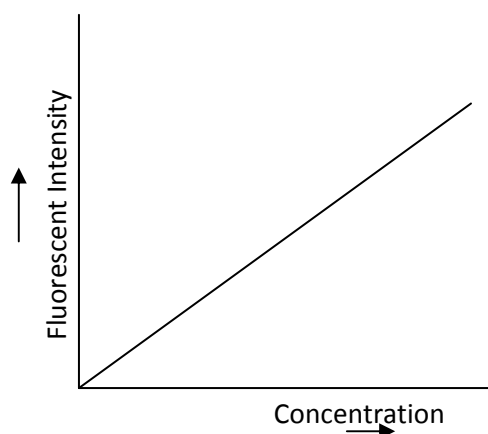
$$\frac{I}{I_0} = 10^{-\epsilon bc}$$

where  $\epsilon$  is the molar absorptivity of the fluorescing molecule and  $\epsilon bc$  is the absorbance ( $A$ )

Generally,  $F \propto C$  or

$$F = KC$$

Thus, a plot of the emitted fluorescence intensity against the concentration of the solution gives a linear graph. This is because for dilute solution, Beer's law is obeyed.

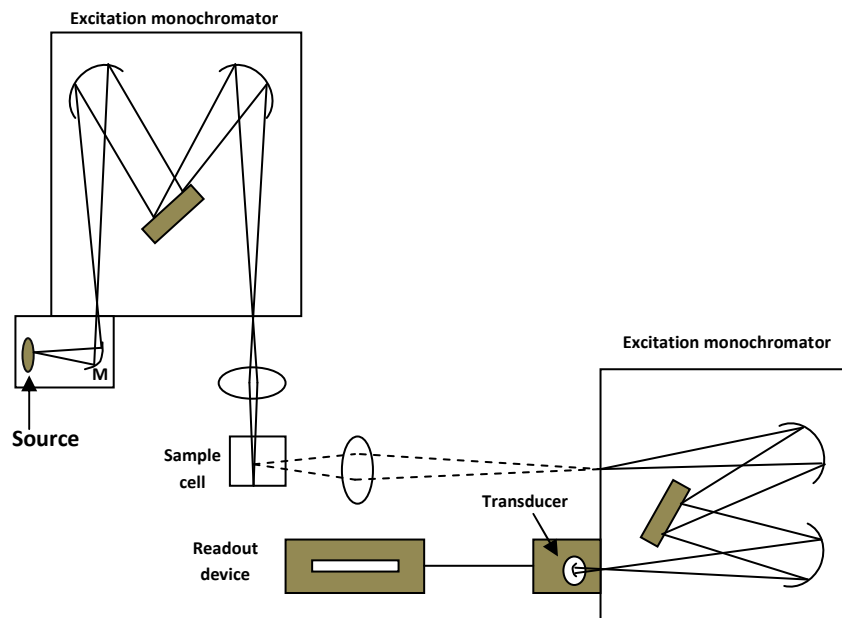


**Fig. 3.3: A graph of emitted fluorescence intensity against the concentration of solution**

Note: At high concentration, the relationship is not linear. This effect is as a result of primary absorption in which the incident beam is absorbed so strongly that fluorescence is no longer proportional to concentration. At very high concentrations,  $F$  reaches a maximum and may even begin to decrease with increasing concentration because of secondary absorption. This process occurs due to absorption of the emitted radiation by other analyte molecules.

### 3.6 Instrumentation

Different types of fluorescence instruments are available. The optical diagram of the typical instrument for fluorescence measurement is shown below:



**Fig. 3.4: A typical Fluorescence Spectrometer**  
Source: Fundamentals of Analytical Chemistry by Skoog et al (2004).

The source of radiation is a mercury lamp. The emission is measured at right angle to the mercury arc lamp source. Fluorescence radiation is emitted in all direction and the 90 degrees geometry avoids the detector viewing the source. The instrument uses two grating monochromators which allow the scanning of excitation spectra and emission spectra (emission wavelength scanned at a fixed excitation wavelength). It also synchronizes the two spectra together.

### 3.7 Applications of Fluorescence Methods

Fluorescence methods are used to study chemical equilibria and kinetics in the same way as absorption spectrophotometry is used. It is often possible to study chemical reactions at lower concentrations due to higher sensitivity of fluorescence technique. Fluorescent probes or tags can be attached covalently to specific site in molecules such as proteins for easy detection and monitoring. These tags can also be used to provide information about energy transfer processes.

Also, quantitative fluorescence methods have been developed for the analysis of inorganic, organic and biochemical molecules. The inorganic fluorescence methods could be done directly or indirectly. The direct methods are based on the reaction of the analyte with a

complexing agent to form a fluorescent complex, while the indirect method depends on the decrease in fluorescence as a result of interaction of the analyte with a fluorescent agent.

With respect to organic and biochemical applications, fluorescence method is used to determine amino acids, proteins, coenzymes, vitamins, nucleic acids, alkaloid, flavonoids, steroids and many other metabolite. Also, fluorescence is widely used as a detection technique for liquid chromatographic methods, for fluoro-analysis methods and electrophoresis.

#### **4.0 Conclusion**

From what we have learnt, it is clear that fluorescence is a radiation produced by an atom or a molecule that has been excited by photons to a singlet state and the fluorescent compounds contain multiple conjugated bond systems or aromatic rings. The technique is very important for both qualitative and quantitative analysis.

#### **5.0 Summary**

In this unit, we have learnt that:

- Molecular fluorescence involves the emission of radiation as excited electrons return to the ground state.
- The wavelengths of the radiations emitted are different from those absorbed and useful in the identification of a molecule.
- The intensity of the emitted radiation can be used in quantitative method while the wavelength of maximum emission can be used qualitatively.
- Fluorescent compounds contain multiple conjugated bond systems or aromatic rings with associated delocalized electrons.
- Most molecules that fluoresce have rigid planer structures.

#### **6.0 Tutor Marked Assignment**

1. (a) How would you distinguish between excitation and fluorescence spectra?  
(b) Why do some absorbing compounds fluoresce while others do not?
2. (a) Explain why molecular fluorescence usually occurs at a longer wavelength than the exciting radiation?  
(b) Describe the components of a fluorometer and state why the instrument is double-beam in design.

#### **7.0 Further reading/Reference**

1. Willard, H.H., Merrit, L.L., Dean, J.A. and Settle, F.A. (1988) *Instrumental Method of Analysis, 7<sup>th</sup> Edition* Wadsworth Publishing, CO., USA
2. Douglas, A.S., Donald, M.W., James, F.H., and Stanley, R.H. (2004). *Fundamentals of Analytical Chemistry, 8<sup>th</sup> Edition*.

## **Unit 4                      Fluorimetry**

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## **Unit 4          Fluorimetry**

### **1.0    Introduction:**

Molecules of some substances under certain conditions absorb radiant energy and after an interval of  $10^{-7}$  or  $10^{-8}$  seconds, the excited electrons return to a lower energy level, and the energy being emitted as visible light. This radiation is termed fluorescence. Under the conditions of biological and biochemical analysis, this energy has a greater wavelength that is lower energy than the exciting radiation. In fluorimetry, there is absorption and emission.

### **2.0    Objectives:**

By the end of this unit, the student should be able to:

- describe a Fluorimeter;
- explain the mode of operation of the instrument;
- explain quenching and sensitivity of the instrument;
- state the application of a Fluorimeter; and
- enumerate the various precautionary measures to be observed when using Fluorimeters.

### **3.0    Definition:**

An instrument that is designed to measure the intensity of fluorescence is called Fluorimeter.

### **3.1    Mode of Operation of the Instrument**

In photoelectric Fluorimeters, a beam of ultraviolet light ( $< 400\text{nm}$ ) of regulated uniform intensity from a mercury vapour lamp passes through a diaphragm to fall on either an incident wavelength selector or an exciter filter. This filter or selector transmits only such UV wavelengths which are close to the absorption maximum of the fluorescent substance to be estimated, and cuts off all other wavelength including those above  $400\text{nm}$ . The UV wavelengths emerging from this selector or filter are focused by a lens system to the test solution in a transparent cuvettes or sample cell. The fluorescent solute in the solution emits long wavelengths of visible fluorescent light on absorbing UV rays. To avoid unabsorbed and undeflected UV rays being transmitted through the solution, long wavelength fluorescent rays, emerging from the sample cell at only an angle to the path of the exciting beam, are made to pass through an emitted wavelength selector or a barrier filter. This filter cuts off any UV ray which might have been deflected from the sample cell, and transmits only the specific long wavelengths of fluorescent rays to a lens system. The system focuses the fluorescent wavelengths to a photo cell which consequently generates a current in direct proportion to the intensity of fluorescence. This current pulse is amplified and measured.

A schematic diagram of a fluorometer is shown below:

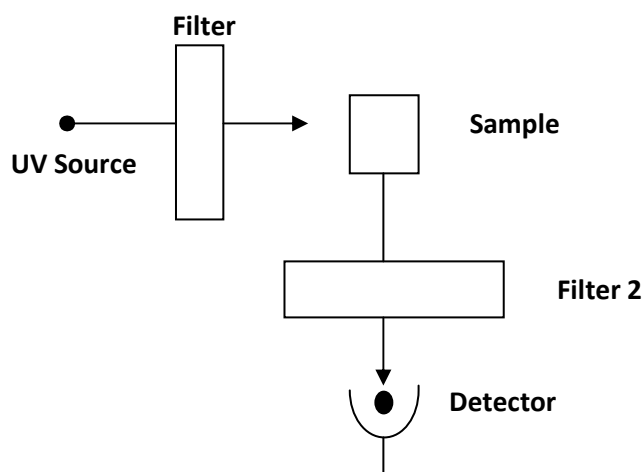


Fig. 4.1 A Simple Fluorimeter design

Source: Analytical Chemistry, Sixth Edition by Gary D. Christian

### 3.2 Quenching:

When there is a reduction in the intensity of fluorescent due to the specific effects of constituents of the solution itself quenching occurs. For example, phosphate buffers quench fluorescence. When the fluorescent substance is responsible for the absorption of fluorescence, it is known as self-quenching. Quenching can also be caused by the transfer of energy by the collision of the excited molecules of the fluorescent material with molecules of solvents or solute.

### 3.3 Sensitivity:

For very low concentration of substances, the sensitivity of fluorescence is proportional to concentration, provided the exciting energy remains constant. At higher concentrations of solutions, this linear relationship tends to be destroyed.

Fluorescence is sensitive to such variables as solvent, pH, temperature, impurities and the presence of ions such as  $\text{CNS}^-$ ,  $\text{I}^-$ ,  $\text{Br}^-$ ,  $\text{Cl}^-$ , which have all marked quenching effect, e.g.  $\text{Cl}^-$  quenches the fluorescence of quinine.

Nevertheless, with more sensitive equipment, fluorimetric procedures can be more sensitive than spectrophotometric methods by an order of magnitude of at least  $10^3$  to  $10^4$ .

### 3.4 Applications:

The instrument can be used for the analysis of:

1. Thiamine, vit B1 and Riboflavin
2. Ruthenium in the presence of other platinum metals.
3. Quinine in dilute sulphuric acid solution.
4. Riboflavin (vitamin B<sub>2</sub>) using their native fluorescence.
5. Histamine and histidine by reacting with o-phthalaldehyde.
6. Magnesium by reacting with 8-hydroxyquinoline and sulphuric acid.

7. Amphetamines by reacting with formaldehyde and acetyl acetone.

### 3.5 Precautions for usage:

For practical purposes, the following precautions must be observed when using Fluorimeters:

1. The solvent used must be non-fluorescent and must not absorb radiation in the spectral regions used in the particular assay.
2. Pyrex or quartz cuvettes must be used because of the slight fluorescence or ordinary glass.
3. Filters should be checked for contribution to blank readings.
4. Stopcock and other greases give rise to problems and should be avoided.
5. pH and temperature must be carefully controlled.
6. Solutions must be free from gas bubbles, suspended solids and turbidity.
7. Exciting radiant energy sometimes done slowly changes the colour or intensity of fluorescence reading and hence must be made rapidly.
8. Internal standards should be used to detect quenching or alteration in colour of fluorescence – comparison should be made between an internal and external solution.

### 4.0 Conclusion

Fluorimetry is a method of analysis which measures the intensity of fluorescence that can be used in estimating the concentration of a fluorescent substance in solution.

### 5.0 Summary

In this unit we learned that:

- Fluorometry involves the measurement of the intensity of fluorescence radiation emitted by fluorescent substance.
- Fluorimeter is an instrument used to measure the intensity of the emitted fluorescence radiation from a given sample.
- Fluorometer consists of light source, filters and detector.
- Fluorimetric method can be used to analyse a variety of substances such as thiamine, riboflavin, quinine, etc.

### 6.0 Tutor marked Assignment (TMA)

1. What is meant by each of the following:
  - i. Fluorimetry
  - ii. Fluorescent filter.
  - iii. Quenching
2. (a) With the aid of a suitable schematic diagram, describe the mode of operation of a fluorimeter.

(b) Explain why pyrex or quartz cell must be used instead of a normal glass while measuring the emitted fluorescent radiation from a given sample.

#### **7.0 Further Reading/References**

1. Harris, D.A. and Bashford, C.L. (1987) *Spectroscopy and Spectrofluorimetry – A Practical Approach*, IRL Press, UK
2. Christian, G.D. and O'Reilly, J.E., (1986). *Instrumental Analysis, 2<sup>nd</sup> Edition*. Boston, Allyn and Bacon.



## **Unit 5:                    Fourier Transform Spectroscopy (Interferometry)**

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## **Unit 5:        Fourier Transform Spectroscopy**

### **1.0 Introduction:**

Fourier transform spectroscopy is a non dispersive technique which uses an array of detectors to measure the entire spectrum at once or simultaneously. The spectrum is spread into its individual component wavelengths and each small band of wavelength is directed onto one detector. Thus, Fourier analysis is a mathematical way to decompose a signal into its component wavelengths that can be determined at once.

### **2.0 Objectives:**

By the end of this unit, you should be able to:

- explain the meaning of interferometry;
- Describe the instrumental design of an interferometer;
- State the application of interferometry; and
- List the advantages of FTIR in relation to interferometer device.

### **3.0 Definition:**

The Fourier transform infrared (FTIR) instruments do not contain any dispersing device and all wavelengths are detected and measured simultaneously. In this case, instead of monochromator, a device called interferometer is used to produce interference patterns that contain the infrared spectral information. Hence, the technique is called interferometry.

### **3.1 Interferometer:**

Interferometer is a non-dispersive device that obtains spectral information through constructive and destructive interference used in Fourier Transform Infrared instrument.

An interferometer is the heart of a FTIR spectrophotometer. It consists of a collimated light source, a stationary mirror at the top, a movable mirror at the right, a beam splitter and a detector. The light source may be a laser or sodium arc lamp. The mirrors are precision polished ultra-flat glass with a reflecting coating vapour deposited on their surface. The movable mirror is mounted on a very precise linear bearing that allows it to move along the direction of the light beam while remaining perpendicular to it.

The beam splitter is the key heart to the operation of the interferometer. It allows a fraction of the light to pass through the mirror and reflect the other fraction or part. The device works in both directions, so that light falling on either side of the beam splitter is partially reflected and partially transmitted.

### 3.2 Instrumentation:

The instrumental representation of an interferometer is shown below:

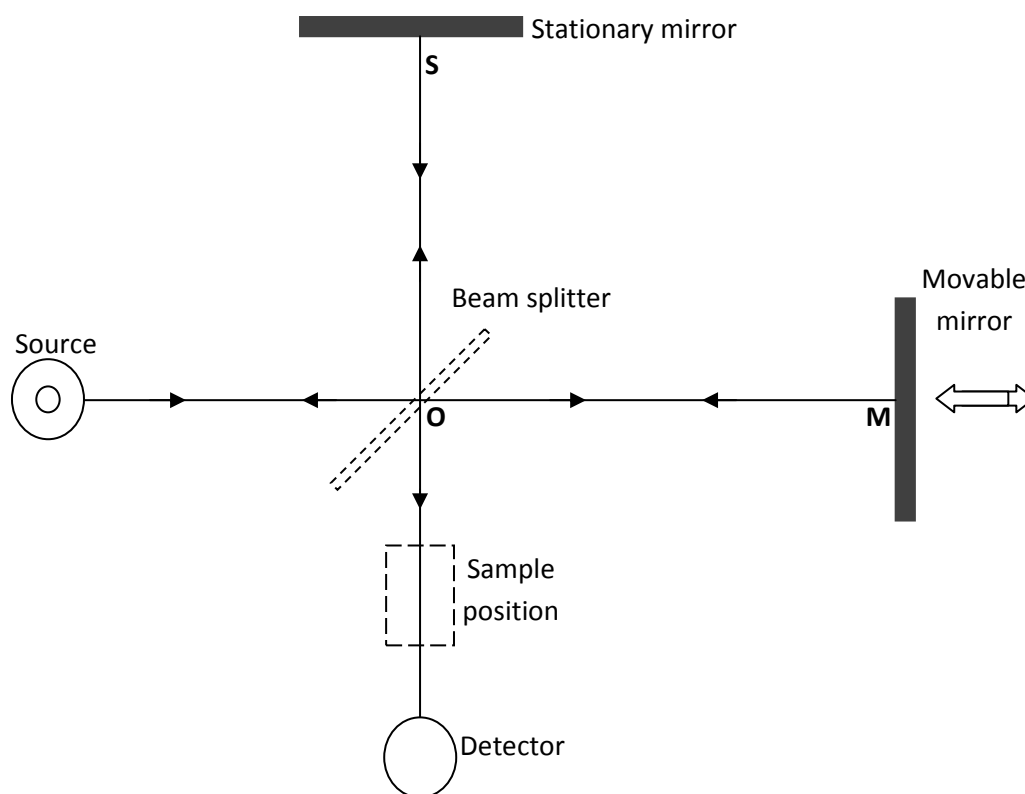


Fig. 5.1 A schematic diagram of interferometer

As seen in the above diagram, a beam from the light source falls on the splitter which then splits the beam into two parts. The two beams travel in separate paths and converge on the detector. The two beams (OS and OM) converge in the same region of space and form an interference pattern. As the movable mirror in the right is moved, the interference pattern shifts across the detector and modulates the optical signal. The resulting reference interferogram which is a plot of output light intensity against the wave number ( $1/\lambda$ ) is recorded. This is used as a measure of the power of the incident beam at all wavelengths. An absorbing sample is then inserted into the beam, and a sample interferogram is recorded. The two interferograms are then used to compute the absorption spectrum of the sample.

Example,

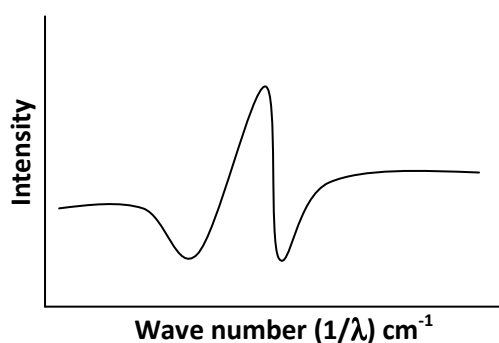


Fig: 5.2 an interferogram of the light source

### 3.3 Sample Application

In a Fourier spectrophotometer, the sample in a cell is usually placed between the output of the interferometer and the detector. As the light is passing through the sample, it then absorbs certain wavelengths of the incident light. Since the sample absorbs certain wavelengths of light, the interferogram contains the spectrum of the source minus the spectrum of the sample. An interferogram of a reference sample (blank) containing only the solvent is first recorded and transformed into a sample spectrum. The two interferograms are then used to compute the absorption spectrum of the sample.

### 3.4 Advantages of FTIR

The FTIR spectrophotometer is a very sophisticated instrument. It has advantages of : Speed, frequency accuracy, more efficient use of radiation by the interferometer, improved signal, a built data handling capabilities.

These advantages cause the rapid displacement of traditional dispersion instruments.

### 4.0 Conclusion

Interferometry is a technique which uses interferometer instead of a monochromator to produce an interference pattern that contains the infra red spectral information.

### 5.0 Summary

In this unit, we have learnt that:

- Interferometry involves the use of a device called interferometer.
- Interferometer is the heart of FTIR instrument.
- An interferometer consists of a beam splitter, a stationary mirror and a movable mirror. Reflection of the light from the two mirrors creates an interferogram.
- Fourier analysis is a mathematical way to decompose a signal into its component wavelengths.

### 6.0 Tutor Marked Assignment

1. (a) Explain briefly, the modifications made on Fourier Transform Infra-Red Spectrometry.  
(b) A typical FTIR spectrometer covers a wavelength range from 3 - 15 $\mu$ m. How would you express this in: (i) wave number and (ii) hertz?
2. How would you distinguish an infra red spectrometer from FTIR spectrometer.

### 7.0 Further Reading/References

1. Daniel, C.H. (1982). *Qualitative chemical Analysis*, 2<sup>nd</sup> Edition
2. Douglas, A.S., Donald, M.U., James, F.H. and Stanley, .C. (2004). *Fundamentals of Analytical Chemistry*, 8<sup>th</sup> Edition

# Module 3: Electroanalytical and Other Optical Techniques

## Unit 1 Polarography

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## **Unit 1          Polarography**

### **1.0    Introduction**

Voltametry encompasses a sophisticated collection of analytical techniques in which the relationship between voltage and current is observed during electrochemical processes. The major subdivision of voltametry is polarography, a sensitive electro-analytical technique that is especially useful for trace analysis. The second major classification within voltametry is amperometry.

In polarography, the current flowing through the cell is measured as a function of the potential of the working electrode. Usually, this current is proportional to the concentration of the analyte. The most sensitive polarographic procedures have a detection limit near  $10^{-9}$  M and a precision around 5%. Less sensitive polarographic methods operating with  $\sim 10^{-3}$  M analyte are capable of a precision of a few tenths of percent, though 2.3% is most common.

### **2.0    Objectives**

By the end of this unit, you should be able to

- define polarography;
- explain the mode of operation of a simple polarograph;
- describe the shape of a polarogram; and
- enumerate the applications of polarography.

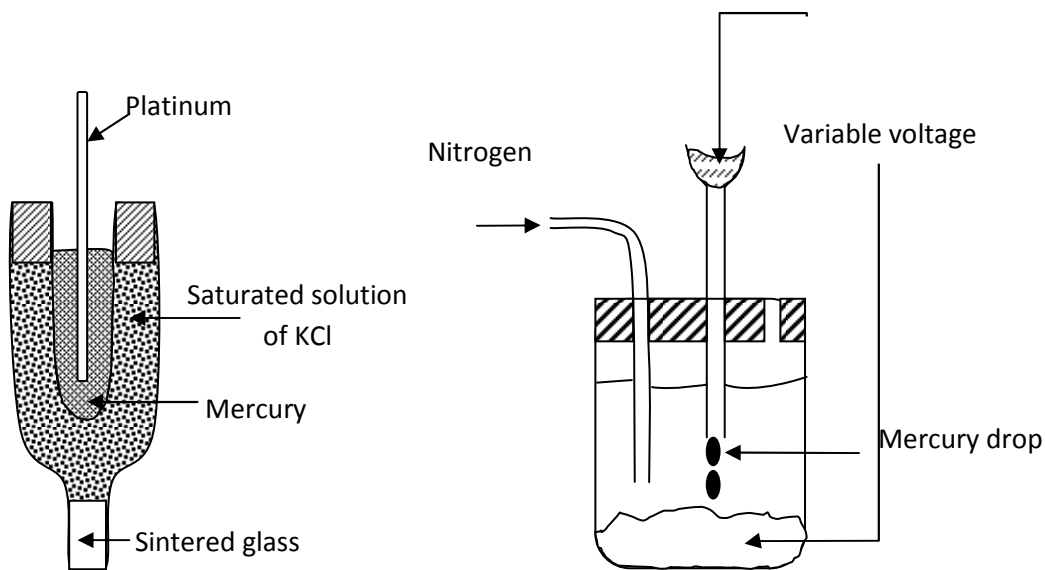
### **3.0    Definition**

Polarography is a technique in which the current flowing into an electrolysis cell is measured as a function of the applied potential.

#### **3.1    Mode of Operation of a Simple Polarograph**

Apparatus for a direct-current polarograph experiment is shown below. The mercury pool provides a large area anode with little polarization. The attached mercury drop is the cathode and the steady detachment of the drops minimizes contamination. The nitrogen keeps the solution free from oxygen which affects the performance adversely. Potassium chloride electrolyte is used to increase the conductivity because of large negative discharge potential of the potassium ion.

The applied voltage is gradually increased and the variation in current is measured. The potential of the cathode is determined by inserting into the solution, a standard electrode. Thus:

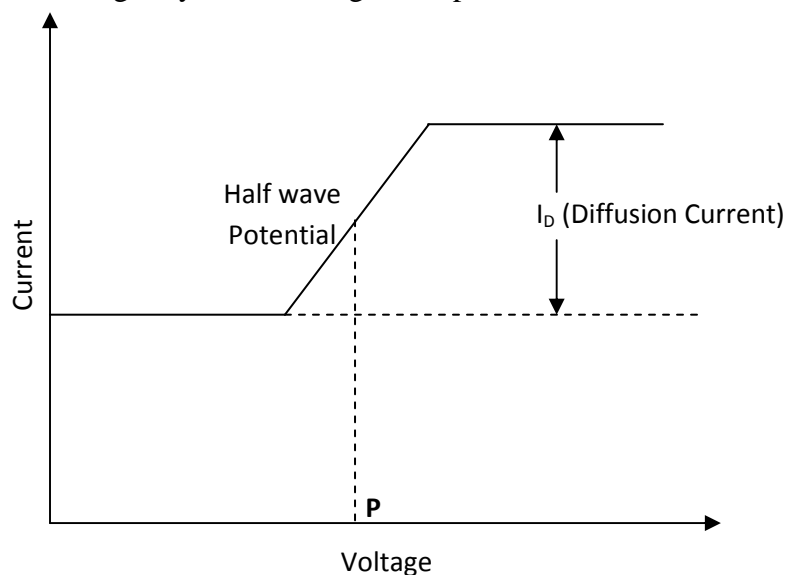


**Fig. 1.0 A Polarography Apparatus**

Source: *Advances in Analytical Methods* by G.R. Palin, Page 207

### 3.2 Shape of a Polarogram

A graph of current versus potential in a polarographic experiment is called a polarogram, and for a solution containing only one dischargeable species,  $M^{n+}$ , it has the form shown below:



**Fig. 1.1 A Polarogram**

Source: *Quantitative Chemical Analysis 2<sup>nd</sup> Edition* by Daniel C. Harris, Page 454

Theoretically, the current should be zero when the voltage is insufficient to produce the discharge potential in the cathode, but in practice, there will be some small current. When the

discharge potential of the ion  $M^{n+}$  is reached, the current increases rapidly as the voltage is increased until the concentration polarization becomes so great that the limiting current is attained. Any further increase in the applied voltage will produce little or no change in the current. It has been shown that the half wave potential  $P$  is independent of the ionic concentration, and for a given potassium chloride concentration, there is a fixed value of  $P$  which corresponds to a given ionic species. This enables qualitative determinations to be carried out.  $I_D$  is known as the limiting diffusion current which is dependent on the concentration of  $M^{n+}$ . Hence, quantitative determinations can also be possible.

### 3.3 Applications of Polarography

- The technique is effective in the qualitative identification of an unknown.
- Polarographic technique can be used to study the rates of chemical reactions that compete with the electrode reactions.
- It can also be used to determine more than one ionic species in solution; but this requires a series of steps of the same form which occurs when the discharge potential of each species is reached.
- The technique is also effective in the determination of very dilute solutions.

### 4.0 Conclusion

Polarography is a method in which the amount of current flowing into an electrolytic cell is measure as a function of the applied potential.

### 5.0 Summary

In this unit, we have learnt that:-

- Polarographic method measures the amount of current flowing through an electrolytic cell.
- The amount of current is directly proportional to the concentration of the analyte.
- A plot of current against the electrical potential produces a polarogram.
- Polarographic method is used in qualitative identification of compounds and to study the kinetic parameters of some chemical reactions.



## **6.0 Tutor Marked Assignment (TMA)**

1. (a) Define the term polarography.  
  
(b) With the aid of a diagram, describe the mode of operation of typical polarographic equipment.
2. (a) State the application of polarography in analytical research.  
  
(b) Give an illustration of a polarogram and explain the meanings of (i) halfwave potential, (ii) diffusion current.

## **7.0 Further Reading/References**

1. Koryata J. (1993) *Ions, Electrodes and Membrane* 2<sup>nd</sup> Editon Wiley-Liss Inc., USA
2. A.J. Bard & L.R. Faulkner (1980). *Electrochemical Methods*, New York, Wiley, UK

## Unit 2: Coulometry

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## Unit 2: Coulometry

### 1.0 Introduction

Coulometry is an electro-analytical method of analysis. Faraday's laws of electrolysis form the basis of quantitative coulometric analysis. You should recall that according to this law: The weight of substance liberated during electrolysis is directly proportional to the quantity of electricity passed. Also the weight of substances liberated by the same quantity of electricity is in direct proportion to their equivalent weights. Coulometric methods are performed by measuring the quantity of electrical charge required to convert a sample of analyte quantitatively to a different oxidation state.

### 2.0 Objectives

By the end of this unit, the students should be able to:

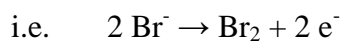
- define coulometry;
- describe the different types of coulometric analysis;
- itemise the steps involved in coulometric titrations; and
- Carry out some coulometric calculations.

### 3.0 Definition

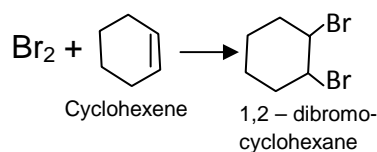
Coulometry is an electrolytic method of analysis that is based on measuring the number of electrons used in a chemical reaction.

#### 3.1 A Typical Example of Coulometric Process

In coulometry, cyclohexene may be titrated with  $\text{Br}_2$  which is generated by electrolytic oxidation of Bromide ( $\text{Br}^-$ ).



Then;



In the above reactions, enough of bromine is generated to react with all of the cyclohexene. Thus, the number of moles of electrons liberated is twice that of  $\text{Br}_2$  and hence, twice the moles of cyclohexene.

In coulometry, the reaction between  $\text{Br}_2$  and cyclohexene is carried out using a coulometric apparatus. During the process, enough  $\text{Br}_2$  is generated by the platinum electrode positioned at the left side. The generated  $\text{Br}_2$  then reacts with cyclohexene. If all of the cyclohexene has been reacted, the concentration of  $\text{Br}_2$  in the solution suddenly rises which indicates the end of the reaction.

The rise in concentration of  $\text{Br}_2$  is detected by measuring the electric current that is applied in the process. Thus, a small voltage is applied between the two electrodes but this is not enough to electrolyze the solute. When the concentration of  $\text{Br}_2$  suddenly increases, a current flows as a result of the reaction taking place in both cathode and anode. Thus, the sudden increase in current is taken as the end point of cyclohexene titration.

### 3.2 Types of Coulometry

There are two common types of coulometric methods namely: Controlled potential and Constant current.

#### 3.2.1 Constant Current Coulometry

In this type of analysis, a constant current is applied whenever the power is connected to the electrodes. This method is called coulometric titration. If the current is known, the time required for complete reaction is measured in order to count the coulombs.

$$\text{i.e. } q = It \quad (1)$$

The power supply is built to automatically measure the time of operation. When the current is multiplied by the time taken, the number of coulombs used in the titration is obtained.

#### 3.2.2 Controlled Potential Coulometry (Potentiostatic)

In this method, the potential of the working electrode is kept at constant level in such a way that only the analyte is responsible for conducting charge across the electrode and solution interface. The charge required to transform the analyte into product is determined by recording and integrating the current against time curve during the electrolytic process.

$$\text{i.e. } q = \int_0^t I dt \quad (2)$$

In this case the current is initially high and decreases exponentially as the concentration of the analyte sample decreases. Thus, the current decreases with time. Also the power supply

contains a circuit that automatically integrates equation (1) to calculate the number of coulombs applied.

Controlled potential coulometry is more selective than the constant current. As the current decays or decreases exponentially, the equivalence point is never reached. But the equivalence point can be obtained by setting the current decay to an arbitrary value. Example, the current will be 1% of its initial value when 99% of the analyte sample is consumed. On the other hand, the current will be 0.1% when 99.9% of the analyte has been used up.

### 3.3 A Typical example of Coulometric Calculations

Assuming that a 2000 cm<sup>3</sup> of solution containing 0.7113 mg of cyclohexene/cm<sup>3</sup> is to be titrated against bromine. If the coulometer is operated at a constant current of 5.820mA, what would be the time required for complete titration?

(Molar mass of cyclohexene = 82.146; 1F = 96485C)

In this case, the quantity of cyclohexene is calculated as follows:

$$\frac{2000 \text{ ml} \times 0.7113 \text{ mg/ml}}{82.146 \text{ mg /mmol}} = 17.318 \text{ mol} \cong 0.0173 \text{ m mol}$$

Since 82.146 is the molecular weight of cyclohexene

and each mole of cyclohexene requires one mole of Br<sub>2</sub>, which in turn requires two moles of electrons to flow through the circuit.

∴ for 0.01732 mmol of hexane to react, 0.03463 mmol of electrons must flow.

$$\text{Moles of } e^- = \frac{I t}{F}$$

$$\therefore t = \frac{\text{moles of } e^- \times F}{I}$$

$$\therefore t = \frac{0.03463 \times 10^{-3} \times 96485 \text{ C/mol}}{5.820 \times 10^{-3} \text{ C/s}} = 574.102 \text{ s/mol}$$

### 3.4 Current Efficiency requirements

In all coulometric methods 100% current efficiency is required. That is each Faraday of electricity must bring about a chemical change in the analyte equivalent to one mole of electron. Note that 100% current efficiency can be obtained without direct participation of the analyte in electron transfer at an electrode.

For example, Chloride ion (Cl<sup>-</sup>) may be easily determined using controlled potential coulometry or coulometric titrations with silver ion (Ag<sup>+</sup>) at a silver anode. The silver ion then reacts with chloride ion to form a precipitate or deposit of silver chloride. The quantity

of electricity required to complete the silver chloride formation serves as analytical variable. In this case, 100% current efficiency is realized because the number of moles of electrons is essentially equal to the number of moles of chloride ion in the sample, even though these ions do not react directly at the electrode surface.

### 3.5 Instrumentation

The equipment required for a coulometric titration consists of a source of constant current, a titration cell, a switch, a timer and a device for monitoring current. When the switch is moved to position 1, the timer starts and initiates a current in the titration cell. When the switch is moved to position 2, the electrolysis and the timer are stopped. With the switch in this position, the current continues to be drawn from the source and passes through a dummy resistor  $R_D$  that has about the same electrical resistance as the cell. This arrangement ensures continuous operation of the source, which aids in maintaining a constant current.

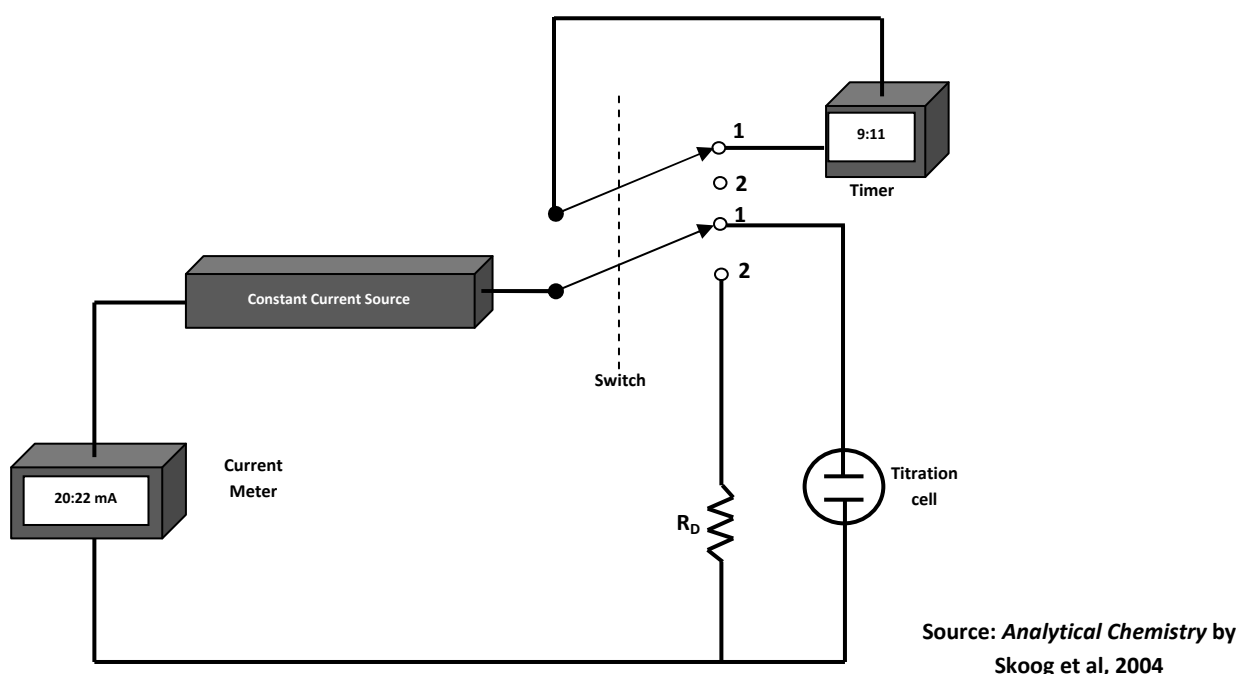


Fig. 2.1 Conceptual diagram of a coulometric titration apparatus.

But the instrument used for controlled potential coulometry consists of an electrolytic cell, a potentiostat and a device for determining the change consumed by the analyte sample.

Two types of cells are used. The first consists of a platinum gauze working electrode, a platinum wire counter electrode and a saturated calomel reference electrode. The counter electrode is separated from the analyte solution by a salt bridge, which contains the same electrolyte as the solution being analyzed. This bridge is needed to prevent the reaction products formed at the counter electrodes from diffusing into the analyte solution and interfering.

The second cell is made of mercury cathode. This is useful for separating easily reduced elements as a preliminary step in analysis. This electrode is also useful in the coulometric

determination of several metallic cations that form metals that are soluble in mercury. In this application, there is only little evolution of H<sub>2</sub> gas even at high applied potentials.

### 3.6 Advantages of Coulometric Titration Over the Conventional Titrations

Coulometric titration has a lot of advantages over a normal volumetric titration. Among the advantages is the elimination of the problems associated with the preparation, standardization and storage of standard solutions. This advantage is particularly significant with reagents that are not stable such as chlorine, bromine, etc. Another advantage is that coulometric method excels when small amounts of analyte is to be used in the titration. But with conventional titration, it is inaccurate and inconvenient to use very dilute solutions and small volumes.

In coulometric procedure, a single constant current source provides reagents for precipitation, complex formation, and neutralization or redox titrations. But this is not possible in normal volumetric titration. Finally coulometric titrations are more versatile and more accurate than the conventional volumetric process.

### 3.7 Application

Coulometric methods have been used to determine several elements in inorganic compounds and synthesize organic compounds.

But generally, coulometric titration is applied in:

- Neutralization titration
- Precipitation and complex formation reactions e.g coulometric titration with EDTA are carried out by reduction of the ammine mercury (II) EDTA chelate at mercury cathode.
- Oxidation – reduction (redox) titration – coulometric titrations have been applied for many redox titrations

### 4.0 Conclusion

Coulometry is a method of analysis which is based on measuring the number of electrons used in chemical reactions.

### 5.0 Summary

In this unit, we have learnt that:

- Coulometry comprises a series of analytical techniques in which the quantity of electricity needed to carry out a chemical reaction is used to measure the quantity of analyte present.
- Coulometric titrations are carried out at a constant current.
- Controlled potential coulometry is operated at constant potential and is more selective than coulometric titration.

- Measuring the electrons consumed in the reaction is achieved by electronic configuration of the current versus time curve.

## 6.0 Tutor Marked Assignment

1. (a) Differentiate between controlled – potential coulometry and constant – current coulometry.  
(b) Why is it necessary to isolate the working electrode from the counter electrode in a controlled – potential coulometric analysis?
2. In a certain coulometric experiment, 5.32 mA of current is passed for 964s for complete reaction of a 5.00 ml of a liquid of unknown cyclohexene solution
  - (a) How many moles of electrons passed through the cell?
  - (b) How many moles of cyclohexene reacted?

## 7.0 Further Reading/References.

1. Koryata J. (1993) *Ions, Electrodes and Membranes*, 2<sup>nd</sup> Edition, Willey – Liss Inc., USA
2. Koryata J., Dvorak and L. Kavan (1996) *Principles of Electrochemistry*, John Wiley, UK
3. Eggins, B.R. (1996) *Biosensors*, John Wiley, UK



## **Unit 3                      Conductimetry**

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## Unit 3 Conductimetry

### 1.0 Introduction

Conductimetry analysis involves the application of electrolytic cell to measure the conductance of a given solution. This is based on the electrical properties of ions present in the solution to be analyzed. When a current is passed through a solution containing negative and positive ions, such ions move towards the electrodes carrying the current into the solution. Positively charged ions migrate towards the cathode and negative ions towards the anode. But, the rate at which the ions move is influenced by a lot of factors such as the degree of the solvation of the ions, temperature and viscosity of the medium. Since the movement of ions is responsible for the conduction of electricity through the solution, the extent of flow of current is dependent on the number of ions per unit volume of solution (concentration), the ionic charge and the rate of migration towards the electrodes. Also, the extent of conduction depends on the potential difference across the solution.

### 2.0 Objectives

By the end of this unit, students should be able to:

- define conductimetric analysis;
- explain the principle of conductimetric analysis; and
- list the basic applications of conductimetric analysis.

### 3.0 Definition

Conductimetry is an electrolytic technique that is used to measure the conductance of sample solution based on the electrical properties of the constituent ions.

### 3.1 Basic Concepts of Conductance Analysis

The current passing between the two electrodes is carried by the ions in the solution and is related to the number of ions present. This is as a result of both the molar concentration of the compound and its extent of ionization. The anions donate electrons to the anode while the cations accept electron from the cathode. It is this transfer of electrons that determines the amount of current flowing and the contribution of each ionic species is determined by its ionic mobility.

The current flowing through a conductor is defined by Ohm's law which states that the electric current flowing through a conductor is directly proportional to the applied voltage and inversely proportional to the resistance of the conductor.

$$\text{i.e. Current (I)} = \frac{\text{Voltage (V)}}{\text{Resistance (R)}}$$

But in terms of conductance (C),

$$I = C \times V$$

### 3.1.1 Specific and Molar Conductance

The specific conductance ( $K$ ) of a given solution is defined as the conductance per centimeter of a solution that has a cross-sectional area of  $1 \text{ cm}^2$ , and is measured in  $\text{Scm}^{-1}$  (or in non SI unit of  $\Omega^{-1}\text{cm}^{-1}$ ).

The molar conductance ( $\Lambda$ ) is the specific conductance of a solution corrected for the concentration of ions in the solution.

That is, Molar Conductance = Conductance  $\times$  Volume of solution which contains 1 gram mole

NOTE: The value of conductance decreases as the concentration of the solution decreases, but the value for molar conductance will increase. This is due to increased dissociation of molecules in dilute solutions.

### 3.2 Conductimetric Measurements

The basic instrument used for conductimetric measurements is called conductivity meter. This instrument has a basic arrangement of a Wheatstone bridge. In this case, the conductance of solution is measured using an alternating current rather than a direct one. This is to prevent the accumulation of charges at the electrodes which would change the composition of the solution at the electrodes and introduce substantial errors in the measurement.

The basic circuit diagram of the instrument is given below:

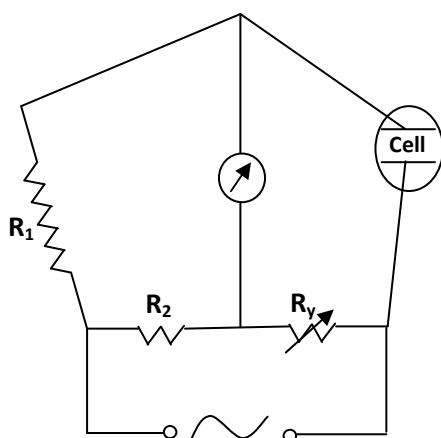


Fig 3.1 : A Wheatstone

With fixed resistance,  $R_1$  and  $R_2$ , the variable resistance  $R_v$  is adjusted until current just flows through the galvanometer. Under these conditions,

$$R_1 \times R_v = R_{\text{cell}} \times R_2$$

This gives a value for resistance of the cell which can be converted to conductance by calculating the reciprocal.

The electrodes conductivity cells are usually made of platinum coated with platinum black with a known area. Despite that in many cells the distance between the electrodes is

adjustable, but in any experiment it must be held constant and for many calculations the precise value is required. The cells must be thermostatically controlled because any changes in temperature will cause significance alteration of conductivity values.

### 3.3 Experimental Precautions

- Solvent purity

The presence of ions in the water used in conductivity measurement can lead to serious errors particularly in the analysis of a very dilute solution where the measured conductance may be of nearly the same magnitude as the conductance of the dissolved impurities. Thus, in conductivity measurement de-ionized water has a specific conductance (conductivity) of  $5.5 \times 10^{-8} \Omega^{-1} \text{cm}^{-1}$

- Titrant Volume

During conductimetric titrations, the concentrations of the conducting species should not change significantly; otherwise errors will be introduced into the measurements. Hence, the concentration of the titrants should be about 10 times that of the solution in the conductivity cell so that the increase in volume at, and beyond, the end point will be small.

- Temperature

The specific conductance of most ions usually increases by about 2% for each degree rise in temperature. It is therefore very important to control the temperature accurately in such measurements. For example, for titrations and other routine analysis, the temperature may be controlled for  $\pm 0.25^{\circ}\text{C}$  which ensure a precision of  $\pm 0.5\%$ . But, for other purposes, such as the determination of dissociation constants, more accurate temperature control is required.

- Extraneous ions

The presence of large concentrations of extraneous ions in solution to be analyzed is usually not required. This is because any change in conductance is being masked by such ions. This happens particularly if the limiting equivalent conductance of the foreign ions is similar to those of the ions to be analyzed. Such problem may be overcome by using high precision conductivity meters or bridges.

### 3.4 Applications

Conductimetry is a very useful phenomenon for the determination of various physical constants, such as dissociation and solubility constants. But its major application is for monitoring titrations.

- Solubility determination

Conductimetry is applied in the determination of solubilities of sparingly soluble salts. It involves the measurement of the conductivity ( $k$ ) for the saturated solution and then followed by the calculation of the concentration ( $C$ ) from the equation:  $\Lambda = \frac{1000K}{C}$

where  $\Lambda$  is the equivalent conductance or specific conductance of 1 cm<sup>3</sup> solution containing 1 gram-equivalent of solute (molar conductance)

- **Dissociation constant**

This is determined by calculating the degree of dissociation from the equivalent conductance of the analyte.

For a weak acid HA,

i.e.

$$K_c = \frac{[H^+][A^-]}{[HA]} = \frac{\alpha^2 C}{1 - \alpha}$$

where  $\alpha$  is the degree of dissociation given by:

$$\alpha = \frac{\Lambda^c}{\Lambda^d}$$

where  $\Lambda^c$  is the equivalent conductance before dissociation while  $\Lambda^d$  is equivalent conductance after full dissociation.

- **Precipitation titrations**

In these titrations, the conductance of a solution is measured as function of the volume of a titrant. The shape of the titration curve depends on the conductance of the ion in the cell ( $i_c$ ) and that of the ion in the titrant ( $i_t$ ). If  $i_c$  is greater than  $i_t$ , the conductance decreases at first, then increases after the endpoint as the concentration of the titrant ion increases. The end point is the intersection of the two lines.

- **Acid-base titration**

As an acid is titrated with an alkali, the ionic composition of the mixture changes and this is reflected in the conductivity of the solution.

In conductimetric titration, the conductivity can be plotted against the volume of the alkali that is being used. This gives rise to different titration curves as below:

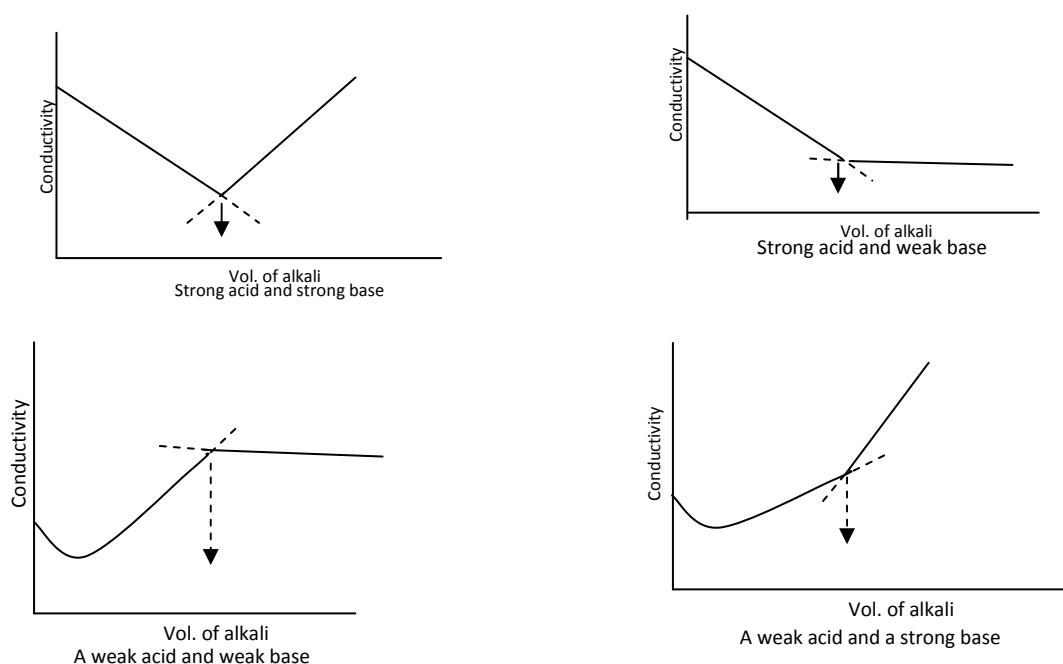


Fig: 3.2

Note: the end point is where the two lines intersect each other.

#### 4.0 Conclusion

From what we have learnt, it is clear that conductimetric analysis involves the application of electrolytic process to measure the conductance of a given solution.

#### 5.0 Summary

In this unit, we have learnt that:

- Conductimetry is an electrolytic technique that is used to measure the conductance of a sample solution based on electrical properties of the constituent ions.
- The electric current flowing through a conductor is directly proportional to the applied voltage and inversely proportional to the resistance of the conductor.
- Specific conductance of a given substance, is the conductance per cm of a solution that has a cross-sectional area of  $1\text{cm}^2$  and is measured in  $\text{Scm}^{-1}$  or S.I. unit of  $\Omega^{-1}\text{cm}^{-1}$ .
- The basic instrument used for conductivity measurement is called a conductivity meter which has a basic arrangement of a Wheatstone bridge.
- Conductimetry is very useful in determining various physical constant such as dissociation constant and solubility constant. It is also very useful in titrimetric analysis.

#### 6.0 Tutor Marked Assignment

1. (a) Explain the basic concept of conductimetric analysis.  
(b) With the aid of a good diagram, describe the basic instrumental design of a conductivity meter.
2. Illustrate the different titration curves obtainable from conductimetric titration and indicate the end point of each titration.

#### 7.0 Further reading/References

1. Douglas, A.S., Donald, M.W., James, F.H. and Stanley, R.C. (2004). *Fundamentals of Analytical Chemistry*, 8<sup>th</sup> Edition.
2. David, J.H. and Hazel, P. (1998). *Analytical Biochemistry*, 3<sup>rd</sup> Edition, Longman.
3. Daniel, C.H., (1982). *Qualitative Chemical Analysis*, 2<sup>nd</sup> Edition.

## **Unit 4                      Polarimetry**

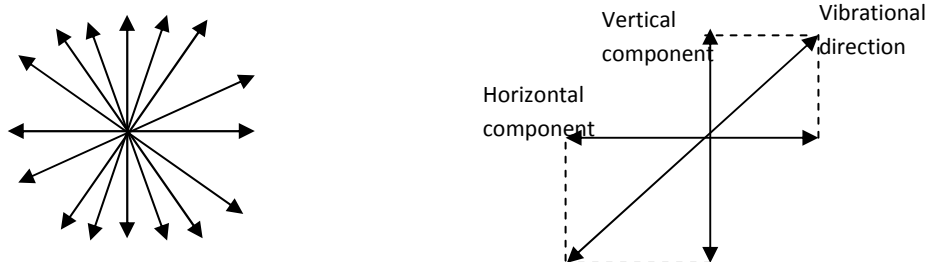
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## Unit 4      Polarimetry

### 1.0      Introduction

Polarimetry is the method of analysis which is based on the ability of a given compound in solution to rotate a plane polarized light to a certain direction.

An ordinary light beam consists of waves oscillating in random directions. Each of the vibrational directions can be resolved into two mutually perpendicular directions. But the actual vibrational direction is the vector sum of the two components



If the ordinary light is collimated and passed through a crystal which allows only one vibrational orientation to be transmitted, the transmitted light is said to be plane polarized. Many natural crystals produce polarized light, but it is most conveniently obtained with commercially available Polaroid materials.

### 2.0      Objectives

By the end of this unit, you should be able to:

- Explain what a polarimeter is;
- draw a schematic diagram of a polarimeter;
- explain the mode of operation of a polarimeter; and
- enumerate the uses of a polarimeter.

### 3.0      Definition

An instrument used in measuring the optical rotation of a substance in solution is called a polarimeter and the phenomenon, polarimetry.

### 3.1      Basic Principle of Polarimetry

Some compounds in solution have the power of rotating the plane polarized light to a certain direction. Such compounds are said to be optically active and, in the case of organic compounds, are found to have one or more asymmetric carbon atoms within the molecule. An asymmetric carbon atom is one with four different groups attached to it. The degree or



direction of the rotation is either to the right (called dextrorotatory, +) or to the left (called laevorotatory, -).

The extent of the degree of rotation of the plane polarized light is dependent on the:-

- nature of the compound;
- nature of the solvent;
- depth of the solution through which the light passes;
- concentration of the solution;
- temperature; and
- wavelength of the light used.

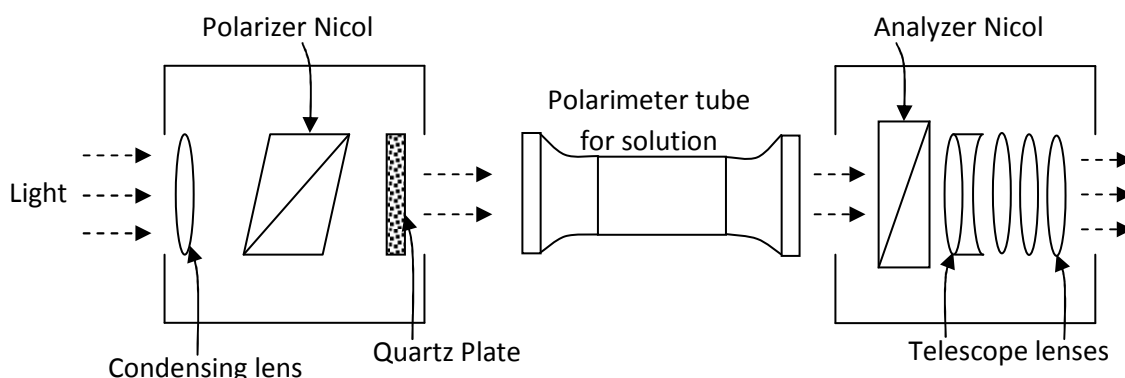
The wavelength of the light normally used is that of sodium D line (589.3 nm), and the temperature is kept constant at 20 or 25°C within the instrument.

### 3.2 Mode of Operation of a Polarimeter

Before operation, the analyzer prism of the polarimeter is rotated to make both halves of the field of view equally bright; the circular scale reading for this position of the analyzer prism is noted as its zero position. The polarimeter tube is then filled with the experimental solution, the jacket around the tube is filled with water at a given temperature and the solution is allowed to stand in the tube for some time to attain that temperature. On passing the monochromatic light again through the polarimeter, the two halves of the field of view now look unequally illuminated due to the rotation of the plane polarized light by the solution in the tube. The analyzer prism is rotated until both halves look equally bright again. The difference in the circular scale reading between the final position of the analyzer prism and its zero position recorded initially, gives the angle of rotation of the polarized light.

### 3.3 Instrumentation

Optical rotation is usually measured by a polarimeter or polariscope and the phenomenon is called **Polarimetry**. The polarimeter consists of a long hollow barrel carrying at its opposite ends, a fixed polarizer Nicol prism and a rotating analyzer Nicol prism respectively. A hollow polarimeter tube, encircled by a jacket, can be placed inside the barrel between the two prisms. This tube of a given length is intended for carrying the solution under investigation while the jacket around it may be filled with water at a given temperature. Between the polarizer prism and the polarimeter tube, a quartz plate is located for obscuring half the field of view. Beyond the analyzer prism are situated the telescopic eyepiece lenses. That end of the polarimeter barrel carries a fixed circular scale graduated in degrees radian, the position of the analyzer prism may be read on this scale. A schematic diagram of a polarimeter is given below:



**Fig: 3.4 A Polarimeter**

**Source: A guide to the choice of Optical Equipment and Reagents for Immunofluorescence Technique by Taylor, C.E.D. et al, Page 165.**

There are three essential parts:

- The polarizer (a lens and nicol prism assembly to polarize the incident light)
- The polarimeter tube (a thick-walled glass tube with a screw on both ends incorporating optical glass windows) which contains the solution under examination.
- The analyzer (a nicol prism, and eye piece assembly for crossing the plane of the emergent polarized light) which is filled with a degree scale and pointer.

### 3.4 Uses of Polarimeter

Below are some of the uses of the instrument:

- From the angle of rotation “ $\alpha$ ” of the polarized light determined by the polarimeter, the length,  $l$ , of the solution column in the polarimeter tube and the concentration  $C$  of the solution, the specific rotation  $\alpha$  of the solute may be calculated by the formula given below:

$$[\alpha]_D^{25} = \frac{\alpha}{l \times C}$$

- An optically active substance may be identified in a solution of a known concentration by measuring its optical rotation.
- From the optical rotation of a mixed solution of two substances having known specific rotations (e.g. sucrose and glucose), their respective concentrations in that solution can be estimated.
- If  $\alpha$  is already known for the solute, its concentration  $C$  in the solution can be calculated using the estimated angle of rotation,  $\alpha$ , and the length,  $l$ , of the solution column.

### 4.0 Conclusion

Polarimetry is a method which is used to determine the optical rotation of a given compound based on the ability to rotate a plane polarized light to a certain direction.

## 5.0 Summary

In this unit, we have learnt that:

- Polarimetry is a method of analysis which is based on the ability of a given optically active compound to rotate a plane polarized light.
- The degree of rotation could be either to the right (D) or to the left (L)
- Sodium lamp is normally used to provide a specific wavelength (589.3 nm) for optical rotation.
- Optically active compounds contain one or more asymmetric carbon or chiral centre.

## 6.0 Tutor Marked Assignment (TMA)

1. (a). Distinguish between dextrorotatory and laevorotatory  
  
(b). With the aid of a schematic diagram, describe the instrumental arrangement of a polarimeter and state the function of each part.
2. (a) A solution of 3 – phosphoglycerate ( $0.25 \text{ g/cm}^3$ ) in water has an observed rotation of  $-2.16^\circ$  in a 1 dm tube at  $20^\circ\text{C}$ . What would be the specific rotation of the compound? State whether the compound is L or D and give your reason.

## 7.0 Further Reading

1. Philip, M. (2003). *Advanced Chemistry Physical and Industrial*. 1<sup>st</sup> Edition, Cambridge
2. Allen, M.S., Barbara, A.G. and Melvin, L.D. (2000). *Microscale and Miniscale Organic Chemistry Laboratory Experiments*. McGraw-Hill Higher Education.

## **Unit 5**

## **Refractometry**

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## Unit 5      Refractometry

### 1.0      Introduction

In addition to absorption and emission, there are other interaction phenomena which are useful for both identification and structural studies. Hence, refraction is one of these important non-absorptive processes. When radiation passes from one medium to another, it is partially reflected and partially transmitted. The transmitted radiation retains its characteristic frequencies in the new medium, but both the velocity and direction of propagation may change. This can be illustrated by allowing a radiation to pass from air to glass medium. To explain this behavior, a 'wave front' perpendicular to the direction of propagation of the ray is considered. Thus if the ray strikes the surface of glass at an angle of incidence ( $\phi$ ), then one side of the wave front reaches the interface and enters the glass before the other. Therefore, while one side of the wave front is travelling in glass, the other side is still travelling in air.

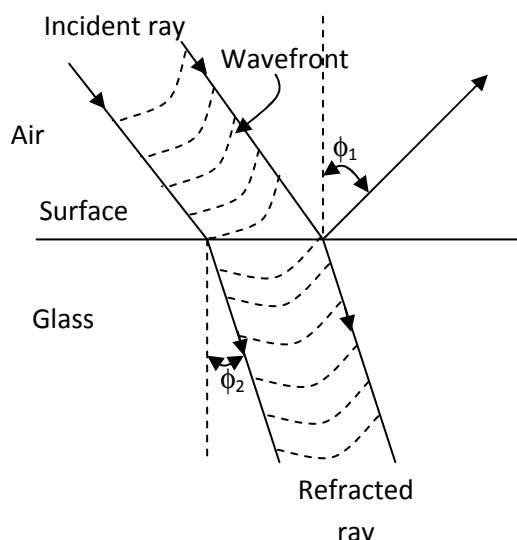


Fig. 3.4

### 2.0      Objectives

By the end of this unit, students should be able to

- explain the meaning of both refractive index and refractometry;
- describe the basic concept of refractometry;
- calculate the exact refractive index of a substance based on the given data; and
- explain the procedure for practical determination of refractive index.

### 3.0      Definition

Refractometry is a method which measures the refractive index of a substance.

The refractive index measures the angle at which a light ray will be bent when passing from one medium to another.

### 3.1 Basic Concept of Refraction

Refraction is the change in the direction of propagation of light waves as they cross the interface between two media of differing densities. When a ray of light passes from air into a dense medium (such as liquid), it is bent or refracted towards the normal. The ratio of the sine of the angle of incidence to the sine of that of refraction is constant and characteristic of that medium. This constant ratio ( $n$ ) is called the refractive index. The refractive index,  $n$ , for a liquid or an isotropic solid is defined as the ratio of the phase velocity of light in vacuum to that in another medium.

$$n = \frac{V_{air}}{V_{medium}} = \frac{\sin \phi_{air}}{\sin \phi_{medium}}$$

Refractive index of a given substance is practically determined by the use of refractometer, which operates on the concept of the critical angle. This is the angle of incidence ( $\phi_{medium}$ ) for which the angle of refraction ( $\phi_{air}$ ) is exactly  $90^\circ$ . The determination of refractive index involves only the determination of the two angles. These angles may be measured quite accurately and thus  $n$  may be determined with high degree of precision.

### 3.2 How to Calculate the Exact Refractive Index

The refractive index is an important physical property of inorganic liquids. The refractive index for many organic liquids can be found in chemical reference books such as *The Handbook of Chemistry and Physics*. The literature values are referenced to the D line of sodium lamp (589.3 nm) at  $20^\circ\text{C}$ . Thus, a correction factor must be applied if the refractive index ( $n_{obs}$ ) is measured at temperatures,  $t$ , other than  $20^\circ\text{C}$ .

This correction factor is :

$$n_D^{20} = n_{obs} + 0.00045 (t - 20)$$

where  $n_{obs}$  is the observed refractive index from the refractometer,  $n_D^{20}$  is the correct determined refractive index.

Example: A student measures the refractive index of an organic liquid. The value obtained at  $18^\circ\text{C}$  is 1.2821. What is the corrected refractive index?

$$\text{Using } n_D^{20} = n_{obs} + 0.00045 (t - 20)$$

$$\therefore n_D^{20} = 1.2821 + 0.00045 (18 - 20)$$

$$= 1.2821 + (0.00045 \times -2)$$

$$= 1.2812$$

### 3.3 How to Operate a Refractometer

The following procedures are used for determining the refractive index of a sample with Abbe Refractometer:

1. Check the sodium arc to see that it is operating properly.
2. Check the temperature reading by the thermometer attached to the prism housing.
3. Rotate the illuminating prism downwards and away from you. Wipe both the prism surfaces gently with a fresh swab of cotton wool dampened with acetone. When the prism surfaces are cleaned and dried, rotate the illuminating prism upwards and towards you to restore the closed position.
4. Introduce a few drops of the sample into the entrance hole.
5. Rotate the prism until the boundary between light and dark fields appears in the field of view. Adjust if necessary, the light source or the mirror to obtain the best illumination.
6. Also rotate if necessary, the prisms to eliminate the colour fringe and sharpen the boundary.
7. Make any necessary fine adjustments to bring the boundary between light and dark fields into coincidence with the intersection of the cross-hairs. Then turn the lamp that illuminates the scale and note the value of the refractive index of the sample.
8. Clean the prism surfaces with a fresh swab of cotton wool moistened with acetone and close the prism when clean and dry.

**Application:** Refractometer is essentially an analytical instrument used to determine the composition of binary mixtures or to determine the purity of compounds. Its most common industrial application is in the food and confectionery industry where it is used for the determination of the concentration of sugar in syrup. In addition to these, refractometry can be used to estimate molecular weights, molecular sizes and shapes, and to calculate properties such as reflectivity and optical dispersion.

### 3.4 Measuring the Refractive Index of a Substance

The sample of the unknown is obtained and recorded accordingly. Put about four drops of the liquid sample between the plates of the refractometer. Measure and record the refractive index of the liquid. Record the room temperature. If the temperature differs from 20<sup>0</sup>C, apply a correction factor to the refractive index. Record the corrected refractive index. Then identify the unknown liquid by comparing the refractive index with the standard.

The refractive indices of some organic liquids are shown below:

Organic liquid	Refractive index ( $n_D^{20}$ )
Acetonitrile	1.3442
Hexane	1.3751
1-propanol	1.3850
1-Butanol	1.3993
Cyclohexane	1.4266
Toluene	1.4961

#### 4.0 Conclusion

Refractometry is a method of analysis which is based on determining the refractive index of a substance. The method is valuable in ascertaining the purity of a substance.

#### 5.0 Summary

In this unit, we have learnt that:

- Refractometry is a technique which measures the refractive index of a substance.
- Refractive index determines the angle at which a light ray is bending when passing from one medium to another
- A correction factor  $n_D^{20} = 0.00045 (t-20)$  is used to obtain the exact refractive index of a substance.
- The technique is applied in food and confectionery industry to determine the concentration of sugar in syrup or other allied products.

#### 6.0 Tutor Marked Assignment (TMA)

1. (a) What is meant by refraction and refractive index?  
(b) A student measures the refractive index of an oil suspected to be of plant origin. The value obtained is 1.3871 at 25<sup>0</sup>C. Calculate the exact refractive index of the oil.
2. (a) How does the impurity affect the refractive index of a substance?  
(b) If two samples of organic liquids give the same value of refractive index, are the liquids similar? Explain.

#### 7.0 Further reading/References

1. A.M. Schoftstall, B.A. Gaddis & M.L. Druelinger (2000) – *Microscale and Miniscale Organic Chemistry laboratory Experiment.*