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Review Article

SPECTROPHOTOMETRIC STUDY – A REVIEW

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ABSTRACT

This article reviews about the principles of spectrophotometry, types of visible spectrophotometers, and discusses about the instrumentation and applications of infra red spectroscopy.

Keywords: Spectrophotometry, Visible spectrophotometers, Infra-red Spectroscopy.

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INTRODUCTION

Spectrophotometry is the quantitative study of electromagnetic spectra. Spectrophotometry deals with visible light, near-ultraviolet, and near-infrared. Spectrophotometry involves the use of a spectrophotometer. A spectrophotometer is a photometer (a device for measuring light intensity) that can measure intensity as a function of the color, or more specifically the wavelength of light. There are many kinds of spectrophotometers. Other important features of spectrophotometer, the spectral bandwidth and linear range. Perhaps the most common applications of spectrophotometers is the measurement of light absorption [1].

Absorption spectroscopy uses the range of the electromagnetic spectra in which a substance absorbs. This includes atomic absorption spectroscopy and various molecular techniques, such as infrared spectroscopy in that region and nuclear magnetic resonance (NMR) spectroscopy in the radio region. Emission spectroscopy uses the range of electromagnetic spectra in which a substance radiates (emits). Molecular luminescence techniques include spectrofluorimetry Scattering spectroscopy measures the amount of light that a substance scatters at certain wavelengths, incident angles, and polarization angles [2].

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ELECTROMAGNETIC RADIATION

EMR may be considered as an electromagnetic wave traveling at the speed of light. The radiation consists of discrete particles (quanta) of energy, known as photons. The term radiation includes electromagnetic radiations ranging from the electric waves of low frequency through UV rays, visible spectrum, IR rays to the high frequency X-rays and p-rays. E is also true that EMR requires no supporting medium for its transmission and can pass through vacuum.)

SPECTROSCOPY

It could be defined as the branch of science which deals with the study of interaction of EMR with matter. Spectroscopy is one of the most powerful tools available for the study of wide range of samples. The study of spectroscopy can be carried out under atomic spectroscopy and molecular spectroscopy [3].

INTERACTION OF EMR WITH MATTER

The interaction of radiation and matter takes place throughout the entire electromagnetic spectrum. The electromagnetic spectrum extends from cosmic rays to radio waves. Within these extremes are the rays such as gamma rays, X rays, far, middle and near ultraviolet rays, the visible spectrum, IR rays and microwaves.

These radiations when they react with matter, the effects produced will vary in its nature. In the IR region, the sorption of radiation causes changes in rotational and rotational-vibrational energy states. In the visible and UV region the absorption of radiation, can change the energy of the valence electrons. Xrays cause the ejection of inner electrons from matter and the gamma rays on absorption of radiation cause changes in the nucleus.

When EMR passes from Vacuum to the surface of a portion of matter, the radiation may be absorbed, transmitted, reflected or scattered [4].

ABSORPTION: is a process in which certain frequencies may be selectively removed when a beam of radiation is allowed to pass through a transparent layer of a solid, liquid or gas.

When an atom or molecule absorbs energy, they will move to higher energy state or excited state. Excited atoms or molecules are short lived and tend to come back to their ground state after 10-8 sec, with emission of certain amount of energy.

When the emission of light is instantaneous, it is called fluorescence but when there is some time lag it is called phosphorescence. When the absorbed energy stored by the atom or molecule and used in producing some chemical reaction, it results in photo-chemical reaction

OPTICAL METHODS

Instruments that measure the transmittance or absorbance of solution contain five basic components which includes a stable light source, a monochromator, sample containers for sample and solvent, a radiation detector, a signal indicator.

Radiation Source

Sources of visible radiation: Tungsten filament lamp. Sources of Ultraviolet radiation: H2 and Deuterium lamps Sources of IR radiation: A coil of nichrome wire can be used as IR source. Wavelength Selection It can be done by 1) The use of filters,2) Dispersion by means of prism or diffraction grating.

Absorption filters consist of colored glass and an interference fitter consists of transparent CaF2 that occupies the space between two semitransparent metallic films coated on the inside surface of two glass plates.

Monochromator:

It is used to separate polychromatic radiation into a suitable monochromatic form. It is a device that resolves radiation into its component wavelength and permits the isolation of any desired portion of the spectrum from the remainder. Such devices may be prisms or gratings.

In a prism monochromator light administered through an entrance slit is collimated by a lens and then strikes the surface of the prism at an angle. This deflects the beam through an angle depending on the wavelength. The dispersed radiation is then focused on a curved surface containing the slit. Radiation of the desired wavelength may be caused to pass through the exit slit by rotation of the prism and with grating it is by rotating the grating [5].

Sample Containers

Cells or cuvettes used are made up of different substances as follows. Quartz and / or fused silica in UV region and glass is used in visible region. For solid samples in IR region, they are either dissolved in liquid paraffin or mixed with alkali halide e.g. KBr.

Detectors

The common detection devices are photocells, photo multipliers, photographic plates, thermocouples and photo conductive cells. Essential characteristics of a detector 1. It should be sensitive to low levels of radiant power. 2. It should respond rapidly to the radiation 3. It should produce an electrical signal that can be amplified. E.g. Thermocouple Thermistor.

Thermocouple: is the most widely used Infra red detector and operates on the basis of heat detection. **Bolometer:** is a miniature resistance thermometer with a tiny platinum wire as sensing element. **Thermistor:** is made by sintering together several metallic oxides. **Photomultipliers:** operate on the principle of photon amplification. A photon striker a photo cathode gets multiplied by striking a series of anodes resulting in electron multiplication [6].

Photovoltaic cells: in which the radiant energy generates a current at the interface of a semi-conductor and a metal are used for the detection and measurement of radiation in the visible region. Photographic Plate: has an advantage that it integrates radiant energy over a period of time.

Instrumentation:

Spectrophotometers are preferred rather than filter photometer. As filters have disadvantages, they are replaced by monochromator.

Light Source: Incandescent tungsten lamp in the visible region 320 .110mu is used as the source of light [7].

Monochromator: Monochromator such as prism, diffraction grating may be used. In some monochromator lenses, mirror or other optical components are required.

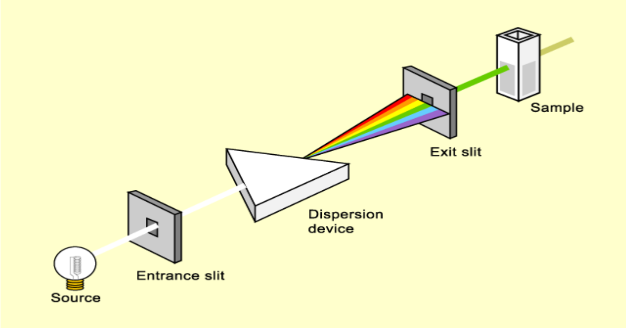
Lenses and prisms of ordinary glass can be used for visible region.
Detectors: A detector is a transducer that converts EMR into an electron flow and subsequently into a current flow or voltage in the read out circuit. The detectors commonly used include solid state.
'S' is a source of radiation which is a tungsten lamp. Light from the radiation source S is allowed to pass, by means of a lens L, through a narrow slit T and then by means of a mirror u to an optical grating G which divides light into narrow spectral regions corresponding to different wavelengths. The light of a desired wavelength emerging from the grating is allowed to pass through the cuvette B containing the solution under examination. The light further passes to photo electric cell D which is in contact with galvanometer. The intensity of light can be measured with the help of cell D. The cuvette B is now replaced by another cuvette C containing pure solvent and the same light is allowed to pass through it and then to cell D. When log b / I is plotted against, maximum absorption will be given by the maximum in the curves. Absorption curve for benzene has a maximum absorption around 2550Ao wavelength. That wavelength at which the maximum absorption of light takes place is called as a max [8].

TYPES OF VISIBLE SPECTROPHOTOMETERS.

For the sake of convenience they are divided into 3 groups. They are, those with glass optics. sensitive from 300-800m, those with quartz optics sensitive from 200-100mu , those covering the range from 100 mu, They can be, 1) Manual or non-recording. 2) Automatic or recording,

UV SPECTROSCOPY

All atoms absorb in the UV region because these photons are energetic enough to excite outer electrons. If the frequency is high enough, photoionisation takes place. UV spectroscopy is also used in concentration as well as the ratio of protein to DNA concentration in a solution.The utilization of near ultraviolet absorption spectra as an analytical tool has increased in recent years, due to their efficiency**.**



PRINCIPLE OF UV SPCTROMETRY

The instrument used in ultraviolet-visible spectroscopy is called a UV/Visible spectrophotometer. It measures the intensity of light passing through a sample (4, and compares it to the intensity of light before it passes through the sample (L.). The ratio I/ L is called the transmittance, and is usually expressed as a percentage (%T). The absorbance, A, is based on the transmittance: A = -log (%T).

INSTRUMENTATION

LIGHT SOURCE

Both high and low voltage hydrogen lamps give rise to continuous spectrum in the region between 180-375m. A deuterium lamp produces very high radiation intensity than H2 lamp. The basic parts of a spectrophotometer are a light source (often an incandescent bulb for the visible wavelengths, or a deuterium arc lamp in the ultraviolet), a holder for the sample, a diffraction grating or monochromator to separate the different wavelengths of light, and a detector

MONOCHROMATOR

They must not have glass optics so prisms or quartz or fused silica must be used as dispersive device. The performance of spectrophotometers related to the design of the monochromator is evaluated by taking 1. The amount of stray radiant energy and 2. Resolution into condition.

CUVETTE

Quartz or fused silica may be used for UV - regions. Two cells may be identical but their absorption characteristics may be different in the UV region. Hence it is necessary to use one cell as reference cell and the other as sample cell always Samples for UV/Vis Spectrophotometry are most often liquids, although the absorbance of gases and even of solids can also be measured. Samples are typically placed in a transparent cell, known as a cuvette. Cuvette is typically rectangular in shape, commonly with an internal width of 1 cm. (This width becomes the path length, L in the Beer-Lambert law.) Test tubes can also be used as cuvette in some instruments. The best cuvettes are made of high quality quartz, although glass or plastic cuvettes are common. (Glass and most plastics absorb in the UV, which limits their usefulness to visible wavelengths.)

DETECTORS

UV spectrophotometers use photo multiplier cells or vacuum photo emissive photo tubes as detectors. . The detector is typically a photodiode or a CCD. Photodiodes are used with monochromator, which filter the light so that only light of a single wavelength reaches the detector. Diffraction gratings are used with CCDs, which collects light of different wavelengths on different pixels.

SPECIAL METHODOLOGY IN UV SPECTROMETRIC ANALYSIS

A spectrophotometer can be either single beam or double beam. In a single beam instrument (such as the Spectronic 20), all of the light passes through the sample cell. I must be measured by removing the sample. This was the earliest design, but is still in common use in both teaching and industrial labs. In a double-beam instrument, the light is split into two beams before it reaches the sample. One beam is used as the reference; the other beam passes through the sample. Some double-beam instruments have two detectors (photodiodes), and the sample and reference beam are measured at the same time. In other instruments, the two beams pass through a beam chopper, which blocks one beam at a time. The detector alternates between measuring the sample beam and the reference beam.

APPLICATIONS OF ULTRA VIOLET SPECTROSCOPY

It is used for identification of vitamins, sterols, hydrocarbons, enzymes, pharmaceuticals.

Vitamin A can be assayed by measuring its absorbance at 324mu .It is used to determine inorganic substances e.g. Lead measurement in bone ash. They are used for identifying compounds and to decide the constituents of compounds. They are used to measure the concentration of solutions, to study H+ ion concentration and to study the structure of inorganic complexes [9].

INSTRUMENTATION AND APPLICATIONS OF IR SPECTROSCOPY

INTRODUCTION

It was first discovered by William Herschel in 1800. Later it was developed by William coblentz. The spectrum of EMR seen in IR region is called IR spectra. They provide valuable information about the basic characteristics of the molecule, nature of atoms, spatial arrangement etc.

PRINCIPLE OF INFRARED SPECTROSCOPY

Consider a diatomic molecule AB. They have 2 nuclei and electrons. These atoms can exist in a number of energy levels. When excited, energy is absorbed and transition occurs. The absorbed energy may set the whole molecule + rotating or causes the vibration of atoms of A and B within the molecule. The changes can occur due to absorption of IR radiation. These vibration or rotation helps in identifying the functional groups.

TYPES OF INFRARED SPECTROSCOPY

They may be of 2 types, Single beam IR Absorption instrument and Double beam IR Absorption instrument

INSTRUMENTATION OF INFRARED SPECTROSCOPY

Source of radiation

Source of IR radiation may be

1) Nernst glowers: are constructed from a fused mixture of oxides of zirconium, yttrium and thorium molded in the form of hollow rods 1-3 mm in diameter and 2 -5 cm long. These glowers are fragile.2) Globar: bar of sintered silicon carbide 6 - 8 mm in diameter and 50 mm long has characteristics intermediate between heated wire coils and the Nernst glower. It is self starting and has an operating temperature near 1300°C

Monochromator

Prism monochromator are most effective in IR radiation. According to wavelength it should be made up of CaF2, KBr, Nacl. The surface should be smooth.

Grating monochromator: They are very popular. They are made of Al and are not attacked by moisture.

Cuvette: The cuvette is made up of Nacl.

Detector:

At the short wave length below 1.2 um, the preferred detection methods are the same as those used for Visible and Ultraviolet radiation. The detectors used at longer wavelengths can be classified into two groups ie, thermal detectors and photon detectors.)

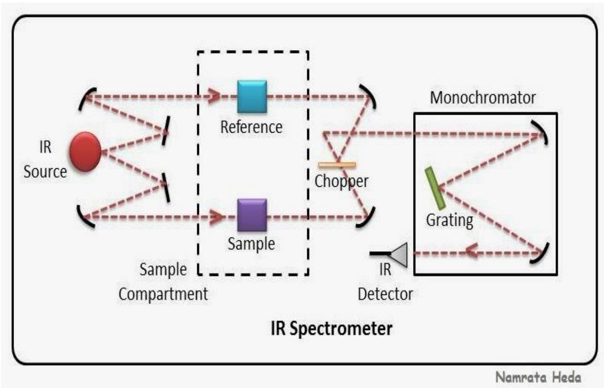
WORKING PRINCIPLE OF IR SPECTROSCOPY

The compounds can be examined in vapour phase, as liquids, in solution and in solid state. In solution: Here the compound is dissolved in chloroform and taken in special cell made of Nacl.

In solid state: Accurately 1 mg of solid is ground well with 1 drop of hydrocarbon and used for the analysis. In vapour state: Vapour of the sample is introduced into a special cell made of Nacl.

A beam of infrared light is produced and split into two separate beams. One is passed through the sample, the other passed through a reference which is often the substance the sample is dissolved in. The beams are both reflected back towards a detector, however first they pass through a splitter which quickly alternates which of the two beams enters the detector. The two signals are then compared and a printout is obtained. Single beam IR absorption Instrument ie here the radiation passes through the sample and then through the entrance slit to monochromator. The detector measures the amount of radiation absorbed by the sample.

Double beam IR absorption Instrument Here the source beam is separated into sample beam and reference beam. The two systems combine and pass along optical system to the detector. An Oscillating signal is produced, and the degree of oscillation becomes a measure of lo/It, the absorbance of solution



APPLICATIONS OF IR SPECTROSCOPY

Infrared spectroscopy is widely used in both research and industry as a simple and reliable technique for measurement, quality control and dynamic measurement.

The instruments are now small, and can be transported, even for use in field trials. With increasing technology in computer filtering and manipulation of the results, samples in solution can now be measured accurately (water produces abroad absorbance across the range of interest, and thus renders the spectra unreadable without this computer treatment). Some machines will also automatically tell you what substance is being measured from a store of thousands of reference spectra held in storage. By measuring at a specific frequency over time, changes in the character or quantity of a particular bond can be measured. This is especially useful in measuring the degree of polymerization in polymer manufacture. Modern research machines can take infrared measurements across the whole range of interest as frequently as 32 times a second. This makes the observations of chemical reactions and processes quicker and more accurate. Techniques have been developed to assess the quality of tea-leaves using infrared spectroscopy. Infrared spectroscopy has been highly successful for applications in both organic and inorganic chemistry. Infrared spectroscopy has also been successfully utilized in the field of semiconductor microelectronicshttp://en.wikipedia.org/wiki/Infrared spectroscopy -note 1# note-1: for example, infrared spectroscopy can be applied to semiconductors like silicon, gallium arsenide, gallium nitride, zinc selenide, amorphous silicon, silicon nitride, etc [7-9].

CONCLUSION

Spectrophotometry which deals with visible light, near-ultraviolet, and near-infrared uses a spectrophotometer that can measure intensity as a function of the color, or more specifically the wavelength of light. Thus spectroscopy is one of the most powerful tools available for the study of wide range of samples.

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