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RECENT ADVANCEMENT IN PHARMACEUTICAL SOPHISTICATED INSTRUMENTS AND ITS APPLICATION

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Abstract:

Spectroscopy is often used in physical and analytical chemistry for the identification of substances through the spectrum emitted from or absorbed by them. Spectroscopy is also heavily used in astronomy and remote sensing most large telescope have spectrometer, which are used either to measure the chemical composition and physical properties of astronomical objects or to measure their velocity from the doppler shifts of their spectral lines.

There are some types of spectroscopy we will discuss here i.e. UV Spectroscopy, IR Spectroscopy, HPLC Spectroscopy. The overview and study of that instruments are also important.

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INTRODUCTION:

Seeing is spectroscopy: we perceive the world via the interaction of visible light with the light receptors in our eyes. The light is emitted from the sun or from other light sources. It is then reflected from (or transmitted through) the objects in our surroundings. In these processes, the color changes because some of the light is absorbed by the objects. How much and what spectral regions are absorbed depends on the atoms and molecules in these objects. The light not absorbed reaches our eye. It carries the information of the molecular structure of our surroundings with it. In our eyes its color is analysed by 3 different types of photoreceptors which absorb different light in spectral regions. In this way we perform a spectroscopic experiment every time we look at things. There is a light source, and object that reflects, transmits, scatters and absorbs light and a wavelength dependent detector in our eyes. An apparatus for spectroscopic studies is called spectrometer and a plot of a particular property of matter against wavelength, frequency or energy of radiation is called spectrum(1).

UV Spectroscopy

Introduction:

Ultraviolet-visible (UV-Vis) spectroscopy is a widely used technique in many areas of science ranging from bacterial culturing, drug identification and nucleic acid purity checks and quantitation. to quality control in the beverage industry and chemical research. This article will describe how UV-Vis spectroscopy works, how to analyse the output data, the technique's strengths and limitations and some of its applications(2).

UV-Vis spectroscopy is an analytical technique that measures the amount of discrete wavelengths of UV or visible light that are absorbed by or transmitted through a sample in comparison to a reference or blank sample. This property is influenced by the sample composition, potentially providing information on what is in the sample and at what concentration. Since this spectroscopy technique relies on the use of light, let's first consider the properties of light.

Light has a certain amount of energy which is inversely proportional to its wavelength. Thus, shorter wavelengths of light carry more energy and longer wavelengths carry less energy. A specific amount of energy is needed to promote electrons in a substance to a higher energy state which we can detect as absorption. Electrons in different bonding environments in a substance require a different specific amount of energy to promote the electrons to a higher energy state. This is why the absorption of light occurs for different wavelengths in different substances. Humans are able to see a spectrum of visible light, from approximately 380 nm, which we see as violet, to 780 nm, which we see as red.' UV light has wavelengths shorter than that of visible light to approximately 100 nm. Therefore, light can be described by its wavelength, which can be useful in UV-Vis spectroscopy to analyze or identify different substances by locating the specific wavelengths corresponding to maximum absorbance (see the Applications of UV-Vis spectroscopy section)(3).

Instrumentation-

The principle of measurement for UV Visible Spectroscopy or UV Vis spectrophotometer is relatively straightforward and consists of a light source, a wavelength dispersive element, sample, and detector(4).

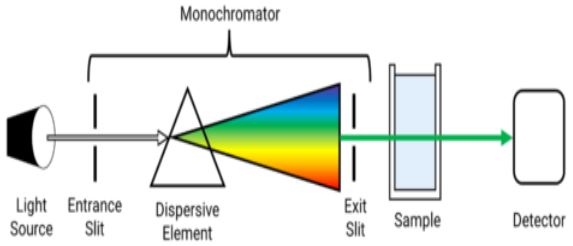

Fig- 1

UV Vis Spectrophotometer Monochromator The monochromator itself houses the mirrors, slits, and grating. panchromatic light from a light source is introduced into the monochromator through the entrance slit and collimated onto a diffraction grating which is rotated to select discrete wavelengths. The light is then refocused by another mirror onto the exit slit so that can be adjusted to control the spectral bandwidth (SBW). The light is then refocused by another series of mirrors and directed to the sample where it is either transmitted, absorbed, or reflected(5).

Light source

JASCO PHOTOMETROMETER use deuterium and halogen light sources. The deuterium lamp is used for the UV region from 190 to 350 nm while the halogen lamp covers a much broader spectral range from 330 and 3200 nm. Both the deuterium and halogen lamps used are continuous sources, although the D2 is also a line source. In continuous sources, the arc created excites the molecules enclosed in the vacuum to a higher energy state. The relaxation of the electrons back to the ground state emits photons and as the electrons return to the ground state, the excitation process restarts, providing a continuous source of light. Continuous sources therefore provide a uniform

amount of light through the monochromator to the sample. While this constant output of light can potentially lead to photobleaching of light sensitive samples, shutters can be implemented so the sample is only irradiated with light during the measurement itself(6).

Grating

The grating is a dispersive element used to select the wavelengths required to probe the electronic transitions of a sample's chromophores. It is rotated to the wavelengths selected and diffracts the light into several beams. The direction that light is diffracted depends on the angle and wavelength of the incident beam, and the grating's groove (or line) frequency, or the number of grooves on the grating per millimeter(7).

Bandwidth

While the monochromator is set to a specific wavelength, the light emerging is not perfectly monochromatic, but contains a range of wavelengths. As seen in figure 4, the total energy at the exit slit of the monochromator at a specific wavelength has the intensity distribution of an isosceles triangle. The peak of this triangle is the target wavelength and the spectral bandwidth is the full width half max (FWHM) of the triangle. The bandwidth should be set to 1/10 of the sample peak's FWHM(8).

UV Vis Spectrophotometer Detectors

Detectors are used to measure the transmitted or reflected light from a sample and convert it into a signal. The type and material of the detector will determine the sensitivity and wavelength range of the data that can be acquired. While photomultiplier tubes and silicon photodiodes are sensitive in the ultraviolet and visible wavelength ranges, Lead sulfide (PbS) photoconductive cells and indium gallium arsenide (InGaAs) photodiodes are used to measure the near-infrared region of the spectrum. However, all the detectors mentioned below exploit the photoelectric effect where light or photons that are incident on a material result in the emission of electrons(9).

Types of Detectors

A detector is an important instrument in the UV-Vis spectrophotometer, used in the conversion of light into proportional electrical signals, which provides the response of a spectrophotometer(10).

Nowadays, detectors used in UV-Visible spectroscopy are classified into four types, namely

 Photomultiplier tube Phototube Diode array detector Charge coupled device

Photomultiplier Tube

Photomultiplier tube is the popular detector nowadays used in UV-Vis spectrophotometers. It has an anode, cathode, and many dynodes.Photon when entering the tube, strikes the cathode, resulting in the emission of electrons. The emitted electrons are accelerated towards the first dynode, which is 90 V more positive than the cathode. An electron striking the first dynode will result in the production of several electrons(10).

Phototube

Phototube is also known as a photoelectric cell. It is filled with gas under low pressure. It contains a light-sensitive cathode and anode inside an evacuated quartz envelope. Between the electrodes, a potential difference of 100 V is applied. A photon entering the tube strikes the cathode and leads to the ejection of an electron, which strikes the anode and results in the flow of current. The resulting current is of low intensity and requires amplification. The response in the phototube depends on the wavelength of incident light(10).

Diode Array Detector

A diode array detector is a multichannel photon detector, which is capable of measuring all wavelengths of dispersed radiation simultaneously. On a single silicon chip, there is an array of silicon photodiodes. Subsequently, Individual diodes are scanned for a response(10).

Charge Coupled Device

Charge coupled device (CCD) is a highly susceptible detector. Hence, it is used for the detection of extremely lower light intensity signals. It is similar to diode array detectors except that it has photo capacitors instead of diodes. They contain an array of photo capacitors arranged in single or two dimensions(10).

Applications-.The applications of UV-Vis spectroscopy are enormous. The following are the main fields in which UV-Vis spectroscopy is used=

DNA & RNA analysis

Uv-Vis spectroscopy deals with the purity of nucleic acids.Quick verification of concentration and purity of DNA and RNA.This is essential before preparation of DNA and RNA in downstream applications like sequencing(11).

Pharmaceutical analysis

UV-Vis spectroscopy is an indispensable equipment in production of pharmaceutics. Overlap of absorbance peaks in uv spectra can be used to find out the

pharmaceutical compounds using mathematical derivatives. Chlortetracycline (antibiotic) and benzocaine (anaesthetic) are identified simultaneously in veterinary powder formulation using first mathematical derivative(11).

Bacterial culture
UV-Vis spectroscopy is essential in the biomass growth curve studies. Routine OD measurements are taken at 600 nm for estimation of cell concentration and growth tracking. 600 nm is chosen due to the optical properties of media in which bacteria is growing and to avoid damage to the cells when there is need for experimentation(11).

IR- Spectrosopy
Introduction
Infrared (IR) spectroscopy is an absorption method widely used in both qualitative and quantitative analyses. The infrared region of the spectrum includes electromagnetic radiation that can alter the vibrational and rotational states of covalent bonds in organic molecules.The IR spectrum of an organic compound is a unique physical property and can be used to identify unknowns by interpretation of characteristic absorbances and comparison with spectral libraries.

IR spectroscopy is also used in quantitative techniques because of its sensitivity and selectivity. It can be used to quantitative analytes in complex mixtures and is used extensively in detection of industrial pollutants in the environment.

The IR technique is discussed here primarily for application in identification of organic compounds and will focus on the mid-infrared region. Instrumental operating procedures are not given because they will vary depending on instrument design. A brief discussion of the theory will be followed by a discussion of instrumentation, sample handling techniques, and qualitative analysis (12).

Instrumentation-

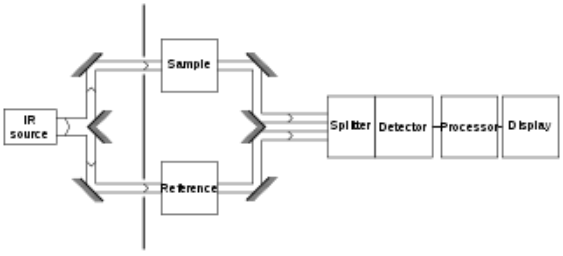

Fig -3

Radiation source
IR instruments require a source of radiant energy which emits IR radiation which must be steady, intense enough for detection, and extend over the desired wavelength (13). Various sources of IR radiations are as follows. 1. Nernst glower 2. Incandescent lamp 3. Mercury arc 4. Tungsten lamp 5. Globber source 6. Nichrome wire

Sample cell and sampling of substances
IR spectroscopy has been used for the characterization of solid, liquid, or gas samples.

Solid – Various techniques are used for preparing solid samples such as pressed pellet technique, solid run in solution, solid films, mull technique, etc.

Liquid – Samples can be held using a liquid sample cell made of alkali halides. Aqueous solvents cannot be used as they will dissolve alkali halides. Only organic solvents like chloroform can be used.
Gas– Sampling of gas is similar to the sampling of liquids (14).

Monochromators
Various types of monochromators are prism, gratings and filters. Prisms are made of Potassium bromide, Sodium chloride or Caesium iodide. Filters are made up of Lithium Fluoride and Diffraction gratings are made up of alkali halides (15).

Detectors
Detectors are used to measure the intensity of unabsorbed infrared radiation. Detectors like thermocouples, Bolometers, thermisters, Golay cell, and pyro-electric detectors are used (16).

Applications-
Infrared spectroscopy is widely used in industry as well as in research. It is a simple and reliable technique for measurement, quality control and dynamic measurement. It is also employed in forensic analysis in civil and criminal analysis (17). Identification of functional group and structure elucidation Entire IR region is divided into group frequency region and fingerprint region. Range of group frequency is 4000-1500 cm-1 while that of finger print region is 1500-400 cm-1.In group frequency region, the peaks corresponding to different functional groups can be observed. According to corresponding peaks, functional group can be determined.Each atom of the molecule is connected by bond and each bond requires different IR region so characteristic peaks are observed. This region of IR spectrum is called as finger print region of the molecule. It can be determined by characteristic peaks (17). Identification of substances IR spectroscopy is used to establish

whether a given sample of an organic substance is identical with another or not. This is because large number of absorption bands is observed in the IR spectra of organic molecules and the probability that any two compounds will produce identical spectra is almost zero. So if two compounds have identical IR spectra then both of them must be samples of the same substances (17). Studying the progress of the reaction Progress of chemical reaction can be determined by examining the small portion of the reaction mixure withdrawn from time to time. The rate of disappearance of a characteristic absorption band of the reactant group and/or the rate of appearance of the characteristic absorption band of the product group due to formation of product is observed (17). Detection of impurities IR spectrum of the test sample to be determined is compared with the standard compound. If any additional peaks are observed in the IR spectrum, then it is due to impurities present in the compound (17). Quantitative analysis The quantity of the substance can be determined either in pure form or as a mixure of two or more compounds. In this, characteristic peak corresponding to the drug substance is chosen (17).

HPLC

Introduction

HPLC is an abbreviation for high-performance liquid chromatography. Chromatography refers to the measurement method, chromatogram refers to the measurement results, and chromatograph refers to the instrument. Chromatography separates components in a particular substance and performs qualitative and quantitative analyses on those components. Qualitative analysis refers to "what kind of compound each component is", and quantitative analysis refers to "how much of each component is present"

HPLC is distinguished from traditional ("low pressure") liquid chromatography because operational pressures are significantly higher (50–350 bar), while ordinary liquid chromatography typically relies on the force of gravity to pass the mobile phase through the column. Due to the small sample amount separated in analytical HPLC, typical column dimensions are 2.1–4.6 mm diameter, and 30–250 mm length. Also HPLC columns are made with smaller adsorbent particles (2–50 µm in average particle size). This gives HPLC superior resolving power (the ability to distinguish between compounds) when separating mixtures, which makes it a popular chromatographic technique (18).

Instrumentation –

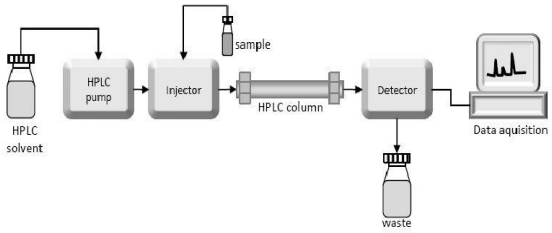

Fig - 4

The Pump

The development of HPLC led to the development of the pump system. The pump is positioned in the most upper stream of the liquid chromatography system and generates a flow of eluent from the solvent reservoir into the system. High-pressure generation is a "standard" requirement of pumps besides which, it should also to be able to provide a consistent pressure at any condition and a controllable and reproducible flow rate. Most pumps used in current LC systems generate the flow by back-and-forth motion of a motor-driven piston (reciprocating pumps). Because of this piston motion, it produces "pulses"(19).

Injector

An injector is placed next to the pump. The simplest method is to use a syringe, and the sample is introduced to the flow of eluent. The most widely used injection method is based on sampling loops. The use of the autosampler (auto-injector) system is also widely used that allows repeated injections in a set scheduled-timing(20).

Column

The separation is performed inside the column. The recent columns are often prepared in a stainless steel housing, instead of glass columns. The packing material generally used is silica or polymer gels compared to calcium carbonate. The eluent used for LC varies from acidic to basic solvents. Most column housing is made of stainless steel since stainless is tolerant towards a large variety of solvents(21).

Detector

Separation of analytes is performed inside the column, whereas a detector is used to observe the obtained separation. The composition of the eluent is consistent when no analyte is present. While the presence of analyte changes the composition of the eluent. What detector does is to measure these differences. This difference is monitored as a form of an electronic signal. There are different types of detectors available(22).

Recorder

The change in eluent detected by a detector is in the form of an electronic signal, and thus it is still not

visible to our eyes. In older days, the pen (paper)-chart recorder was popularly used. Nowadays, a computerbased data processor (integrator) is more common. There are various types of data processors; from a simple system consisting of the in-built printer and word processor while those with software that are specifically designed for an LC system which not only data acquisition but features like peak-fitting, baseline correction, automatic concentration calculation, molecular weight determination, etc(23).

Degasser

The eluent used for LC analysis may contain gases such as oxygen that are non-visible to our eyes. When gas is present in the eluent, this is detected as noise and causes an unstable baseline. Degasser uses special polymer membrane tubing to remove gases. The numerous very small pores on the surface of the polymer tube allow the air to go through while preventing any liquid to go through the pore(24).

Column Heater

The LC separation is often largely influenced by the column temperature. In order to obtain repeatable results, it is important to keep consistent temperature conditions. Also for some analysis, such as sugar and organic acid, better resolutions can be obtained at elevated temperatures (50 to 80°C). Thus columns are generally kept inside the column oven (column heater)(25).

Types of high-performance liquid chromatography

1. Normal phase: Column packing is polar (e.g silica) and the mobile phase is non-polar. It is used for watersensitive compounds, geometric isomers, cis-trans isomers, and chiral compounds(26).
2. Reverse phase: The column packing is non-polar (e.g C18), the mobile phase is water+ miscible solvent (e.g methanol). It can be used for polar, non-polar, ionizable, and ionic samples(26).
3. Ion exchange: Column packing contains ionic groups and the mobile phase is buffer. It is used to separate anions and cations(26).
4. Size exclusion: Molecules diffuse into pores of a porous medium and are separated according to their relative size to the pore size. Large molecules elute first and smaller molecules elute later(26).

Applications of high-performance liquid chromatography –

The HPLC has developed into a universally applicable method so that it finds its use in almost all areas of chemistry, biochemistry, and pharmacy. Analysis of drugs. Analysis of synthetic polymers. Analysis of pollutants in environmental analytics. Determination of drugs in biological matrices. Isolation of valuable products. Product purity and quality control of industrial products and fine chemicals. Separation and purification of biopolymers such as enzymes or nucleic acids. Water purification. Pre-concentration of trace components. Ligand-exchange chromatography. Ion-exchange chromatography of proteins. High-pH anion-exchange chromatography of carbohydrates and oligosaccharides(27,28).

NMR Spectroscopy

Introduction

Nuclear magnetic resonance (NMR) spectroscopy is the study of molecules by recording the interaction of radiofrequency (Rf) electromagnetic radiations with the nuclei of molecules placed in a strong magnetic field. Zeeman first observed the strange behaviour of certain nuclei when subjected to a strong magnetic field at the end of the nineteenth century, but the practical use of the so-called "Zeeman effect" was only made in the 1950s when NMR spectrometers became commercially available. Nuclear magnetic resonance spectroscopy is a powerful analytical technique used to characterize organic molecules by identifying carbon-hydrogen frameworks within molecules. Two common types of NMR spectroscopy are used to characterize organic structure: 'H NMR is used to determine the type and number of H atoms in a molecule; 13C NMR is used to determine the type of carbon atoms in the molecule. The source of energy in NMR is radio waves which have long wavelengths, and thus low energy and frequency(29).

Instrumentation

The NMR spectrophotometer consists of following components:-

1. Sample holder
2. Permanent Magnet
3. Magnetic coil
4. Radio frequency generator
5. Radio frequency receive
6. Read out system

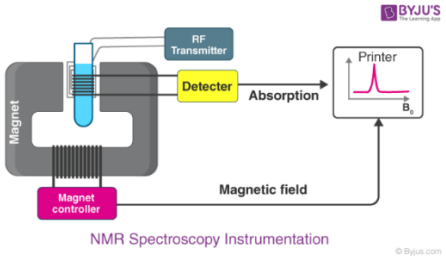

Fig 5

1.Sample holder

The sample holder in NMR is normally tube- shaped and is therefore called the sample tube.The tube must be transparent to RF radiation, durable, and chemically inert. Glass or Pyrex tubes are commonly used. These

are sturdy, practical, and cheap. They are usually about 6-8cm long and 0.3- 0.5cm in diameter, with a plastic cap to contain the sample. This type of tube is used for obtaining spectra of bulk samples and solutions(30).

2.Permanent Magnet

Permanent Magnet or electromagnet can be used in a NMR instrument. It should give stable and homogeneous magnetic field i.e. the strength and direction of magnetic field should not change point to point. Strength field should very high i.e. 20,000 Gauss (G). Because the chemical shifts are proportional to the field strength. The magnet size is 15 inches in diameter(31).

3. Magnetic coil

There is a relationship between the resonance frequency of nucleus and the strength of the magnetic field in which the sample is placed. Relationship is- V = Constant x Ho For the nucleus to resonate, the precessional frequency of the nucleus must equal to the applied RF radiation. If the Ho is constant, the precessional frequency is fixed(32).

4. Radio frequency generator

In order to generate radio frequency radiation, radio frequency oscillator is used. To achieve the maximum interaction of the RF radiation with the sample, the coil of oscillator would be around the sample container. The oscillator irradiates the sample with a RF radiation.The oscillator coil is perpendicular to the applied magnetic field(33).

5. Radio frequency receiver

It is installed perpendicular to both magnetic field and the oscillator coil. It is tuned to the same frequency as transmitter. When precession frequency is match with RF radiation the nucleus induces (emf) in detector coil and this signal is amplified and sent to read out system(34).

6. Read out system

The read out system gives a spectrum as a plot of strength resonance signal on Y axis & strength of magnetic field on X axis. The strength of resonance signal is directly proportional to number of nuclei resonating at that particular field strength (35).

APPLICATION OF NMR SPECTROSCOPY

Hydrogen bonding

Drug screening and design Particularly useful for identifying drug leads and determining the conformations of the compounds bound to enzymes, receptors, and other proteins (36). Native membrane protein Solid state NMR has the potential for determining atomic-resolution structures of domains of membrane proteins in their native membrane environments, including those with bound ligands. Metabolite analysis A very powerful technology for metabolite analysis. Chemical analysis A matured technique for chemical identification and conformational analysis of chemicals whether synthetic or natural(37).

Some of the applications of NMR spectroscopy are listed below:

It determine the bio macromolecules in aqueous solutions under near physiological conditions.

It determine the residual structures of unfolded proteins and the structures of folding intermediates It determine the chemical properties of functional groups in bio macromolecules such as the ionization states of ionizable groups at the active sites of enzymes(38).

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