

A REVIEW ON UV-VIS SPECTROSCOPY – THEORY & APPLICATIONS

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ABSTRACT

Ultraviolet and visible spectrometers have been in general use for the last 35 years end over this period have become the most important analytical instrument in the modern day laboratory. UV – Visible spectroscopy is useful as an analytical technique for two reasons; first, it can be used to identify some functional groups in molecules; secondly, it can be used for assaying. The second role is used to determine the content and the strength of a substance is very useful. Spectroscopic techniques emphatically UV – Visible spectroscopy is used to provide many answers in chemical and bio – chemical laboratories. In many applications other techniques could be employed but none competing UV-Visible spectrometry for its simplicity, versatility, speed, accuracy and cost-effectiveness.

Keywords- UV – Visible spectroscopy, principle, applications

INTRODUCTION

Spectroscopy is the study of the interaction between matter and electromagnetic radiation. Historically, spectroscopy originated through the study of visible light dispersed according to its wavelength, by a prism. Later the concept was expanded greatly to include any interaction with radiative energy as a function of its wavelength or frequency. Physicists classify light waves by their energies (wavelengths). **Ultraviolet-visible spectroscopy** or **ultraviolet-visible spectrophotometry** (UV-Vis or UV/Vis) refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral

region. This means it uses light in the visible and adjacent ranges. The absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, atoms and molecules undergo electronic transitions. Absorption spectroscopy is complementary to fluorescence spectroscopy, in that fluorescence deals with transitions from the excited state to the ground state, while absorption measures transitions from the ground state to the excited state. Ultraviolet and visible radiation interacts with matter which causes electronic transitions (promotion of electrons from the ground state to a high energy state). The ultraviolet region falls in the range between **190-380nm**, the visible region fall between **380-750nm**. [1,2]

UV-visible spectroscopy of microscopic samples is done by integrating an optical microscope with UV-visible optics, white light sources, a monochromator, and a sensitive detector such as a charge-coupled device (CCD) or photomultiplier tube (PMT). As only a single optical path is available, these are single beam instruments. Modern instruments are capable of measuring UV-visible spectra in both reflectance and transmission of micron-scale sampling areas. The advantages of using such instruments is that they are able to measure microscopic samples but are also able to measure the spectra of larger samples with high spatial resolution. As such, they are used in the forensic laboratory to analyze the dyes and pigments in individual textile fibers, microscopic paint chips and the color of glass fragments. They are also used in materials science and biological research and for determining the energy content of coal and petroleum source rock by measuring the vitrinite reflectance [3-5].

The ultraviolet - visible spectroscopy has very important applications in the qualitative and quantitative study of organic compounds. The system of conjugated dienes can be distinguished from isolated double bonds. The hydrogen bonding in a molecule can be established. The identity of a compound can be ascertained.

UV/Vis spectroscopy is routinely used in analytical chemistry or the quantitative determination of different analytes, such as transition metal ions, highly conjugated organic compounds, and biological macromolecules [6-9]. Spectroscopic analysis is commonly carried out in solutions but solids and gases may also be studied. Some of the specific applications are as follows:-

1. Detection of Impurities:

UV absorption spectroscopy is one of the best methods for determination of impurities in

organic molecules. Additional peaks can be observed due to impurities in the sample and it can be compared with that of standard raw material. By also measuring the absorbance at specific wavelength, the impurities can be detected. Benzene appears as a common impurity in cyclohexane. Its presence can be easily detected by its absorption at 255nm.

2. Structure elucidation of organic compounds:

UV spectroscopy is useful in the structure elucidation of organic molecules, the presence or absence of unsaturation, the presence of hetero atoms. From the location of peaks and combination of peaks, it can be concluded that whether the compound is saturated or unsaturated, hetero atoms are present or not etc.

3. Quantitative analysis:

UV absorption spectroscopy can be used for the quantitative determination of compounds that absorb UV radiation. This determination is based on Beer's law which is as follows.

$$A = \log I_0 / I_t = \log 1/T = -\log T = abc = \epsilon bc$$

Where ϵ is extinction co-efficient, c is concentration, and b is the length of the cell that is used in UV spectrophotometer.

4. Qualitative analysis:

UV absorption spectroscopy can characterize those types of compounds which absorb UV radiation. Identification is done by comparing the absorption spectrum with the spectra of known compounds. UV absorption spectroscopy is generally used for characterizing aromatic compounds and aromatic olefins. UV - visible spectrophotometry may be used to identify various classes of compounds in both pure state and as well as in biological preparations. This is done by plotting the absorption spectrum curves. These curves represent specific class of compounds and a knowledge these curves will help in identification of any substance.

5. Dissociation constants of acids and bases: Dissociation constant of acids and bases is an important parameter to get valuable information. From the equation given below:

$$\text{pH} = \text{pK}_a + \log \frac{[\text{A}^-]}{[\text{HA}]}$$

the pK_a value can be calculated if the ratio of [A⁻] / [HA] is known at a particular pH and the ratio of [A⁻] / [HA] can be determined spectrophotometrically from the graph plotted between absorbance and wavelength at different pH values.

Chemical kinetics: Kinetics of reaction can also be studied using UV spectroscopy. The UV radiation is passed through the reaction cell and the absorbance changes can be observed.

In addition to the above some structural applications [10] of UV-Visible Spectrophotometry involves :

Control of Purification:

This is one of the most important application of UV-visible spectrophotometry. Impurities can be detected very easily by testing if the compound is not showing its characteristic absorption spectrum. Example: Benzene impurity in absolute alcohol can be detected by this method. This can be detected by measuring the absorbance at 280nm. As at 280nm, benzene will absorb, whereas alcohol (210nm) will not absorb.

Study of Cis-Trans Isomerism:

The trans-isomer is more elongated as compared to its counterpart cis-isomer. Hence, this structural difference will be reflected in absorbance spectrum. The trans-isomer will have a higher wavelength of maximum absorption. The graph at the adjacent shows the absorption spectrum of the azobenzene dye, 4-n-butyl-4'-methoxyazobenzene (BMAB) where both cis-BMAB and trans-BMAB have different absorption spectrum.

Molecular Weight Determination:

If a compound forms a derivative with a reagent which has a characteristic absorption band. Suppose a compound forms a derivative with a reagent; now it will give the absorption band of a high intensity at a wavelength where the compound does not absorb, then the

extinction coefficient of the derivative is usually the same as that of the reagent. Although the extinction coefficient will remain same for any of the derivatives formed, the optical density is different for the compounds of different molecular weight.

The molecular weight of the compound can then be calculated readily on the basis of absorption data.

$$M = awb/OD$$

where a – absorption coefficient

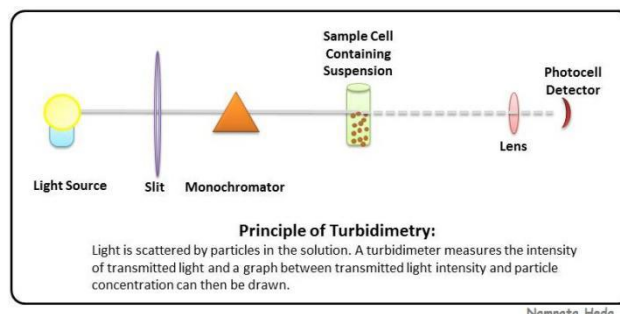
w – weight of the compound in g/l

b - path-length.

OD- optical density

Turbidimetry:

Any particulate matter (or even bacteria) makes the solution look turbid. This is due to Tyndall effect which is because of the light scattering by the colloidal particles. The particles in this solution will absorb at a particular wavelength and these particles will scatter the incident light. If this happens, then the radiation of a wavelength which is not absorbed by the solution is made to pass through the suspension and the apparent absorption will be solely because of the scattering by the particles. So, the transmitted light will have lower intensity as compared to that of the incident light. As a result, if the intensity of the transmitted light is measured, it will give an idea of the number of particles in the suspension. This technique is turbidimetry. By using this technique, we can find out an approximate number of particles in a given suspension. This technique is turbidimetry[11]. By using this technique, one can find out an approximate number of particles in a given suspension.



Identification of a compound:

The absorption spectrum of a compound is its characteristic property. The values of λ_{\max} i.e., the wavelength at which maximum absorption takes place, for no two compounds are the same. This property of the compound is used for their identification. The absorption spectrum of the given compound is compared with the standard spectrum. This method of identification of compound is called finger printing[12]. By this method the compound can be identified from its mixture relative amount can also be determined in the mixture. This is the most sensitive and reliable amount of the identification of organic compound.

Biochemists and molecular biologists often determine the concentration of a DNA sample by assuming an average value of $\epsilon = 0.020 \text{ ng}^{-1} \times \text{mL}$ for double-stranded DNA at its λ_{\max} of 260 nm (notice that concentration in this application is expressed in mass/volume rather than molarity: ng/mL is often a convenient unit for DNA concentration when doing molecular biology). Because the extinction coefficient of double stranded DNA is slightly lower than that of single stranded DNA, we can use UV spectroscopy to monitor a process known as DNA melting[13].

A UV/Vis spectrophotometer may be used as a detector for **HPLC**. The presence of an analyte gives a response assumed to be proportional to the concentration. For accurate results, the instrument's response to the analyte in the unknown should be compared with the response to a standard; this is very similar to the use of calibration curves. The response (e.g., peak height) for a particular concentration is known as the response factor. The wavelengths of absorption peaks can be correlated with the types of bonds in a given molecule and are valuable in determining the functional groups within a molecule. The Woodward-Fieser rules, for instance, are a set of empirical observations used to predict λ_{\max} , the wavelength of the most intense UV/Vis absorption, for conjugated organic compounds such as dienes and ketones. The

spectrum alone is not, however, a specific test for any given sample. The nature of the solvent, the pH of the solution, temperature, high electrolyte concentrations, and the presence of interfering substances can influence the absorption spectrum. Experimental variations such as the slit width (effective bandwidth) of the spectrophotometer will also alter the spectrum. To apply UV/Vis spectroscopy to analysis, these variables must be controlled or accounted for in order to identify the substances present. In the present study, most of the application of UV-VIS spectroscopy has been taken into account. However such studies are very extensive.

CONCLUSION

Ultraviolet and visible spectrometers have been in general use for the last 35 years and over this period have become the most important analytical instrument in the modern day laboratory. UV-Visible spectroscopy is useful as an analytical technique for two reasons. First, it can be used to identify some functional groups in molecules; secondly, it can be used for assaying. This second role-determining the content and the strength of a substance- is extremely useful. UV-Visible spectroscopy is used extensively in chemical and bio-chemical laboratories for a variety of tasks. In many applications other techniques could be employed but none rival UV-Visible spectrometry for its simplicity, versatility, speed, accuracy and cost-effectiveness.

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