ISSN 2395-650X

International Journal of Life Sciences Biotechnology Pharma Sciences

IJLBPS

www.ijlbps.org

E-mail: editorijlbps@gmail.com editor@ijlbps.org

International Journal of Life Sciences Biotechnology Pharma Sciences
Volume 6, Issue 4, 2018

QUANTITATIVE APPLICATION OF UV SPECTROSCOPY

[1]Mr. A. Ramesh,[2]Dr. K. Rathnakar Reddy,[3]Mr. P. Rakesh

[1,2]Associate Professor,[3]Assistant Professor

[1,2,3]Department of Pharm.Chemistry

Vaagdevi Institute of Pharmaceutical Sciences, Bollikunta, Warangal. Telangana.

ABSTRACT

The phrase UV-VIS Spectroscopy refers to the analytical analysis of different substances and solvents. UV-visible spectrometers have been in widespread usage for the last 37 years. Spectroscopy is a popular choice since it requires less expensive equipment and requires less maintenance, particularly for small businesses. Measuring the absorption of monochromatic light by colorless molecules in the near-ultraviolet (200–400 nm) spectrum is the analytical method. Pharmaceutical analysis is the process used to ascertain the "identity, strength, quality, and purity" of such substances. It also entails inspecting the intermediates and raw materials utilized in the manufacturing of pharmaceuticals. It is common knowledge that the dissociation constant is a crucial factor to consider while creating and refining a novel molecule for efficient formulation development.

KEYWORDS: Electrochemical approaches, UV-VIS spectroscopy, Optimization techniques.

1. INTRODUCTION

All analytical techniques used to collect physicochemical Data obtained by absorbing, transmitting or reflecting incident Radiant energy in sample are called spectrophotometry. Among these analytical techniques, there is light absorption Spectroscopy in Ultraviolet and Visible region (UV-Vis) (200-800nm) as one of most used technique for characterization And determination of several organic and inorganic substances, UV-Visanalytical method has become very important And widespread in different scientific areas around world Due to its availability, simplicity, flexibility and wide applicability In several areas, including biochemistry and analytical chemistry. Currently, it is necessary to reduce sample and reagents quantity to Develop analytical measurement, especially for scarce samples or toxic solvents; therefore, UV-Vis micro volume spectrometric Instrumentation has been developed.

Currently, spectroscopy techniques and chemometric methods Are largely used in food industry analyses to improve quality Control of foods and beverages, such as: detection of falsification Or adulteration, identification of origin,[4] differentiation of Caffeinated and decaffeinated coffee, origin and variety of wine Or origin of olive oils, and others. This study presents Bibliographic review in order to evaluate effectiveness and relevance of using analytical technique of molecular Spectrophotometry in Ultraviolet and Visible region in food Industry.

2. METHODOLOGY

The study objectives, methodology applying UV-Vis analysis, And main results. It could be noted various objectives of using UV-Vis spectroscopy in food industry, highlighting analyses to

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Identificate possible frauds in general foods and beverages.

The Origin of UV-Vis Spectra

Spectroscopy is interaction between waves originated in electromagnetic spectrum And molecules present in sample matrix under analysis.[29,30] two main spectroscopic Techniques used in food analysis are atomic and molecular spectroscopy.

Development And implementation of these spectroscopy methods in field of food analysisare based on interactions between matter and light that resulted in absorption, emission, and scattering events Characteristic of sample. In food analysis, these applications are based on variety of Spectroscopic methods and techniques that use benefits of different wavelength ranges including Ultraviolet and visible (UV-Vis), near-infrared (NIR), mid- infrared (MIR), far infrared (FIR), Raman, Microwaves, radio waves, and nuclear magneticresonance. UV-Vis spectroscopy is sensitive method in molecular spectroscopy that uses ultraviolet and visible light in wavelength range between 200 and 780 nm. As stated above, This spectroscopic method is based on absorption, scattering, diffraction, refraction, and reflection Properties of sample analysed. Absorption of UV and Vis light isrestricted to Certain molecular functional groups called chromophores, in which electrons are excited at different Frequencies. As in many spectroscopic applications, Beer– Lambert law describes correlation between Light absorption by molecule, light path length of sample, and concentration of absorbing molecules in liquid medium. Therefore, on basis of absorption Measurement, presence and concentration of analytes in food matrix as consequence of its Chemical and physical properties can be determined and quantified.

Principle of UV spectroscopy

Principles of absorption spectroscopy[4]

The greater number of molecules capable of absorbing light of given wavelength, greater extent of light absorption. Furthermore, more effectively molecule absorbs light of given Wavelength, greater extent of light absorption. From these guiding ideas, following Empirical expression, known as Beer–Lambert Law, may be formulated.

There are two types of absorbance instruments used to collect UV-Visible spectra

- Single beam spectrometer
- Double beam spectrometer

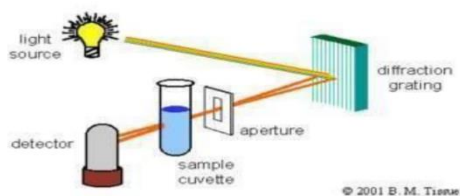

Figure 1: Single beam spectrometer.

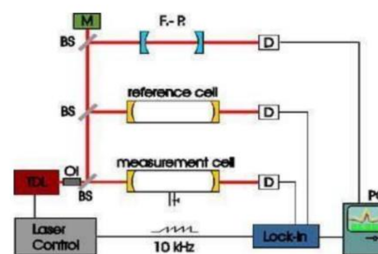

Figure 2: Double beam spectrometer.

Methods of UV-Vis Spectroscopy[5-9]

Methods applied on zero order absorption spectra

- Methods based on dual-wavelength ¬ Induced dual wavelength method (IDW) ¬ Dual wavelength resolution technique (DWRT) ¬

Absorption correction method (ACM)

- Methods based on absorptivity points ¬ Absorbance subtraction (AS)
- Advanced absorbance subtraction ¬ Absorptivity factor spectrophotometric method (a-Factor method) ¬ Methods based on area under the curve (AUC) ¬ Area under the curve correction method (AUC-CM) ¬ Compensated area under the curve method (CAUC)
- Spectrum subtraction
- Methods applied on derivative spectra ¬ Amplitude subtraction (AS) ¬ Modified amplitude subtraction (MAS) ¬ Amplitude Factor (P-Factor) ¬ Amplitude summation method (A-Sum) ¬ Simultaneous derivative ratio spectrophotometry (S1DD)
- Derivative compensation ratio via regression equation ¬ Differential dual wavelength (D1DWL) ¬ Differential derivative ratio (D1DR) ¬ Successive derivative subtraction coupled with constant multiplication (SDS-CM) ¬ Derivative transformation (DT)
- Method applied on the ratio spectra
- Methods based on subtraction of the amplitudes of ratio spectra ¬ Ratio subtraction method (RSM) ¬ Successive ratio subtraction (SRS) ¬ Extended ratio subtraction method (EXRSM) ¬ Simultaneous ratio subtraction method (SRSM)
- Methods based on amplitude difference of the ratio spectra ¬ Ratio difference spectrophotometric method (RDSM)
- Constant center spectrophotometric method (CCSM) ¬ Constant center coupled with spectrum subtraction (CC-SS) ¬ Constant value via amplitude difference (CV-AD) ¬ Constant value (CV) ¬ Amplitude center method (ACM)
- Method based on modulation of the amplitudes of ratio spectra ¬ Amplitude modulation method (AM) ¬ Advanced amplitude modulation method (AAM) ¬ Induced amplitude modulation method (IAM)
- Methods based on computed geometrical representation of amplitudes of ratiospectra ¬ Geometrical amplitude modulation method (GAM) ¬ Geometrical induced amplitude modulation method (GIAM) ¬ Ratio H-point standard addition method (RHSAM)
- Methods based on mean centering of amplitudes of ratio spectra ¬ Pure component contribution algorithm (PCCA) ¬ Continuous wavelet transform (CWT)

The expression of Beer-Lambert

law is A = log (I0/I) = Ecl Where, = absorbance I0 = intensity of light incident upon sample cellI = intensity of light leaving sample cell C = molar concentration of soluteL = length of sample cell (cm.) E = molar absorptivity

The term log (I0/I) is also known as absorbance (or optical density in older literature) andmay Be represented by A. molar absorptivity (formerly known as molar extinction coefficient) is property of molecule undergoing electronic transition and is not function of variable Parameters

involved in preparing solution. Size of absorbing system and probability that electronic transition will take place control absorptivity, which ranges from0 to 106. Values Above 104 are termed high-intensity absorptions, while values below 103 are low-intensity absorptions. Forbidden transitions have absorptivities in range from0 to 1000. Beer–Lambert Law is rigorously obeyed when single species gives rise to observed Absorption. Law may not be obeyed, however, when different forms of absorbingmolecule Are in equilibrium, when solute and solvent form complexes through some sort of association, when Thermal equilibrium exists between ground electronic state and low- lying excited state, or When fluorescent compounds or compounds changed by irradiationare present.[4]

From Beer-Lambert law it is clear that greater number of molecules capable of absorbinglight of given wavelength, greater extent of light absorption. This is basic principle of UV spectroscopy.[4]

The Beer-Lambert law[10]

It is linear relationship between absorbance and concentration, molar absorption coefficient and optical coefficient of solution: The molar absorption coefficient is sample dependent property and is measure of how strong absorber sample is at particular wavelength of light. concentration is simply molesL-1 (M) of sample dissolved in solution, and length is length of cuvette used for absorbance measurement and is typically 1 cm. = log (I0/I) = Ecl

The Beer-Lambert law states that there is linear relationship between concentration and absorbance of solution, which enables concentration of solution to be calculated by measuring its absorbance. To demonstrate this linear dependence five solutions of Rhodamine B in water were measured using DS5 Dual Beam Spectrophotometer and from these absorption spectra, linear calibration curve of absorbance versus concentration was created. Using this calibration curve concentration of unknown Rhodamine B solution can be determined by measuring its absorbance which is main utility of Beer-Lambert Law.

.7 UV-Visible Spectroscopy instrumentation

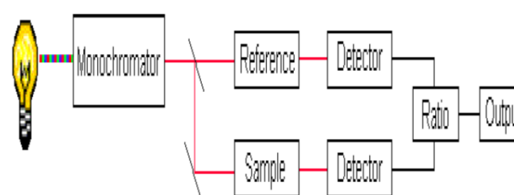

Figure 3: Instrumentation of UV-Visible Spectroscopy.

3. Instrumental design

Components

A spectrophotometer is an instrument for measuring the transmittance or absorbance of a sample as a function of the wavelength of electromagnetic radiation. The key components of a spectrophotometer are:[8] • A source that generates a broad band of electromagneticradiation • A dispersion device that selects from the broadband radiation of the source a particular wavelength (or, more correctly, a waveband) • A sample area • One or more detectors to measure the intensity of radiationated/VIS/NIR.[7] • Other optical components, such as lenses or mirrors, relay light Through the instrument. Sources The ideal light source would yield a constant intensity over all Wavelengths with low noise and long-term stability.

Unfortunately, however,such a source does not exist.[7]

Two sources Commonly used in UV-visible spectrophotometers.

The first source, the deuterium arc lamp

Yields a good intensity Continuum in the UV region and provides useful intensity in the Visible region. Although modern deuterium arc Lamps have low noise, noise from the lamp is often the limiting Factor in overall instrument noise performance. Over time, the Intensity of light from a deuterium arc lamp decreases steadily. Such a lamp typically has a half-life (the time required for the Intensity to fall to half of its initial value) of approximately 1,000 h.

The second source, the tungsten-halogen lamp[19]

Yields good intensity over part of the UV spectrum and over the Entire visible range. Thistype of lamp has very low noise and Low drift and typically has a useful life of 10,000 h. Most spectrophotometers used to measure the UV-visible range Contain both types of lamps. In such instruments, either a source Selector is used to switch between the lampsas appropriate, or The light from the two sources is mixed to yield a single Broadband source.

Dispersion devices

Dispersion devices cause different wavelengths of light to be Dispersed at different angles. When combined with an Appropriate exit slit, these devices can be used to selecta Particular wavelength (or, more precisely, a narrow waveband) of Light from a continuous source. Two types of dispersion devices, Prisms and holographic gratings, are commonly used in UVvisible spectrophotometers. A prism generates a rainbow from sunlight. This same principle Is used in spectrophotometers. Prisms are simple and Inexpensive, but the resulting dispersion is angularly nonlinear Moreover, the angle of dispersion is Temperature sensitive. For these reasons, most modern spectrophotometers contain Holographic gratings instead of prisms. These devices are made From glass blanks, onto which very narrow grooves are ruled. Traditionally, this task was done mechanically, but modern Production methods use a holographic optical process. The Dimensions of the grooves are of the same order as the Wavelength of light to be dispersed. Finally, an aluminum coating Is applied to create a reflecting source. Light falling on the Grating is reflected at different angles, depending on the Wavelength. Holographic gratings yield a linear angular dispersion withwavelength and are temperature insensitive.

However, they reflect light in different orders, which overlap. As a result, filters must be used to ensure that only the light from the desired reflection order reaches the detector. A concave grating disperses and focuses light simultaneously. A monochromator consists of an entrance slit, a dispersion device, and an exit slit. Ideally, the output from a monochromator is monochromatic light. In practice, however, the output is always a band,optimally symmetrical in shape. The width of tIBWband at half its height isthe instrumentalbandwidth (IBW).

Detectors

A detector converts a light signal into an electrical signal. Ideally, it should give a linear response over a wide range with low noise and high sensitivity. Spectrophotometers normally contain

either a photomultiplier tube detector or a photodiode detector.

Configuration

Various configurations of spectrophotometers are commercially Available.

- Single-beam design
- Dual-beam design
- Split-beam design
- Dual-wavelength design

Analysis of liquid samples

UV/VIS/NIR spectroscopy is for the most part used to decide analyte focuses or synthetic transformation of part in arrangement. Strategy estimates ingestion of light across wanted optical reach. Test is apportioned into cuvette and put in way between optical light sourceand locator. As per Beer-Lambert law, with consistent light way length and known assimilation coefficient (subject to frequency), convergence of compound being referredto still up in the air from light consumed at that frequency.

UV/VIS/NIR Analysis of solid samples

Estimating conveyance of strong example: An example is set before incorporating circle. Light from optical light source is communicated through example and enters incorporating circle. light isthen reflected by innersurface of circle and arrives at identifier.Both by and large conveyance and direct conveyance can be estimated. From these two boundaries, diffuse conveyance can be inferred by: Tdiff = Toverall – Tdirect

Estimating reflectance of strong example

Similarly as with conveyance, coordinating circle is expected to quantify by and large reflectance of strong materials. test is put behind coordinating circle. Light from optical light source is reflected by test and accordingly by inside surface of incorporating circle, after which it arrives at locator. Notwithstanding by and large reflectance, diffuse reflectance can likewise be estimated. Specular reflectance information can be determined from by and large and diffuse reflectance information>[6] Rspec = Roverall – Rdiff

Working out absorbance of strong example

The absorbance rate is characterized as level of episode shaft consumed by test, for example that piece of pillar which is neither reflected nor sent. Absorbance can be determined from reflectance and conveyance: %A = 100% – %Roverall – %Toverall UV-vis optical framework guidelines discovery moduleOptical framework in Lambda 1050 spectrometer.

Table 1: Illustrates the relationship between light absorption and radiation (nm)

Colour Absorbed	Colour Absorbed	Absorbed Radiation (nm)
Violet	Yellow-green	400-435
Blue	Yellow	435-480
Green-blue	Orange	480-490
Blue-green	Red	490-500
Green	Purple	500-560
Yellow-green	Violet	560-580
Yellow	Blue	580-595
Orange	Green-blue	595-605
Red	Blue-green	605-750

Advantages and Disadvantages and use of UV-Spectrophotometry:

Advantages[11,14]

♣ UV Derivative Spectroscopy has expanded affectability and selectivity. It enjoys various benefits viz., single part investigation and concurrent assurance of a few parts in combination, assurance of follows in lattice, protein and amino corrosive examination, natural investigation, recognizable proof of natural and inorganic mixtures. [5]

♣ Explicit advantages of subsidiary unearthly investigation remembers viz; Even for little frequency range, in presence of at least two covered pinnacles, absorbance groups can be recognized.[5]

♣ In presence of solid and sharp absorbance top, powerless and little absorbance pinnacle can be recognized.[5]

♣ Wide absorbance range gives clear thought regarding specific frequency at that most extreme range.[5]

♣ Indeed, even in presence of existed foundation ingestion, quantitative examination can concentrated asthere isstraight connection between subordinate qualities andfocus levels.[5].

Disadvantages

♣ Despite the fact that it is delicate strategy still it is exceptionally vulnerable to different boundaries.

♣ Technique is restricted to specific framework just and has restricted applications because of its less reproducibility.

♣ The technique is subsequent option while existing instrumental strategy (which estimates signal) is missing.

♣ It is less exact in estimating zero-intersection spectra. There is resemblance fit asa fiddle of subsidiary spectra and zero request range, so little variety in fundamental range can unequivocally change subordinate range.

♣ Helpless reproducibility can change brings about way when various spectrophotometers utilized for zero request spectra gives comparative outcomes yet derivatization of them show distinctive. [5].

1.9 Applications

Single part examination: Derivative spectrophotometry investigations single part alongside Area under Curve n drug plan.

UV –vis spectroscopy has many different application also

• Detection of impurities

• Structural elucidation of organic compounds • Quantitative analysis

• Qualitative analysis

• Chemical analysis

• Quantitative analysis of pharmaceutical substance

• Dissociation constant of acids and bases

• Molecular weight determination

• As HPLC detector

• Deviations from the Beer-Lambert law

4. CONCLUSION

When estimating small amounts of drugs in the presence of numerous potentially influencing compounds, the derivative sign handling approach is typically used. As a result of this obstruction, analytical indications become weak, loud, and superimposed on massive foundation signals. Deficient cuvette transmission coordination, non-reproducible cuvette positioning, soil or fingerprints on cuvette dividers, ambiguous broadband interference assimilation, and arrangement turbidity all contribute to debased estimation accuracy, which is measured in terms of test-to-test pattern shifts.[4] Standard movements could be the result of reasonable errors, such as weak frequency dependence (low turbidity of molecules) or

frequency independence (light obstruction caused by large suspended particles or air pockets). Separating significant retention from these sources of benchmark change is therefore necessary.

It is believed to inhibit broad foundation by separating to the extent that it reduces variations in foundation abundance from experiment to experiment. This leads to improved estimate and accuracy in many situations, especially when there is some uncontrolled variation in the foundation and when the analyte signal is not significantly different from the foundation.

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