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RECENT PERSPECTIVES ON UV-VISIBLE SPECTROPHOTOMETRY FOR PHARMACEUTICAL ANALYSIS: PRINCIPLES, INSTRUMENTATION, APPLICATIONS, AND QUANTITATIVE DRUG ESTIMATION

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Abstract

UV-Visible (UV-Vis) spectrophotometry is among the most widely used analytical methods in pharmaceutical analysis due to its simplicity, speed, affordability, and applicability to routine quantitative measurements. The technique relies on the absorption of ultraviolet and visible radiation by molecules, which causes electronic transitions between molecular energy levels. The review summarizes the basic concept, significant electronic transitions, the Beer-Lambert law, instrumentation, pharmaceutical applications and quantitative methods of UV-Visible spectrophotometry. The influence of chromophores, auxochromes, solvent characteristics, pH, and molecular interaction on the spectra is discussed. The basic elements of UV-Visible spectrophotometers such as radiation sources, monochromators, sample cells, detectors, and signal-processing systems are also discussed, particularly the single-beam designs. Pharmaceutical applications: Identification and Structure Elucidation, Active Pharmaceutical Ingredients Analysis, Impurity Analysis, Multicomponent Analysis, Dissolution and Stability Analysis, Reaction Monitoring and Coupling with Chromatographic and Process Analytical Technologies. Special emphasis is placed on quantitative drug estimation by direct spectrophotometry, Q-absorbance ratio and dual-wavelength methods. The review also emphasises the growing application of chemometric methods for enhancing selectivity and thus the analysis of complex and multicomponent samples. Despite some drawbacks of spectral overlap, matrix effects, properties of solvents, concentration-dependent deviations and instrumental factors, UV-Visible spectrophotometry is still an important analytical technique in pharmaceutical research, quality assurance, formulation development, and data-processing techniques are still improving and increasing the scope of UV-Visible spectrophotometry in contemporary pharmaceutical analysis.

Keywords: UV-Visible spectrophotometry, pharmaceutical analysis, Beer-Lambert law, electronic transitions, quantitative estimation of the drug, derivative spectrophotometry, multicomponent analysis, chemometrics, quality control.

1. Introduction

UV-Visible (UV-Vis) spectroscopy is among the most popular analytical methods for qualitative and quantitative analysis of pharmaceutical compounds [1-5]. It is based on the interaction of ultraviolet (200-400 nm) and visible (400-800 nm) light with matter, where molecules absorb certain wavelengths of light associated with electronic transitions between energy levels [4,5]. UV-Visible spectroscopy is a reliable method for estimation of drug, because the amount of light absorbed is proportional to the concentration of

the analyte, as described by the Beer-Lambert law [1,2,5]. Spectroscopy is the interaction of electromagnetic radiation with matter in terms of absorption, emission or transmission of energy. UV-Visible spectroscopy is one of the most useful of the spectroscopic techniques due to its simplicity, quick detection, low cost and sensitivity [1-5]. It can be used for the analysis of colourless compounds that absorb in the ultraviolet region and coloured compounds that absorb in visible region. The absorption spectrum can give clues to the chemical composition, molecular structure, purity and concentration of a sample [4,5]. UV-Visible spectroscopy is also used extensively in pharmaceutical analysis for drug development, quality control and for daily analytical testing [1-4]. It is widely utilised for quantitative determination of active pharmaceutical ingredients, for dissolution studies, for stability testing, foe evaluation of impurities and for the assay of dosage forms [1-3]. The method is also applicable to many aspects of chemistry, biochemistry, environmental research and molecular biology, such as the estimation of proteins, nucleic acids, microbial growth, and other biologically significant entities besides pharmaceuticals [4,5,14,15]. Due to its accuracy, cost-effectiveness and user-friendly nature, UV-Visible spectroscopy is an essential analytical technique in the research laboratory and the pharmaceutical industry [1-4].

2. Principle

UV-Visible spectroscopy is an analytical method which relies on the interaction of molecules with ultraviolet and visible light [4,5]. The molecules within a sample absorb certain wavelengths of the light, which are equal to the amount of energy needed to raise electrons to the higher energy (excited) state [4,5]. The amount of energy absorbed will vary with the molecular structure and with the presence of bonding (σ and π) and non-bonding (n) electrons [4,5]. The main electronic transitions in the UV-spectroscopy are σ→σ*, n→σ*, n→π* and π→π* transitions [4,5]. Groups that absorb UV or visible radiation are called chromophores, and groups that modify the intensity or wavelength of absorption by the chromophore are called auxochromes [4,5]. The Beer-Lambert law, which states that the absorbance of the solution is proportional to the concentration of the absorbing species and the path length of the light through the sample, is the basis used for quantitative analysis [1,2,5]. Over the years UV-Visible spectroscopy has emerged as one of the common methods used in routine QC and research of pharmaceuticals due to its simplicity, accuracy, sensitivity, and speed of analysis [1-4].

3. Electronic transition

In the UV-Visible region, electronic transitions can occur with a variety of energies-energies ranging from high to low [4,5].

Absorption of ultraviolet or visible radiation causes electrons to move up to higher-energy, unoccupied, antibonding molecular orbitals, The type of electronic transitions is dependent on the type of chemical bond and the nature of the electrons, the intensity and the wavelength of the absorption [4,5]. In UV-Visible spectroscopy, the four main electronic transitions are σ→σ*, n→σ*, π→π*, and n→π* [4,5].

Transition from a bonding sigma (σ) orbital to an antibonding sigma (σ*) orbital is the σ→σ*. It is the most energetic transition and is found in the vacuum ultraviolet region, in saturated hydrocarbons with only single bonds [4,5]. A transition from n to σ* is a transition in which non-bonding electrons of heteroatoms, e.g., oxygen, nitrogen, sulphur, or halogens are promoted into σ* orbitals [4,5].

The π→π* transition happens when electrons are promoted from bonding π orbitals to antibonding π* orbitals, Typical in unsaturated and conjugated molecules, such as alkenes, aromatic compounds and carbonyl-containing molecules, and typically gives strong absorption bands [4,5]. The transition of non-bonding to π* orbital is called the n→π* transition and is the lowest energy of the common transitions. It is generally seen at longer wavelengths in compounds with carbonyl, nitro, azo and other unsaturated functional groups having lone pair electrons [4,5].

The probability of an electronic transition is not the same for every transition. Usually transition of π→π* are very intense and permitted, but other ones are formally forbidden such as n→π* and d→d in complexes of

transition metals and are sometimes observed with a low intensity [4,5]. Apart from the functional groups which cause the molecule to absorb light but affect the wavelength and the intensity of absorption, such as -OH, $–NH_2$, $–CH_3$, $–NO_2$ [4,5]. Also, the position and shape of absorption bands are affected by molecular interactions, which can be influenced by solvent polarity and solution pH; therefore, the choice of solvent is an important aspect in UV-Visible analysis [4,5].

4. According to Beer-Lambert law

The Beer-Lambert law is the fundamental law of UV-Visible spectrophotomet yand is fundamental for quantitative analysis of pharmaceutical compounds[1,2,5]. It is a relation between light absorption and concentration of the absorbing substance. This principle is known as the Beer-Lambert law, which states that absorbance is directly proportional to the absorbing species, concentration and optical path length [1,2,5]. The equation is:

$$\mathbf{A = \varepsilon lc}$$

The value of the absorbance, A, is directly proportional to the molar absorptivity or molar extinction coefficient, ε, the path length of the sample cell, l (cm), and the concentration of the analyte, c [1,2,5]. The law is a combination of the ideas that were put forward independently by Beer showed that the absorbance is proportional to the concentration of the molecules that absorb light, while lambert showed that absorbance is proportional to the path the light travels through the sample [1,5]. These principles can be combined to allow the calculation of unknown concentration of a substance by measuring its absorbance at a chosen wavelength, usually the wavelength of maximum absorbance (λmax) [1,2,5].

The relationship enables the quantitative estimation of drugs, biomolecules and other chemical substances within a desired concentration range [1,2,5]. The Beer-Lambert law is therefore the main factor upon which building calibration curves and routine analytical measurements in pharmaceutical quality control units and other analytical laboratories are based [1-5]. The Beer-Lambert law is very accurate under ideal conditions, but may be violated in circumstances of high concentration of the analyte, chemical interactions, the effect of the solvent, light scattering, the turbidity of the sample, stray light from the instrument, or the use of polychromatic radiation [1,2,5]. The careful preparation of samples, the correct dilution of samples and correct calibration of the instruments are therefore necessary for obtaining accuracy and repeatability of analytical results [1,2,5].

5. Types of UV Spectrometers

There are two types of UV spectrophotometers, single-beam and double-beam, that measure the absorbance or transmittance of a sample, but differ in optical design and operation [1,4,5].

5.1.1 Single-beam UV spectrometer

It utilizes a single optical path consisting of a light source and a monochromator that filters the desired wavelength from the source and then sends the light to the sample. The transmitted light is measured and converted to an electrical signal, and absorbance is calculated based on the Beer-Lambert law [1,4,5]. This instrument is very popular for regular laboratory analysis because of its easy construction and ease of use [1,4].

5.1.2 Double-beam UV spectrometer

Its splits the monochromatic light into two beams, one of which passes through the sample, and the other a refence or blank solution [1,4,5]. It is used to continuously compare the intensities of both beams, which reduces errors due to fluctuations in the light source and increases the accuracy of analysis [1,4,5]. This setup provides speedy measurements, higher precision and stability, leading to its use for advanced pharmaceutical

and research applications [1,4]. In general, single-beam instruments cost less and are suitable for routine measurement applications, but double-beam instruments provide greater reliability and accuracy because they allow a simultaneous comparison of both the sample signal and the reference signal [1,4,5].

6. Instrumentation

UV-Visible spectrophotometry is a common technique used for qualitative and quantitative analysis of pharmaceutical compounds based on their absorption of UV and visible light [1-5]. The conventional spectrophotometer is composed of a radiation source, wavelength-selection system, sample cell, detector and signal-processing system [1,4,5].

6.1 Radiation Sources

The radiation sources should be stable and continuous over the wavelength range of interest. Deuterium lamps are typically used in the ultraviolet region, and tungsten-halogen lamps are primarily used in the visible region [4,5], Xenon lamps are good for broad UV-visible radiation, and LEDs can be used as stable, narrow-band, long-life sources at selected wavelengths [4,5,]. Quartz and fused-silica optical components are preferred for UV applications because of their UV transparency [4,5].

6.2 Monochromator

The monochromator is used to select a specific wavelength in polychromatic radiation. It typically includes collimating optics, focusing optics, an exit slit, an entrance slit and a dispersing element [4,5]. Separation of wavelength can be achieved using diffraction gratings and prisms, with diffraction gratings widely used in modern instruments [4,5]. Monochromators enable narrower spectral bandwidth and continuous scanning of wavelength [4,5].

6.3 Sample Cells

Sample cells contain the sample and reference solutions during measurement. Glass cells generally can be used for measurements in the visible region, while quartz or fused-silica cells are preferred for UV analysis due to their high UV transparency [4,5]. The typical optical path length of a cuvette is 1 cm [4,5].

6.4 Detector

The detector must be highly sensitive, have good linearity, low noise, be stable and reproducible and respond quickly to transmitted radiation [4,5]. Photovoltaic cells, photo emissive tubes, photomultiplier tubes (PMTs), and photodiode arrays (PDAs) are common detectors [4,5]. PMTs are sensitive and respond quickly and can be used for low-intensity radiation, PDAs can simultaneously measure over a spectrum od wavelengths and are especially convenient for rapid spectral acquisition [4,5].

The electrical signal from the detector is typically very faint and is amplified and processed before being displayed. Modern instruments use digital processing and computer-based systems to present spectra as absorbance or transmittance as a function of wavelength [4,5].

UV-Visible spectrophotometers can be configured in either single-beam or double-beam systems. A single-beam instrument is simpler, whereas a double-beam instruments uses sample and reference beams to compensate for variations in lamp intensity and enhance baseline stability and measurement reliability [1,4,5].

7. Applications of UV-Visible Spectrophotometry

UV-Visible spectrophotometry is a general analytical method that has a wide range of applications in pharmaceutical, biochemical, environmental and food analysis [3-5,14,15]

7.1 Qualitative and Structural Analysis

UV-Visible spectra offer characteristic patterns of absorption which can help identify compounds by comparison with reference UV-Visible spectra [4,5]. Absorption maxima and spectral shifts give information on chromophores and conjugated systems and can be used to study structural changes and interactions [4,5].

7.2 Quantitative Pharmaceutical Analysis

This method is very common for the assay and estimation of active pharmaceutical ingredients in bulk drugs and pharmaceutical formulations [1-4]. Absorbance measurements can be used to determine the concentration of a drug based on the Beer-Lambert relationship and calibration curves [1,2,5].

7.3 Impurity Detection

Pharmaceutical substances can be used to detect and estimate the presence of impurities that absorb UV-Visible light [2,3]. The presence of impurities can be detected by differences in the absorption maximum or the spectral profile [2,3]. When there is spectral overlap, the use of appropriate solvent, Ph adjustment, derivatives and chemometric methods can help to improve selectivity [7-9,13].

7.4 Analysis of Multicomponent Systems

The direct determination of components of mixtures is sometimes complicated by overlapping absorption spectra [7-9,13]. Mathematical derivative spectroscopy and multivariate chemometric methods such as partial least-squares regression (PLSR) and multivariate curve resolution (MCR) can enhance spectral resolution and allow simultaneously of several analytes without complex separation methods [7-9,13].

7.5 Determination of Dissociation Constants

The dissociation constants (pKa) of ionizable compounds can be determined by using UV-Visible spectroscopy. Variations in absorbance are measured due to changes in pH, and the spectral data obtained are used for estimating the ionisation behaviour of acidic and basic compounds [4,5].

7.6 Nucleic Acid Analysis

The absorbance of UV light is a common method of measuring the concentration and purity of DNA and RNA. Nucleic acids have a high absorbance at 260nm; absorbance at 280nm and 230nm can be used to characterise protein and chemical contamination [4,5].

7.7 Microbiological Applications

Monitoring microbial growth is common with the use of spectrophotometry in the ultraviolet and visible regions. Optical density, typically at 600 nm for bacterial culture, is used to obtain a quick estimate of cell density and can be used to follow growth kinetics [4,5].

7.8 HPLC and Processing Analytical Applications

UV-Visible detectors are commonly used in high-performance liquid chromatography (HPLC) to detect compounds which absorb in the UV or visible region [1-3]. The method is also suitable for processing analytical applications for monitoring drug concentration and other critical quality attributes in pharmaceutical manufacturing [3,4].

7.9 Chemical Kinetics and Reaction Monitoring

Decreases in absorbance as a function of time can be followed to study reaction progress and reaction kinetics [4,5]. UV-Visible spectroscopy can be used to monitor changes in concentration during chemical and biochemical reactions [4,5].

7.10 Biological and Biochemical Analysis

It can be applied to estimating biomolecules and analysis of biological samples. It has been used to study proteins, amino acids, nucleic acids, haemoglobin and other biological components that absorb in the UV [4,5,15].

7.11 Food and Beverage Analysis

Food and beverage quality assessment uses UV-Visible spectroscopy to quantify compounds like caffeine, pigments, phenols and other chromophore components [4,5]. It can also help to verify the quality, authenticity and possibility adulteration of the product by comparing characteristic absorption profiles [4,5].

7.12 Environmental & Industrial Applications

Pollutants, dyes and other substances that absorbs UV are monitoring using UV-Visible spectrophotometry [14]. It can be used to track degradation of contaminants during wastewater treatment and to monitor water quality changes [14].

7.13 Drug Discovery and Research

UV-Visible spectroscopy is used in drug discovery to obtain preliminary information about candidate compounds, their absorption and molecular interactions [3,4]. It can also help characterise bioactive compounds and study changes in their spectral properties during formulation or experimental studies [3,4].

8. Methods for Quantitative Estimation of Drug Using UV-Visible Spectrophotometry

Several techniques for quantitative estimation of drugs using UV-Visible spectrophotometry are described [1,2,7-12]. Quantitative estimation of drugs can generally be divided into single-component and multicomponent analysis. The choice of method depends on the absorptive properties of the drugs, the presence of interfering materials and formulation complexity [1,2,7-12].

8.1 Single-Component Analysis

A. Direct Spectrophotometric Analysis

Direct analysis can be used for compounds which show significant absorption in the UV or visible region. This method is especially well suited for molecules that have chromophores, such as aromatic rings and conjugated double bonds [1,2,4,5].

In this method, the drug is first dissolved in an appropriate solvent and suitably diluted, after which its absorbance is measured at the chosen analytical wavelength, typically the wavelength of maximum absorbance (λmax) [1,2,5]. The concentration is the determined by the Beer-Lambert law or by a calibration curve [1,2,5].

Benefits: The method is simple, fast, economical and generally does not require chemical derivatisation [1,2].

B. Indirect or Derivatives Spectrophotometric Analysis

Indirect analysis means changing the chemical composition or spectral property of the analyte to obtain a measurable response if direct analysis is not possible [7,8,12]. It is especially beneficial when the compound if interest absorbs at a wavelength that is not detected by direct UV-Visible measurements, or when the spectrum is interfered with by other components in the sample [7,8,12]. In samples with overlapping absorption bands, derivative spectrophotometry can improve resolution of the spectrum and increase selectivity [7-9,12].

8.2 Multicomponent Analysis

Multicomponent spectrophotometry is the method for simultaneously estimation of two or more absorbing substance without separation of them [2,7-11]. These commonly used methods involve simultaneously equation, difference spectrophotometry, Q-absorbance ratio method and dual-wavelength [2,7-11].

A. Simultaneous Equation Method

The simultaneous Equation Method (Vierordt's method) is usually employed to determine the simultaneous concentration of two drugs whose absorption spectra overlap [2]. The absorption of the mixtures is measured at two wavelengths, and the concentration of each in the mixture is calculated from the simultaneous equations based on the Beer-Lambert law [1,2].

Thia technique is founded on the principle that the absorbance of a mixtures at a particular wavelength is the sum of the absorbance of its constituent substances. Thus, the amount of each drug can be determined if the absorptivity values at these selected wavelengths are known [1,2].

B. Difference Spectrophotometry

The increased selectivity of difference spectrophotometry is possible because it subtracts the absorbance of one form of the analyte from another that has a different spectral shape [7,12]. A suitable change in pH, such as acid, base or buffer treatment, is often used to achieve the required spectral changes [7,12].

The method is especially applicable if there are interfering substances which are not affected by the treatment selected, and the selected treatment causes a reproducible spectral change in the analyte [7,12]. The difference in absorbance is then related to the concentration of the drug [7,12].

C. Q-Absorbance Ratio Method

The Q-absorbance ratio method is a modified approach used for the analysis of two-component mixtures [11]. It uses absorbance measurements at two selected wavelengths: one at which both components have equal absorption, known as the iso absorptive wavelength, and the λmax of one component [11]. These absorbances are measured for the mixture, and the concentration of each component is determined using the respective absorptivity and Q-values [11].

D. Dual-Wavelength Method

The dual-wavelength method is employed to estimate one of the drugs when another one is present that has a similar absorption spectrum [10]. Two wavelengths are chosen at which the absorbance of the interfering component [10]. The absorbance of the substance of interest is, however, different at the selected wavelength [10].

The difference in absorbances between the two is thus proportional to the desired drug concentration. Such a method is an easy way to remove spectral interference and is applicable for quantitative analysis without separation [10].

Conclusion

The simplicity, rapidity, cost-effectiveness and routine quantitative determination make UV-Visible spectrophotometry a very important and versatile analytical technique in pharmaceutical analysis. The technique is useful in providing information by means of characteristic absorption behaviour due to electronic transitions, and it offers qualitative assessment and quantitative estimation, using the Beer-Lambert law.

Technical innovations in the design of spectrophotometers such as more sensitive detectors, single and double beam, digital data processing, and better optical systems have improved analytical accuracy, reproducibility

and operational efficacy. The applications of UV-Visible spectrophotometry are board, ranging from assay of active pharmaceutical ingredients, dissolution studies, impurity detection, stability testing, multi-component analysis, reaction monitoring, to pharmaceutical quality control.

Other spectrophotometric methods, including direct spectrophotometry, derivative spectrophotometry, simultaneous equation, difference spectrophotometry, Q-absorbance ratio and the dual wavelength method, are available as useful alternatives to single and multicomponent formulations, especially when spectral overlap is a problem. Moreover, the use of chemometric and multivariate data analysis techniques has enhanced the selectivity and usefulness of UV-Visible spectroscopy in more complex pharmaceutical samples.

Spectral interference, solvent and pH effects turbidity, stray radiation and deviations from Beer-Lambert behaviour may have an impact on analytical performance, but can be minimised by appropriate method development, sample preparation, calibration and validation. As instrumentation, automation, chemometrics and analytical technologies for the pharmaceutical industry continue to evolve, UV-Visible spectrophotometry is likely to remain a useful and accessible technique in drug research, formulation development, quality control and routine drug estimation.

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