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A Review on recent developments in UV spectroscopy

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A B S T R A C T

Ultraviolet (UV) spectroscopy is a crucial and advanced analytical tool used in the pharmaceutical industry for over 35 years. This method analyzes compounds by measuring the absorption of monochromatic light within the near-ultraviolet spectrum (200-400 nm), particularly for colorless compounds. Pharmaceutical analysis involves procedures to assess the identity, strength, quality, and purity of these compounds, as well as the examination of raw materials and intermediates during drug manufacturing. The basic principle of UV spectrophotometry involves passing light of a specific wavelength range through a solvent-filled cell, where a photoelectric cell converts the radiant energy into electrical energy, measured by a galvanometer. UV-visible spectroscopy is commonly used to obtain the absorbance spectra of compounds in solution or solid form.

Keywords: UV spectroscopy, Detector, Analytical, Spectra.

A R T I C L E I N F O

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C o n t e n t s

1. Introduction:

Ultraviolet (UV) spectroscopy is an optical spectroscopy technique that utilizes light in the visible, ultraviolet, and near-infrared regions of the electromagnetic spectrum. The molecular absorption is typically studied within the wavelength range of 190 to 800 nm, with the ultraviolet region spanning 190 to 400 nm and the visible region covering 400 to 800 nm[1]. When monochromatic radiation passes through a homogeneous solution in a cell, the intensity of the transmitted radiation depends on the path length (b) and the concentration (C) of the solution. (Io) represents the intensity of the incident radiation, while (I) denotes the intensity of the transmitted radiation[2].

The amount of radiation absorbed may be measured in a number of ways:
Transmittance, T = I/I0
Transmittance %T = 100 X T
Absorbance A = 2 – log10 %T
The equation, A= 2 – log10 %T, it allows you to calculate absorbance from percentage transmitted data[3].

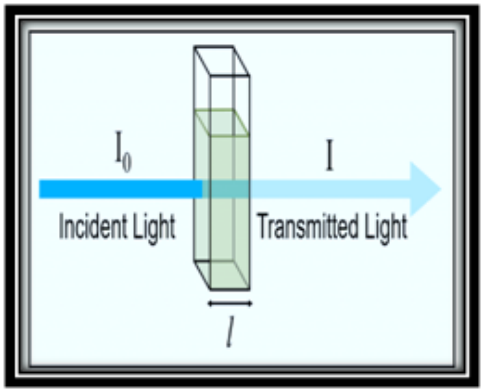
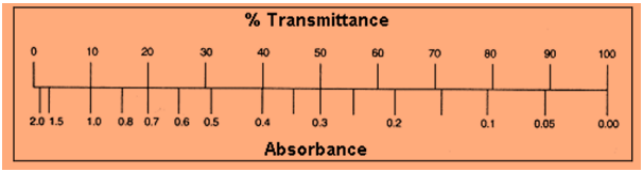


Fig. 1: Mechanism of absorbance



The relationship between absorbance and transmittance is given in following diagram: So, if all the light passes through a solution without any absorption, then absorbance is zero, and percenttransmittance is 100%. If all the light is absorbed, then percent transmittance is zero, and absorption is infinite[4].

Principle:

Beer-Lambert Law:

The Beer-Lambert law states that the absorbance of a solution (A) is directly proportional to the concentration of the absorbing species (c) and the path length (b) of the solution[5].

Absorbance A = molar absorptivity constant x cell length x concentration

A = abc

C = A /a b

Where,

A = absorbance

A = molar absorptivity

b = path length

c = Concentration

Electronic Transitions:

A molecule or ion absorbs visible or ultraviolet light when the radiation induces an electronic transition within its structure. This absorption is associated with a change in the electronic state of the molecules. The energy from the light promotes electrons from their ground-state orbitals to higher energy excited-state or antibonding orbitals[6]. Various electronic transitions can occur due to the absorption of ultraviolet and visible light.

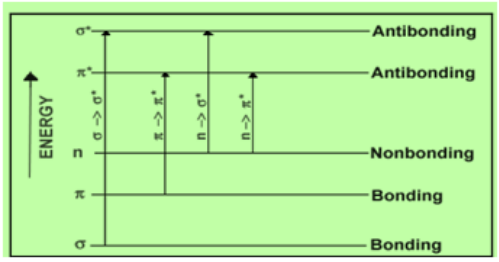


Fig. 2: Electronic transistion of σ, π and n electrons

1. σ to σ* Transitions:

An electron in a bonding σ orbital is excited to the corresponding antibonding orbital. The energy required is large.

For example, methane (which has only C-H bonds, and can only undergo σ to σ* transitions) shows an absorbance maximum at 125 nm. Absorption maxima due to σ to σ* transitions are not seen in typical UV-Vis. spectra (200 -700 nm)[7,8].

2. n to σ* Transitions:

Saturated compounds containing atoms with lone pairs (non-bonding electrons) are capable of n to σ* transitions. These transitions usually need less energy than σ to σ*. Transitions They can be initiated by light whose wavelength is in the range 150 - 250 nm.

3. n to π* and π to π* Transitions:

Most absorption spectroscopy of organic compounds is based on transitions of n or π electrons to the π * excited state. This is because the absorption peaks for these transitions fall in an experimentally convenient region of the spectrum (200 - 700 nm). These transitions need an unsaturated group in the molecule to provide the π electrons. Molar absorptivity from π to π* transitions are relatively low, and range from 10 to 100 L mol-1 cm-1. π to π*transitions normally give molar absorbtivities between 1000 and 10,000 L mol-1 cm-1.[9]

2. Instrumentation

The Essential components of UV-VIS Spectrophotometer are as follows:

1. Sources (UV and visible)
2. Monochromator
3. Sample containers (Cuvette)
4. Detector
5. Amplifier and recorder

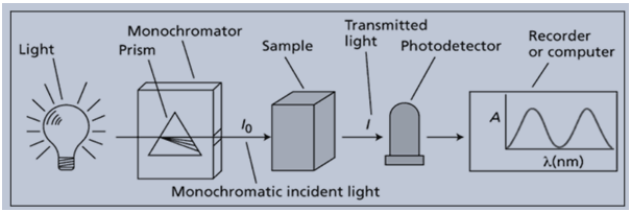


Fig. 3: Schematic diagram of UV Spectrophotometer

1. Sources:

UV-Vis spectroscopy requires a continuous light source that emits radiation over a wide range of wavelengths. The main UV radiation sources include:

1. **Hydrogen Lamp:**

Hydrogen lamps are stable, robust sources that emit continuous radiation in the range of 160-380 nm. They contain hydrogen gas under high pressure, where electrical discharge excites hydrogen molecules, causing them to emit radiation.

2. **Deuterium Lamp:**

Deuterium lamps, commonly used as UV sources, are gas discharge lamps emitting radiation in the range of 160-450 nm. These lamps are more expensive than hydrogen lamps.

3. **Tungsten Lamp:**

Tungsten lamps are the most common light source in spectrophotometers. They consist of a tungsten filament inside a glass envelope and emit light in the visible region, with a wavelength range of approximately 330 to 900 nm.

4. **Xenon Discharge Lamp:**

Xenon lamps are discharge lamps filled with xenon gas, emitting radiation in the range of 250-600 nm.

2. Monochromator:

A monochromator generates monochromatic light by filtering out unwanted wavelengths from a light source. Polychromatic radiation (containing multiple wavelengths) enters the monochromator through an entrance slit. The

beam is collimated and then directed at the dispersing element (such as a grating or prism). This dispersing element splits the beam into its component wavelengths. By adjusting the position of the dispersing element or exit slit, only the desired wavelength leaves the monochromator through the exit slit.

Types of monochromator:

1. Prism Monochromator
2. Grating Monochromator.[11]

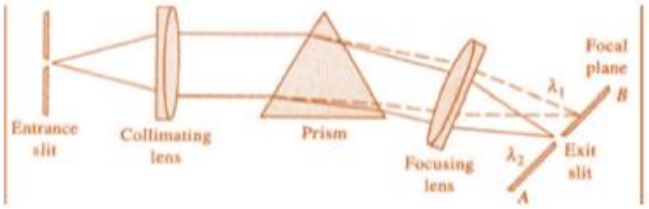


Fig. 4: Prism monochromator

3. Sample Containers (Cuvette):

Cuvettes are sample container which used to hold samples for spectroscopic measurement and which is transparent to all wavelength of light passing through it. The cuvette made of Quartz ,Square shape and having path length 1 cmare selected and can be used for wavelengths ranging from 190 to 200 nm[12, 13].

4. Detectors:

Detector converts light energy into electrical signals that are displayed on readout devices. The transmitted radiation falls on the detector which determines the intensity of radiation absorbed by sample the following types of detectorsare employed in instrumentation of absorption spectrophotometer.

Types of Detectors:

1. Barrier layer cell/Photovoltaic cell
2. Phototubes/ Photo emissive tube
3. Photomultiplier tube.[14]

General Rule for Performing UV Spectroscopy:

1. Drug Must be completely Soluble in Solvent
2. Drug must absorb UV Visible radiation or light.
3. Drug must not interact with solvent
4. Solvent must be selected on consideration of Cutt off wavelength.
5. The solvent should be UV transparent at the measuring wavelength.
6. When using volatile solvents stoppered cells should be employed to avoid evaporation
leading to changes in solution concentration.
7. Absorbtion must be linear.
8. Only Dilute solution obeys Beer-Lambert law.
9. Calibration curve must be linear.
10. In case of Binary drug, both drug must ne soluble in same solvent.
11. Drug and solvent must be free of contamination.[15]

Solvents Cut –off Wavelength:

UV cut off is defined as the wave length where solvent also absorbs light (UV or Visible). In that region, the measurement should be avoided. It is difficult to determine the absorbance comes from your analyte or your solvent[16]. So when choosing a solvent is aware of its absorbance cutoff and where the compound under investigation is thought to absorb. If they are close, chose a different solvent.

The following table provides an example of solvent cut offs.

Table 1: Commonly used solvent and cut –off Wavelength[17]

Solvent	Cut-off (nm)
Is-octane	202
Ethyl alcohol	205
Cyclohexane	200
Acetone	325
Tetrachloroethylene	290
Benzene	280
Carbon tetrachloride	265
Water	180

3. Application of UV-VIS Spectroscopy[18]:

1. Detection of Impurities
2. Structural Illucidation of organic compound
3. Detection of conjugation
4. Detection of functional group
5. Detection of Geomitrical isomer
6. Molucular Weight Determination
7. Distinction of Cis-Trans Isomerism.

Techniques Utilizing Area Under the Curve (AUC)

1. Area Under the Curve Correction Method (AUC-CM)

This innovative approach leverages the spectrophotometric method to calculate the area under the curve (AUC) for determining the concentration of a specific component in a binary mixture. It is especially useful for mixtures with significant spectral overlap. The method measures the absorption spectral area, and corrected AUC values can increase sensitivity, even at low concentrations. By calculating the AUC for each component in the mixture (e.g., X and Y), the need for more complex mathematical rules, like Cramer's rule, is eliminated.

2. Compensated Area Under the Curve (CAUC) Method

This method is an evolution of the derivative compensation technique, using AUC in place of derivative spectrum amplitude. It is particularly useful when two components (X and Y) exhibit overlapping absorption spectra. By measuring the absorbance difference between a mixture (m) and a reference (r), the AUC ratio between two spectral ranges can be used to estimate the concentration of one of the components, often even when minor components are present.

3. Amplitude-Summation Technique (A-Sum)

This method is applied when two substances, X and Y, are combined, and the difference spectrum of Y shows no contribution from X. By subtracting the measured amplitude of Y from the total (X + Y) spectrum, the contribution from X can be determined. This is achieved by calculating the first derivative spectra for the same concentrations.

Techniques Based on Ratio Spectrum Amplitude Differences

1. Ratio Difference Spectrophotometric Method (RDSM): Developed by Lotfy and Hegazy, RDSM is effective for analyzing binary and ternary mixtures. It calculates the amplitude difference at two points on the ratio spectra of a mixture, which correlates with the concentration of the target component while being independent of interfering substances.

2. Constant Center Spectrophotometric Method (CCSM): In this method, the spectrum of a mixture is divided using a divisor (X or Y), allowing for the separate determination of components. The concentration of each component is then calculated using regression analysis, based on the absorbance values of the zero-order absorption spectrum.

3. Constant Center Coupled with Spectrum Subtraction (CC-SS): This method is applied when only one distribution (Y) needs to be determined. It involves subtracting the spectrum of Y from the combined X + Y spectrum to isolate the X component. A regression equation then helps determine the concentration of X.

4. Constant Amplitude Difference (CV-AD) Method

Derived from both the Constant Center Method and the Amplitude Method, this approach calculates the zero-direction absorption spectrum for ternary mixtures. The concentration of component Z is determined using the ratio spectrum's amplitude.

5. Constant Value (CV) Method

The Constant Value method is used for analyzing binary and ternary mixtures. It calculates the concentration of component Z (which overlaps significantly with X and Y) by applying regression equations to the AUC ratio of the difference spectrum.

Spectrum Subtraction Techniques

1. Amplitude Subtraction (AS)

This technique simplifies the two-regression equation method by calculating the peak amplitude at a specific wavelength. The amplitude of component X can be found by subtracting the amplitude of the mixture from the expected value at the same wavelength.

2. Modified Amplitude Subtraction

Used for mixtures of two components (X and Y), this method selects a wavelength where the peak of X overlaps with that of Y. The concentration of component Z can then be determined using the Z/Z' ratio spectrum.

Amplitude Modulation Techniques

1. Amplitude Modulation (AM)

This method regulates the amplitude in a binary mixture by normalizing the spectrum of one component. By dividing the spectrum by the absorbance curve of a continuous product, the amplitude is linked to concentration.

2. Advanced Amplitude Modulation Method (AAM)

An extension of AM, this technique is useful for binary mixtures with significant spectral overlap. It involves calculating the iso-point and using a regression equation to determine both components.

3. Induced Amplitude Modulation (IAM)

IAM is used when there are no isoabsorption points, or where X and Y have low absorptivity. This method uses a regression equation to calculate relative amplitudes and concentrations.

4. Geometric Amplitude Modulation (GAM)

This method calculates the geometric effects of adding component X to a binary mixture of X and Y. It is based on the difference in amplitude at two selected points in the overlapping region.

Techniques Using Mean Centering of Ratio Spectrum Amplitudes

1. Mean Centering of Ratio Spectra (MCRS)

This method calculates the mean-centered ratio spectra to separate components in binary or ternary mixtures. It eliminates the constant from the ratio spectra for accurate concentration determination.

2. Geometric Mean Centering

This technique improves the mean-centering process by using the geometric mean, offering a more accurate representation of central tendency.

3. Pure Component Contribution Algorithm (PCCA)

PCCA separates overlapping signals from components by extracting their pure contributions through mean-centering techniques.

4. Continuous Wavelet Transform (CWT)

The CWT technique utilizes wavelets to compress, smooth, and analyze spectrophotometric data, offering a flexible tool for resolving complex chemical mixtures.

Applications and Conditions

The application of these techniques depends on the spectral characteristics of the components. The strengths and weaknesses of each method are outlined in corresponding tables, which help guide their use based on the type of spectral data being analyzed.

4. Conclusion

UV-Vis spectroscopy is essential in analytical laboratories due to its ability to provide both qualitative and quantitative data on chemicals. It is widely used in fields such as biochemical research, materials science, pharmaceutical analysis, and environmental monitoring. The method's sensitivity, ease of use, and versatility have made it a cornerstone of scientific analysis. Over the past decade (2006-2016), significant advancements in UV spectroscopy have revolutionized its application in analytical chemistry. Initially, derivative spectrophotometry dominated the field, but newer, innovative spectrophotometric techniques have since emerged. These methods are more efficient, cost-effective, and eco-friendly, as they avoid the use of hazardous solvents or reagents. These advancements have led to the development of techniques that can accurately analyze drug combinations in pharmaceutical formulations, making them viable alternatives to more complex and resource-intensive analytical methods.

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