Republic of Iraq
Ministry of Higher Education & Scientific research
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Analytical Chemistry

For

First Year Student

Lecture 8

By

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Ultraviolet-visible spectrophotometric analysis

- Ultraviolet (UV)–visible spectrophotometry is widely used in biochemistry, both for the determination of species and for studying biochemical processes.
- This technique enables the determination of micromolar concentrations of substances and has a broad scope of application in this field since most biochemical compounds absorb in the UV–visible region or can be converted into some absorbing derivative.
- Ultraviolet–visible (UV–visible) spectrophotometry is primarily a quantitative analytical technique concerned with the absorption of near-UV (180–390 nm) or visible (390–780 nm) radiation by chemical species in solution.
- It is routinely used in analytical chemistry for the quantitative determination of analytes, such as transition metal ions, highly conjugated organic compounds, and biological macromolecules

What is UV-Vis Spectroscopy used for?

UV–visible is used to :

1.Identify unknown compounds,

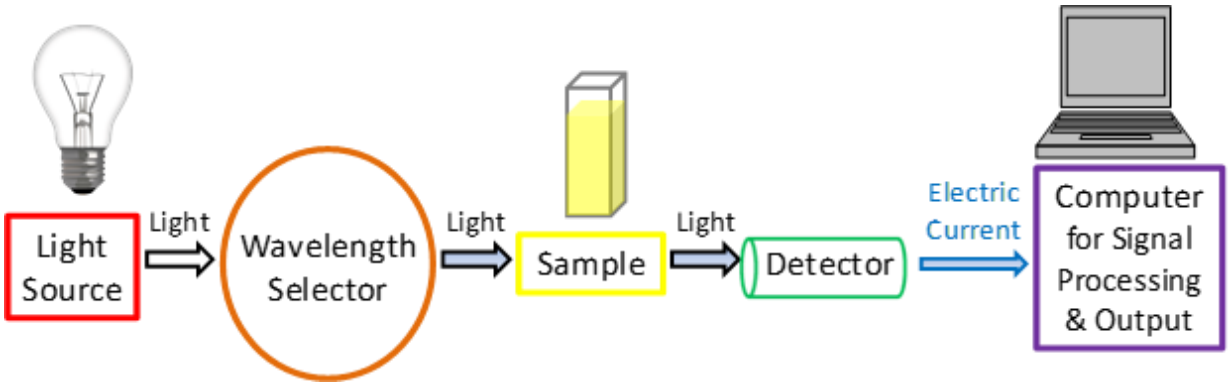
2.Provide information about the physical and electronic structures of organic and inorganic compounds.

3.Determine the size and concentration .

How does a UV-Vis spectrophotometer work?

Whilst there are many variations on the UV-Vis spectrophotometer, to gain a better understanding of how an UV-Vis spectrophotometer works, let us consider

the main components, depicted in Figure 1.



1-Light source: As a light-based technique, a steady source able to emit light across a wide range of wavelengths is essential. A single xenon lamp is commonly used as a high intensity light source for both UV and visible ranges. Xenon lamps are, however, associated with higher costs and are less stable in comparison to tungsten and halogen lamps.

2-wavelength selection: In the next step, certain wavelengths of light suited to the sample type and analyte for detection must be selected for sample examination from the broad wavelengths emitted by the light source. Available methods for this include:

3-Monochromators: A monochromator separates light into a narrow band of wavelengths.

Absorption filters - Absorption filters are commonly made of colored glass or plastic designed to absorb wavelengths of light.

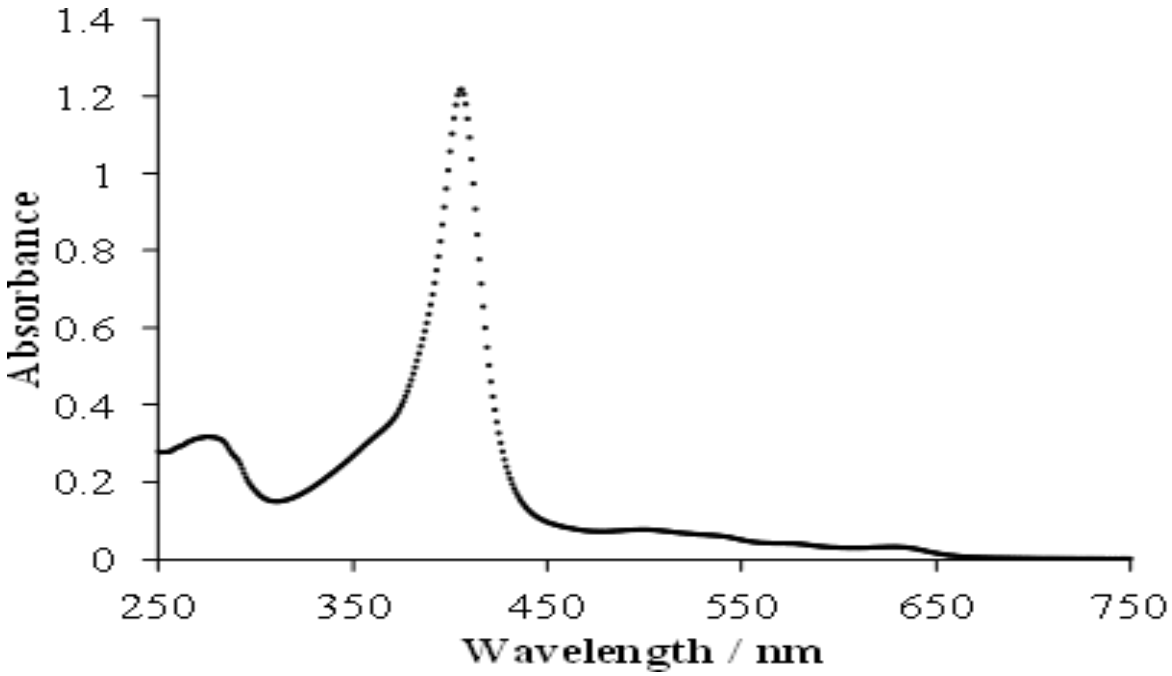
Interference filters - Also called dichroic filters, these commonly used filters are made of many layers of dielectric material where interference occurs between the thin layers of materials.

4-**Sample analysis:** Whichever wavelength selector is used in the spectrophotometer; the light then passes through a sample. For all analyses, measuring a reference sample, often referred to as the "blank sample", such as a cuvette filled with a similar solvent used to prepare the sample, is imperative. If an aqueous buffered solution containing the sample is used for measurements, then the aqueous buffered solution without the substance of interest is used as the reference.

5-**Detection:** After the light has passed through the sample, a detector is used to convert the light into a readable electronic signal. Generally, detectors are based on photoelectric coatings or semiconductors.

UV-Vis spectroscopy analysis, absorption spectrum and absorbance units

UV-Vis spectroscopy information may be presented as a graph of absorbance, optical density or transmittance as a function of wavelength. However, the information is more often presented as a graph of absorbance on the vertical y axis and wavelength on the horizontal x axis. This graph is typically referred to as an absorption spectrum; an example is shown in Figure2.



Strengths and limitations of UV-Vis spectroscopy:

No single technique is perfect and UV-Vis spectroscopy is no exception. The technique does, however, have a few main strengths listed below that make it popular.

1. The technique is non-destructive, allowing the sample to be reused or proceed to further processing or analyses.
2. Measurements can be made quickly, allowing easy integration into experimental protocols.
3. Instruments are easy to use, requiring little user training prior to use.
4. Data analysis generally requires minimal processing, again meaning little user training is required.
5. The instrument is generally inexpensive to acquire and operate, making it accessible for many laboratories.

Applications of UV-Vis spectroscopy

UV-Vis has found itself applied to many uses and situations including but not limited to:

DNA and RNA analysis:

Quickly verifying the purity and concentration of RNA and DNA is one particularly widespread application. A summary of the wavelengths used in their analysis and what they indicate are given in Table 1.

Wavelength used in absorbance analysis in nanometers	What does UV absorbance at this wavelength indicate the presence of?	What causes UV absorbance at this wavelength?
230	Protein	Protein shape[10]
260	DNA and RNA	Adenine, guanine, cytosine, thymine, uracil
280	Protein	Mostly tryptophan and tyrosine

Beer–Lambert law

The relative amount of a certain wavelength of light absorbed (A) that passes through a sample is dependent on:

1. Distance of the light must pass through the sample (cell path length (b).
2. Amount of absorbing chemicals in the sample (analyte concentration (c).
3. Ability of the sample to absorb light (molar absorptivity (E)

$$\boldsymbol{A = \varepsilon b c}$$

Where: A = absorbance (no units)

b = cell path length (cm)

c = concentration of analyte (mol/L)

Ɛ = molar absorptivity (L/mole-cm)

Beer's Law, the relative amount of light making it through the sample (I/Io) is known as the transmit.

$$T = I / I_o$$

$$A = -\log T = -\log \frac{P}{P_0} = \log \frac{P_0}{P}$$

Where:

T: Transmittance

I: Intensity of light

I_o: Initial intensity of light

Beer's Law is followed only if the following conditions are met:

• Incident radiation on the substance of interest is monochromatic.

• Solvent absorption is insignificant compared to solute absorbance.

• Solute concentration is within given limits.

• An optical interferant is not present.

Example 1

A 7.25×10^{-5} M solution of potassium permanganate has a transmittance of 44.1% when measured in a 2.10-cm cell at a wavelength of 525 nm. Calculate (a) the absorbance of this solution and (b) the molar absorptivity of $KMnO_4$.

Solution

(a) $A = -\log T = -\log 0.441 = -(-0.356) = 0.356$

(b) From Equation 24-8,

$$\varepsilon = A/bc = 0.356/(2.10 \text{ cm} \times 7.25 \times 10^{-5} \text{mol L}^{-1})$$

$$= 2.34 \times 10^{3} \text{ L mol}^{-1} \text{ cm}^{-1}$$

Exercise1 :A solution containing 4.48 ppm $KMnO_4$ exhibits85.9 %T in a 1.00-cm cell at 520 nm. Calculate the molar absorptivity of KMnO4 at this wavelength.

Exercise2

Convert the accompanying transmittance data to absorbances.

a) 27.2%

b) 0.579

c) 30.6%

d) 3.98%

e) 0.093

(f) 63.7%