



**Republic of Iraq**
**Ministry of Higher Education & Scientific research**
**Al-Mustaqbal University**
**Science College**
**Biochemistry Department**

# Analytical Chemistry Instrumental Analysis

## For

## Second Year Student/course 1

## Lecture 4

**By**

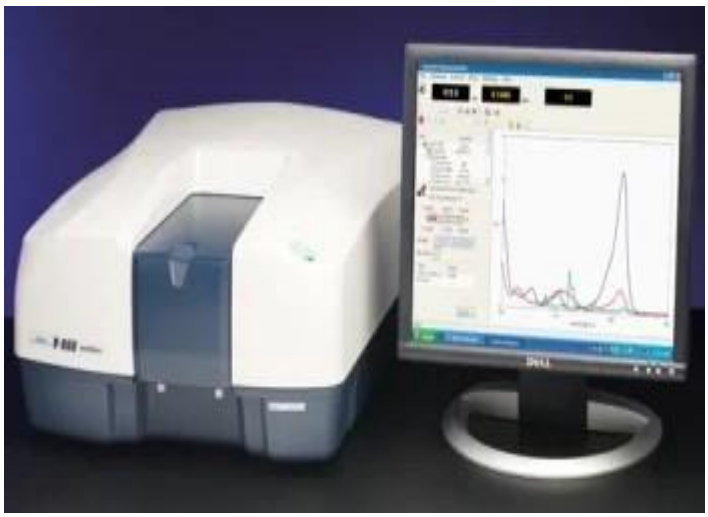
**Dr. Karrar M. Obaid**

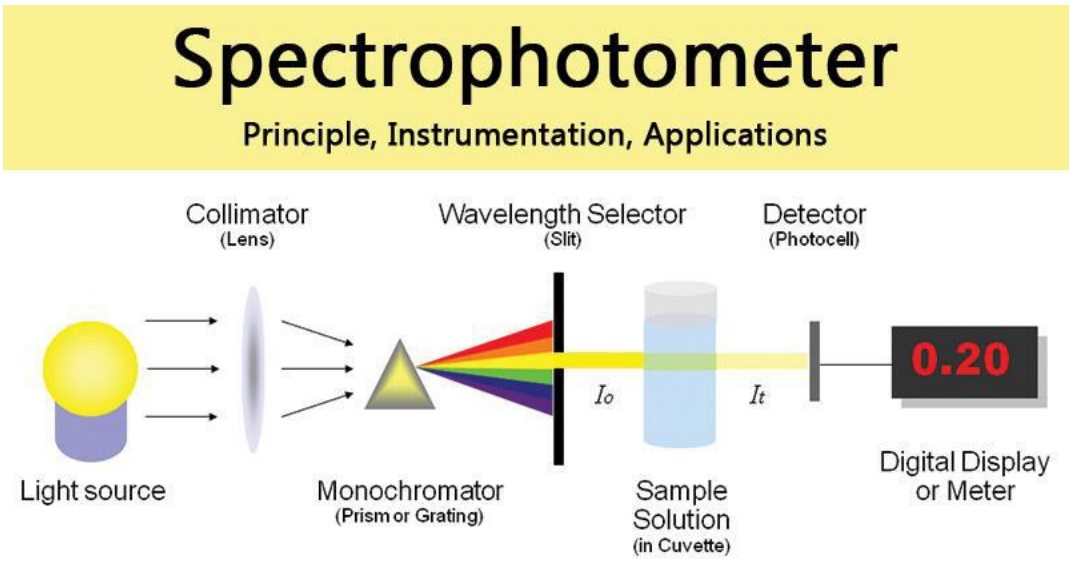
**2025-2026**

# UV/Visible Spectroscopy: Instrumentation

- **Spectrometer** is an instrument which can measure the absorbance of a sample at any wavelength
- Most spectroscopic instruments in the UV/visible and IR regions are made up of five components:

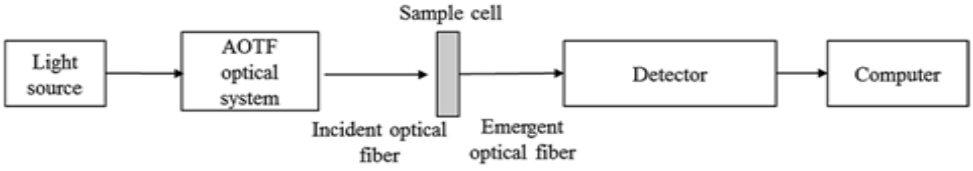
1. A stable source of radiant energy
2. A wavelength selector to isolate a limited region of the spectrum for measurement
3. One or more sample containers
4. A radiation detector, to convert radiant energy to a measurable electrical signal
5. A signal-processing and readout unit consisting of electronic hardware and in modern instruments a computer.



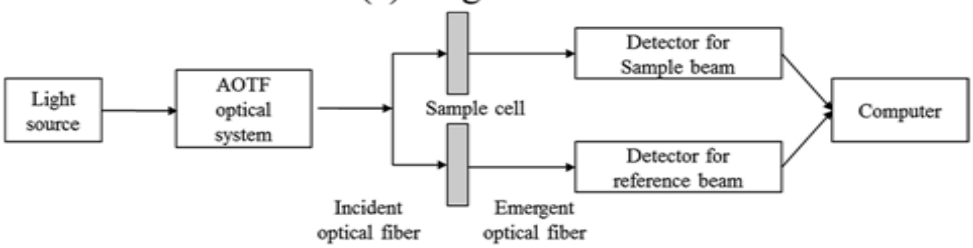



the most common application of spectrophotometers is the measurement of light absorption.

- ✓ The most common spectrophotometers are used in the UV (200 – 400nm) and visible (400 – 800nm) regions of the spectrum, and some of these instruments also operate into the near-infrared region as well.
- ✓ Visible region spectrophotometry is used extensively in colorimetry science .
- ✓ There are two major classes of spectrophotometers; single beam and double beam.




(a) Single beam.




(b) Double beam.

**The single beam spectrophotometer** was the first invented, and all the light passes through the sample.

- ❖ In this case, to measure the intensity of the incident light, the sample must be removed so all the light can pass through (using blank solvent).
- ❖ This type is cheaper because there are less parts and the system is less complicated.

**Later, the double beam spectrophotometer was invented.**

1. In this type, the light source is split by a rotating mirror into two equal Intensity beams before it reaches the sample. One beam passes through the reference compartment containing only solvent and the other passes through the sample.
2. This is advantageous because the reference reading and sample reading can be taken at the same time.
3. In some double beam spectrophotometers, there are two detectors and the sample and reference beams can be measured simultaneously. Other double beam spectrophotometers that have only one detector use a beam chopper. This device inside blocks one beam at a time and the detector alternates between measuring the sample and reference beams.
4. The output of a spectrophotometer is usually a graph of light intensity versus wavelength (Absorption curve).

## Spectrophotometers

A spectrophotometer is a device to measure light intensity at different wavelengths. It produces light with a light source, and after the light passes through a subject, the light is diffracted into a spectrum which is detected by a sensor and interpreted into results we can use.

## Components of Spectrophotometer

1- source of light

2 – monochromator

3 – sample component

4 – detector

5 – meter ( readout device , computer)

If the sample compound does not absorb light of a given wavelength ,**I = Io** However, if the sample compound absorbs light then **I** is less than **Io** and this difference may be plotted on a graph versus wavelength,

- Absorption may be presented as **transmittance (T = I/I**o ) (or **absorbanc *A*** =log **I/Io )**.
- If no absorption has occurred, T = 0 and *A* =0 .
- Most spectrometers display absorbance on the vertical axis, and the commonly observed range is from 0 (100% transmittance) to 2 (1% transmittance).
- The wavelength of maximum absorbance is a characteristic value, designated as **λmax**.

## 1. Radiation Source

The radiation used for spectrometric measurements must be intense and stable

- **For the UV** region, the radiation source is a Hydrogen or deuterium discharge lamp .
- The instrument scans a wavelength region from 150nm to 350 nm.
- **For the Vissble** Region the radiation source must be changed to a tungsten lamp.
- The wavelengths are scanned from 400 nm (violet) to 750 nm (red).

## 2. Monochromator :

dispersion of polychromatic radiation into monochromatic radiation.

Monochromatic light may be obtained by one of the following methods

A – filter :

B – prisms :

C- gratings:

## 3. Sample Compartment

- For UV spectra the sample must be placed in sample compartments made of quartz since quartz will not absorb in the UV region
- For the visble region, compartments composed of simple pyrex glass is sufficient since pyrex will absorb in the UV but will not absorb in the visible region .

4. **detectors**

Convert the radiant energy into an electric current which is proportional to light intensity.

A – photocells :

B – phototubes (photomultiplier tubes) :

5. **Recorder (meters):**

many types are used such as galvanometers, electric recorders or electric cells to give digits.

All may the connected to a compueter which can give final data ( spectrum or concentration).

## 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 |

## Problems on Beer–Lambert Law

**Example 1**

A $7.25 \times 10^{-5}$ 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%