Cihan University - Sulaimaniya

Clinical Biochemistry

Lab 1

Introduction

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Academic Year 2023-2024

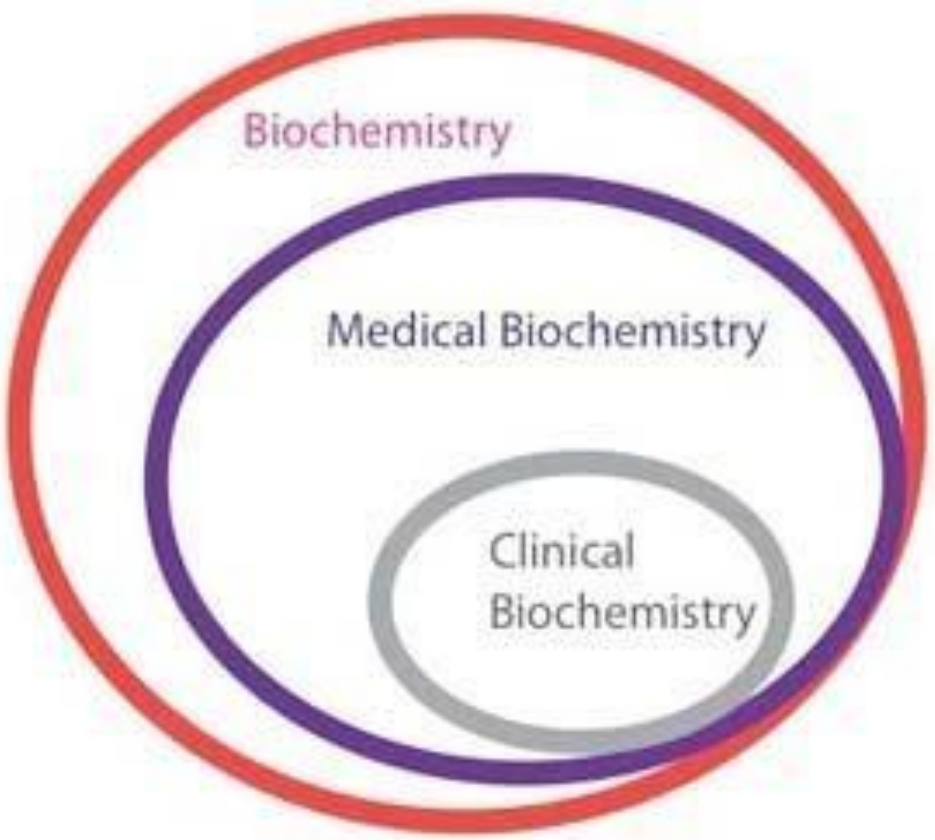
Biochemistry

- **Medical Biochemistry:**

Deals with **chemical basis of human body.**

- **Clinical Biochemistry:**

Deals with clinical **diseases/pathological conditions of human body.**



In the practical module of Clinical Biochemistry we are going to Practice:

Week Number	Subject- Practical
Week #1	First Week Lab-Introduction
Week #2	Spectrophotometry- Determination of Lambda Max
Week #3	Determination of Serum Glucose
Week #4	Determination of Serum Urea
Week #5	Determination of Serum Creatinine
Week #6	Determination of Serum Bilirubin
Week #7	Determination of AST and ALT
Week #8	Determination of serum TG
Week #9	Determination of Serum Cholesterol
Week #10	Determination of ALP
Week #11	Determination of CPK
Week #12	Determination of amylase
Week #13	LDL/ HDL cholesterol Test
Week #14	Review
Week #15	Final Exam

Spectrophotometry

What is light?

Light is a form electromagnetic radiation which exists in tiny "packets" called photons, exhibits properties of both particles and waves(photons travels in waves).

What we mean by the Wavelength?

Light have both frequency and wave length which Is the distance between peaks of the light wave and each light color is defined by its wavelength.

Different wavelengths of light appear to our eyes as different colors

polychromatic light (White)	Monochromatic light
Light containing all wavelengths, including ultraviolet, visible light, and infrared is called white light	On the other hand, each color of light, (e.g., red, blue, etc) is called monochromatic light

Fundamentals of Spectrophotometry

- **Photometric measurement**

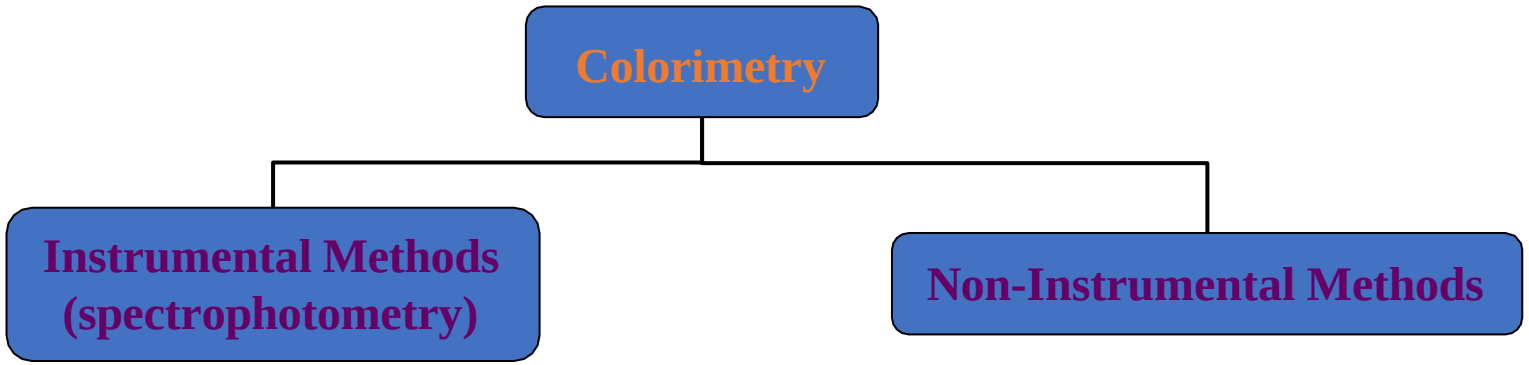
It means the use of light for measurement of the concentration of a substances in solution

- **Colorimetry**

Also known as **Photometry.** It is defined as the use of only the visible portion of light spectrum for measurement of concentration

- **Spectrophotometry**

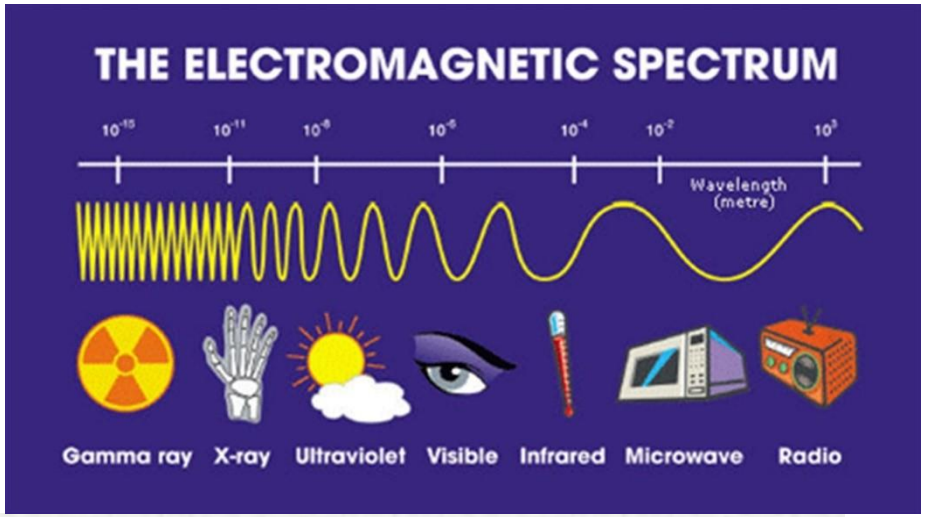
It is defined as the use of the visible and **UV portions of light spectrum** for **measurement of concentration**



Colorimetry

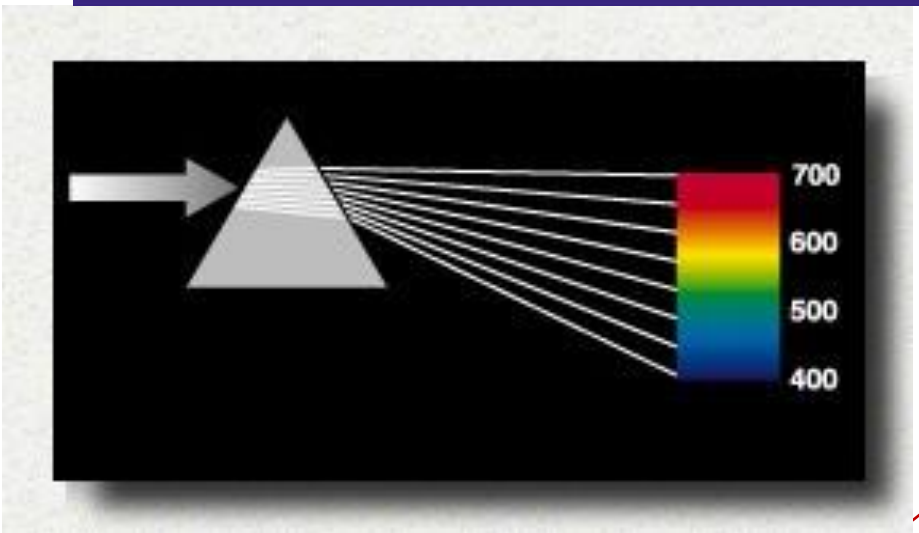
- **Visual Observations** :

  Because **colorimetry** is based on inspection of materials with the **human eye**, it is necessary to review aspects of visible light.



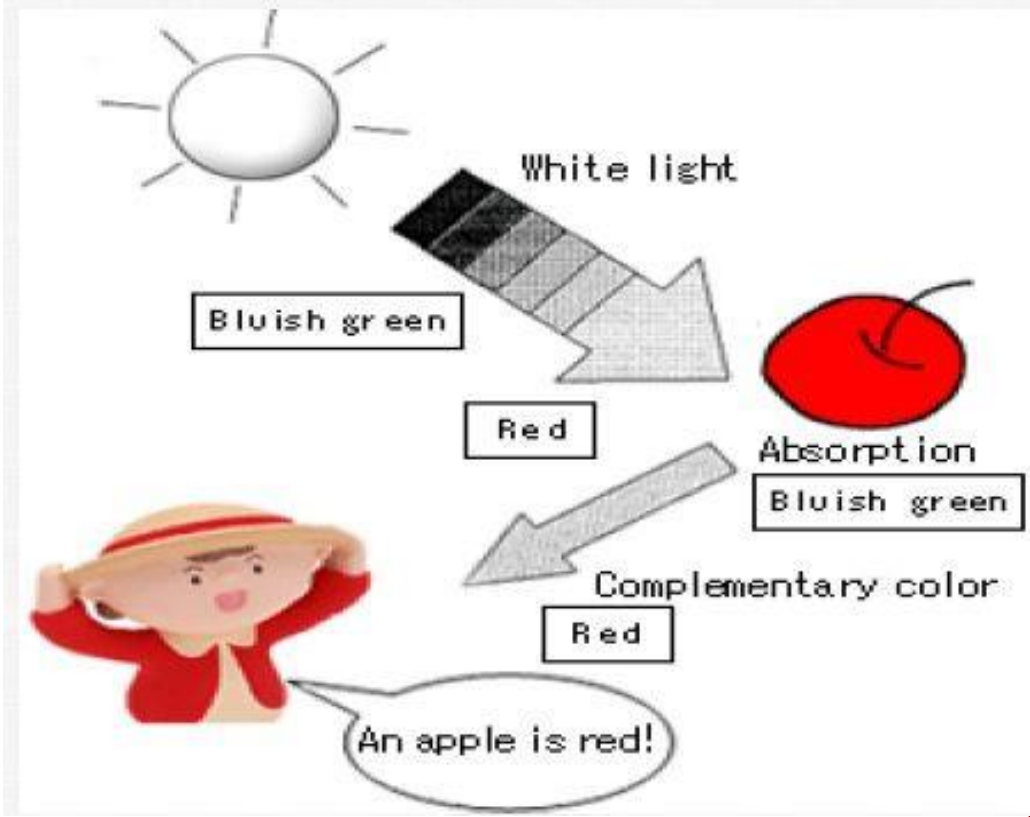
- **Visible light**:

  is the narrow range of electromagnetic waves with the wavelength of **400-700 nm**.



Why an apple looks red to our eye?

When a certain material is exposed to light containing various colors (white light), it takes in and keeps only its favorite colors from this light (a phenomenon called **—absorption of light**). Its disliked color (called a **—complementary color**) is then reflected, and becomes visible to our eyes as the color of the material.



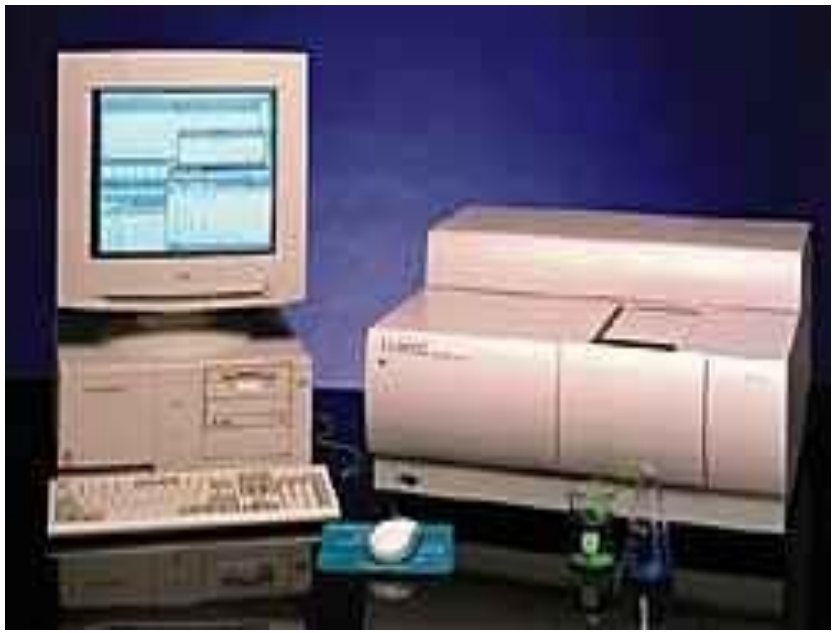
In other words, an apple prefers **blue and green (490-500 nm)** and dislikes **red (610-750 nm)**. When it is exposed to white light, it will absorb blue and green, and look like red, which is the complementary color.

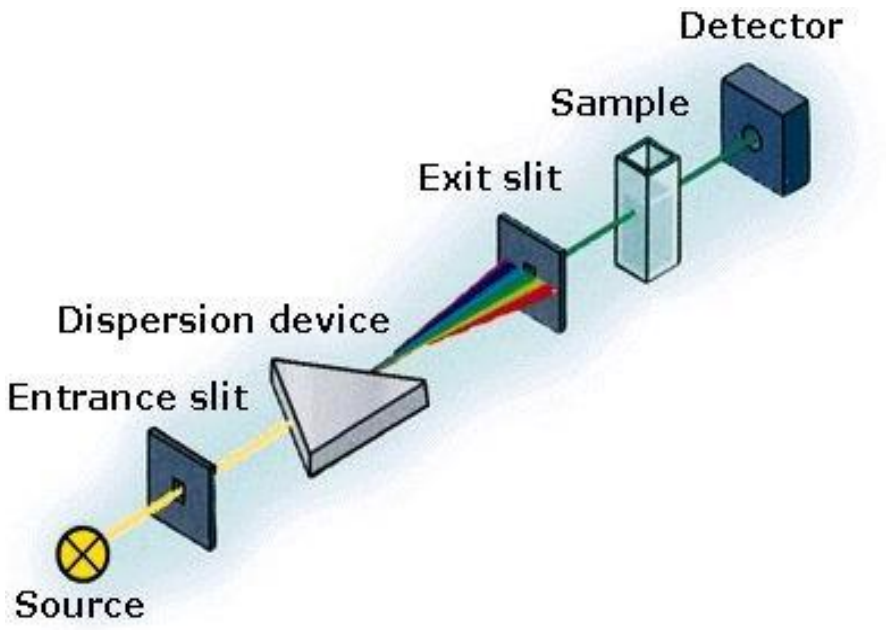
Fundamentals of Spectrophotometry

Spectrophotometer

Basic Design

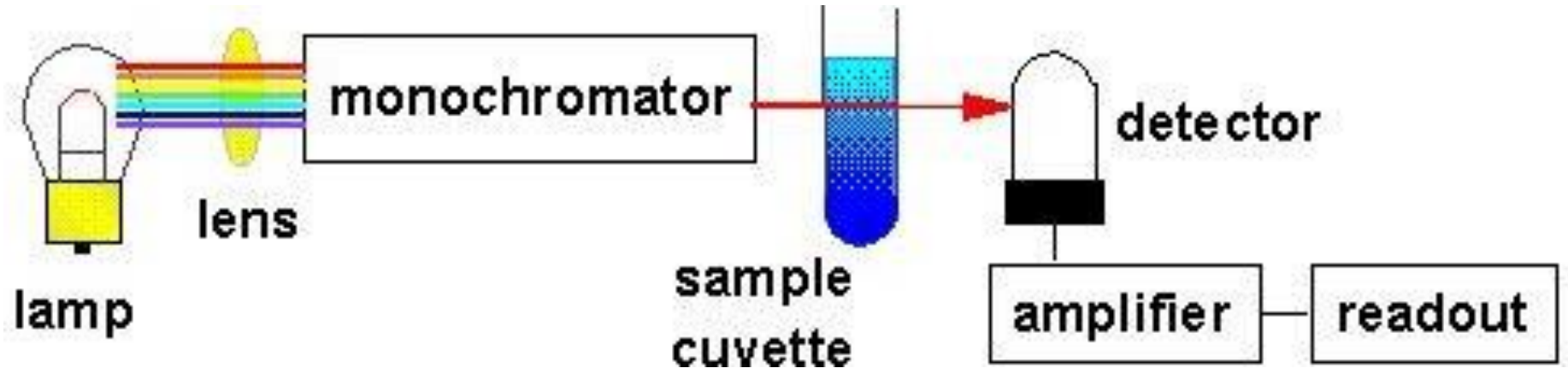
- An instrument used to make **absorbance or transmittance measurements** is known as a spectrophotometer





Simple Spectrophotometer Schematic

- **The lamp:** emits all colors of light (i.e., white light).
- **The monochromator:** selects **one wavelength** and that wavelength is sent through the sample.
- **The detector:** detects the wavelength of light that has passed through the sample.
- **The amplifier:** increases the signal so that it is easier to read against the background noise.



Fundamentals of Spectrophotometry

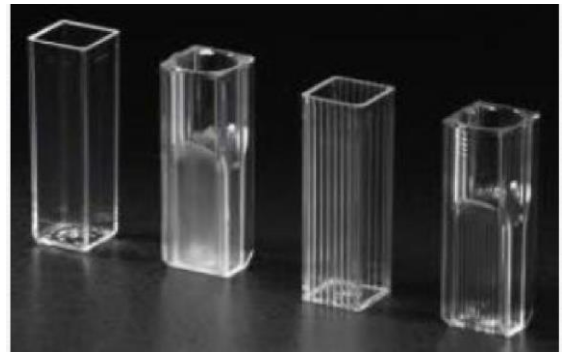
Absorption cell (cuvets)

A cuvette is a kind of laboratory glassware, usually a small tube of circular or square cross section, sealed at one end, made of plastic, glass, or optical grade quartz and designed to hold samples for spectroscopic experiments.

Types of Cells

There are three different types of cuvettes commonly used, with different usable wavelengths:

- Glass, with a wavelength from 380 to 780 nm (visible spectrum)
- Plastic, with a wavelength from 380 to 780 nm (visible spectrum)
- Quartz, with a wavelength below 380 nm (ultraviolet spectrum)



Fundamentals of Spectrophotometry

Precautions

- ✓ Always clean the cells thoroughly and rinse at least once with a portion of the sample, before filling the sample for measurement.
- ✓ Always wipe the exposed surface of the cells dry and free from finger prints, using tissue paper.
- ✓ Clean cells thoroughly immediately after use.
- ✓ Do not leave solutions, particularly strong alkali, in the cells for periods more than an hour.
- ✓ Never use a brush or any tool for cleaning which may scratch the optical surfaces.

Fundamentals of Spectrophotometry

https://www.youtube.com/watch?v=xHQM4BbR040

Absorbance

Absorbance is the measure of the **quantity of light** that **a sample absorb** (neither transmits nor reflects) and is **proportional to the concentration of a substance in a solution**.

The absorbance of a solution depends on:

- ✓ Nature of substance
- ✓ Concentration of substance
- ✓ Wavelength of light
- ✓ Path of light

As Concentration (C) increases, light **Absorption (A)** increases, linearly.

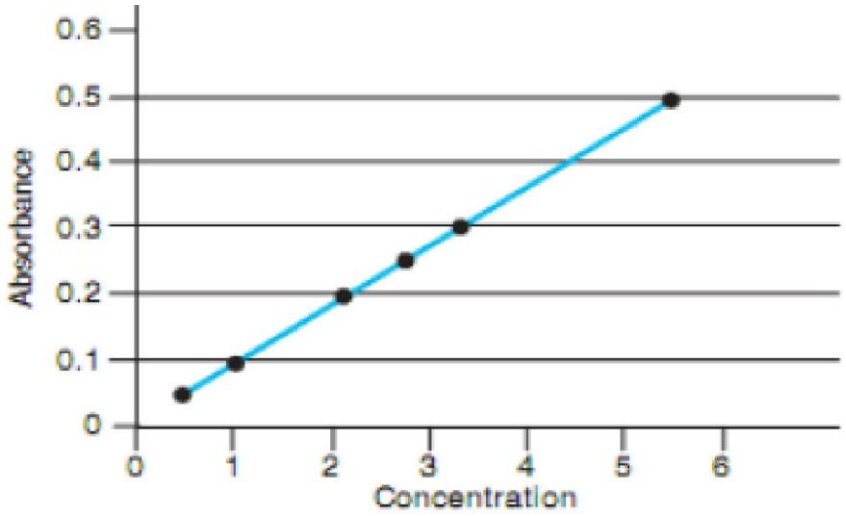
Optical density (OD)

The O.D. is used the same as absorbance and directly proportional to the concentration of the colored compound.

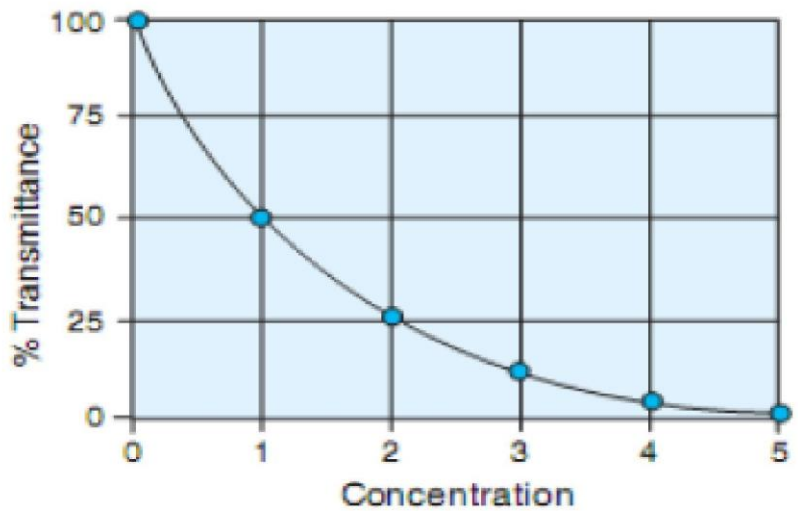
Fundamentals of Spectrophotometry

Transmittance

- The transmittance of a sample is the ratio of **the intensity of the light that has passed through the sample** to the intensity of the light when it entered the sample and displayed as a percentage (%T).



- As Concentration (C) **increases**, light Transmission (%T**)** **decreases.**

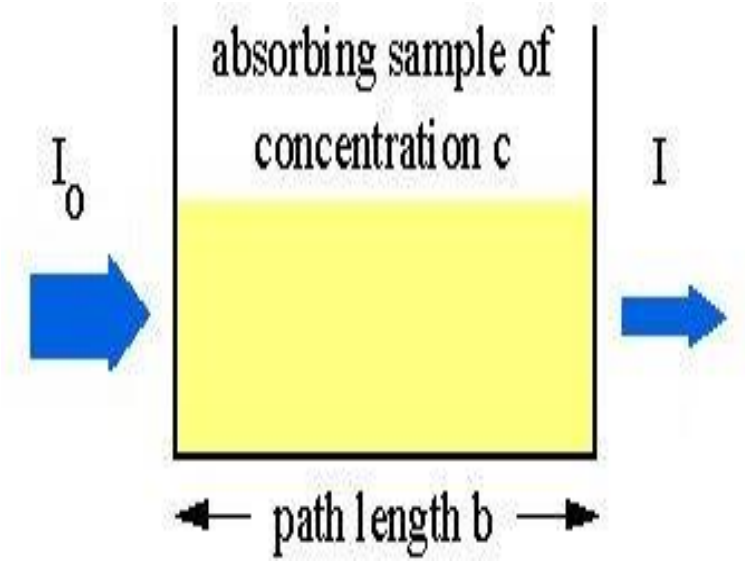


Law of Absorbance

The Lambert Law

- It states that the intensity of light transmitted by a colored solution decrease as the path of light increase
- The absorbance of a solute is directly proportional to the path of light

$$A \alpha b$$



Law of Absorbance

The Beer law

- It states that the intensity of light transmitted by a colored solution decrease as the concentration increase.
- The absorbance of a solute is directly proportional to the concentration of a solution.

$$A \alpha C$$

The Beer-Lambert Law

Also known as Beer's law where the two laws are usually combined together and used in all absorption analysis

A: is the absorbance

C: is the concentration of the compound in solution, expressed in mol/L

b: the path length of the cuvette in which the sample is contained expressed in cm

ε :is the molar absorptivity with units of L mol-1 cm-1

✓ **The Main use of Beer's Law** is to **determine the concentration** of various solutions.

Fundamentals of Spectrophotometry

Example 1

Guanosine has a maximum absorbance of 275 nm. $\epsilon_{275} = 8400 M^{-1} cm^{-1}$ and the path length is 1 cm. Using a spectrophotometer, you find the that $A_{275} = 0.70$. What is the concentration of guanosine?