Spectrophotometric analysis

Spectrophotometer

- **Spectrophotometric techniques** are used to measure the concentration of solutes in solution by measuring the amount of light that is absorbed by the solution in a cuvette placed in the spectrophotometer.

The function of Spectrophotometer

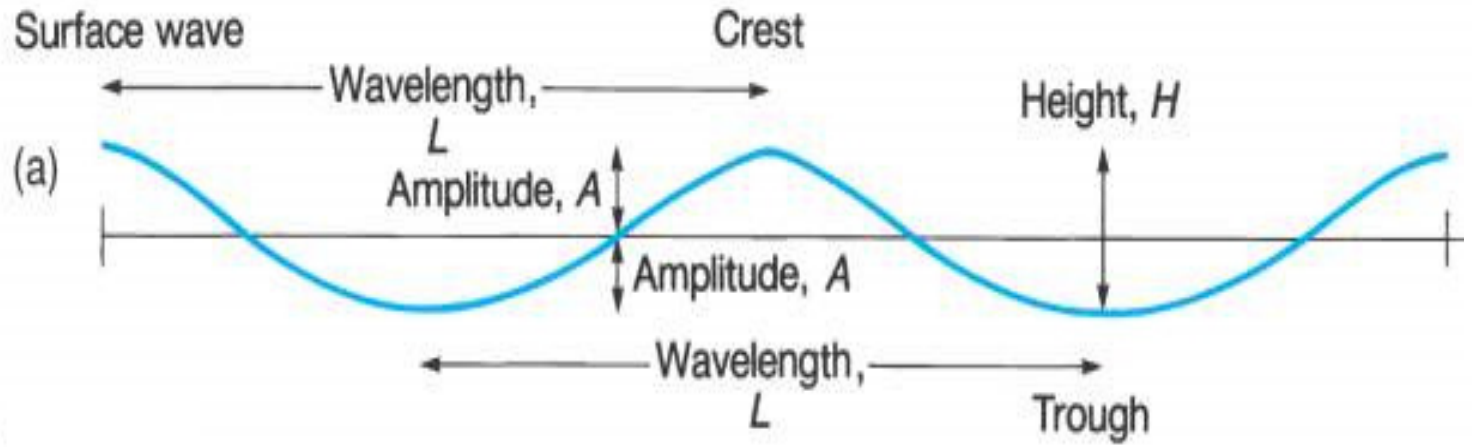
- It can measure the amount of light or electromagnetic radiation (of certain frequency) transmitted or absorbed by the solution.

Wave motion of light

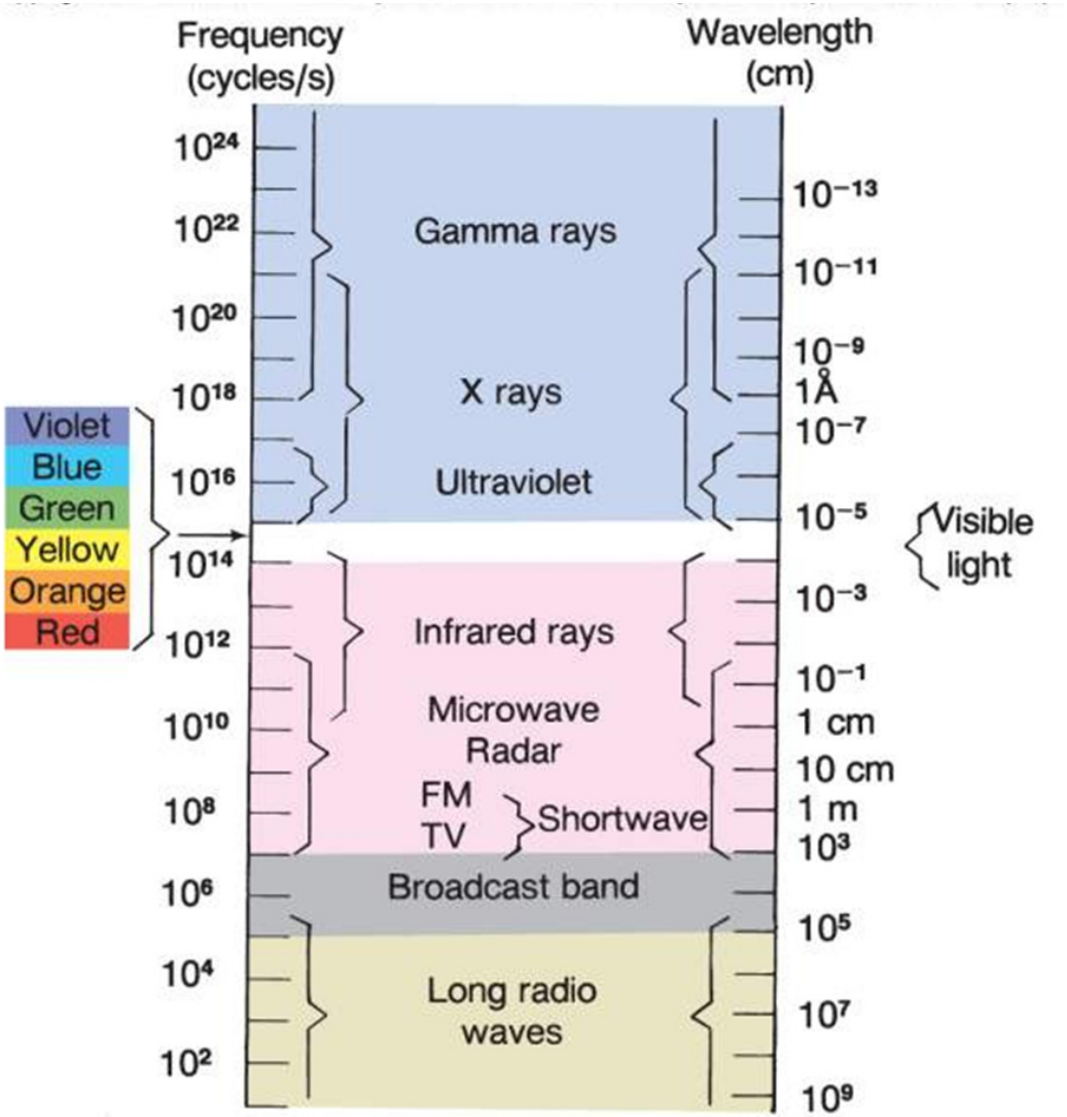
- **Properties of waves:**
  - The wave is described either in terms of its wavelength (λ) or frequency(ν).
  - **wavelength (λ) :** The distance between two successive waves (cm, nm, A^{o}).
  - **frequency(ν) :** The number of oscillation of the field (waves) per second (Hz).

Wave motion of light

- *Electromagnetic radiation* moves in waves



Electromagnetic spectrum



Colors & Wavelengths

COLOR	WAVELENGTH (λ in nm)
Ultraviolet	< 380
Violet	380 – 435
Blue	436 – 480
Greenish-blue	481 – 490
Bluish-green	491 – 500
Green	501 – 560
Yellowish-green	561 – 580
Yellow	581 – 595
Orange	596 – 650
Red	651 – 780
Near Infrared	> 780

What is Spectrophotometry?

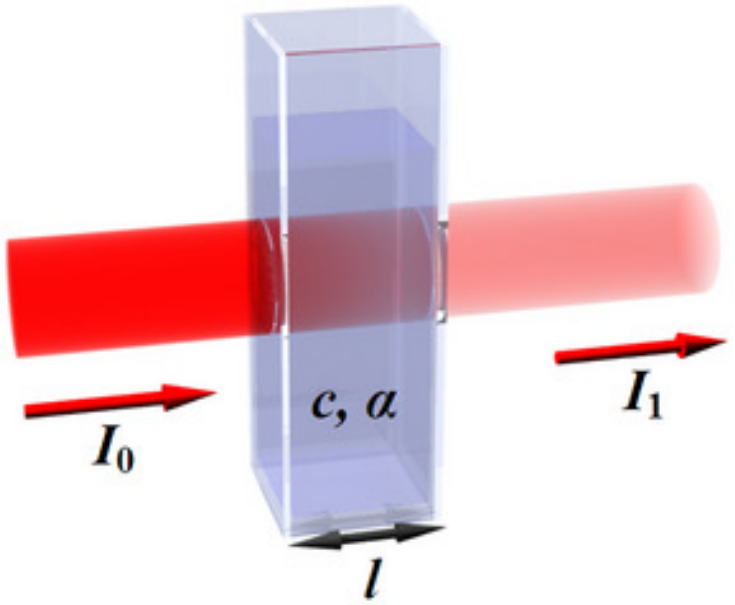
- Light can either be *transmitted* or *absorbed* by dissolved substances.
- Presence & concentration of the dissolved substances is analyzed by passing light through the sample.
- Spectrophotometers measure electromagnetic ***absorption.***
- The solutions of many compounds have characteristic colors.
- The intensity of such a color is proportional to the concentration of the compound.

The absorption process
How does matter absorb radiation

- **When monochromatic light falls upon a homogenous medium:**
  1. A portion of light is reflected (neglected).
  2. Other portion is absorbed in the medium.
  3. The remainder is transmitted.

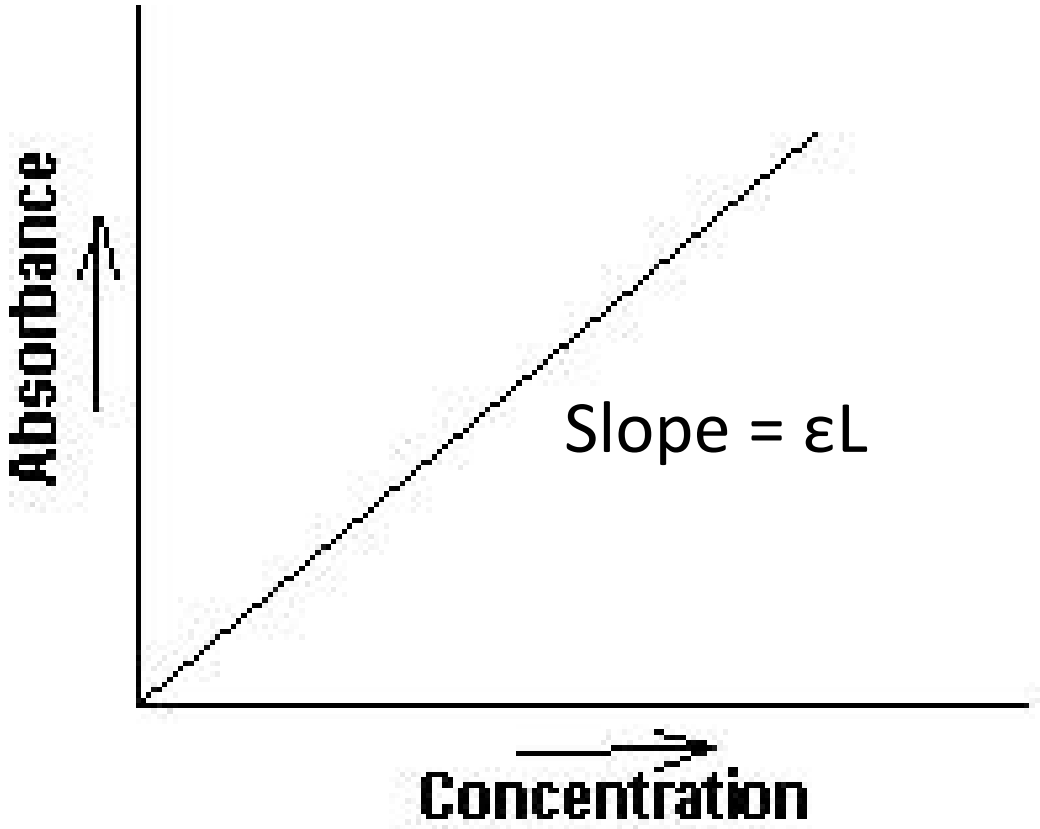
The absorption process

- The intensity of the incident light is expressed by I_o.
- The absorbed light by I_a
- The transmitted light by I_t.
- $I_o = I_a + I_t$

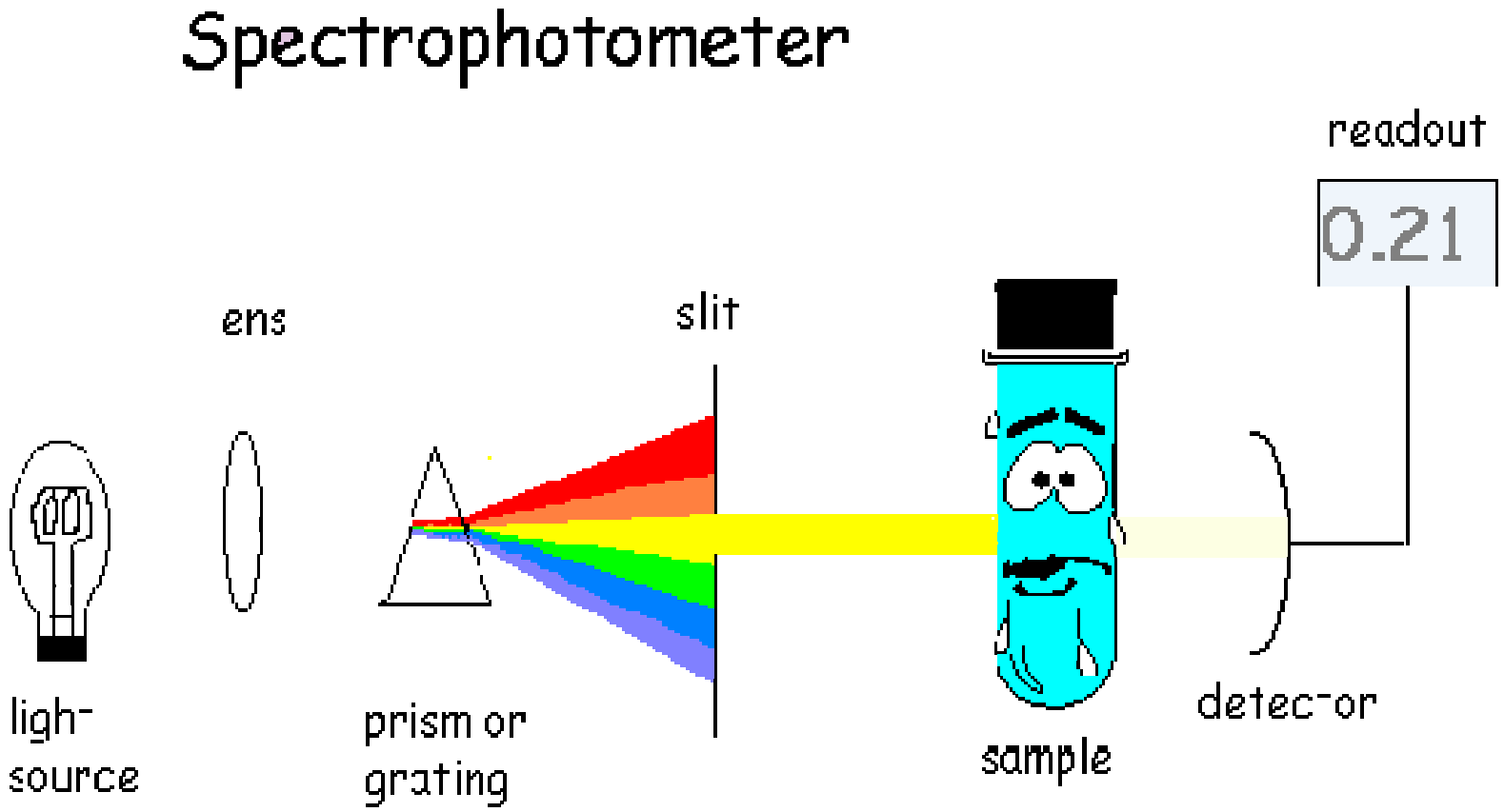


- **Beer's law:**
- $A \alpha C$
- **Lambert's law:**
- $A \alpha L$
- **Beer-Lambert law:**
- $A \alpha CL$, $A = \varepsilon CL$, $A = \text{Log}(I_o/I_t)$
- **Where:**
- A : is the absorbance.
- L : is the thickness of the sample.
- C : is the molar concentration.
- ε : is the molar absorption coefficient.

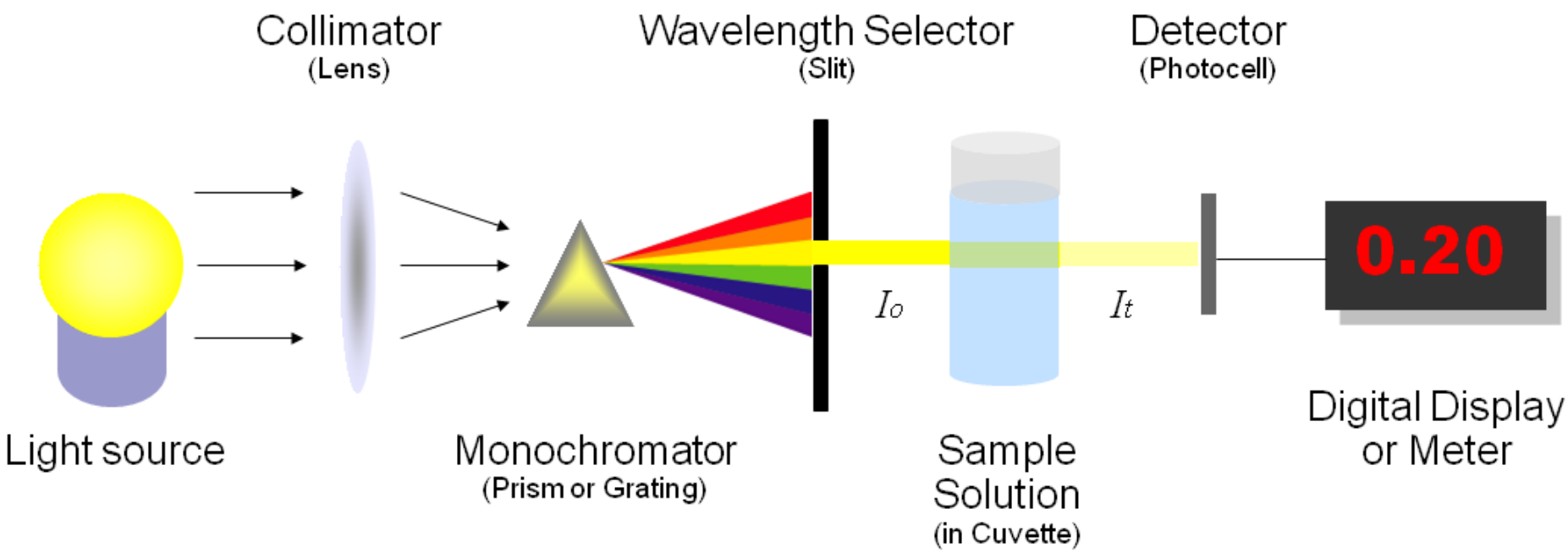
Representing Beer-Lambert law graphically



The Spectrophotometer



Single beam spectrophotometer:



Thank you