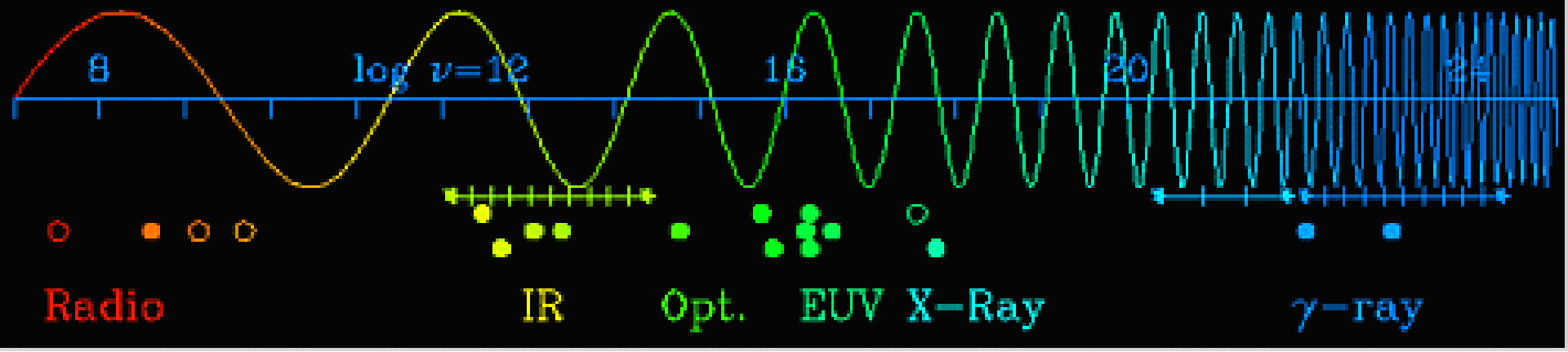
Spectrophotometry & The Beer-Lambert Law



ELECTROMAGNETIC SPECTRUM

High energy: γ rays, x rays — Light: uv, vis, ir — Microwaves: esr, nmr — Radiowaves — Low energy

10^{-12} 10^{-10} 10^{-8} 10^{-6} 10^{-4} 10^{-2} 1 10^{2} 10^{4}

Wavelength (m) on a logarithmic scale

VISIBLE SPECTRUM

Ultraviolet | Violet | Blue | Green | Yellow | Orange | Red | Infrared

400 500 600 700 800

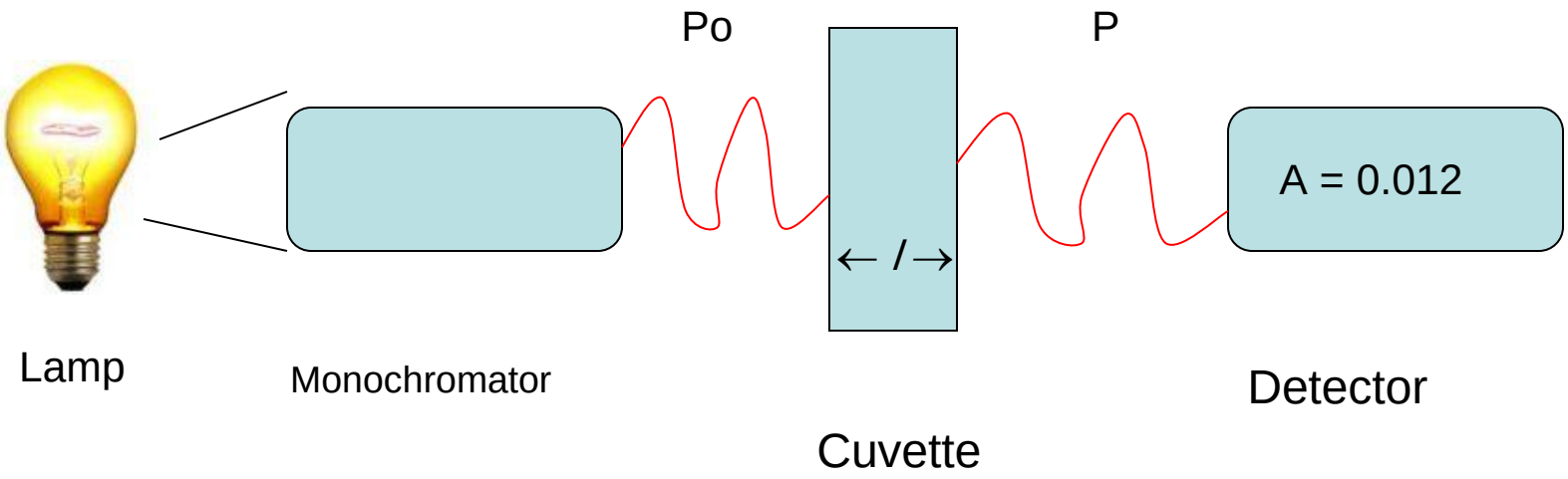
Wavelength (nm) on a linear scale

1 nm = 10^{-9} m = 10^{-7} cm

Fig. 10.1 Wavelength regions of the electromagnetic spectrum.

- **Introduction**
- Many compounds absorb ultraviolet (UV) or visible (Vis.) light.
- **Beer's law**, also called **Lambert-Beer law** or **Beer-Lambert law**, in spectroscopy, a relation concerning the absorption of radiant energy by an absorbing medium. Formulated by German mathematician and chemist August Beer in 1852, it states that the absorptive capacity of a dissolved substance is directly proportional to its concentration in a solution.
- The relationship can be expressed as $A = \varepsilon lc$ where A is absorbance, ε is the molar extinction coefficient (which depends on the nature of the chemical and the wavelength of the light used), l is the length of the path light must travel in the solution in centimeter's, and c is the concentration of a given solution.

Spectrophotometry



- The amount of radiation absorbed may be measured in a number of ways:
- **Transmittance**, $T = P / P_0$
  % Transmittance, $\%T = 100\ T$
- **Absorbance**,
- $A = log_{10}\ P_0 / P$
  $A = log_{10}\ 1 / T$

The Beer-Lambert Law

- Now let us look at the Beer-Lambert law and explore it's significance. This is important because people who use the law often don't understand it - even though the equation representing the law is so straightforward:
- **A=ebc**
- Where
- A is absorbance (no units, since A = log10 P0 / P)
  e is the molar absorptivity with units of L mol-1 cm-1
  b is the path length of the sample - that is, the path length of the cuvette in which the sample is contained. We will express this measurement in centimeters.
  c is the concentration of the compound in solution, expressed in mol L-1

Absorption: The Beer-Lambert Law

- The Beer-Lambert law sortof has the wrong name...

Pierre Bouguer
(1698-1758)

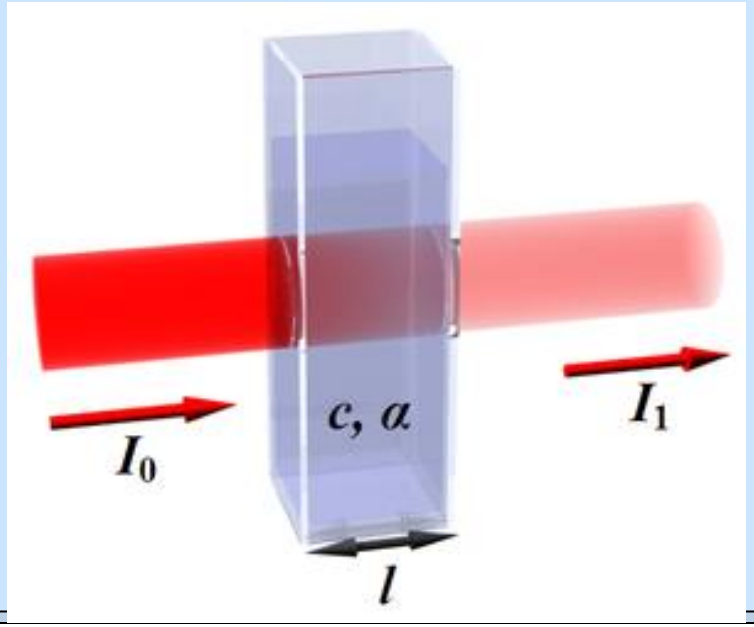
Johan Lambert
(1728-1777)

$$A = -\log(I_1 / I_0) = \varepsilon c l$$

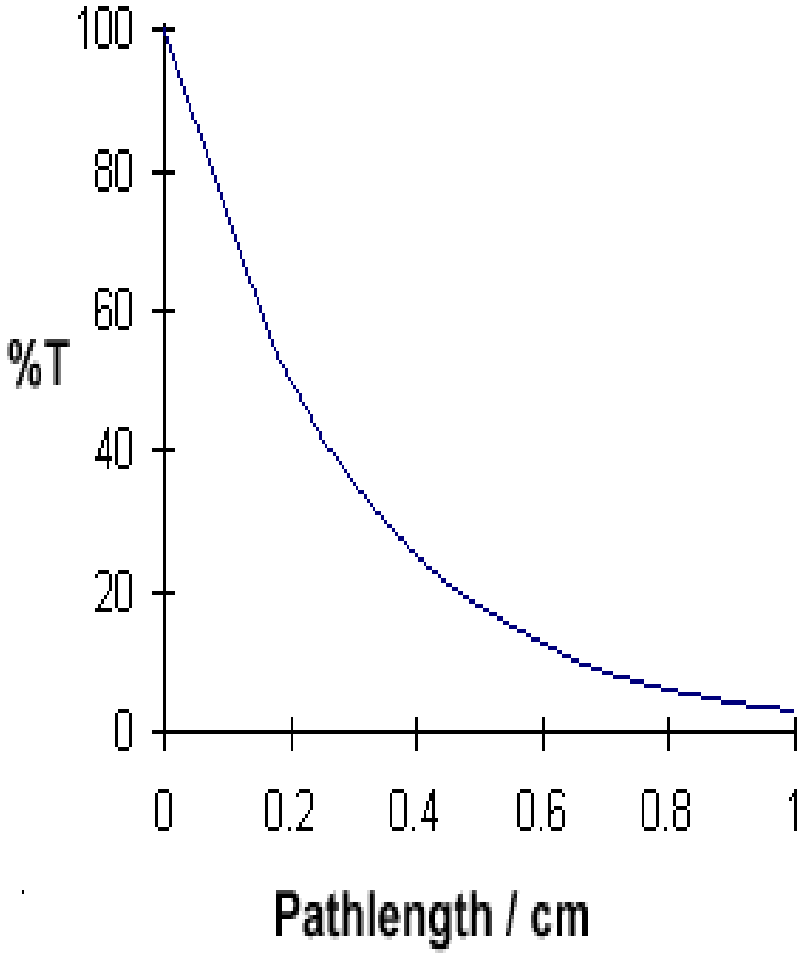
Extinction coefficient

Concentration

Path length



A = ebc tells us that absorbance depends on the total quantity of the absorbing compound in the light path through the cuvette. If we plot absorbance against concentration, we get a straight line passing through the origin (0,0).



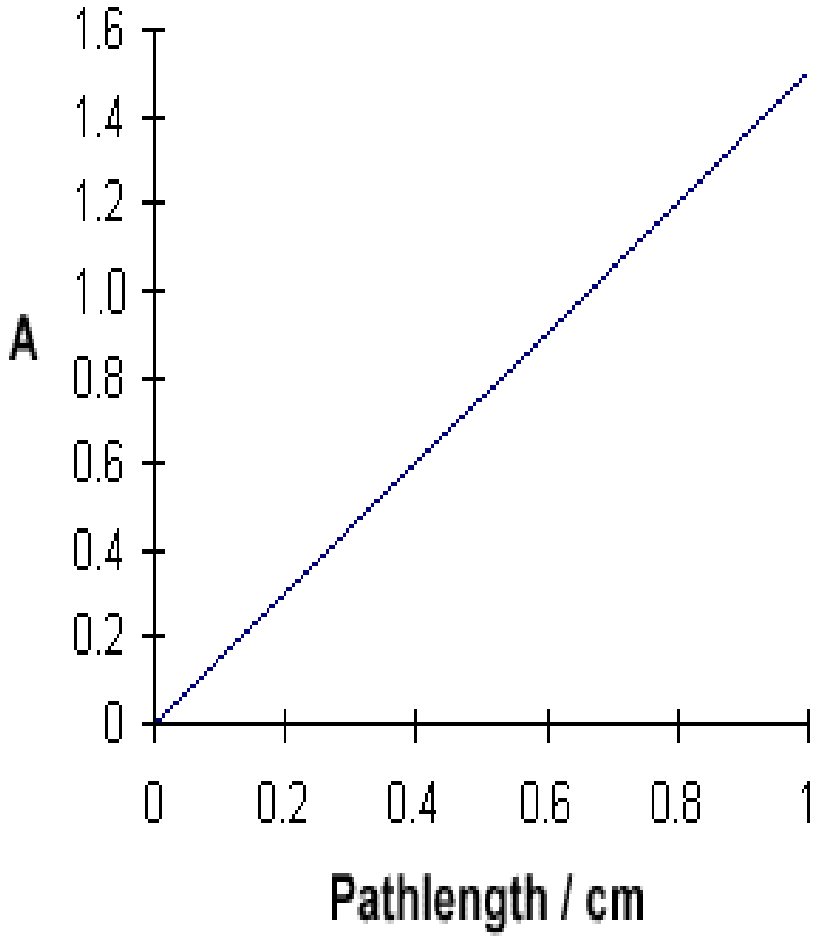
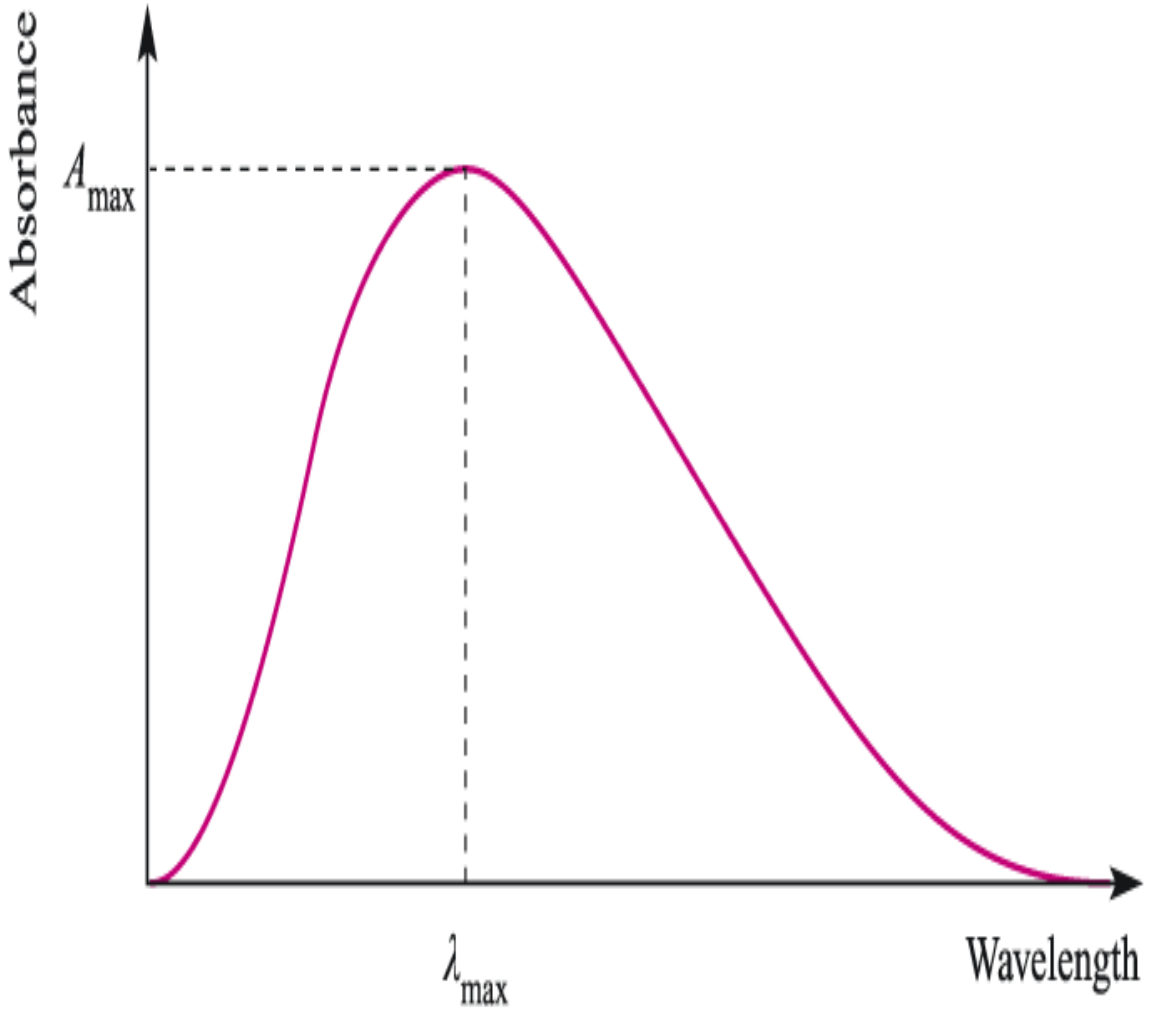


Fig. 2 **Precision and accuracy.**

UV-visible spectrum



The two main properties of an absorbance peak are:

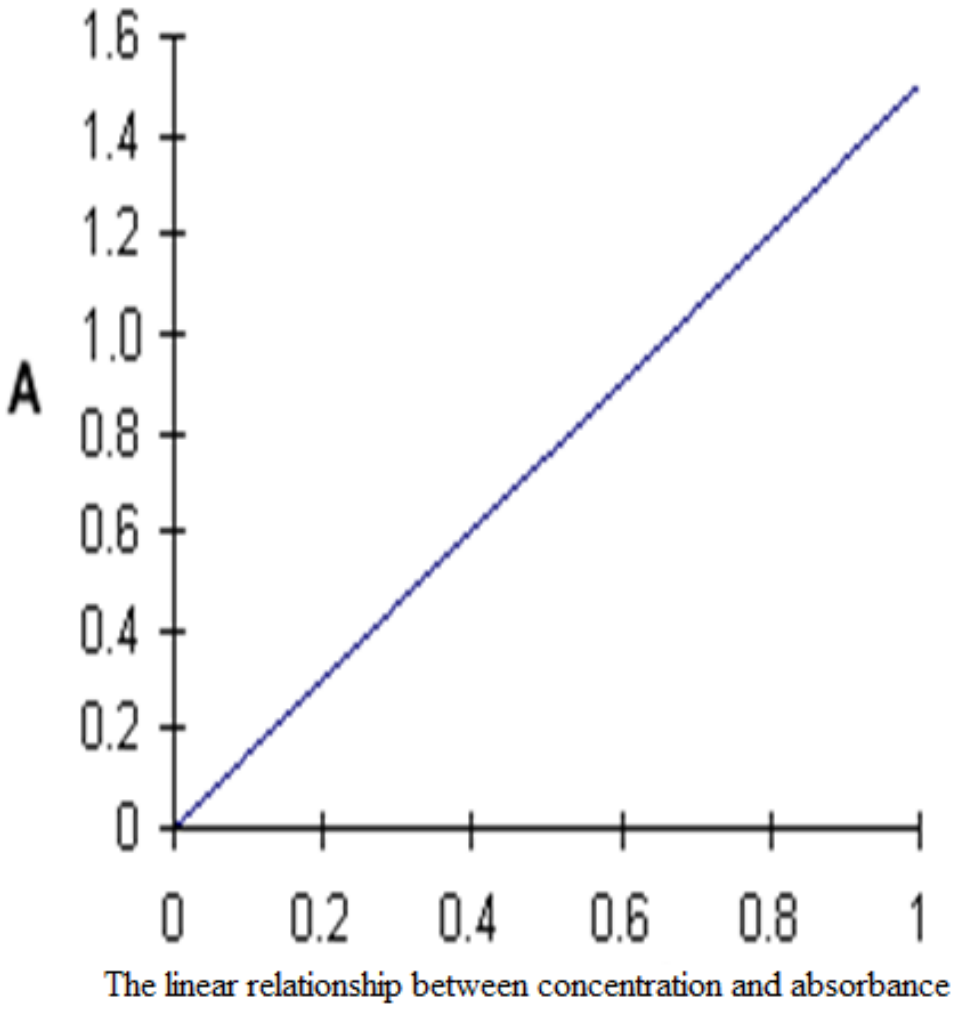
1. Absorption wavelength

   λ_{max}

2. Absorption intensity

   A_{max}

Stander curve



The linear relationship between concentration and absorbance

Absorbance	Concen. mg
0.3	200
0.5	400
0.8	600
1.1	800
1.4	1000
0.7	unknown

Methods:-

Applying Beer's law to find concentration of compounds:-

- Biuret REAGENT

according to Gornall et al. (1949)

- 1. Weigh 1.50 g of cupric sulfate pentahydrate ($CuSO_4$ 5 H_2O) with 6.0 g sodium potassium tartrate tetrahydrate ($NaKC_4H_4O_6$ 4 H_2O).
- 2. Dissolve in 500 ml of H_2O.
- 3. Add 300 ml of 10% NaOH.
- 4. Make up to total volume of 1 liter. Store in a plastic bottle protected from light.
- 5- Albumin 1%

Find unknown concentration of Albumin

Absorbance	Concen. mg
	200
	400
	600
	800
	1000
	unknown