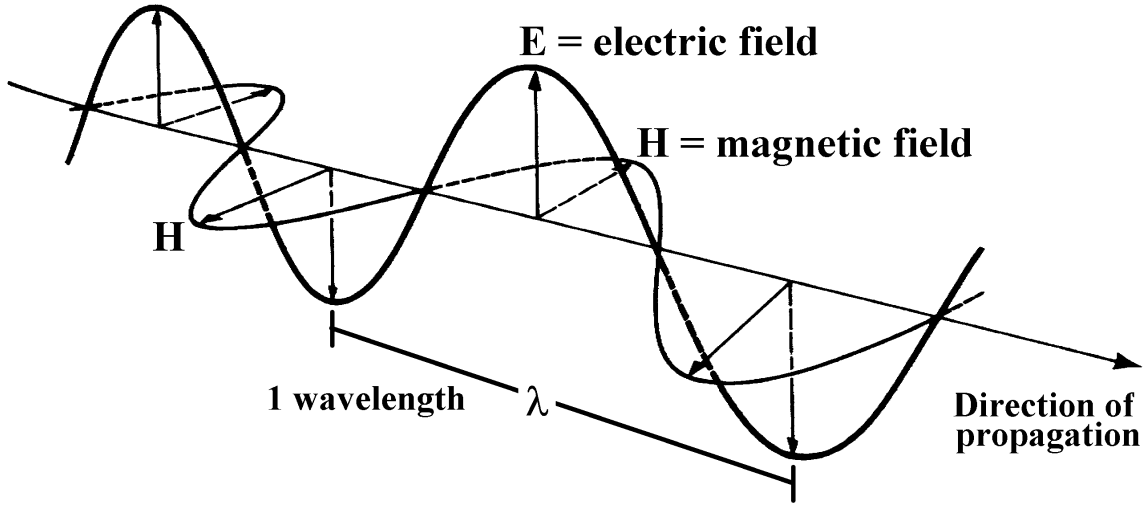
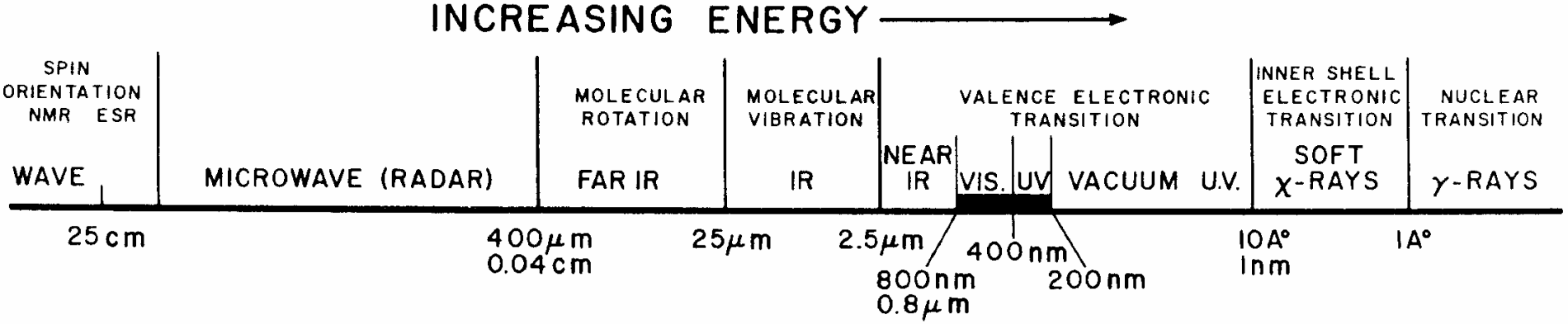
SPECTROPHOTOMETRY

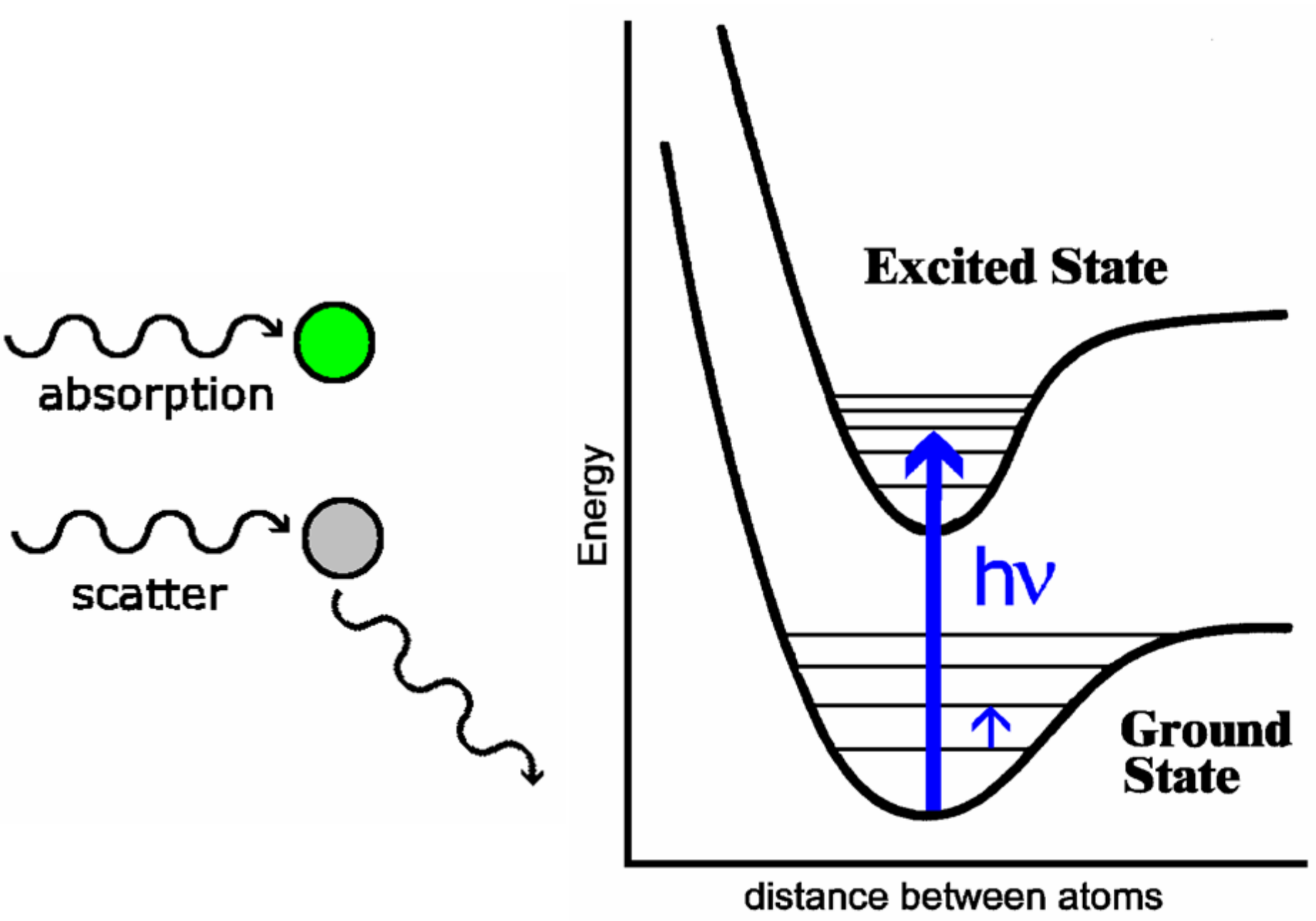
- versatile and widely used analytical tool
- based on how substances affect radiation (i.e., light)
- advantages:
  - often non-destructive
  - can be selective
  - short time interval of measurement (10^{-14} s)

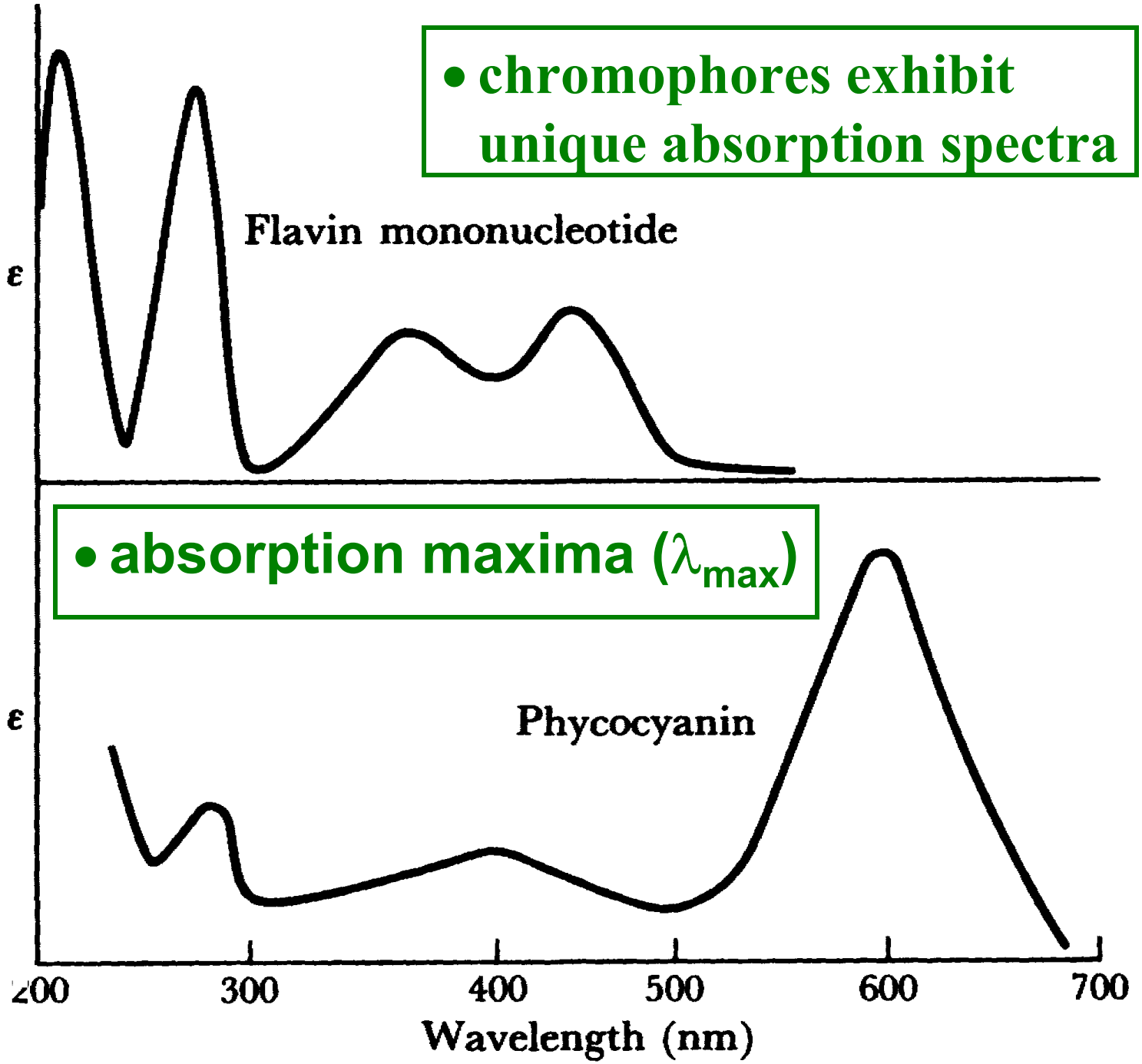




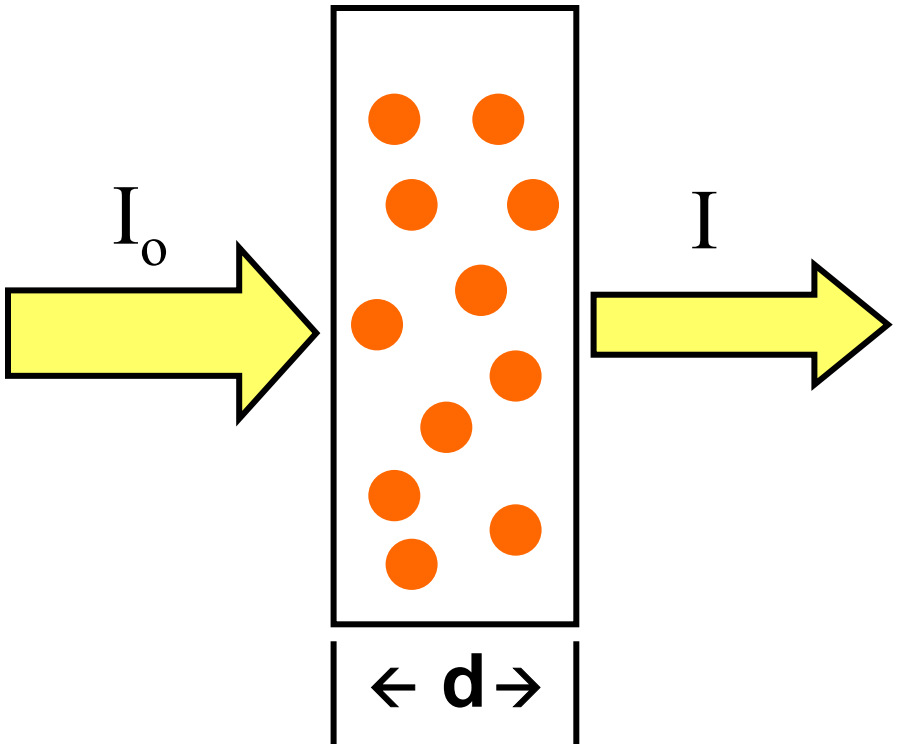
$$E = hc/\lambda$$
$$= h\upsilon$$
(photon)

E = energy
h = Planck's constant
c = speed of light
λ = wavelength
υ = frequency


absorption
scatter
Energy
Excited State
hν
Ground State
distance between atoms


• chromophores exhibit unique absorption spectra
Flavin mononucleotide
ε
• absorption maxima (λ_{max})
Phycocyanin
ε
200
300
400
500
600
700
Wavelength (nm)

Beer-Lambert Law

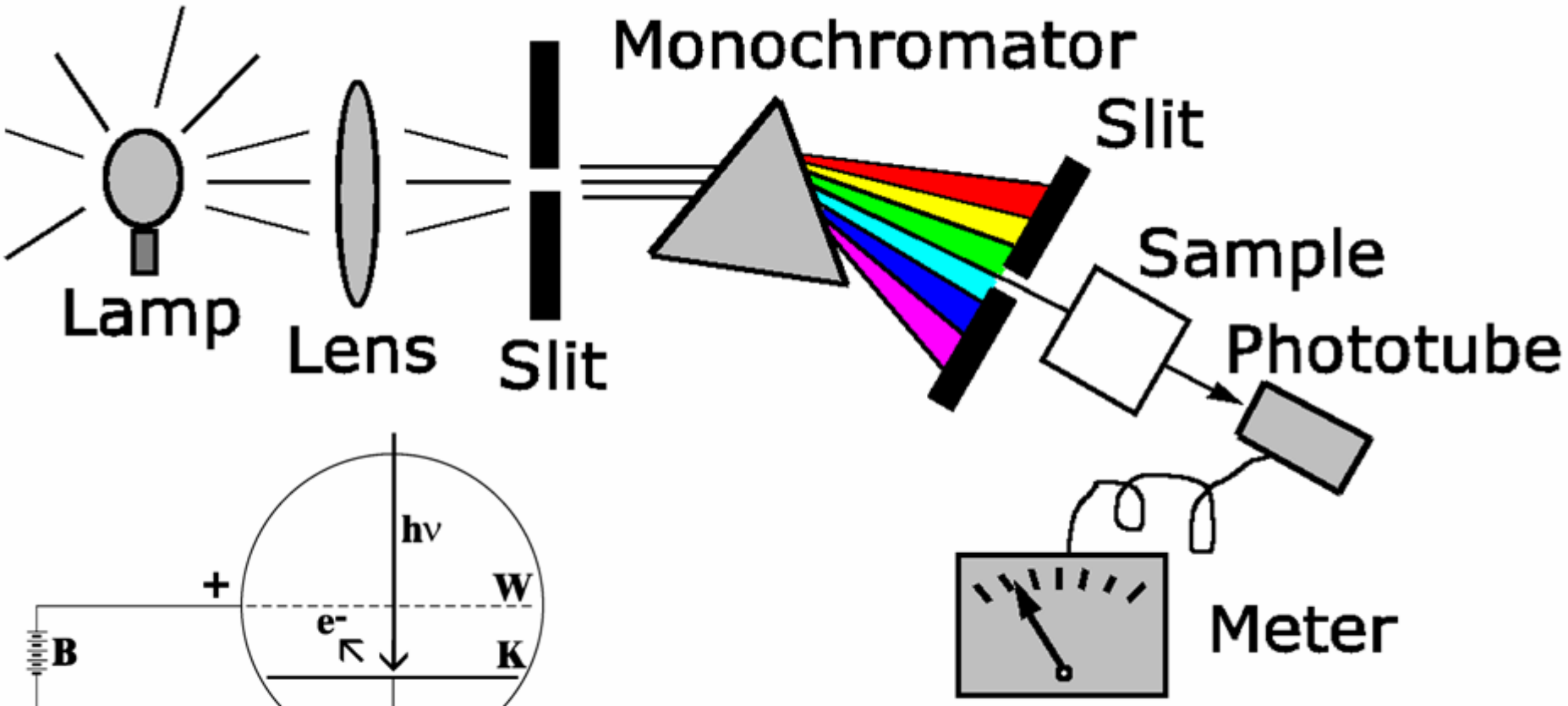


$$I = I_o 10^{-\varepsilon dc}$$

I = light intensity
ε = extinction coefficient
d = thickness
c = concentration

$$\text{Absorption (A)} = -\log(I/I_o) = \varepsilon dc$$

Spectrophotometer



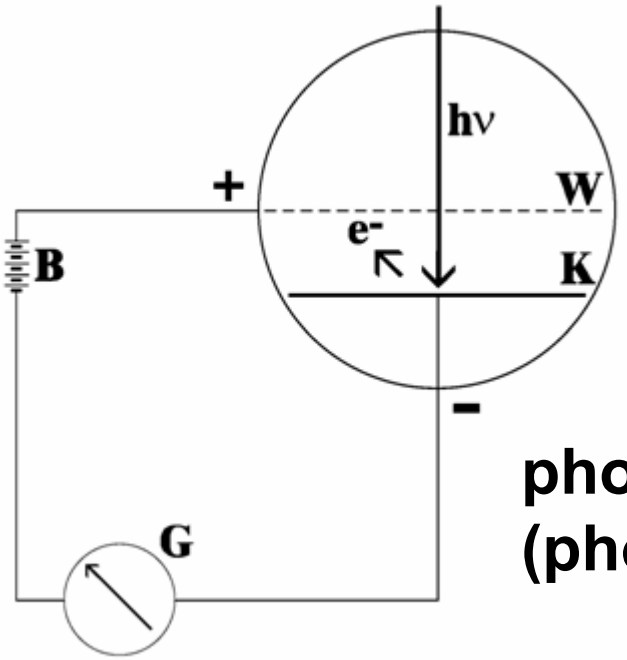
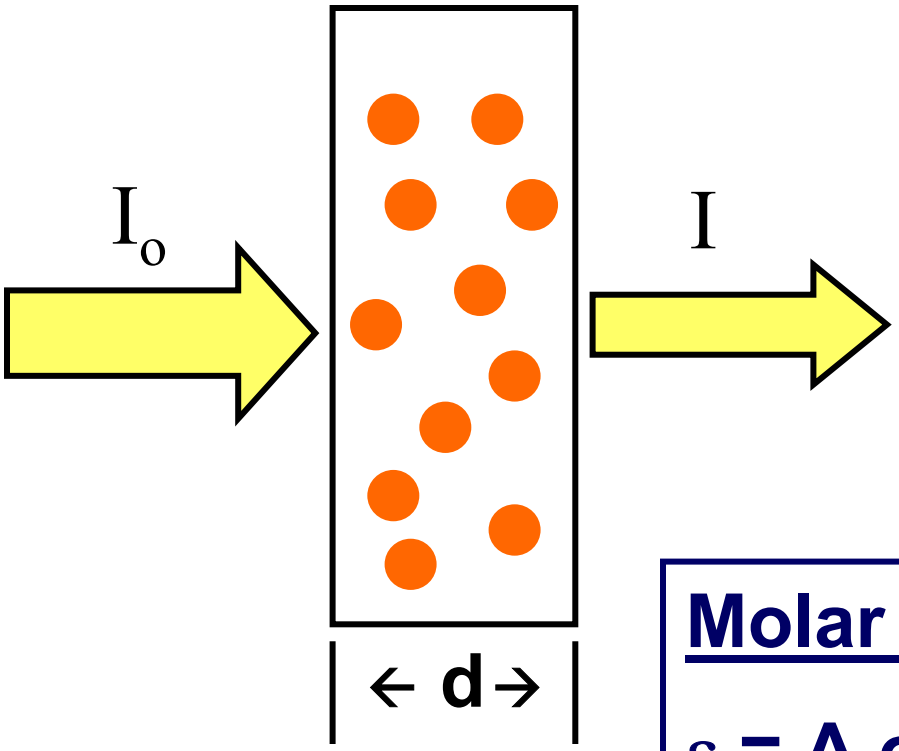


photo multiplier tube
(photo electric cell)

$$-\log(I/I_o) = A$$

Beer-Lambert Law



$$A = \varepsilon dc, \text{ or}$$

$$c = A/\varepsilon d$$

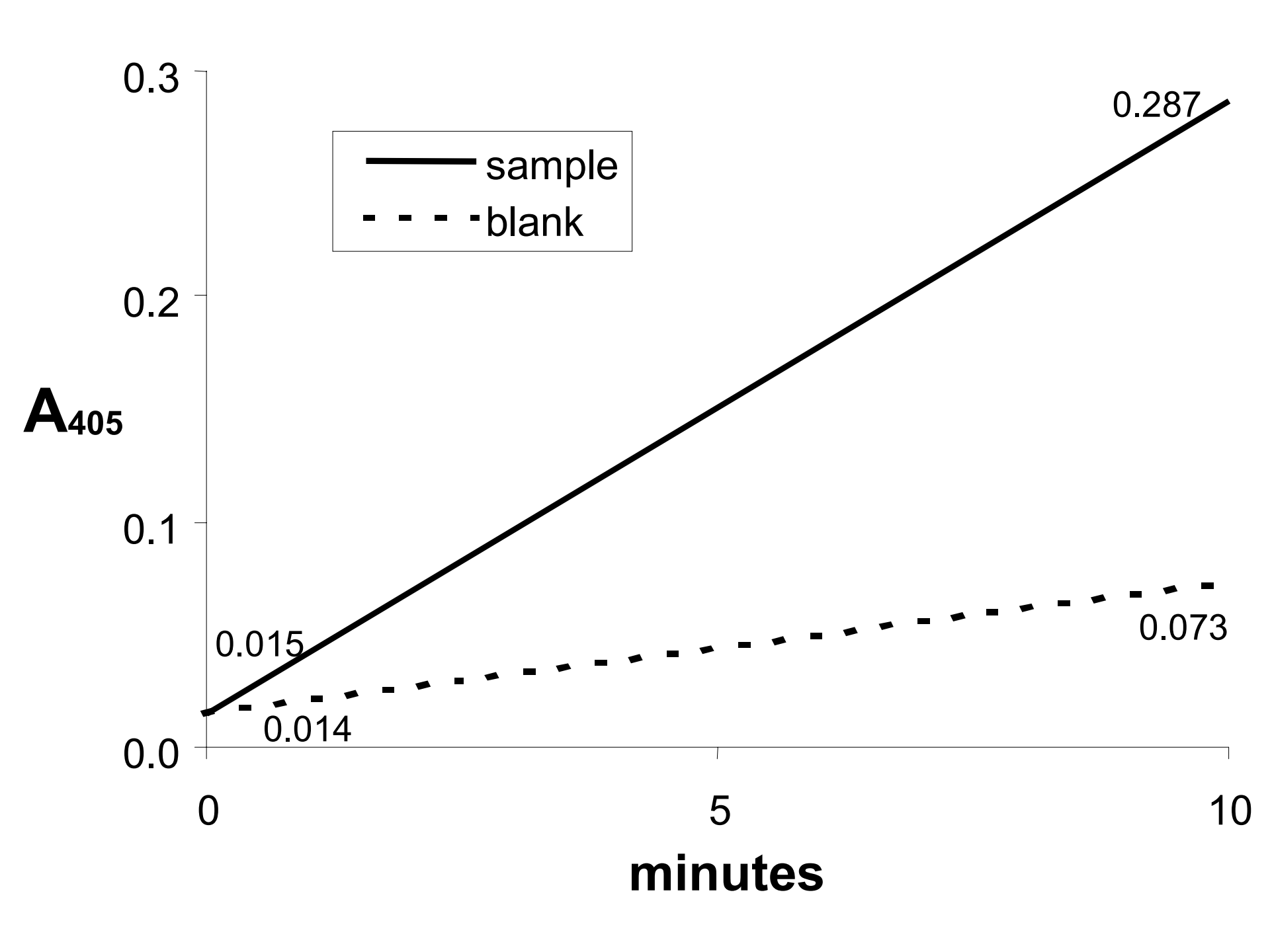
c = concentration
d = thickness (1 cm)
ε = extinction coefficient

Molar Extinction Coefficient

ε = A of 1 M of pure compound*
(liter/mole•cm)

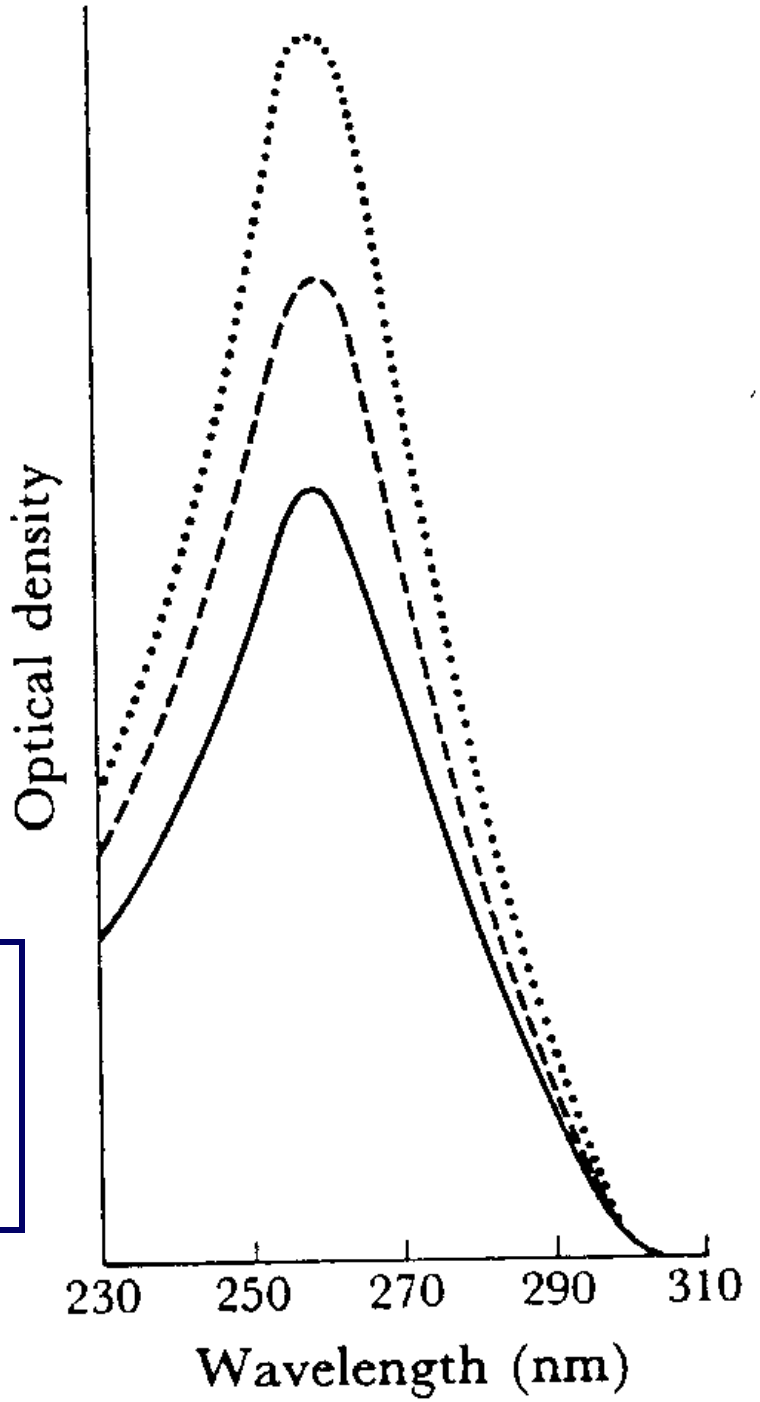
$E^{1\%}$ = A of 1% (w/v) solution*

*(under 'standard' conditions)


0.3
0.2
0.1
0.0
A405
sample
blank
0.287
0.015
0.014
0.073
0
5
10
minutes

Factors Affecting Absorption

- pH (ionization of chromophore)
- redox state
- polarity or solvent effects
- orientation effects



Hypochromism of Nucleic Acids

free nucleotides > single stranded > double stranded

Variations in Spectrophotometry

Spectrophotometry	Comment
Infrared (IR)	vibrational levels
Raman	
Optical Rotary Dispersion (ORD)	polarize light
Circular Dichroism (CD)	
Nuclear Magnetic Resonance (NMR)	magnetic moments
Electron Spin Resonance (ESR)