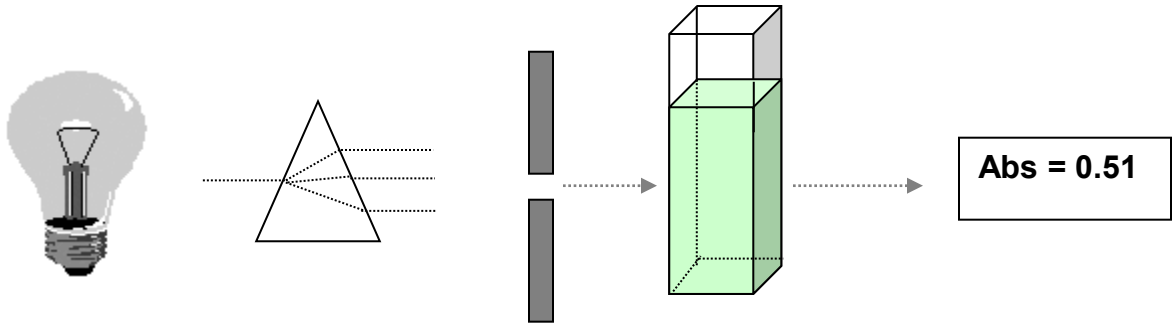
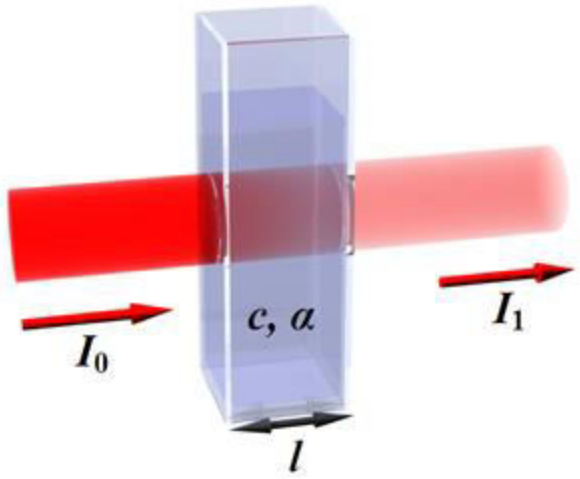
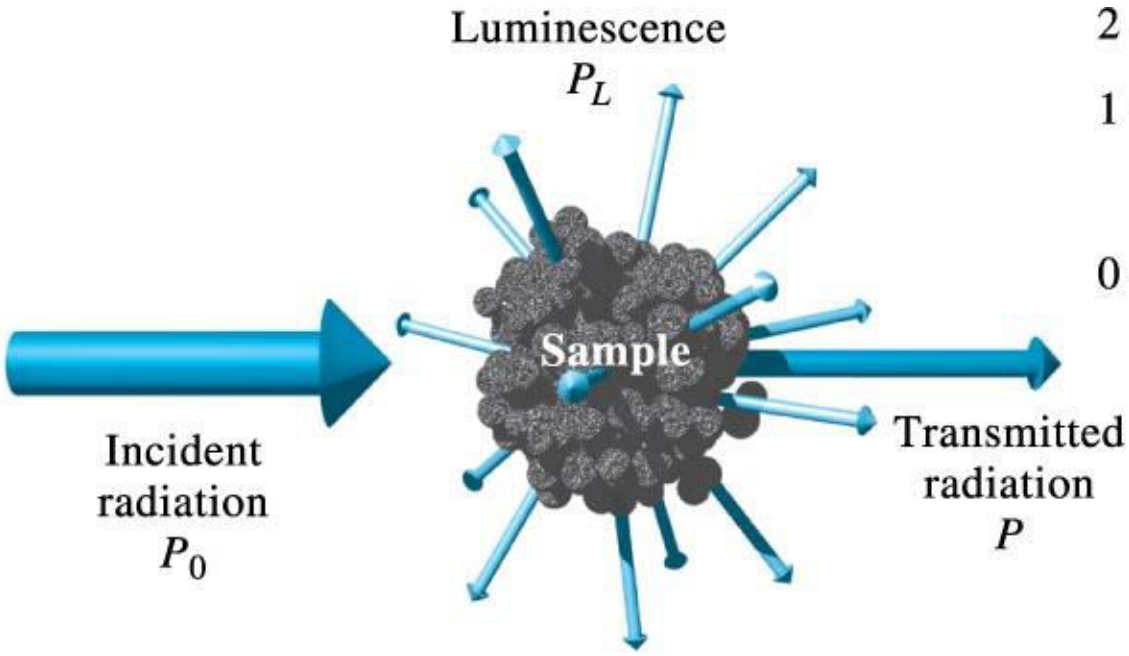
BEER- LAMBERTS LAW

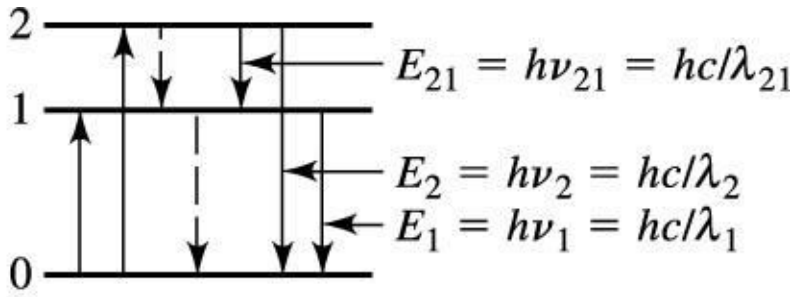
Spectrophotometry



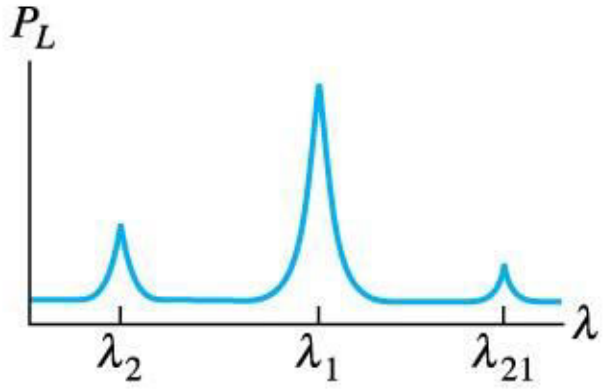




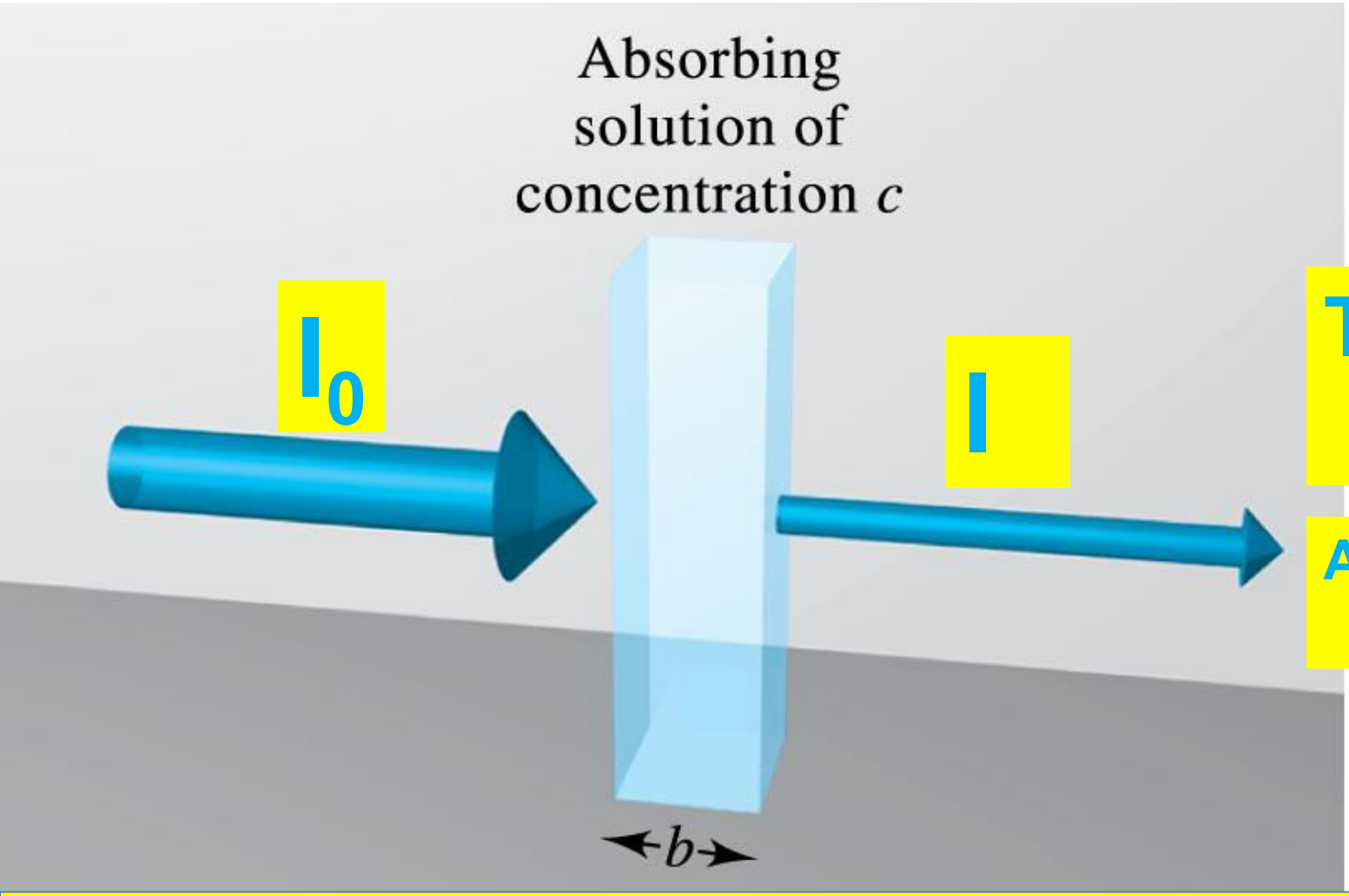
(a)



(b)



(c)



$T = I/I_0$

$A = \text{Log}_{10}(I_0/I$

Intensity of $I < I_0$

Lambert's law

- When a ray of monochromatic light passes throughan absorbing medium its intensity decreases exponentially as the length of the absorbing medium increases.

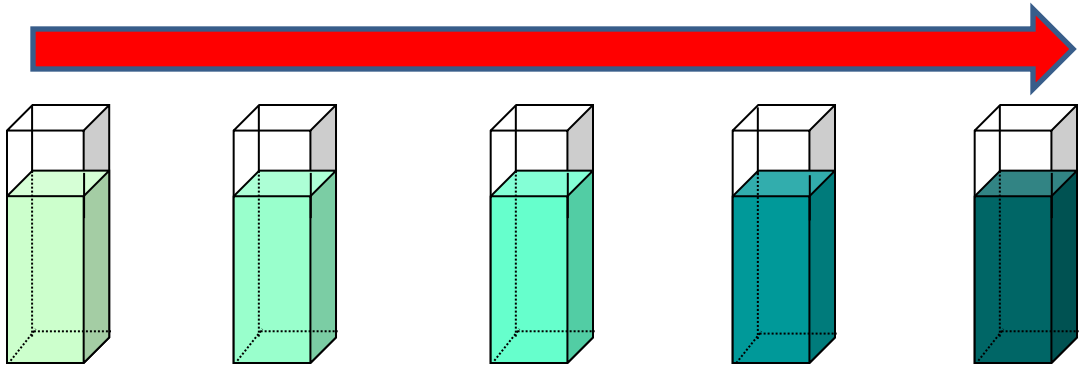
$$I = I_0 e^{-k_1 l}$$

Beer's law :

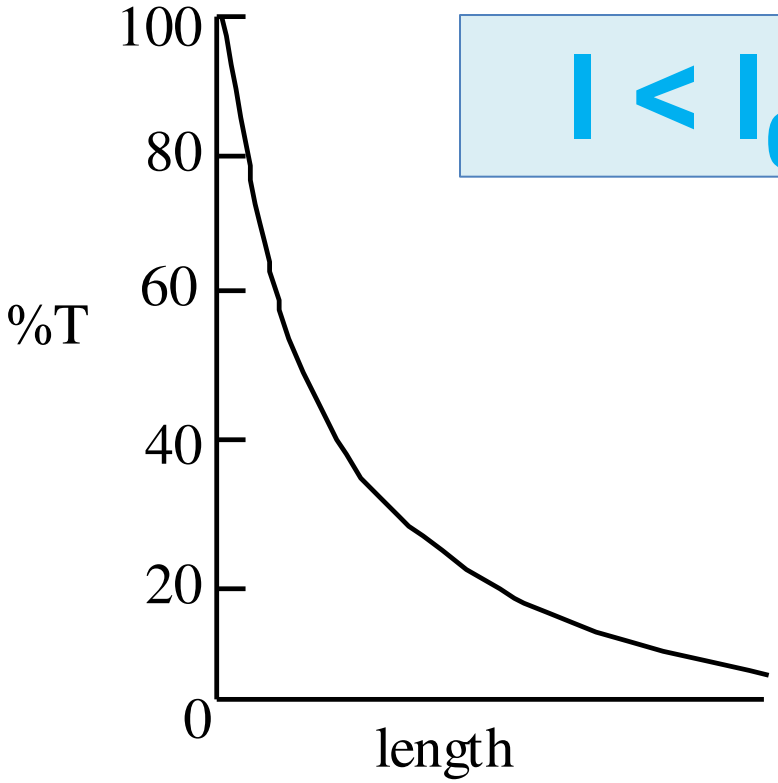
- When a monochromatic light passes through an absorbing medium its intensity decreases exponentially as the concentration of the absorbing medium increases.

$$I = I_o e^{-k_2 c}$$

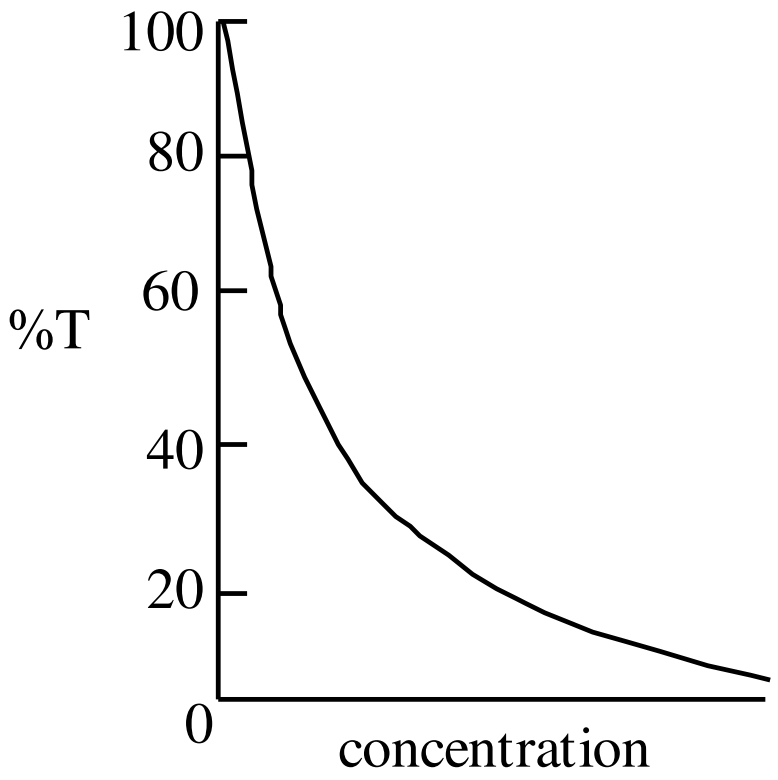
Concentration increases



Relationship between %Transmittance and light path length and concentration



$I < I_0$



Fix *C* vary *l*

Fix *l* vary *c*

1. The two Laws are combined into $I = I_0 e^{-k_3 cl}$

2. The ratio of intensities is also known as the transmittance (T) and is usually expressed as a %

$$i.e., \quad T = I/I_0 = e^{-k_3 cl}$$

Taking –ve Log_e on both sides of the equation

$$- Log_e (T) = - Log_e (I/I_0) = - Log_e e^{-k_3 cl}$$

$$Log_e (1/T) = Log_e (I_0/I) = - - k_3 cl \times (Log_e e)$$

$$Log_e (1/T) = Log_e (I_0/I) = k_3 cl \times (1)$$

$$Log_e (1/T) = Log_e (I_0/I) = k_3 cl$$

$$Log_{10} (1/T) = Log_{10} (I_0/I) = \underline{2.303 \times k_3} cl$$

Absorbance = εcl (The Beer-Lambert Law)

Beer-Lambert Law

Beers Law states that absorbance is proportional to concentration over a certain concentration range

$$A = \varepsilon cl$$

A = absorbance (Optical Density)

ε = molar extinction coefficient ($M^{-1}\,cm^{-1}$ or $mol^{-1}\,L\,cm^{-1}$)

Is an intrinsic value to a given compound can be used in identifying cpd

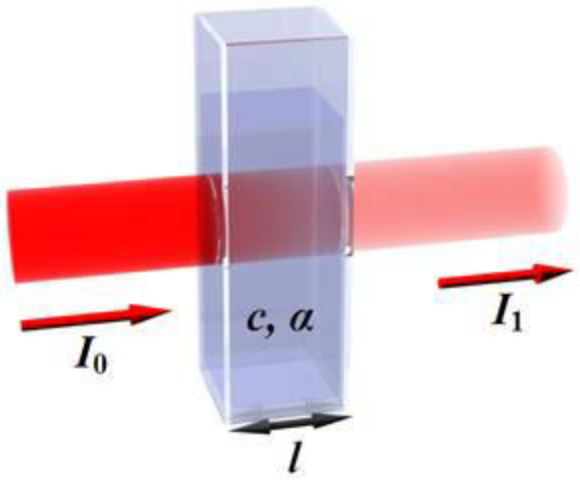
c = concentration (M or mol L^{-1})

l = path length (cm) (width of cuvette)

- **Beer-Lambert Law**

$$A = \log(I_O/I) = \varepsilon cl$$

- If you fix the Concentration the A α l

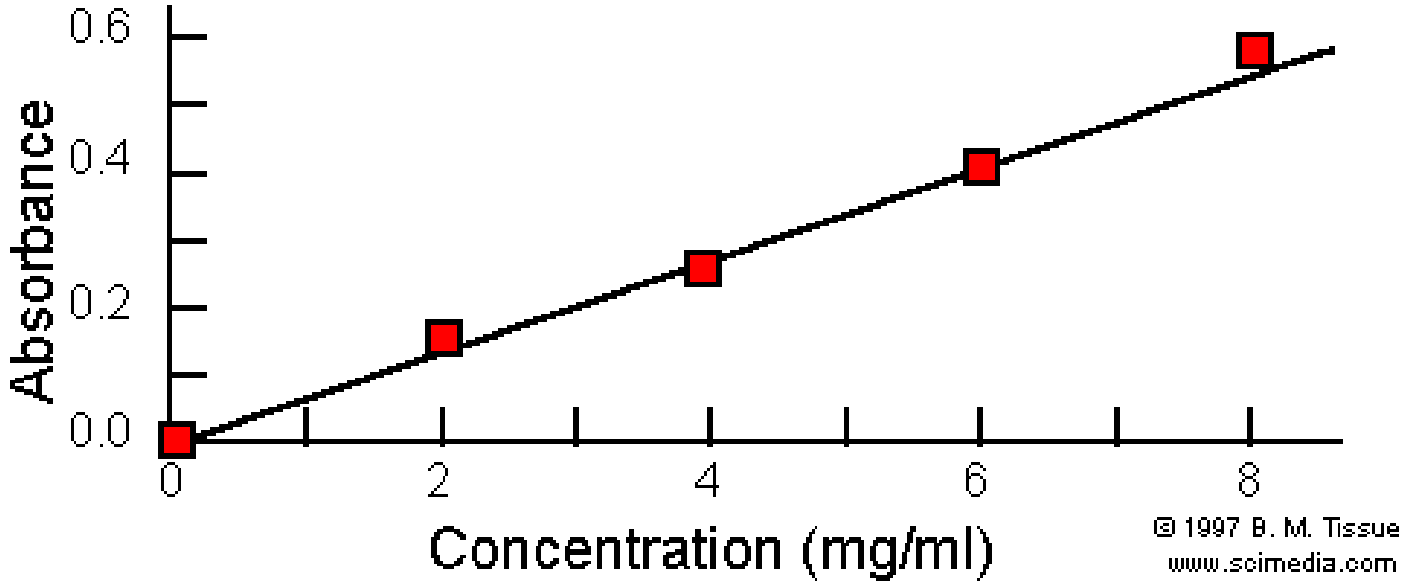


- If you fix the path length A α c

Widely used in biochemistry to determine concentrations of substances and in reaction rate determination

Absorbance

Beer's law - the linear relationship between absorbance and concentration of an absorbing species.

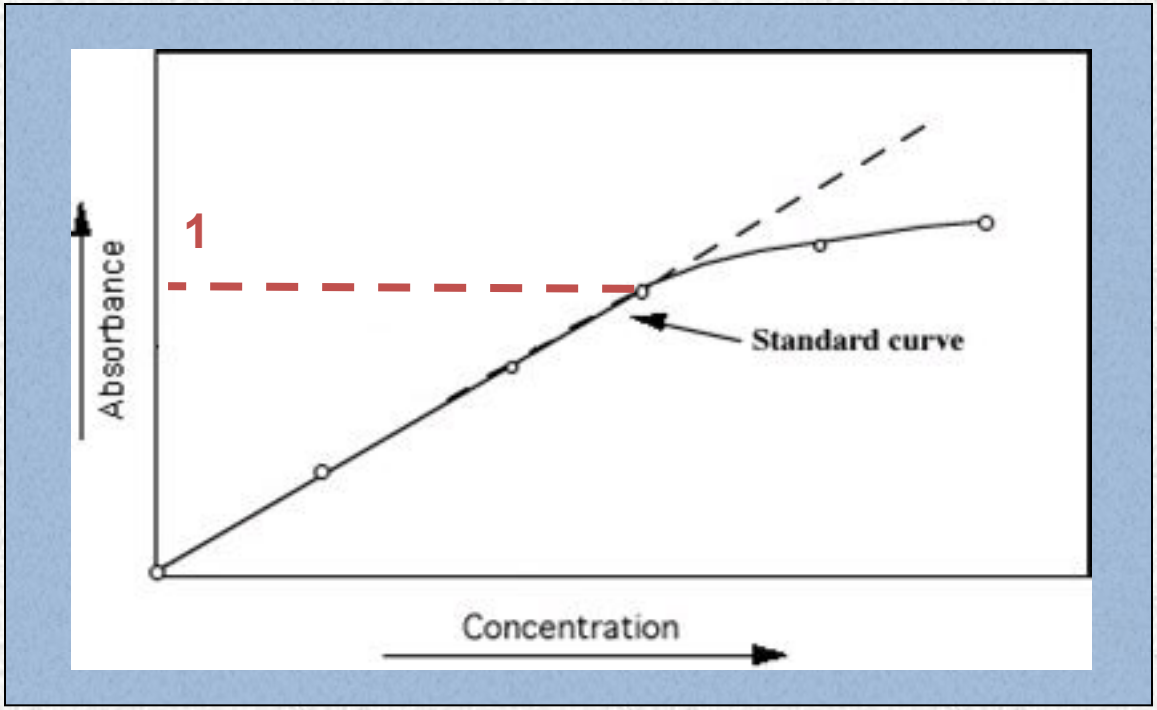


Produce **A Working Curve** by plotting the **Absorbance vs. the Concentration.**

From this we can determine the concentration of an **unknown** sample by **knowing the absorption**.

Beer-Lambert Law

- If your **unknown** has a higher concentration than **your highest standard**, you have to ASSUME that linearity still holds (**NOT GOOD** for quantitative analysis)



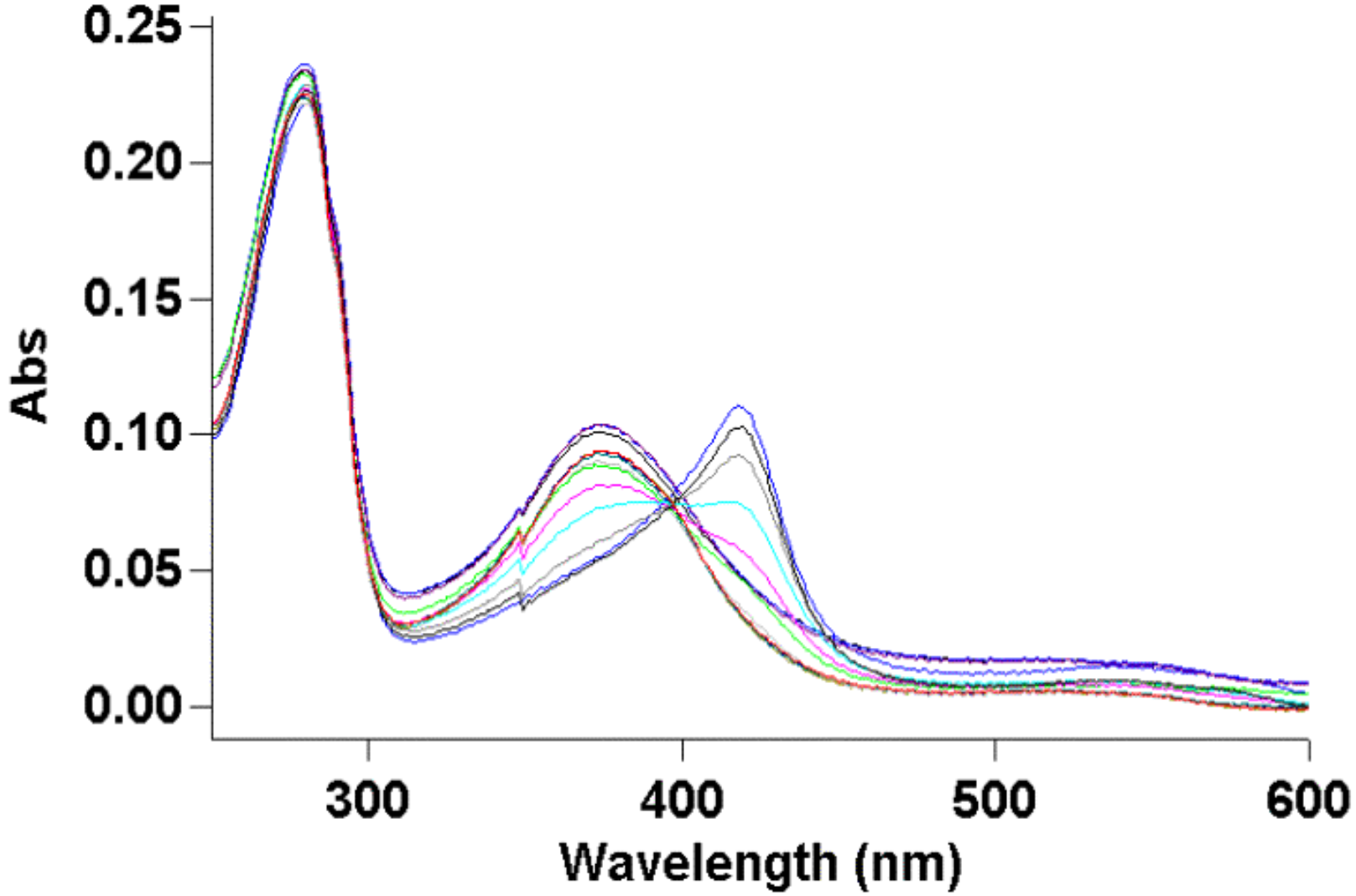
- **Unknowns should ideally fall within the standard range**

DILUTIONS ARE NECESSARY

The Standard Curve

- Although identifying a compound by spectro is a useful property, spectrophometry is used more **often to measure the concentration** of a compound.

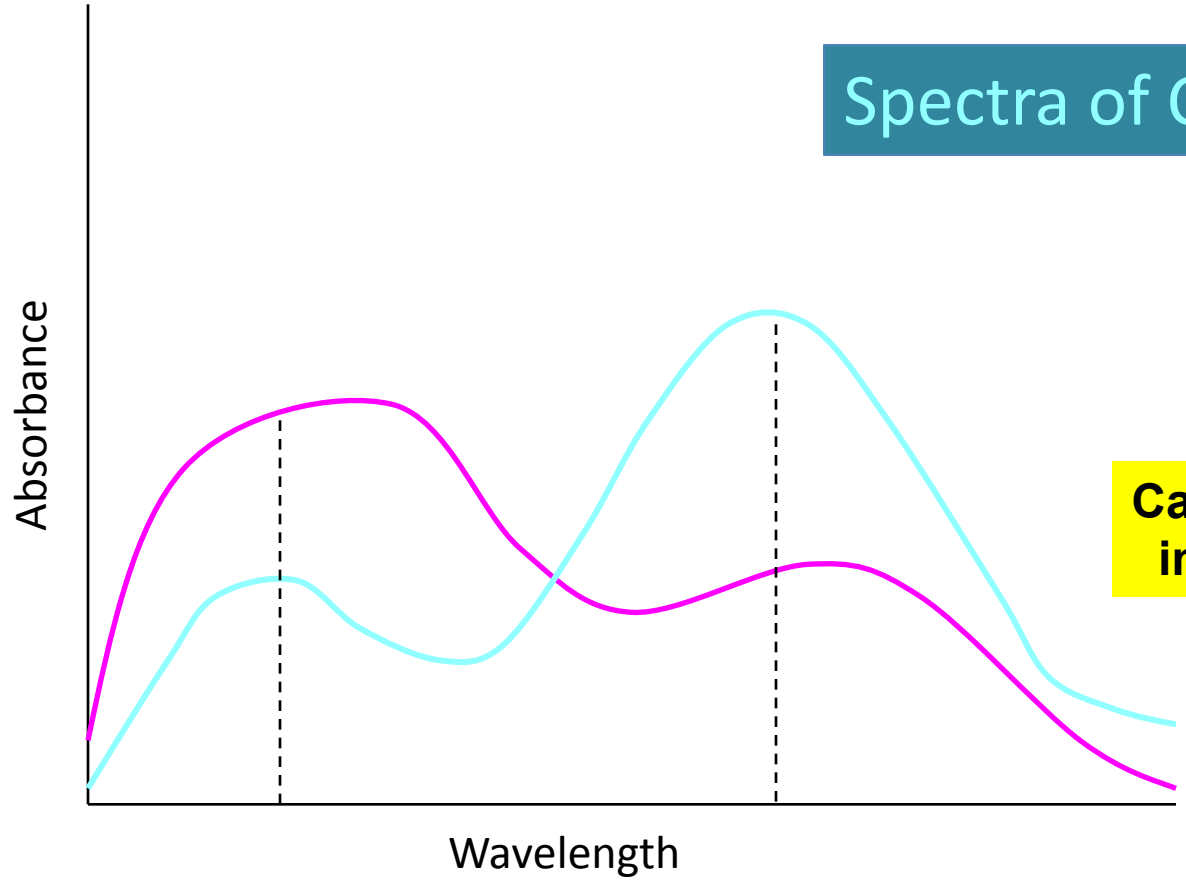
- If the compound of interest does not have its own intrinsic absorbance then a **coloured derivative** must be made by reacting it with reagents. Then a standard curve must be produced.


0.25
0.20
0.15
0.10
0.05
0.00
Abs
300
400
500
600
Wavelength (nm)

Quantification of Two Compounds

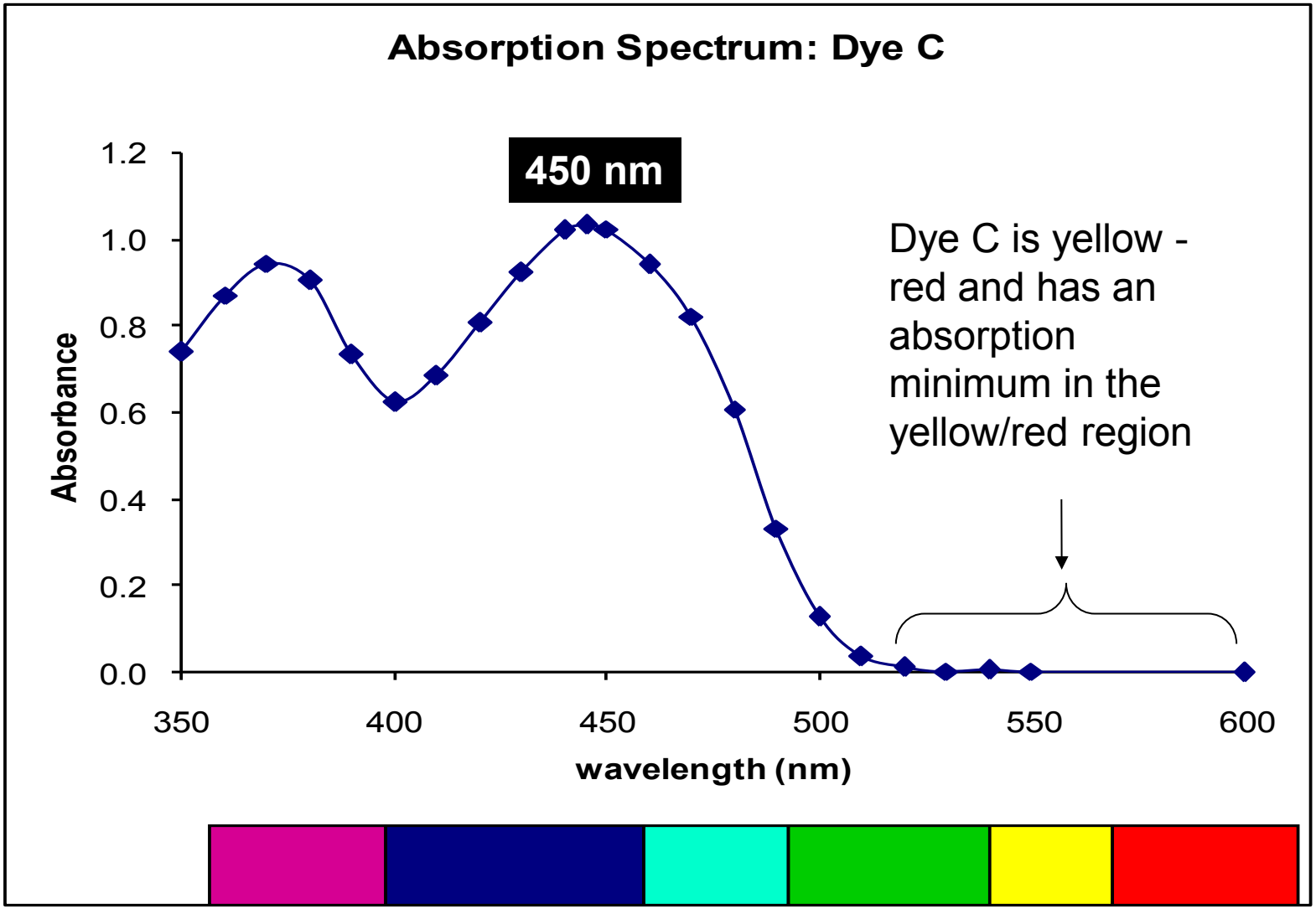
Spectra of Compound 1

Spectra of Compound 2



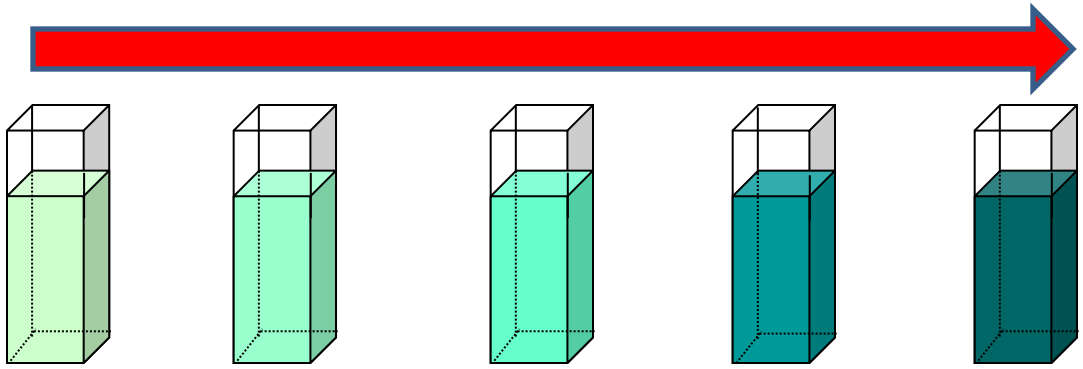
Can easily measure one in presence of 2

Dye C: Riboflavin



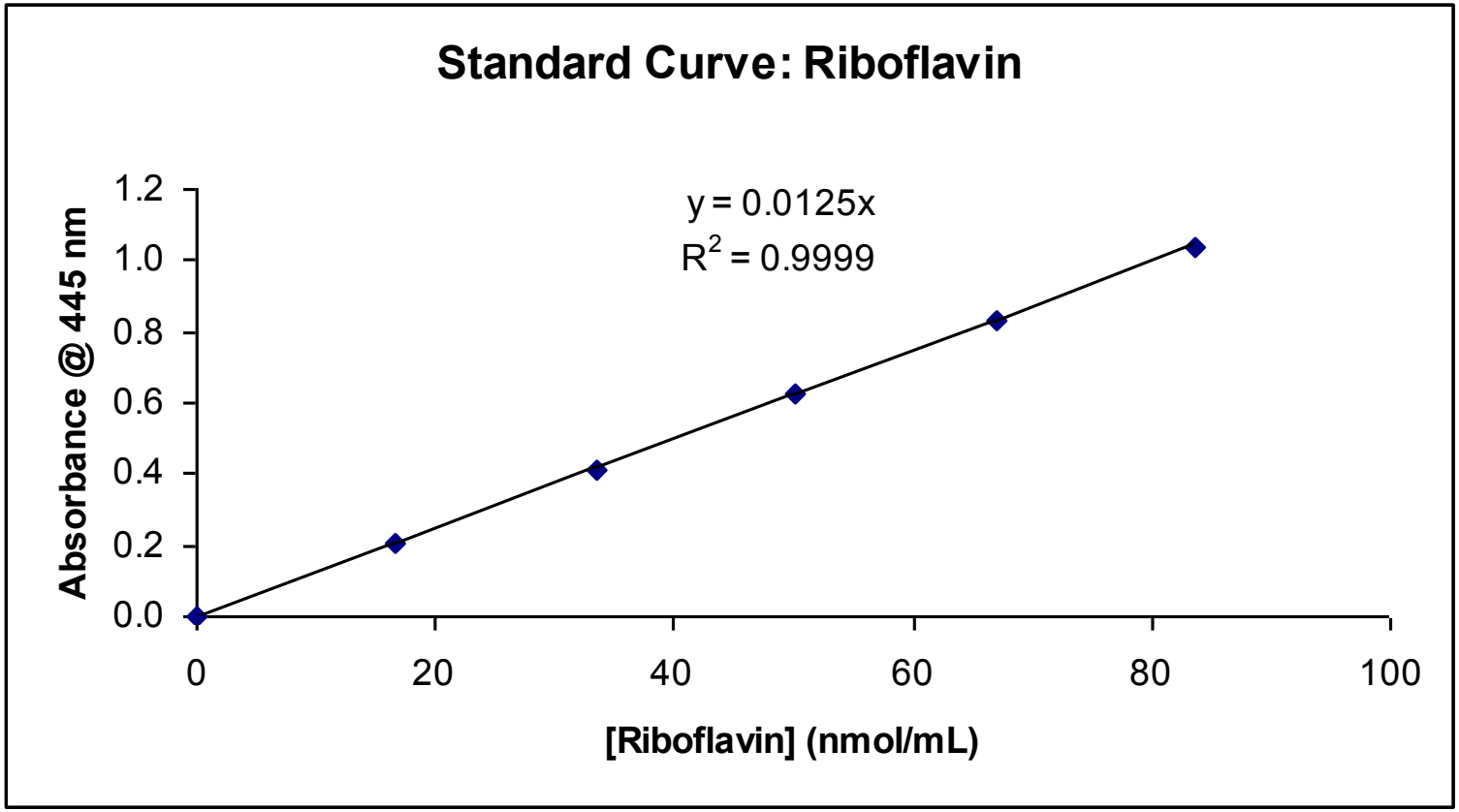
Can be used identifying a compound

Concentration increases



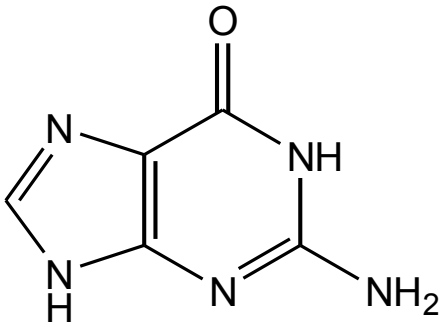
Used in preparation of a standard (reference curve) for determination of unknown concentration of samples

The standard curve

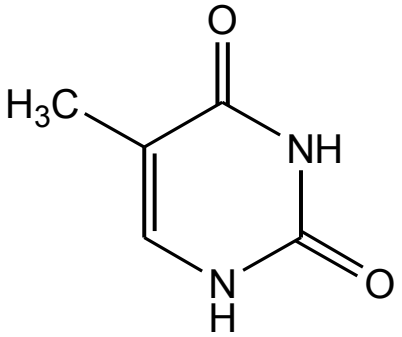


Common Absorbing Biochemicals

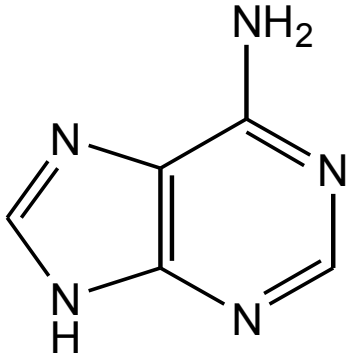
- The bases of nucleic acids



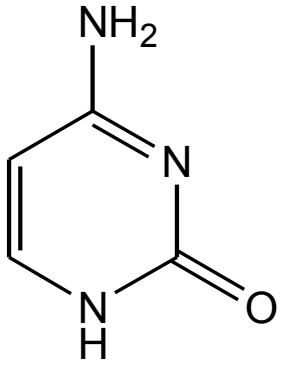
Guanine



Thymine



Adenine



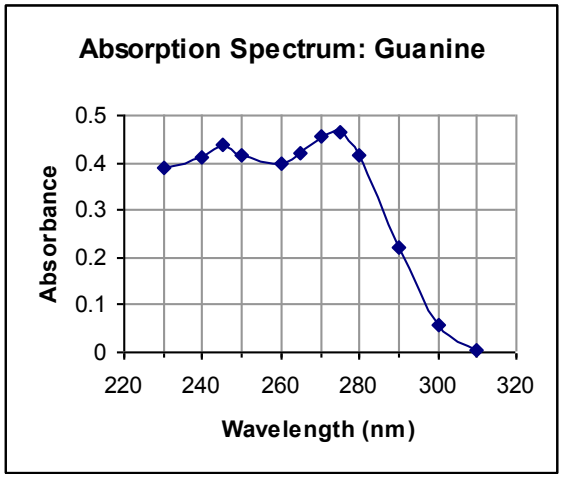
Cytosine

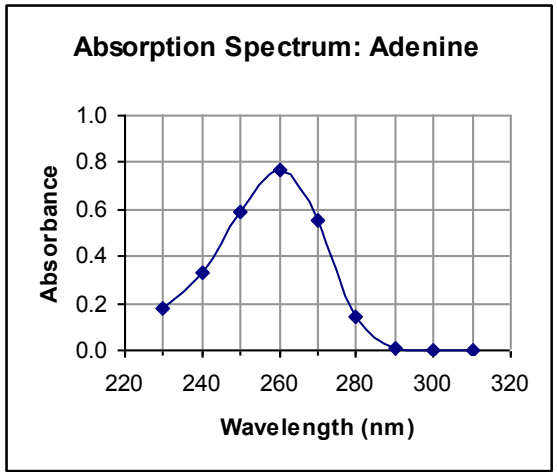
Nucleic Acid Absorption Properties

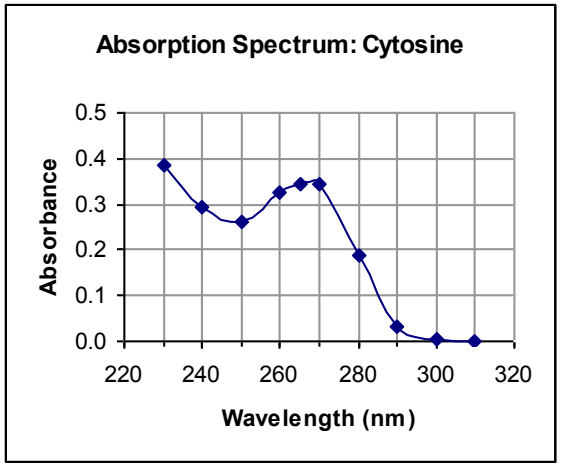
Base	**λmax (nm)**	**ε ($mM^{-1}cm^{-1}$)**
Guanine	275	8.0
Adenine	260	12.9
Cytosine	265	5.8
Thymine	258	8.0

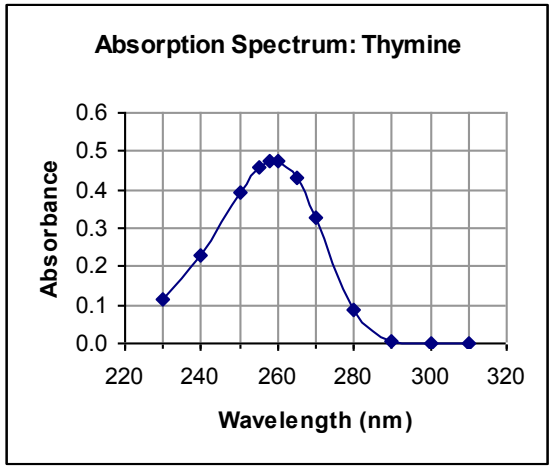
λmax Can be used in identifying compounds present in samples

Nucleic Acid Absorption Properties

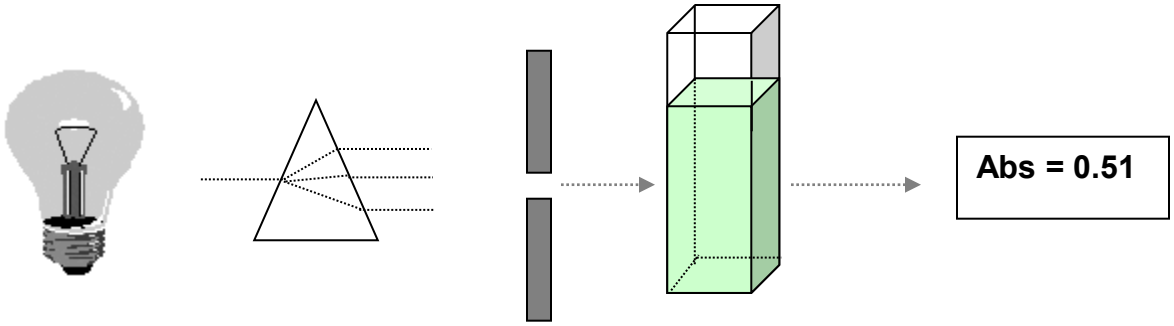




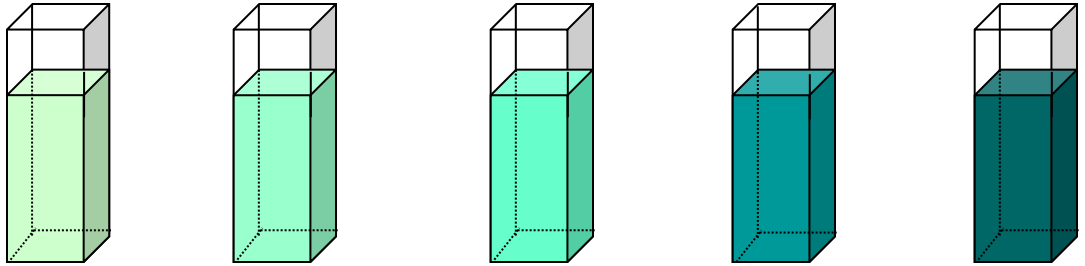




Spectrophotometry



Concentration increases



0.396 0.490 0.594 0.681 0.774

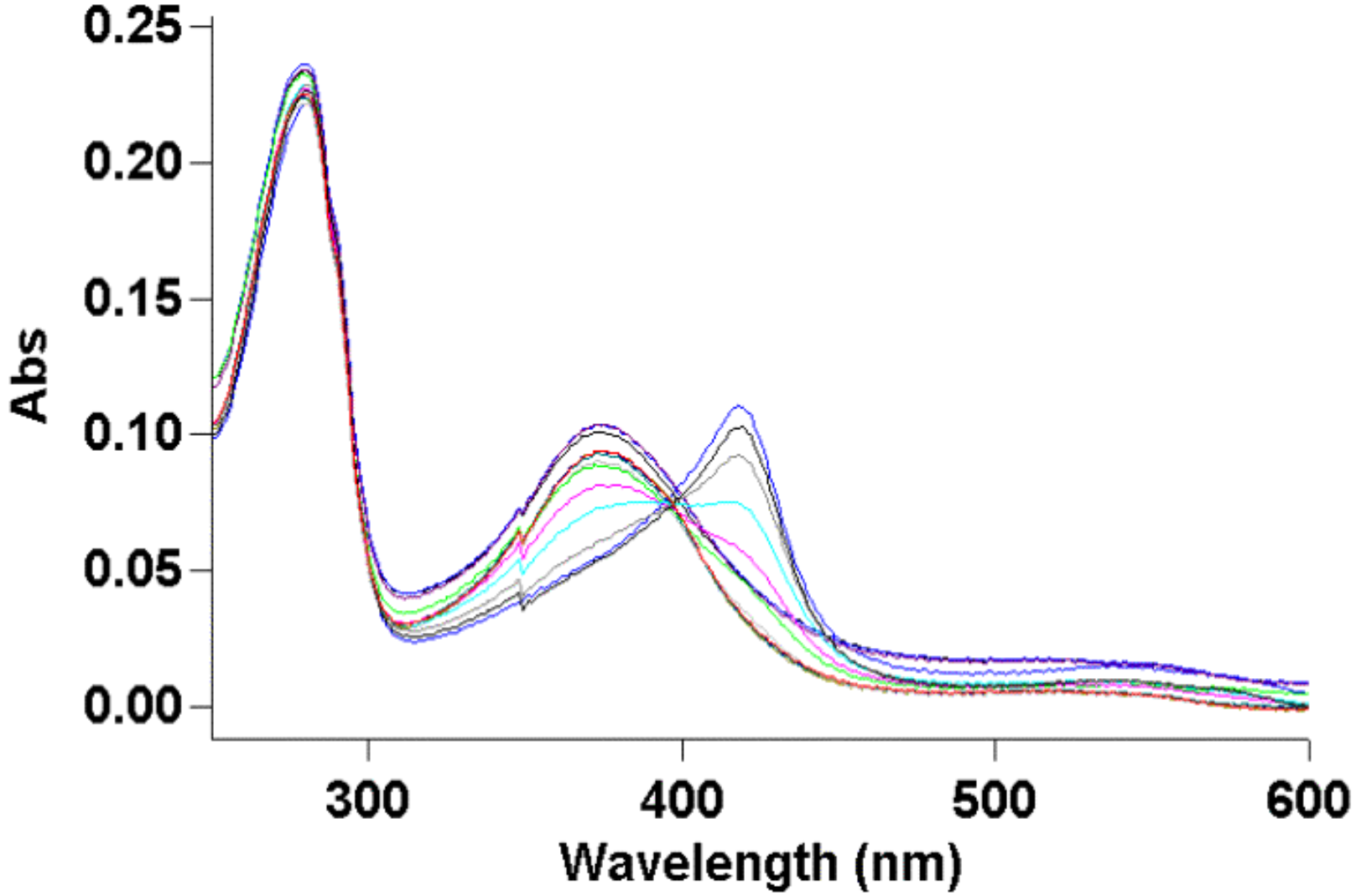
Absorption Intensity

- The absorption efficiency of an analyte is affected by:
  - The nature of the analyte
  - The number of available microstates
  - The solvent (sortof)
- The absorption efficiency of an analyte generally not affected by:
  - Other (low conc.) solutes
  - Temperature (within reason)****
  - Concentration *****
- This makes absorption spectroscopy one of the few bioanalytical methods where the signal intensity is directly proportional to the concentration

UV/Visible Spectroscopy: Instrumentation

- In absorption spectroscopy, we measure **ε** as a function of wavelength **λ**
- The instrument we use to do this is called a UV/visible Spectrophotometer

- The Major Components Are:
  - A light source
  - A monochromator
  - A Sample Compartment
  - A detector


0.25
0.20
0.15
0.10
0.05
0.00
Abs
300
400
500
600
Wavelength (nm)

$$\text{Log}_{10}\,(I_0/I) = A = \text{Log}_{10}\,(1/T)$$

$$A = \varepsilon cl$$

$$\varepsilon = A/cl$$

$$A = \varepsilon \text{ (if } c = 1 \text{ Molar, } l = 1 \text{ cm)}$$

$$\%T = I/I_0$$

$$T = 1/\text{Antilog (A)}$$