CHEMISTRY 5 (INSTRUMENTAL)

AGRICULTURAL BIOTECHNOLOGY, LEVEL 2

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CONTENTS

BEER'S LAW INTRODUCTION

- **Beer's law, also called Lambert-Beer law or Beer-Lambert law, in spectroscopy.**
- **Beer's law study the relation concerning the absorption of radiant energy by an absorbing medium.**
- **Beer's law was formulated by German mathematician and chemist *August Beer* in 1852.**
- **Beer's law states that the absorptive capacity of a dissolved substance is directly proportional to its concentration in a solution.**

BEER'S LAW DEFINITION

- **Beer's Law states that the concentration of a chemical solution is directly relative to its absorption of light.**
- **The principle is that a beam of light becomes weaker as it passes through a chemical solution, which means that the decrease of light occurs either as a result of distance through solution or increasing concentration.**

BEER'S LAW EQUATION

- **Beer's Law may be written simply as:**

$$A = \varepsilon bc$$

where A is Absorbance (no units, $A = \log_{10} P_0 / P$)

ε is Molar absorptivity with units of $L\ mol^{-1}\ cm^{-1}$ (formerly called the extinction coefficient)

b is Path length of the sample, usually expressed in cm

c is Concentration of the compound in solution, expressed in $mol\ L^{-1}$

Beer's Law

$$A = \varepsilon bc$$

Question: In the equation which part are constant and which part are variable?

Answer: ε and b are constant (under the correct condition)

A and c are variables

Beer's Law

Putting this all together we come up with the

Beer – Lambert Law:

For any particular wavelength,

$$A = \varepsilon b C$$

Absorbance

Molar absorptivity → L/(mol cm)

Path length → cm

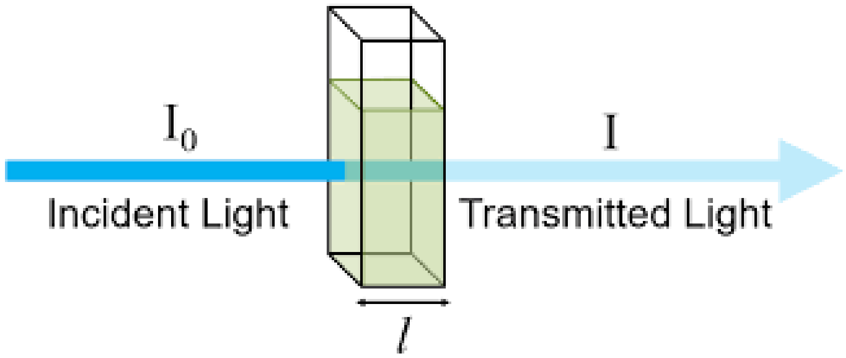
Concentration

Mol/L

Calculating the absorbance of a sample using the equation depends on two assumptions:

1. **The absorbance is directly relative to the path length of the sample (the width of the cuvette).**
2. **The absorbance is directly relative to the concentration of the sample.**

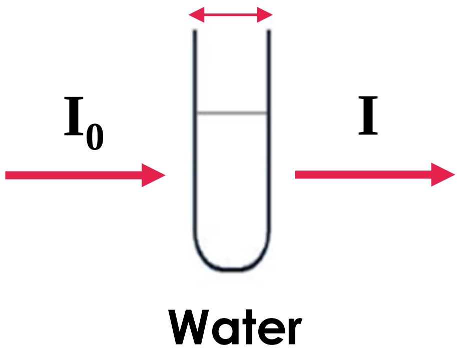
PRECENT TRANSMITTANCE, %T



- **Another measurement that is related to absorbance is %Transmittance.**
- **Precent Transmittance, %T, is a measure of how much light passes through a sample.**

- **Question: If light passes through water, what will be the %T?**

$$T(\%) = 100\frac{I}{I_0}$$



Answer: All the light passes through, so the transmittance is 100%

RELATIONSHIP OF (A) AND (%T)

- **Absorbance and % transmittance are mathematically related through the following equation:**

$$A = -\text{Log}\ \frac{\%T}{100}$$

Question: Calculate the Absorbance of a sample if %T is 56.5%.

Answer: A= - Log (0.565)= 0.248

Notice that the higher the %T the lower the A, but the relationship is not linear

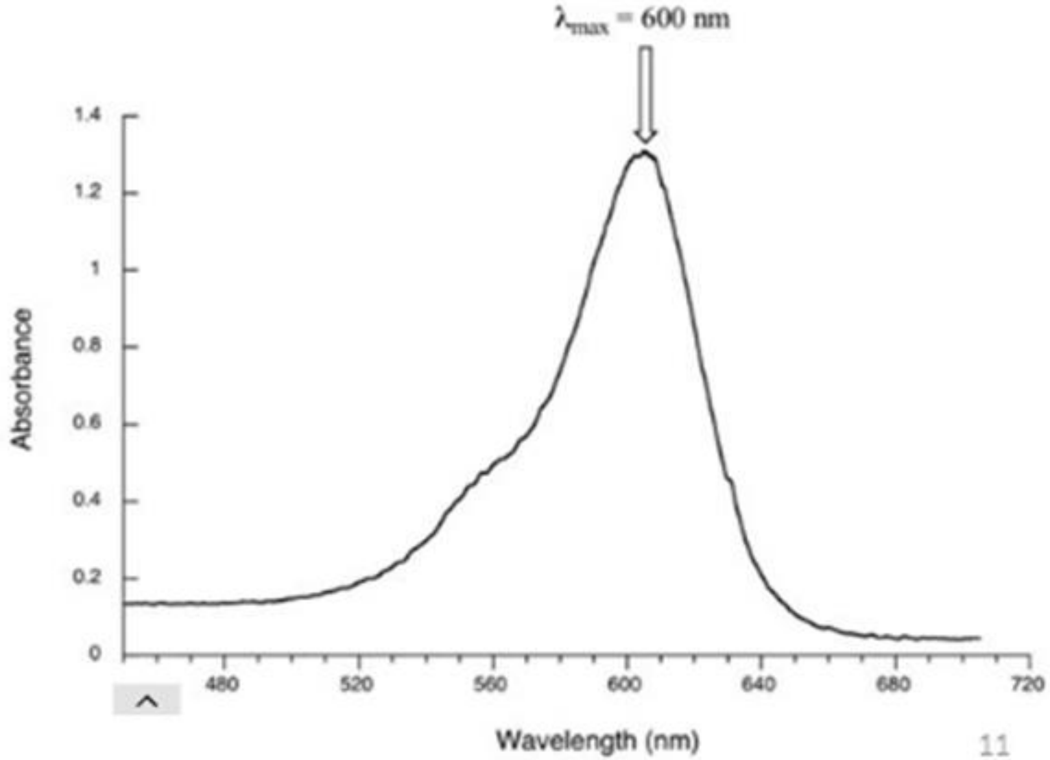
λ_{Max}

The wavelength of maximum absorbance is called λ_{Max}

For best accuracy, when measuring the absorbance of several solutions, it is best to measure as close to λ_{max} as possible.

Question: What would happen if we use a wrong wavelength?

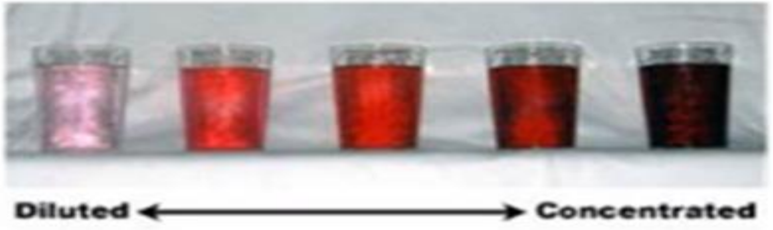
Answer: If the wrong wavelength is selected there will be little to no absorbance



RELATIONSHIP OF CONCENTRATION & ABSORBANCE

Which of these two solutions contains a higher concentration of red dye?

A B



Did you answer solution A?

If so, you are correct!

A higher concentration leads to a darker color.

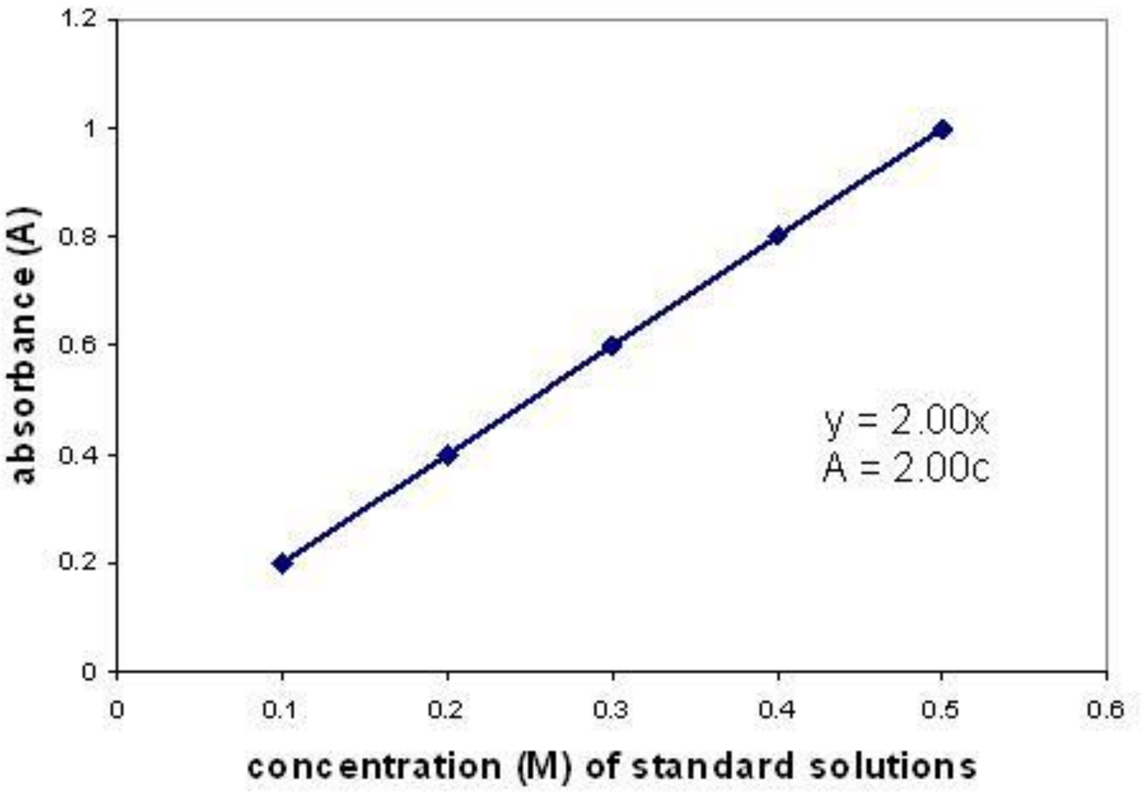
Concentration and Absorbance

Which of these two solutions will have a higher absorbance at λ_{max}?

Did you answer solution A again?

That's right! The higher the concentration, the greater the absorbance.

HOW TO USE BEER'S LAW



Source: https://en.wikipedia.org/wiki/File:Beer%27s_Law_Plot.jpg

- **While many modern instruments perform Beer's Law calculations by simply comparing a blank cuvette with a sample, it's easy to prepare a graph using standard solutions to determine the concentration of a specimen.**

- **The graphing method assumes a straight-line relationship between absorbance and concentration, which is valid for dilute solutions.**

Live Exercise : https://phet.colorado.edu/sims/html/beers-law-lab/latest/beers-law-lab_en.html

BEER'S LAW EXERCISE

- **Guanosine is known to have a maximum absorbance value of 275 nm. Its molar absorptivity is 8400 $M^{-1}cm^{-1}$. The width of the cuvette is 1 cm. A spectrophotometer finds A = 0.70. What is the concentration of the sample?**

- To solve the problem, use Beer's Law:

$$A = \varepsilon bc$$

$$0.70 = (8400\ M^{-1}cm^{-1})(1\ cm)(c)$$

- Divide both sides of the equation by [(8400 M^{-1} cm^{-1})(1 cm)]

Guanosine c = 8.33 x 10^{-5} mol/L

BEER'S LAW EXERCISE

- **A sample is known to have a maximum absorbance value of 275 nm. Its molar absorptivity is 8400 $M^{-1}cm^{-1}$. The width of the cuvette is 0.5 cm. A spectrophotometer finds A = 0.70. What is the concentration of the sample?**
- To solve the problem, use Beer's Law:

$$A = \varepsilon bc$$

$$0.70 = (8400\ M^{-1}cm^{-1})(0.5\ cm)(c)$$

- Divide both sides of the equation by [(8400 M^{-1} cm^{-1})(0.5 cm)]

$$c = 1.67 x\ 10^{-4}\ mol/L$$

BEER'S LAW EXERCISE

- **A sample is known to have a maximum absorbance value of 275 nm. Its molar absorptivity is 8400 $M^{-1}cm^{-1}$. The width of the cuvette is 1.0 cm. the concentration of the sample is 8.33 x 10^{-5} mol L^{-1} What is the Absorbance of the sample?**
- To solve the problem, use Beer's Law:

$$A = \varepsilon bc$$

$$A = (8400\ M^{-1}cm^{-1})(1.0\ cm)(8.33 \times 10^{-5})$$

$$A = 0.7$$

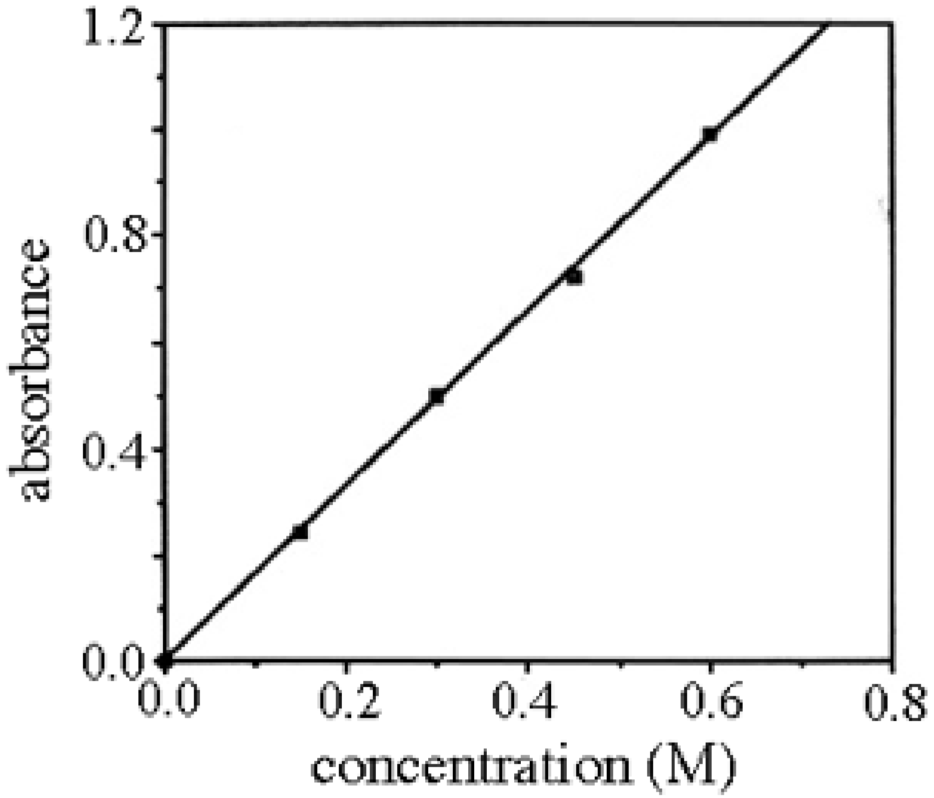
BEER'S LAW EXERCISE

A series of standard solutions containing a red dye was made by diluting a stock solution and then measuring the percent transmittance of each solution at 505 nm (greenish blue). This wavelength was selected by examining its absorption spectrum. If the solution looks red, it is absorbing red's complementary color of light, which is greenish blue. The results, after conversion to absorbance, are shown below.

An absorbance of 0.39 was also determined at 505 nm for a solution with an unknown concentration of the red dye.

solution	concentration	absorbance
blank	0.00 M	0.00
standard #1	0.15 M	0.24
standard #2	0.30 M	0.50
standard #3	0.45 M	0.72
standard #4	0.60 M	0.99
sample	**???? M**	0.39

The equation for the best fit line through the points is



y = 1.64 x - 0.002

Substituting the meaning of x and y into the equation tells us that:

absorbance = 1.64 * concentration - 0.002

To solve for the concentration of the dye in the unknown simply plug in the absorbance value:

0.39 = 1.64 * concentration - 0.002

and solve for the concentration. In this case it is equal to **0.24 M**.

IMPORTANCE OF BEER'S LAW

- Beer's Law is especially important in the fields of **chemistry**, **physics**, and **meteorology**. Beer's Law is used in chemistry to measure the concentration of chemical solutions, to analyze oxidation, and to measure polymer degradation. The law also describes the attenuation of radiation through the Earth's atmosphere. While normally applied to light, the law also helps scientists understand the attenuation of particle beams, such as neutrons. In theoretical physics, the Beer-Lambert Law is a solution to the Bhatnagar-Gross-Krook (BKG) operator, which is used in the Boltzmann equation for computational fluid dynamics.

SOURCES

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THANK YOU