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Generating and Using a Calibration Graph

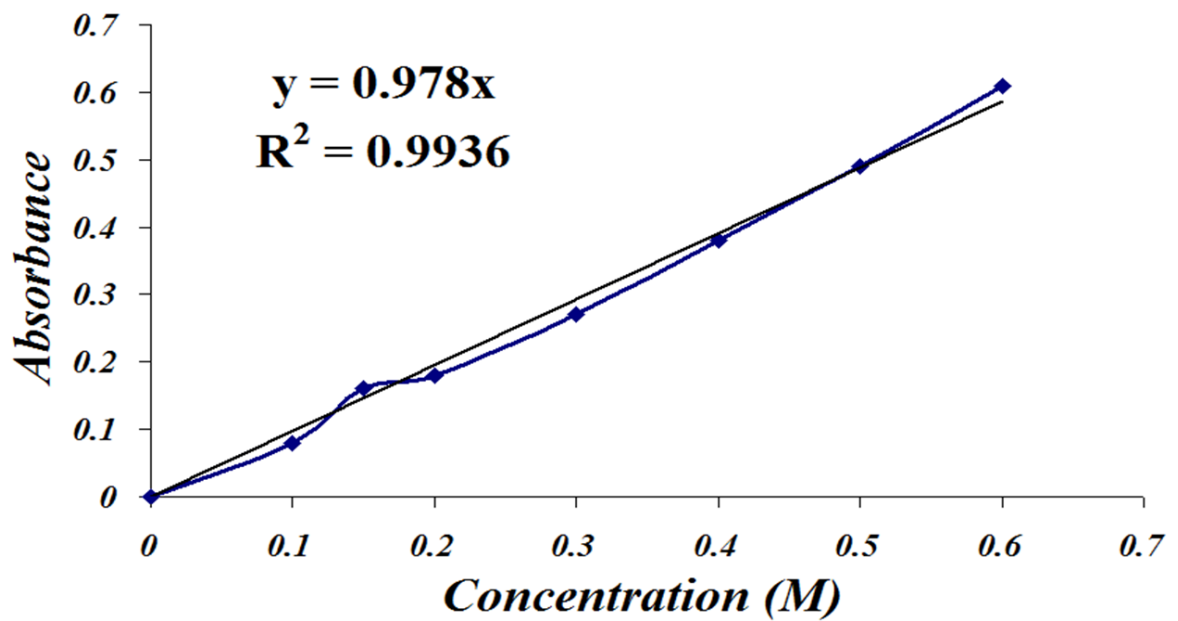
Using your Calibration Graph!

Now for the fun part! Using the calibration plot that YOU made from the data two pages ago. We are going to determine the concentration of an unknown solution. Make sure you have your plot ready, because here we go!

Here's a typical problem. You take 3mL of your unknown sample and 7mL water and mix them together. The diluted sample gives an absorbance of 0.432. What is the concentration of the initial unknown?

Where do you begin?! Well, you have your calibration graph, and it **SHOULD** look something like this, all properly labeled.

Calibration Plot of Solution at 410nm



1). You have an absorbance, and you have a straight line equation that relates absorbance to concentration. This is the line of best fit through your data.

$$y = 0.6717x \Rightarrow Abs = 0.6717(\text{concentration})$$

$$Abs = 0.432$$

$$0.432 = 0.6717(\text{concentration})$$

$$\text{Concentration} = 0.643M$$



2). Now this is the absorbance of your **DILUTED** solution. But what was the concentration of your **ORIGINAL** solution?

Remember you diluted it once, so you can use the Dilution Equation

$$M_1V_1 = M_2V_2$$

Your diluted sample was 0.643M = M_1

The volume of that solution was 3mL Unknown and 7mL Water

$$3\text{mL} + 7\text{mL} = 10\text{mL} = V_1$$

The initial volume of your Unknown was 3mL = V_2

$$\left(0.643\text{M}\right) * \left(10\text{mL}\right) = M_2 \left(3\text{mL}\right)$$

$$\left(6.43\text{M} * \text{mL}\right) = M_2 \left(3\text{mL}\right)$$

$$\frac{(6.43\text{M} * \text{mL})}{(3\text{mL})} = \frac{M_2(3\text{mL})}{(3\text{mL})} = 2.14\text{M} = M_2$$

The original unknown concentration was 2.14M

Common Errors In Calibration Plots

- Spectrophotometer is not calibrated
- Abs readings are incorrect
- Diluted samples are prepared incorrectly or contaminated
- Inappropriate wavelength chosen for calibration graph
- The calibration line is not a "best fit" line

