Experiment 7: Analysis of Phosphate in Colas

For this lab you are going to play the role of a food chemist analyzing phosphate concentrations in colas. You will be working in groups of 4.

In experiment 3 you used the spectrophotometer to measure the absorbance of differently colored solutions. You might remember that when you compared two solutions of the same dye, one concentrated and one more dilute, the absorbance of the concentrated solution was higher (i.e. it absorbed more light) than that of the diluted solution.

Beer's Law tells us that the absorbance of a solution is related to the concentration:

$$A = \varepsilon bc$$

In this equation:
A is the measured absorbance.
ε is the molar extinction coefficient, a value that is constant for a particular analyte at a particular wavelength. (As you might remember from experiment 3, different substances absorb differently at different wavelengths.)
b refers to the path length—that is, how far the light travels through the solution. The farther the light has to travel through a solution, the more light gets absorbed. (Think about going scuba diving. If you are close to the surface, there is a lot of light present. But if you dive down very far, very little sunlight penetrates to where you are.)
c is the concentration of the solution.

The molar extinction coefficient ε is always a constant. When using a spectrophotometer, you will be using the same size of cuvette for all of your samples so the path length will also be a constant. Thus, you can think of Beers' Law this way:

$$\text{Absorbance} = (\text{constant}) \times \text{concentration}$$

This is the equation for a line! Knowing that the relationship is linear, you can use solutions of known concentration to make a **calibration curve** to determine the concentration of an unknown. This is how it works:

1. Make 4 or 5 solutions of known concentration. The lowest concentration should be below the concentration of your most dilute unknown and the highest concentration should be above the concentration of the most concentrated unknown. The remaining 2-3 points should lie in between those two concentrations. They don't need to be evenly spaced, but try to spread the concentrations out as much as possible.
2. Measure the absorbance of each of your known solutions. Then make a graph of absorbance (y-axis) vs. concentration (x-axis) like the one shown below. After you graph the raw data, do a linear fit in Excel and show both the equation for the line and the R^2 value. (The latter is a measure of quality: the closer the value is to 1, the closer to linear your line is.)
3. Use the graph to calculate the concentration of your unknown(s). Use the equation from your linear fit in conjunction with the measured absorbance of your unknown to calculate its concentration.

Week 1

Pre-lab:

Read the section above on Beer's Law as well as the instructions for week 1.

Work out a detailed scheme for creating a calibration curve. You will want five solutions with concentrations between 10^{-3} and 10^{-4} M and will be starting with a 0.1 M phosphate stock solution.

Hints for creating your procedure:

- You may want to dilute the 0.1 M solution once and then use that diluted solution to make the solutions for your calibration curve. (This practice is called "serial dilution.")

- You want to know the concentrations of your calibration solutions as precisely as possible. For this to be true, you must be able to measure both the pre-dilution volume and the post-dilution volume very precisely. What glassware should you use to facilitate this?

This week you are going to prepare the colas for next week's analysis and learn how to make a good calibration curve.

As you might remember from the experiment 3, it's important to get rid of any bubbles in the cuvette before taking a spectrum. This is highly problematic when measuring a carbonated drink such as a cola. You are going to de-carbonate the colas by heating each one at a gentle simmer (NOT to boiling) throughout the lab period.

While the colas are degassing, you are going to make a practice calibration curve (or two) using a stock phosphate solution of known concentration. Phosphate itself is colorless and therefore cannot be measured directly by spectrophotometry. However, when combined with ammonium vanadomolybdate (AVM), it forms a yellow complex whose absorbance is easily measured. When using this method, combine one part AVM solution to two parts of the solution you want to measure.

In general, this method for measuring phosphate works best between the concentrations of 10^{-3} and 10^{-4} M. At concentrations lower than that the absorbance is hard to read; at concentrations higher than that, a precipitate starts to form.

Choose one of your solutions and run a complete absorbance spectrum using your spectrophotometer; select a wavelength where you get very high absorbance for running all subsequent absorbance measurements. Measure the absorbance of your five calibration solutions at that wavelength and graph the data as mentioned in the introduction.

If your graph has a poor R^2 value (<0.97), consider what you can do to improve the linearity of your calibration curve. The graph itself is based on two measurements, absorbance and concentration. Assuming your technique is good, there's not much you can do about the precision of your absorbance measurements. You are more likely to improve the R^2 value by increasing the precision of your concentrations. (If you're not sure how to do this, talk to your professor or SIT.)

Week 2

Pre-lab:

Based on your results from week 1, work out a detailed scheme for creating a very precise calibration curve that you will use to calculate the concentrations of phosphate in your colas. Once again, you will want five solutions with concentrations between 10^{-3} and 10^{-4} M and will be starting with a 0.1 M phosphate stock solution.

Use your calibration curve from week 1 to set up a sample calculation for phosphate in cola. Assume that (a) you had to dilute the cola by a factor of 3; and (b) the absorbance of the diluted cola was 0.2500.

Today you are going to measure the phosphate levels in the colas you degassed last week.

First you will need to make another calibration curve following the procedure you developed last week. Why not just use the one you made last week? Because absorbance measurements may depend on a variety of factors (for example, how warm the machine is) that can change from day to day.

Once you have a good calibration curve, measure the absorbance of each cola. Remember that the concentration of your unknown should lie between the top and bottom concentrations of your calibration curve. If the concentration is higher than the points on your calibration curve, you will need to dilute the cola by an appropriate amount. (If you do this you must dilute precisely so you can go back and calculate the original concentration of phosphate in the cola.) Repeat for each cola in turn.

Lab Write-up

For this lab you are going to do a full lab write-up.

Introduction
Talk about why it is useful to know the phosphate content of a cola.
Discuss Beers' Law and how it can be used to determine the concentration of an unknown solution.
Write a brief statement that explains your goal for this lab.

Procedure
Tell what you did. Be clear enough that your professor or SIT could duplicate your work. Remember that procedure sections are always written in the past tense and passive voice. For example: "The solutions were mixed with a stirring rod." (Don't say, "we mixed the solutions…")

Results

Show all of the calibration plots that you made throughout the course of the experiment.
For the last calibration plot, be sure to show the equation for the linear fit as well as the R^2 value.
Make a table that shows the name of each cola, the amount you diluted it, and the absorbance you measured for that cola. Also show the phosphate concentration that you calculated.

Discussion

Write a paragraph analyzing the errors in your experiment. What errors do (or might) exist? How do you know of their existence, or are you just speculating? How could you minimize this error in future experiments?

Write a paragraph that summarizes the phosphate levels you measured and explain what these results mean to consumers.