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Spectroscopy Lab
Determining the Concentration of an Unknown Species

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Prelab Questions:

1) What is Beer's Law?

2) What is a serial dilution?

3) How would you prepare solutions of concentration 0.50M, 0.40M, 0.30M, and 0.20M from a 1.00 M stock solution?

4) If a solution is green what color light does it absorb? What color light does it transmit?
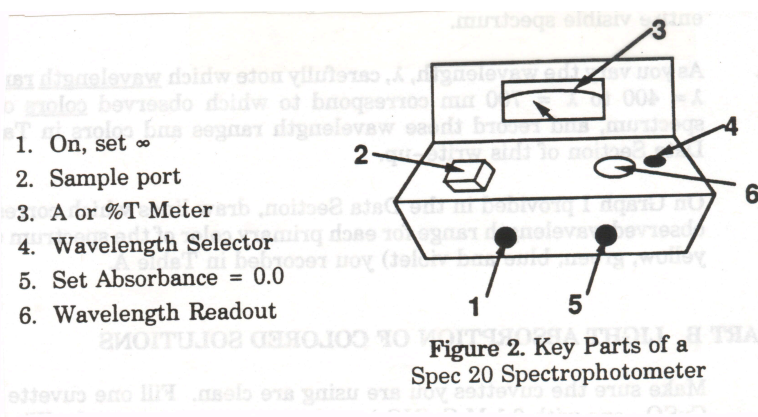
Procedure:

All procedures for this lab are run on a computer simulation supplied by the Journal of Chemical Education: Software division. This is a DOS program and may not run properly on newer operating systems. The program is called Spec 20.

Part I: Determining the Lambda Maximum

You will run two parts to this experiment. They do not have to be done at the same time but it is helpful to do so. In the first part of the experiment you must determine what the lambda maximum is for your sample. In the second part of the experiment you will determine the concentration of an unknown sample using a Beer's Law plot.

You run the program by choosing spec20.exe from the run menu. At the end of the command line type/L to turn on the labels.

Here is your instrument:

1. On, set ∞
2. Sample port
3. A or %T Meter
4. Wavelength Selector
5. Set Absorbance = 0.0
6. Wavelength Readout

Figure 2. Key Parts of a
Spec 20 Spectrophotometer

1. Turn on the instrument. Allow it to warm up. The dial will turn right and left when ready. A red light appears to be "on".

2. Choose your sample from the Sample menu.

3. Record the molar absortivity constant for your sample.

4. Choose the concentration for your sample. This should be the direct inverse of the absortivity constant.

5. Set the lowest wavelength the instrument will allow. Use the wavelength control (figure #6).

6. Start your run by setting the zero transmittance using the on/off switch (figure #1) to zero.
7. Choose the blank cuvette (by pressing B), align it, and wipe it clean. Open the port (figure #2) and insert the cuvette. Close the port.

8. Set the transmittance to 100% using the % transmittance knob (figure #5). Remove the blank and double (or triple) check that closed and empty you read zero and blank inserted and closed you read 100% transmittance.

9. Choose sample # 4 from the five cuvettes and measure it's percent transmittance at this wavelength. Press #4, align, wipe, open the sample port, insert the cuvette, close the port, read the meter, write it in your lab book, and press D on the keyboard. This brings up a data table and you can enter the value into the program. Click ok and remove the sample from the sample port.

10. Reset the wavelength increasing it by 25 nanometers and repeat steps 6 - 9. Do this every 25 nanometers until you maximize what the instrument will allow. Graph the material and determine the so-called lambda max for your sample.

Part II: Determining the Concentration of an Unknown Sample

In this part of the experiment you will determine the concentration of an unknown sample using Beer's law. Make sure you use the same sample as in part I and the same concentration for the sample. If you don't your experiment will not work.

1. Set the wavelength on the Spec20 to the lambda maximum determined in part I. Set the zero transmittance and 100% transmittance as you did in part I.

2. Choose sample one. Align and wipe the cuvette. Insert the sample into the Spec20, close the port, and measure the percent transmittance of your sample. You may insert this into the computers data table if you wish but be sure to record the concentration, transmittance, and color of the sample in your lab book.

3. Remove the sample and repeat step number two for samples two through four.

4. Finally choose the unknown concentration solution and determine its percent transmittance and color. Record this in your lab book.

5. Empty and turn off the instrument. The computer is not able to save any data.

Data and Calculations

Prepare a graph of wavelength versus transmittance. Use this to highlight the best wavelength for testing samples.

Prepare a graph of absorbance versus concentration (a Beer's Law plot) to determine the concentration of the unknown sample.

All of the data you collected should be presented in table and graph form both in your lab book and your formal lab report. This means at least two data tables and two graphs.

Post Lab Questions

1) Is your Beer's Law plot linear?

2) What is the concentration of the unknown?

3) What is the numerical value for the slope of the line? What should the value for the slope of the line be equal to?

4) What is the purpose of finding the Lambda Maximum? What does it tell you?

5) If a fingerprint were left on the cuvette how would it change the calculated value of the unknown? Give concrete reasons and say if it goes up or down.

6) Why is this technique useful for all of the solutions available in the computer program but not for a solution of NaCl?