Experiment 21 Spectrophotometric Analysis

Problem:

Develop a best fit line using 0/10, 1/5, 1/2, 4/5, and 1/1 dilutions of $FeCl_3$. The equation of this line will be used to determine the concentration of unknown dilutions of $FeCl_3$.

Introduction:

UV/VIS Spectrophotometers are used to determine the concentration of colored solutions. Colored ions absorb light from the visible spectrum. A more concentrated solution will absorb a greater amount of light. The spectrophotometer measures the amount of light which is transmitted through a sample (%T). The greater the concentration of a solution, the lower the %T will be. Absorbance and transmittance (T) are related by the following equation.

$A = -\log T$ A = Absorbance T = Transmittance

A set of standard solutions of known concentrations will be prepared, the %T of each will be determined, and the data will be plotted to create a calibration curve. A standard calibration curve may be used to calculate the concentration of an unknown sample.

The Lambert-Beer Law

$A = \varepsilon M C l$

A = Absorbance
εM = molar extinction coefficient (units: $M^{-1}cm^{-1}$)
C = Concentration (mol / L)
l = path length of light through cuvette (cm)

Procedure:

1. Turn on the spectrophotometer to warm up and adjust wavelength to 625 nm.
2. Prepare the standard solutions.
    a. Obtain about 40mL of 0.4M $FeCl_3$ "stock solution."
    b. In a 10mL graduated cylinder, measure 1.0 mL of stock solution. Then fill the graduated cylinder up to the 10mL mark with distilled water. This will make a 0.04 M solution of $FeCl_3$. Mix the solution and transfer as much as you can into a test tube labeled 1/10 (1/10 dilution of $FeCl_3$).
    c. Rinse out the graduated cylinder before creating the following dilutions.
        - 2.0mL of stock diluted up to a total volume of 10mL to make a 0.08M solution of $FeCl_3$. Pour into a test tube labeled 1/5.
        - 5.0mL of stock diluted up to a total volume of 10mL to make a 0.2M solution of $FeCl_3$. Pour into a test tube labeled 1/2.
        - 8.0mL of stock diluted up to a total volume of 10mL to make a 0.32M solution of $FeCl_3$. Pour into a test tube labeled 4/5.
        - For the final test tube, fill with undiluted stock solution. This will be a 0.4M solutions of $FeCl_3$. Label this tube 1/1.
3. Calibrate the spectrophotometer.
    ***Run samples as quickly as possible after calibrating to avoid error due to drift.**
    a. Set the %T to zero with the sample compartment empty.
    b. Fill a cuvette with distilled water, wipe it clean with a kimwipe and place it into the sample compartment. Close the lid.
    c. Set the %T to 100% with the cuvette containing distilled water in the compartment.
    d. Pour out the water and pour the the 1/10 dilution into the cuvette, wipe it clean, place in the compartment, close the lid and record the %T value.
    e. Repeat this procedure for the 1/5, 1/2, 4/5 and 1/1.
4. Measuring the unknowns. Using the same technique used to determine the %T for the known dilutions, determine the %T of the two unknown solutions.

Data and Calculations:

Tube	Vol. stock	Vol. water	Conc. (M)	%T	Absorbance
1	1.0 mL	9.0 mL	0.04 M		
2					
3					
4					
5					
Unknown A					
Unknown B					

1. Create a best fit line plotting solution concentration (M) on the x-axis and Absorbance on the y-axis using standard solutions only.
2. Using the Lambert-Beer Law, write the best fit line equation in the form which solves for concentration (x) in terms of absorbance (y).
3. Use this equation to calculate the molarity (M) of the unknown samples, A and B.
4. Obtain the actual molarities for each unknown and calculate the percent error for each.
5. Write a two paragraph conclusion which discusses your results. Include the importance of making a best fit line, discuss possible sources of error in the experiment, and ways to improve the experiment.