Spectrophotometer (Spectronic 20D+) Lab (Biotech Series)

Pre-Lab: 1). View the spectrophotometer podcast on the Biotech website (click here).
2). Review the spectrophotometer protocol and SOP thoroughly (click here).
3). Complete the SMART quiz over the podcast, the protocol, and the pre-lab.

Purpose: To learn how to calibrate and operate a Spectronic 20D+ spectrophotometer, which is a commonly used machine in Chemistry, Biology and Biotechnology. In this lab, you will continue to practice making dilutions and calculating molarity. Lastly, you will determine unknown concentrations by results from the Spec 20D+ and from a graph of percent transmittance and molarity.

Materials: 1 M $CuSO_4$ stock solution, 2- 5 mL serological pipettes, pipette motor, 7 Spectronic 20 cuvettes, 7 small test tubes, 1- test tube rack, Kimwipes, a 50 mL beaker, 100 mL beaker,1-unknown concentration tube, water cuvette blank, dH_2O wash bottle, swabs and a Spectronic 20D+ machine.

Introduction: The spectrophotometer is a tool used by scientists to retrieve information about atoms, molecules, ions. It is an instrument designed to measure the wavelength and the amount of electromagnetic radiation absorbed or transmitted by a sample. When a sample is exposed to a source of electromagnetic radiation, it will emit a spectrum. The shape of the resulting spectrum is determined by the energy transition which occurs as the electrons in the sample move between their excited and ground states. Since every substance has a unique electron arrangement, the shape of the spectrum emitted by a substance will also be unique. Therefore, scientists can use the spectrophotometer to identify unknown substances and analyze the purity of known substances. The detector in the spectrophotometer measures the intensity of a continuous beam of light that has passed through the sample. A scale on the spectrophotometer records the detector's measurements as absorbance, A, or percent transmittance, %T. They are related by the equation [$A = 2 - \log(\%T)$].

Procedure: You have been provided with a detailed protocol for the spectrophotometer (click here). Clean all test tubes, cuvettes, and beakers with dH_2O. Shake dry all tubes and dry inside of cuvettes with swabs, use paper towels to dry beakers if needed. Next you will need to make 40 mL of 0.750 M $CuSO_4$ from the stock solution (place into the 50 mL beaker) and you will need to show all of your work in your Biotech notebooks. Next you will prepare a set of dilutions from the standard solution of $CuSO_4$. Use your 100 mL beaker to put about 40 mL of dH_2O so you can pipette from the beaker. Using your small test tubes and 2- 5 mL serological pipettes, you will prepare the following dilutions; 5/5, 4/5, 3/5, 2.5/5, 2/5, 1/5, 0.5/5. You will make these by adding the amount of $CuSO_4$ into the test tube and then add the remaining amount of dH_2O needed to make 5 mL of solution. Be careful not to cross contaminate the pipettes. One pipette will be for the H2O only. After making these dilutions, transfer about 4-5 mL (enough to fill 3/4 full) into the Spec 20 cuvettes. Make sure you keep the cuvettes in order. Go to the Spec 20. Be sure to check the blank tube is set to 100% transmittance. Next you will find the %T for your 7 tubes from the Spec 20 following the protocol and techniques modeled in the podcast. You will also find the %T for the unknown sample at this time. If all of the machines are occupied, begin filling in your data table with the molarity concentrations for your dilutions. Also you can begin setting up your graph and added information to your notebooks. After recording your %T from the Spec 20, dump all solutions in the waste container labeled "copper sulfate waste". Clean all test tubes and Spec tubes with tap water three times. Return all equipment to its proper places.

Data/Observations: Record your data and observations carefully in your Biotech notebooks adhering to the protocol and SOP. Be sure to include a description of all chemicals used in the experiment. Observations should be both qualitative and quantitative and should follow the guidelines listed below.

Suggested Template for Data Table:

	Standard Solutions							Unknown #1
Test Tube	**1**	**2**	**3**	**4**	**5**	**6**	**7**	
Dilution	**5/5**	**4/5**	**3/5**	**2.5/5**	**2/5**	**1/5**	**0.5/5**	
Molarity								
%T								
Absorbance								

Analysis/Calculations: SHOW ALL WORK next to the data table in your notebooks. All data and answers should have the proper number of significant digits and labels/units. Molarity for your dilutions can be found by using the formula: $\mathbf{C_1V_1} = \mathbf{C_2V_2}$, where V = volume and C = concentration in molarity... example if a 2/10 dilution is made from a 0.10 M solution, then the calculation would be (0.10 M) (2 mL) = (**x** M) (10 mL) and **x** = 0.020 M (note that answer contains two sig figs). Fill in all parts of your data table, including absorbance. Remember A = 2 – log (%T). Remember to fill in the molarity for your standards after your calculations. Set up a graph for A vs. molarity. Put A (0- about 0.5) on the Y axis and molarity (0-0.75 M) on the X axis. Draw in the best fit line. Graph only the seven standard solutions. Put a title on your graph. Calculate the concentrations for your unknown by using $\mathbf{A}_{unk}/\mathbf{A}_{stnd} = \mathbf{C}_{unk}/\mathbf{C}_{stnd}$, where A = absorbance, C = concentration, unk = unknown, and stnd = standard. The standard solution should be the one with the least error. Fill those values in on your data table. Attach your graph (if computer generated) and make sure your calculations are shown for all work in your notebook per protocol. Groups that finish early are encouraged to further analyze these data using inferential statistics.

Questions: All questions should be answered in complete sentences when applicable. Please include these as part of your conclusion entry in your notebook.

1. From your graph**,** determine the A and %T for: (SHOW WORK)

A **%T**

for a 0.650 M solution = _______________ = _______________

for a 0.570 M solution = _______________ = _______________

for a 0.280 M solution = _______________ = _______________

2. What was the molarity of unknown #1 (from your calculations)?

3. Using a different pencil color, circle on your graph where your unknown absorbance crosses your line. Also write in what the concentration is at this point from the graph, using the axis (not your calculated values). Compare those values to your calculations (from #2).

a. Do they differ? Calculate the percentage difference between the two values.

$$\%Difference = \left| \frac{V_1 - V_2}{V_1 + V_2 / 2} \right| x100$$

b. Why would the graph give "better" (more accurate) answers? Justify your answers