10B

Spectrophotometric Analysis

One of the most important skills a chemist must acquire is knowing how to prepare a solution of a substance with a precise volume and molarity (concentration). In a wide variety of laboratories, including medical, teaching, research, manufacturing, and testing laboratories, solutions of known concentration are regularly required. Solutions whose concentrations are known are referred to as *standard* solutions. Another important skill is to be able to measure the unknown concentration of a solution quickly and easily.

In Part I of this experiment, you will prepare a solution of cobalt(II) nitrate hexahydrate, $Co(NO_3)_2{\bullet}6H_2O$, having a molarity of 0.160*M*. In Part II, you will make several solutions of lower concentrations by making appropriate dilutions. Then in Part III, the known concentration solutions will be placed in an instrument called a spectrophotometer that will be used to determine their color intensities. Finally, you will use the spectrophotometer to analyze an unknown cobalt(II) nitrate solution in order to determine its molarity.

A spectrophotometer makes use of the fact that in any given colored solution, light of a particular frequency is absorbed. When a suitable frequency is selected, the light is passed through the solution (which should be in a special type of tube called a cuvette having walls of uniform thickness which is designed for this purpose) and detected by a photo cell. The intensity of the light is shown by a needle on a dial and can be read either as absorbance or as percent transmittance. The value of this reading depends upon the concentration of the colored material in the solution.

Your instructor may set up the spectrophotometer in advance or selected students may be asked to do it. (The instructions below refer to a Bausch and Lomb Spectronic 20® spectrophotometer. If your instrument is a different one, your instructor will advise you of any modifications to the procedure.) The procedure is as follows:

1. Turn on the instrument by the front left-hand knob. Allow it to warm up for 15 min.
2. Select an appropriate wavelength to use by rotating the dial on the top right of the instrument. In this experiment, use 510 nm.
3. With the receptacle lid closed, adjust the left-hand knob on the front of the instrument until the needle reads 0% transmittance.
4. Place a cuvette three quarters full of distilled water in the receptacle on top and close the lid. Adjust the right-hand knob on the front of the instrument until the needle reads 100% transmittance. The spectrophotometer is now ready for use.

The following are some important points to note regarding the use of this instrument:

a. Make sure the receptacle lid is always closed before taking any readings, since stray light will affect the results.

b. Don't touch any of the knobs unless specifically directed by your instructor to recheck the 0% and 100% transmittance calibration.

c. The scale may have a mirror behind it—make sure the needle and its mirror image are lined up with one another before taking the reading to ensure greater accuracy.

d. If you are using the special tubes (cuvettes) for the spectrophotometer, make sure that the lines marked on them line up with the mark on the receptacle.

Your instructor may tell you to read either of the two scales (percent transmittance or absorbance) or both. There are advantages and disadvantages to each type of reading. The percent transmittance scale is uniform and therefore easier to read, but the calibration graph produced will be a curve and a graph must be drawn in order to obtain the concentration of the unknown. The absorbance scale is more difficult to read; it increases as you go from right to left and the size of the unit changes across the scale. However, the resulting calibration graph will usually be a straight line in the range of concentrations used; therefore, the concentration of an unknown may be obtained by calculation as well as from the graph.

OBJECTIVES

1. to prepare a standard solution of known concentration of cobalt(II) nitrate
2. to prepare various dilutions of the standard solution
3. to measure the percent transmittance, or absorbance, or both, of the solutions using a spectrophotometer, and to construct a calibration graph from the data
4. to determine the concentration of an unknown cobalt(II) nitrate solution using the calibration graph

SUPPLIES

Equipment

beaker (100 mL)
centigram balance
volumetric flask (100 mL)
 or graduated cylinder
 (100 mL)
funnel
wash bottle
5 test tubes (18 mm × 150 mm)
test-tube rack
graduated cylinder (10 mL)
graduated cylinder (25 mL)
dropping pipet
spectrophotometer
5 cuvettes (use 13 mm × 100 mm
 test tubes instead if necessary)
lab apron
safety goggles

Chemical Reagents

cobalt(II) nitrate hexahydrate, $Co(NO_3)_2 \cdot 6H_2O$
solution of above substance of unknown concentration
distilled water

PROCEDURE

Part I: Preparation of a Standard $Co(NO_3)_2$ Solution

1. Before coming to the laboratory, calculate the mass of cobalt(II) nitrate hexahydrate $Co(NO_3)_2{\bullet}6H_2O$, needed to make 100.0 mL of a 0.160*M* solution. Check with other students or your instructor to make certain that you have calculated the correct mass before proceeding.

2. Put on your lab apron and safety goggles.

3. Obtain a centigram balance and measure the mass of a clean, dry 100 mL beaker. Next weigh out the precise amount of $Co(NO_3)_2{\bullet}6H_2O$ (calculated in Step 1 above) in the beaker.

4. Dissolve the compound in about 50 mL of water in the beaker.

5. Transfer the solution to a 100 mL volumetric flask by means of a funnel. Then, using a wash bottle, wash the beaker several times with small amounts of water, adding the washings to the flask. Be careful not to add so much water that you go beyond the mark. (Note: If you do not have a 100 mL volumetric flask, use a 100 mL graduated cylinder instead.)

6. Remove the funnel and add water from the wash bottle until the volume is exactly at the 100 mL mark. (Add the water drop by drop when you are close.)

7. Stopper the flask and shake to ensure thorough mixing. You now have a 0.160*M* $Co(NO_3)_2$ solution. Chemists refer to such an initially prepared solution of known molarity as a *stock solution.*

CAUTION

Cobalt(II) nitrate is toxic. Do not get any in your mouth.

Part II: Preparation of Dilute Solutions of $Co(NO_3)_2$

1. Obtain 5 test tubes (18 mm × 150 mm) and place them in a rack. Label them A to E.

2. In test tube A, place approximately 10 mL of your stock solution (0.160*M*).

3. Prepare a diluted solution by placing 12.0 mL of the stock solution in a 25 mL graduated cylinder and adding water until the solution is up to the 16.0 mL mark. Transfer this solution to test tube B.

4. Repeat Step 3 using 8.0 mL of stock solution and making it up to 16.0 mL. (If the graduated cylinder is wet from previous washings, rinse it out with about 4 mL of your stock solution first and discard the rinsing liquid.) Transfer the diluted solution to test tube C.

5. In the same manner, prepare a dilution with 4.0 mL of stock solution made up to 16.0 mL for test tube D and 2.0 mL of stock solution made up to 16.0 mL for test tube E.

6. Calculate the new molarities of the diluted solutions and enter them in your copy of Table 1 in your notebook.

Part III: Measuring the Concentration with a Spectrophotometer

1. Make sure you understand thoroughly how to use the spectrophotometer, as outlined in the introduction to this experiment.
2. Transfer solutions A to E into five cuvettes. (If cuvettes are not available, use 13 mm × 100 mm test tubes that are clean and scratch-free instead.) If the tubes are wet inside, rinse each with about 4 mL of the solution which will go into it, discard the rinsings, then fill each about three quarters full with the appropriate solution.
3. Make sure the tubes are clean and dry on the outside, place each in turn in the receptacle on top of the spectrophotometer, and then close the lid. Read off the value of either the percent transmittance or the absorbance or both, as directed by your instructor. Enter the values in Table 1.
4. Obtain a $Co(NO_3)_2$ solution of unknown concentration from your instructor and note any identifying letter or number on it. Fill a tube three quarters full as before. Read either percent transmittance or absorbance, depending on which scale you read for your standards. Enter this value in Table 1 as well.
5. Before you leave the laboratory, wash your hands thoroughly with soap and water.

REAGENT DISPOSAL

Rinse all solutions down the sink with copious amounts of water, unless your instructor asks you to save them.

POST LAB CONSIDERATIONS

The accuracy of your answer for the concentration of the unknown molarity $Co(NO_3)_2$ solution will be a good measure of your experimental skills. It is important to have taken all your readings carefully and have double-checked them to be sure you had the correct values. Graphs must now be plotted carefully and accurately.

The spectrophotometer results from the known molarity solutions will be used to create a curved and/or straight calibration line for the $Co(NO_3)_2$ solution. Then, the unknown molarity of the given $Co(NO_3)_2$ solution can be determined from this calibration line. Your instructor may ask you to average the molarities determined from each of the two lines.

EXPERIMENTAL RESULTS

Table 1

Test Tube	Original Molarity	Dilution	New Molarity	Absorbance (A)	Percent Transmittance (%)
A	0.160*M*	—	0.160*M*		
B	0.160*M*	12 mL to 16 mL			
C	0.160*M*	8 mL to 16 mL	COMPLETE IN YOUR NOTEBOOK	COMPLETE IN YOUR NOTEBOOK	COMPLETE IN YOUR NOTEBOOK
D	0.160*M*	4 mL to 16 mL			
E	0.160*M*	2 mL to 16 mL			
	Unknown	—	—		

ANALYSIS OF RESULTS

1. Calculate the molarity of the Co^{2+} ion in each of solutions A–E. The new molarity is given by the molarity of the stock solution multiplied by the dilution factor (ratio of original volume to final volume).
2. Plot a graph of absorbance versus concentration using proper graphing techniques. (Substitute percent transmittance for absorbance if that is what you measured.) Your instructor may ask you to plot both graphs.
3. Determine the unknown concentration of your given solution by reading from your graph the concentration that is equivalent to the absorbance or percent transmittance you recorded. If your instructor asked you to plot both graphs, you may need to report the average of the two values. Be sure to state in your report which unknown you used if more than one was available.

FOLLOW-UP QUESTIONS

1. Why does a volumetric flask have the shape it does?
2. Looking at your results and the shape of your graph, do you think your results could have been improved by the use of graduated pipets instead of graduated cylinders?
3. Why is it a good idea to wash out the tubes with the solution you are using before refilling them to read in the spectrophotometer?
4. In order to analyze the waste water containing Co^{2+} from a manufacturing process, 1.0 L of water was evaporated to 10.0 mL and then placed in

a spectrophotometer tube. The absorbance was found to be 0.30 A (63% transmittance). Using your calibration curve, calculate the number of milligrams of Co^{2+} in 1.0 L of waste water.

CONCLUSION

State the results of Objective 4.