Experiment # 7: Quantitative Absorption Spectroscopy

When radiant energy passes through a solution containing an appropriate absorbing species, some of the EMR is absorbed. Absorption of energy in this manner has been recognized for more than two centuries and accounts for the fact that colorimetric and spectrophotometric methods are the most prevalent analytical techniques. Colorimetry refers to determinations which employ only visible light, while photometry implies the use of photoelectric instruments. Spectrophotometric methods often utilize radiation outside the visible region, particularly EMR in the infrared and ultraviolet ranges.

Two elementary laws make up the basic foundations of quantitative absorption spectroscopy. The first law was formulated by Bouguer in l729 (and later restated by Lambert in 1760). It says: each absorbing substance layer of the same thickness absorbs an equal fraction of the EMR passing through it. In mathematical terms, absorption increases exponentially with the thickness of the absorbing species.

The second basic law is that of Beer: the absorption of a monochromatic EMR beam increases exponentially with the concentration of the absorbing species. Both laws state the same fundamental aspect of absorption spectroscopy: namely, that absorption is proportionate to the number of absorbing species with which the EMR beam comes in contact. Thus, neither law is functionally complete without the other. No deviations from Bouguer's Law are known, however, deviations from Beer's law are fairly common.

The two laws are usually combined into a single relationship commonly referred to as "Beer's Law" and are expressed in the following mathematical relationships.

$$-\text{Log}\,(P/P_o) = -\text{Log}\,(T) = a\,b\,C = A,$$

where "P" is the emergent EMR intensity passing through the absorbing medium, "P_o" is the incident EMR intensity going into the absorbing medium, "T" is defined as transmittance, "a" is the absorptivity constant whose value depends upon the EMR wavelength and the chemical nature of the absorbing species, "b" is the cell width or length of the light path though the absorbing medium, "C" is the concentration of the absorbing species in the medium, and "A" is defined as absorbance.

It should be obvious from the above relationships that if all variables

Thus, absorption spectroscopy is a direct quantitative analysis method for materials which obey B Law within acceptable limits.

The divalent copper cation, Cu

$^{2+}$, forms a coordinate covalent complex with ammonia, as: Cu $(NH_3)_4^{2+}$. This complex species absorbs strongly in the visible region of the EMR spectrum from

Preparation of standard copper complex series

A 1.000 mg Cu^{2+} /mL standard solution is prepared by dissolving 3.928 grams of $CuSO_4 \cdot 5H_2O$ to 1.000-liter with deionized water. The copper standard and a 7.5 N $NH_{3\,(aq)}$ solution are provided in labeled bottles on the reagent table. Burets will be used to deliver measured portions of these two reagents as well as deionized water (from the large storage bottle on the lab benchtop).

Each group of students working together should assemble six clean test tubes (15 x 125 mm) and two clean Spectronic 20 cuvettes. Using the burets, each filled with the proper reagent, prepare the solution mixtures listed in Table I. Run the specified volume of each reagent into a labeled test tube, cap the tube with a rubber stopper, and thoroughly mix the solutions. Rinse a cuvette with the copper complex standard, and then fill the cuvette about half-full with the solution. Standard sample #6 will be used as the blank or reference solution to calibrate the Spectronic 20 to read 100%T at 610 nm.

Table I

Sample #	mL Cu^{2+} standard	mL deionized H_2O	mL 7.5 M $NH_{3\,(aq)}$
1	9.00	0.00	1.00
2	7.00	2.00	1.00
3	5.00	4.00	1.00
4	3.00	6.00	1.00
5	1.00	8.00	1.00
6	0.00	9.00	1.00

Note that 1.00-mL of the 7.5 M $NH_{3\,(aq)}$ is used in each of the solutions: this provides an excess of ammonia to insure that all complexes are forced into the $Cu(NH_3)_4^{2+}$ species. Also, the total volume of each solution is 10.00mL.

Calculate the theoretical concentration of the copper-ammonia complex, using the simple dilution equation:

$$C_{STD}(\text{mg Cu/mL}) = C_{STOCK\ STD}(\text{mg Cu/mL}) \times \frac{\text{mL } Cu^{2+} \text{ Stock Std.}}{10.00 \text{ mL}}$$

Record the complex concentration of each solution in Table II.

Preparation of unknown copper complex solution

Several soluble copper (II) salts are provided in numbered bottles on the reagent shelf. Your instructor will tell you whether each member of a group should analyze a different salt to determine the copper content, or whether only one salt will be analyzed by the group. When selecting an unknown solid, record the unknown # written on the container for later inclusion in the experiment report.

Using the technique of weighing-by-difference (as explained by the lab instructor), accurately we1dh8(h)8 382.92 T

On an 18 x 24 cm sheet of graph paper, plot C_{STD} (mg Cu/mL) for the standard series on the abscissa (long, horizontal axis) versus calculated A on the ordinate (short, vertical axis). Select the axis scales to maximize use of the graph paper for the actual data points recorded starting both axes at zero. Use a ruler to draw the best straight line through the plotted data points.

Calculate an average A value for the three unknown sample readings, and determine the concentration of copper complex in the unknown solution by a graphical interpretation of the Beer's Law plot. Mark the position of the average unknown A value on the ordinate, draw a horizontal straight line to intersect the Beer's Law plot, and drop a perpendicular to the abscissa to determine unknown copper complex-concentration, C_{UNK}(mg Cu/mL). Record the numerical value of the unknown complex concentration on the graph paper beside the perpendicular line which intersects the abscissa.

Since 9.00mL of the unknown copper solution in the vol