

## 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 1729 (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.

$$-\text{Log}(P/P_0) = -\text{Log}(T) = a b C = A,$$

where "P" is the emergent EMR intensity passing through the absorbing medium, "P<sub>0</sub>" 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 through 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 except absorber concentration are held constant (i.e., the same wavelength, cell length, light source, etc., is used in all measurements), the absorbances of a solution series will be directly related to the absorber concentrations of the solutions. Thus, absorption spectroscopy is a direct quantitative analysis method for materials which obey Beer's Law within acceptable limits.

The divalent copper cation, Cu<sup>2+</sup>, forms a coordinate covalent complex with ammonia, as: Cu(NH<sub>3</sub>)<sub>4</sub><sup>2+</sup>. This complex species absorbs strongly in the visible region of the EMR spectrum from 550—650 nm. The wavelength of maximum absorption lies between 600—620 nm, nominally at 610 nm. However, the strongly absorbing species is formed only in an excess of the complexing agent, NH<sub>3</sub>. If an excess of ammonia is not present in the solution, non-absorbing complexes may be formed instead of the tetraamminecopper (II) species, and the Beer's Law relationships will not be observed.

### Preparation of standard copper complex series

A 1.000 mg Cu<sup>2+</sup> /mL standard solution is prepared by dissolving 3.928 grams of CuSO<sub>4</sub>·5H<sub>2</sub>O to 1.000-liter with deionized water. The copper standard and a 7.5 N NH<sub>3</sub> (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 <sup>2+</sup> standard	mL deionized H <sub>2</sub> O	mL 7.5 M NH <sub>3</sub> (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<sub>3</sub> (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<sub>3</sub>)<sub>4</sub><sup>2+</sup> 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:

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

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 weigh out 0.4-0.6 grams of an unknown copper (II) salt into a clean beaker (150-mL or larger). Record the sample mass as precisely as the balance is capable of measuring, that is, to the third decimal place.

Add about 100 mL of deionized water to the solid in the beaker, and make sure the copper salt dissolves completely. Transfer the salt solution into a clean, 250.00-mL volumetric flask. Rinse the beaker with several portions of deionized water, collecting the washings in the flask. These rinses are made to insure quantitative transference of the copper solution to the volumetric flask. Add deionized water to the flask until the solution level reaches the calibration mark on the flask neck. Stopper the flask, and mix the solution thoroughly.

*Note: Since this procedure prepares 250 mL of unknown copper solution, you may be asked to provide unknown solution for other students in other groups, or you may be asked to acquire unknown solution from another group. This determination will be made by the lab instructor.*

Each group will need three test tubes for the copper unknown. Pour a small amount of the unknown copper solution into a clean buret in order to rinse it, then fill the buret with the unknown copper solution. Withdraw three 9.00-mL samples of the copper salt solution each into three clean, dry test tubes. Add 1.00-mL of the 7.5 M  $\text{NH}_3$  (aq) solution (measured from a buret) to each of the test tubes cap with stoppers, and mix the solutions thoroughly. The resulting copper-ammonia complex solution is now ready for use in the spectroscopic analysis.

### Spectroscopic measurements for Beer's Law plot

If the Spectronic 20 is not already turned on, using the left control to turn it on and allow at least five minutes for it to warm up. Adjust the wavelength dial to 610 nm. With the cell compartment empty and closed, adjust the left control to set the meter needle (or digital display) at 0% T.

Place the cuvette containing the blank/reference solution (standard sample # 6) into the cell compartment, close the cover, and adjust the right control to position the meter needle (or digital display) at 100% T. The instrument is now calibrated at the wavelength setting of 610 nm.

Working in groups of no more than three students, place each copper complex standard in turn into the cell compartment. Read and record the values in terms of % T and A values, listing the data in Table II. It is good practice to measure experimental values for each solution at least twice to insure that no errors are made. Such readings should show reproducibility to within  $\pm 0.2$  %T. After all the standard solutions have been measured, each group should read/record the %T and A values of his/her unknown copper samples, listing the data in Table II. Theoretical values for A should be calculated, using the equation:  $A = 2 - \log \% T$ .

**Table II**

Sample	CSTD (mg Cu/mL)	Measured A	Measured %T	Calculated A

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 volumetric flask is diluted by the addition of 1.00 mL of the 7.5 M  $NH_3$  solution (which provides an excess of ammonia to complex all the copper as the tetraammine species), the corrected complex concentration necessary to give the true copper concentration in the volumetric flask is given by the dilution equation:

$$C_{FLASK} \text{ (mg Cu/mL)} = C_{UNK} \text{ (mg Cu/mL)} \times \frac{10.00 \text{ mL}}{9.00 \text{ mL}}$$

Using this corrected concentration of copper in the original 250.00 mL solution, the mass of copper, in grams, in the salt sample is given by:

$$\text{grams Cu}^{2+} = C_{FLASK} \text{ (mg Cu/mL)} \times (250.0 \text{ mL}) \times (0.001 \text{ g/mg})$$

Thus, the percent copper in the soluble copper (II) salt equals:

$$\% \text{ Cu} = \frac{\text{grams Cu}^{2+}}{\text{grams salt}} \times 100\%$$

Each student will submit a two-page report, each page containing a complete heading. The first page should include a neat, titled, completed copy of—Table II. Additional information to be listed are: the unknown number on the copper (II) salt container, the mass of the salt in grams, the concentration of the stock copper standard solution, absorbances of the three unknown complex solutions and the average  $A$  value, the unknown copper complex concentration from the Beer's Law plot, the corrected concentration of copper in the flask, the calculated mass in grams of copper in the salt sample, and the calculated percent copper in the salt sample.

The second page should consist of a neat, complete Beer's law plot on the proper type of graph paper. The graph should have a title and a complete heading. The axes should be properly labeled and scaled, and the best straight line should be drawn through the data points of the standard concentration series. All specific instructions must be carried out in the interpretations and operations performed on the experimental data.