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Chem 506
7/31/2008

Colorimetric Determination of Iron

Introduction

In this lab, the absorbance of various solutions with known concentrations of iron (bound in a colored iron-phenanthroline complex) were measured to determine a calibration curve. The absorbance of an unknown was then determined both quantitatively (with a spectrometer) and qualitatively (by sight comparison) to assess the amount of iron in the unknown. The relationship that links absorbance with concentration and path length is given by Beer-Lambert's law:

$$A = Ecl \qquad (1)$$

where A = absorbance of the sample
E= extinction coefficient (a.k.a constant of proportionality)
c = concentration
l = pathlength of sample

The following equations show the two reactions that take place in the lab: the conversion from ferric iron to ferrous iron (rxn. 1) and the complexing of ferrous iron with o-phenanthroline (rxn. 2):

$$4\ Fe^{+3}_{(aq)} + NH_2OH_{(aq)} \rightarrow 3\ Fe^{+2}_{(aq)} + N_2O_{(aq)} + 4\ H^{+}_{(aq)} + H_2O_{(l)} \qquad \text{(rxn. 1)}$$

$$Fe^{+2}_{(aq)} + 3\ C_{12}H_2N_{2\ (aq)} \rightarrow [(C_{12}H_2N_2)_3Fe]^{+2}_{(aq)}\ \text{(orange-red complex)} \qquad \text{(rxn. 2)}$$

Procedures:
For details, see pp. 4-7 of "Pre-Lab Notes: Colorimetric Determination of Iron" MCEP UPenn Chem 506 Lab. 2-mL of the unknown solution was diluted to 50-mL in accordance with lab procedure (1/25 dilution) and then 10-mL of that solution was diluted out to 50-mL again (1/5) before measurements were made. Therefore, measurement of the unknown are upon a 1/125 dilution of the original solution containing all of the unknown measured.

Materials used (beyond common lab glassware):
Spec 20, set at 510 nm

Total amount needed	Chemical
50-mL	Stock Fe solution (0.050 mg Fe^{+3}/ ml)
5-mL	1 M ammonium acetate ($NH_4C_2H_3O_2$), buffer to maintain pH = 3.5
5-mL	10% Hydroxylamine HCl (NH_3OHCl), reducing agent
50-mL	0.3% o-phenanthroline ($C_{12}H_2N_2$)
10 drops	3 M H_2SO_4
~0.1 g	Unknown #217

Safety:
Detailed hazard information can be found at http://www.flinnsci.com/search_MSDS.asp

Ammonium acetate solution is not considered hazardous. Hydroxylamine hydrochloride is moderately toxic by ingestion and corrosive to body tissues. O-phenanthroline is highly toxic by ingestion and a mild skin irritant. Sulfuric acid is corrosive to eye, skin, and all other body tissues, and generates considerable heat when diluted with water.

Since solutions used are dilute and students are wearing gloves and goggles, the hazards posed by exposure are minimized. If exposed to solutions in lab, students should rinse area of contact with soap and water. Solutions may be disposed of safely in the sink with plenty of running water.

Data:

Table 1: Calibration curve data

Concentration	Absorbance (at λ = 510 nm)
0.0005 mg Fe/ mL	0.108
0.0010 mg Fe/ mL	0.203
0.0015 mg Fe/ mL	0.275
0.0020 mg Fe/ mL	0.393

Table 2: Unknown #217 Data and Results

Unknown Number	#217
Mass of unknown	0.1036 g
Absorbance of diluted unknown solution (1/125 of original solution)	Trial 1: 0.173 Average: 0.1755 Trial 2: 0.178
Qualitative sight comparison data	Absorbance (color intensity) was the same when: 0.0005 mg/ mL: 10.6 cm pathlength in test tube 1/125 dilute unknown: 6.10 cm pathlength in test tube
% of Fe in unknown	Quanititatively (using calibration curve from Spec20 absorbances) : 5.41% Qualitatively (using sight inspection and pathlength variation): 5.24%

Calculations:

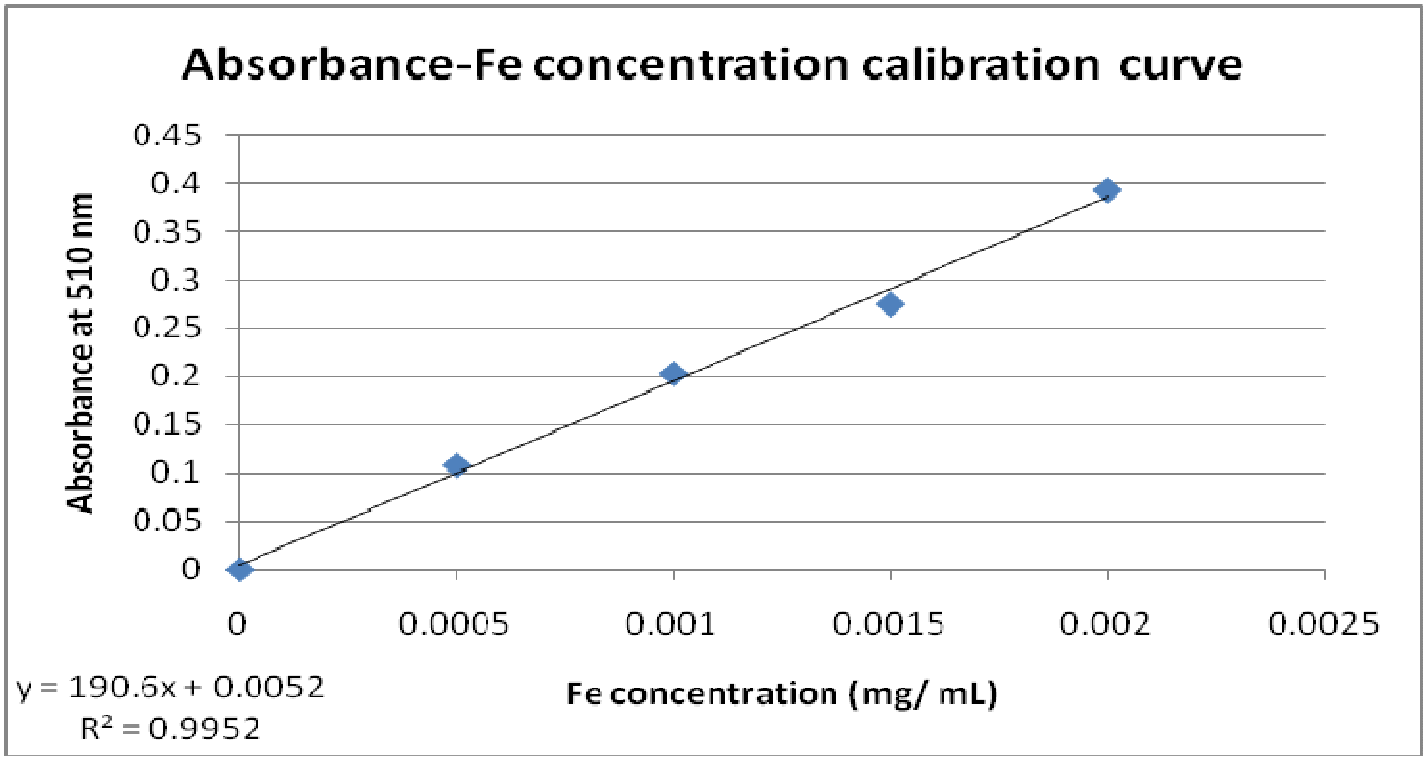
Calculation of Fe in unknown using visual inspection and pathlength variation:

For the unknown dilute solution (1/125 of original solution in the second solution),
$A_1 = A_2$ $Ec_1l_1 = Ec_2l_2$
$c_1 = c_2l_2/l_1$ = (0.0005 mg/mg)(10.6 cm)/(6.1 cm) = 8.69×10^{-4} mg/mL in second dilution

Concentration in first dilution (5x final dilution): $M_1V_1 = M_2V_2$ so M_1V_1/V_2
(8.69×10^{-4} mg Fe/mL)(50 mL)/(10mL) = 0.00434 mg Fe/mL in first dilution

Concentration in original solution (25x first dilution) : $M_1V_1 = M_2V_2$ so M_1V_1/V_2
(0.00434 mg Fe/mL)(50mL)/(2 ml) = 0.109 mg Fe/mL in original solution

Mass of Fe in original solution: (0.109 mg Fe/mL)(50.0 mL) = 5.43 mg Fe = 0.00543 g Fe
% Fe in unknown = 0.00543 g Fe/ 0.1036 g unknown x 100 = 5.24%



The trendline for the data is: Absorbance at 510 nm = 190.6 (Fe concentration in mg/ mL) + 0.0052.
This can be rearranged so that Fe concentration in mg/ mL = (1/190.6)(Absorbance at 510 nm – 0.0052)

Calculation of Fe in unknown using calibration curve:

For the unknown dilute solution (1/125 of original solution in the second solution),

Fe concentration in mg/ mL = (1/190.6)(**0.1755** – 0.0052) = 8.93 x 10^{-4} mg Fe/ mL in second dilution

Concentration in first dilution (5x final dilution): $M_1V_1 = M_2V_2$ so M_1V_1/V_2
(8.93 x 10^{-4} mg Fe/mL)(50 mL)/(10mL) = 0.00447 mg Fe/mL in first dilution

Concentration in original solution (25x first dilution) : $M_1V_1 = M_2V_2$ so M_1V_1/V_2
(0.00447 mg Fe/mL)(50mL)/(2 ml) = 0.112 mg Fe/mL in original solution

Mass of Fe in original solution: (0.112 mg Fe/mL)(50.0 mL) = 5.60 mg Fe = 0.00560 g Fe
% Fe in unknown = 0.00560 g Fe/ 0.1036 g unknown x 100 = 5.41%

Conclusions/ Discussion:

Unknown #217 was determined to have 5.41% Fe with the calibration curve method and 5.24% Fe with the visual inspection/ pathlength variation method.

The calibration curve method is more likely to be accurate because it uses absorbances precisely determined by a spectrometer and because it uses four point of measurement and a blank to determine the linear relationship between absorbance and concentration rather than a single observation. Using a precise instrument and increasing the number of observations decreases error and makes this method more accurate unless a single observation skews the trendline.

The visual inspection/ pathlength variation method is fairly accurate, but there is significant error if the pathlengths of the test tube are not long enough to be far away from the curved bottom of the test tube, which will disproportionately affect the color intensity observed of the sample with the shorter pathlength. Also, care must be taken to select two test tubes that are similar enough that differences do not affect the color intensity observed.

Since the unknown was diluted twice, there is an introduction of error with each dilution. This error, however, cannot be avoided since to take a direct $1/125^{th}$ dilution of the original solution would make the aliquot used so small that the % error introduced by the use of a 10-mL graduated cylinder would be significant.

Post-lab Questions:

1. $Fe^{+3} + 3\ H_2O \rightarrow Fe(OH)_3$
   Iron (III) hydroxide precipitates out of solution, decreasing the amount of iron dissolved in solution that is available to complex with the o-phenanthroline.
2. The hydroxylamine HCl is the reducing agent that intercepts oxygen and prevent oxidation of ferrous iron to ferric iron. If omitted, a different complex with a different color and optimum absorption could form. Or, perhaps no colored complex would form.
3. $A_1 = A_2$ $\quad Ec_1l_1 = Ec_2l_2$ $\quad c_1 = c_2l_2/l_1$
   The visual inspection demonstrates this law because absorbances can be made equal by adding or removing colored solution (increasing or decreasing path length). When the color intensities are the same, the absorbances are assumed to be equal. Since the extinction coefficient E is the same, the concentration of the unknown solution can be determined by calculation using the known concentration and the pathlengths measured.
4. The rounded bottoms on the test tubes may have made the % calculated in this lab less than it should have been. While the absolute error introduced by the rounded error is the same for both test tubes, the % error (% the absolute error comprises of the measurement) is greater in the sample with the smaller pathlength and the rounded bottom would disproportionately affect the sample with the smaller path length. This would make the sample seem lighter in color intensity than it should. In this lab, this may help explain the minor discrepancy between 5.41% (quantitative) and 5.24% (qualitative).