

# Experiments

To Accompany  
*Quantitative Chemical Analysis*, 6<sup>th</sup> Edition (2002)  
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# Experiments

Experiments described here illustrate major analytical techniques described in the textbook, *Quantitative Chemical Analysis*. Procedures are organized roughly in the same order as topics in the text. You are invited to download these instructions and reproduce them freely for use in your laboratory. References to many additional experiments published in recent years in the *Journal of Chemical Education* are listed at the ends of many chapters in the textbook.<sup>1</sup> Some of the experiments refer to the availability of standards or unknowns from Thorn Smith.<sup>2</sup>

Although these procedures are safe when carried out with reasonable care, *all chemical experiments are potentially hazardous*. Any solution that fumes (such as concentrated HCl), and all nonaqueous solvents, should be handled in a fume hood. Pipetting should never be done by mouth. Spills on your body should be flooded immediately with water and washed with soap and water; your instructor should be notified for possible further action. Spills on the benchtop should be cleaned immediately. Toxic chemicals should not be flushed down the drain. Your instructor should establish a procedure for disposing of each chemical that you use.

## 1. Calibration of Volumetric Glassware

An important trait of good analysts is the ability to extract the best possible data from their equipment. For this purpose, it is desirable to calibrate your own volumetric glassware (burets, pipets, flasks, etc.) to measure the exact volumes delivered or contained. This experiment also promotes improved technique in handling volumetric glassware. The procedure for calibrating a 50-mL buret is described at the end of Chapter 2 of the textbook.

Other volumetric glassware can also be calibrated by measuring the mass of water they contain or deliver. Glass transfer pipets and plastic micropipets can be calibrated by weighing the water delivered from them. A volumetric flask can be calibrated by weighing it empty and then weighing it filled to the mark with distilled water. Perform each procedure at least twice. Compare your results with the tolerances listed in tables in Chapter 2 of the textbook.

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1. You can search for experiments by key words in the index of the *Journal of Chemical Education*, at <http://jchemed.chem.wisc.edu/> on the Internet.
  2. Thorn Smith Inc., 7755 Narrow Gauge Road, Beulah, MI 49617. Phone: 231-882-4672; e-mail: [www.thornsmithlabs.com](http://www.thornsmithlabs.com). The following analyzed unknowns are available: Al-Mg alloy (for Al, Mg), Al-Zn alloy (for Al, Zn), Sb ore (for Sb), brasses (for Sn, Cu, Pb, Zn), calcium carbonate (for Ca), cast iron (for P, Mn, S, Si, C), cement (for Si, Fe, Al, Ca, Mg, S, ignition loss), Cr ore (for Cr), Cu ore (for Cu), copper oxide (for Cu), ferrous ammonium sulfate (for Fe), Fe ore (for Fe), iron oxide (for Fe), limestone (for Ca, Mg, Si, ignition loss), magnesium sulfate (for Mg), Mn ore (for Mn), Monel metal (for Si, Cu, Ni), nickel silver (for Cu, Ni, Zn), nickel oxide (for Ni), phosphate rock (for P), potassium hydrogen phthalate (for H<sup>+</sup>), silver alloys (for Ag, Cu, Zn, Ni), soda ash (for Na<sub>2</sub>CO<sub>3</sub>), soluble antimony (for Sb), soluble choride, soluble oxalate, soluble phosphate, soluble sulfate, steels (for C, Mn, P, S, Si, Ni, Cr, Mo), Zn ore (for Zn). Primary standards are also available: potassium hydrogen phthalate (to standardize NaOH), As<sub>2</sub>O<sub>3</sub> (to standardize I<sub>2</sub>), CaCO<sub>3</sub> (to standardize EDTA), Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub> (to standardize dichromate or permanganate), K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> (to standardize thiosulfate), AgNO<sub>3</sub>, Na<sub>2</sub>CO<sub>3</sub> (to standardize acid), NaCl (to standardize AgNO<sub>3</sub>), Na<sub>2</sub>C<sub>2</sub>O<sub>4</sub> (to standardize KMnO<sub>4</sub>).

## 2. Gravimetric Determination of Calcium as $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$ <sup>1</sup>

Calcium ion can be analyzed by precipitation with oxalate in basic solution to form  $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$ . The precipitate is soluble in acidic solution because the oxalate anion is a weak base. Large, easily filtered, relatively pure crystals of product will be obtained if the precipitation is carried out slowly. This can be done by dissolving  $\text{Ca}^{2+}$  and  $\text{C}_2\text{O}_4^{2-}$  in acidic solution and gradually raising the pH by thermal decomposition of urea (Reaction 27-2 in the textbook).

### REAGENTS

*Ammonium oxalate solution:* Make 1 L of solution containing 40 g of  $(\text{NH}_4)_2\text{C}_2\text{O}_4$  plus 25 mL of 12 M HCl. Each student will need 80 mL of this solution.

*Unknowns:* Prepare 1 L of solution containing 15-18 g of  $\text{CaCO}_3$  plus 38 mL of 12 M HCl. Each student will need 100 mL of this solution. Alternatively, solid unknowns are available from Thorn Smith.<sup>2</sup>

### PROCEDURE

1. Dry three medium-porosity, sintered-glass funnels for 1–2 h at 105° C; cool them in a desiccator for 30 min and weigh them. Repeat the procedure with 30-min heating periods until successive weighings agree to within 0.3 mg. Use a paper towel or tongs, not your fingers, to handle the funnels. An alternative method of drying the crucible and precipitate is with a microwave oven.<sup>3</sup> A 900-W kitchen microwave oven dries the crucible to constant mass in two heating periods of 4 min and 2 min (with 15 min allowed for cooldown after each cycle). You will need to experiment with your oven to find appropriate heating times.
2. Use a few small portions of unknown to rinse a 25-mL transfer pipet, and discard the washings. *Use a rubber bulb, not your mouth, to provide suction.* Transfer exactly 25 mL of unknown to each of three 250- to 400-mL beakers, and dilute each with ~75 mL of 0.1 M HCl. Add 5 drops of methyl red indicator solution (Table 12-4 in the textbook) to each beaker. This indicator is red below pH 4.8 and yellow above pH 6.0.
3. Add ~25 mL of ammonium oxalate solution to each beaker while stirring with a glass rod. Remove the rod and rinse it into the beaker. Add ~15 g of solid urea to each sample, cover it with a watchglass, and boil gently for ~30 min until the indicator turns yellow.
4. Filter each hot solution through a weighed funnel, using suction (Figure 2-15 in the textbook). Add ~3 mL of ice-cold water to the beaker, and use a rubber policeman to help transfer the remaining solid to the funnel. Repeat this procedure with small portions of ice-cold water until all of the precipitate has been transferred. Finally, use two 10-mL portions of ice-cold water to rinse each beaker, and pour the washings over the precipitate.

5. Dry the precipitate, first with aspirator suction for 1 min, then in an oven at 105° C for 1-2 h. Bring each filter to constant mass. The product is somewhat hygroscopic, so only one filter at a time should be removed from the desiccator, and weighings should be done rapidly. Alternatively, the precipitate can be dried in a microwave oven once for 4 min, followed by several 2-min periods, with cooling for 15 min before weighing. The water of crystallization is not lost.
  
6. Calculate the molarity of Ca<sup>2+</sup> in the unknown solution or the weight percent of Ca in the unknown solid. Report the standard deviation and relative standard deviation ( $s/\bar{x}$  = standard deviation/average).

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1. C. H. Hendrickson and P. R. Robinson, *J. Chem. Ed.* **1979**, 56, 341.
  2. Thorn Smith Inc., 7755 Narrow Gauge Road, Beulah, MI 49617. Phone: 231-882-4672; e-mail: [www.thornsmithlabs.com](http://www.thornsmithlabs.com).
  3. R. Q. Thompson and M. Ghadiali, *J. Chem. Ed.* **1993**, 70, 170.

### 3. Gravimetric Determination of Iron as Fe<sub>2</sub>O<sub>3</sub><sup>1</sup>

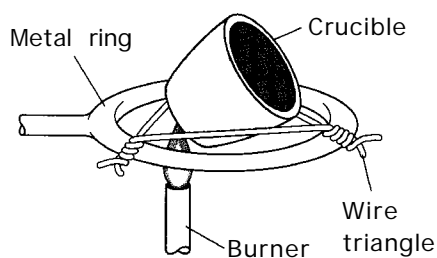
A sample containing iron can be analyzed by precipitation of the hydrous oxide from basic solution, followed by ignition to produce Fe<sub>2</sub>O<sub>3</sub>:



The gelatinous hydrous oxide can occlude impurities. Therefore, the initial precipitate is dissolved in acid and reprecipitated. Because the concentration of impurities is lower during the second precipitation, occlusion is diminished. Solid unknowns can be prepared from reagent ferrous ammonium sulfate or purchased from Thorn Smith.<sup>2</sup>

#### PROCEDURE

1. Bring three porcelain crucibles and caps to constant mass by heating to redness for 15 min over a burner (Figure 1). Cool for 30 min in a desiccator and weigh each crucible. Repeat this procedure until successive weighings agree within 0.3 mg. Be sure that all oxidizable substances on the entire surface of each crucible have burned off.



**Figure 1.** Positioning a crucible above a burner.

2. Accurately weigh three samples of unknown containing enough Fe to produce ~0.3 g of Fe<sub>2</sub>O<sub>3</sub>. Dissolve each sample in 10 mL of 3 M HCl (with heating, if necessary). If there are insoluble impurities, filter through qualitative filter paper and wash the filter very well with distilled water. Add 5 mL of 6 M HNO<sub>3</sub> to the filtrate, and boil for a few minutes to ensure that all iron is oxidized to Fe(III).
3. Dilute the sample to 200 mL with distilled water and add 3 M ammonia<sup>†</sup> with constant stirring until the solution is basic (as determined with litmus paper or pH indicator paper). Digest the precipitate by boiling for 5 min and allow the precipitate to settle. (<sup>†</sup> Basic reagents should not be stored in glass bottles because they will slowly dissolve the glass. If ammonia from a glass bottle is used, it may contain silica particles and should be freshly filtered.)



8. Cool the crucible briefly in air and then in a desiccator for 30 min. Weigh the crucible and the lid, reignite, and bring to constant mass (within 0.3 mg) with repeated heatings.
9. Calculate the weight percent of iron in each sample, the average, the standard deviation, and the relative standard deviation ( $s/\bar{x}$ ).

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1. D. A. Skoog and D. M. West, *Fundamentals of Analytical Chemistry*, 3d ed. (New York: Holt, Rinehart and Winston, 1976).
  2. Thorn Smith Inc., 7755 Narrow Gauge Road, Beulah, MI 49617. Phone: 231-882-4672; e-mail: [www.thornsmithlabs.com](http://www.thornsmithlabs.com).

## 4. Penny Stastics<sup>1</sup>

U.S. pennies minted after 1982 have a Zn core with a Cu overlayer. Prior to 1982, pennies were made of brass, with a uniform composition (95 wt % Cu / 5 wt % Zn). In 1982, both the heavier brass coins and the lighter zinc coins were made. In this experiment, your class will weigh many coins and pool the data to answer the following questions: (1) Do pennies from different years have the same mass? (2) Do pennies from different mints have the same mass? (3) Do the masses follow a Gaussian distribution?

### Gathering Data

Each student should collect and weigh enough pennies to the nearest milligram to provide a total set that contains 300 to 500 brass coins and a similar number of zinc coins. Instructions are given for a spreadsheet, but the same operations can be carried out with a calculator. Compile all the class data in a spreadsheet. Each column should list the masses of pennies from only one calendar year. Use the spreadsheet "sort" function to sort each column so that the lightest mass is at the top of the column and the heaviest is at the bottom. (To sort a column, click on the column heading to select the entire column, go to the DATA menu, select the SORT tool, and follow the directions that come up.) There will be two columns for 1982, in which both types of coins were made. Select a year other than 1982 for which you have many coins and divide the coins into those made in Denver (with a "D" beneath the year) and those minted in Philadelphia (with no mark beneath the year).

### Discrepant Data

At the bottom of each column, compute the mean and standard deviation. Retain at least one extra digit beyond the milligram place to avoid round-off errors in your calculations.

Damaged or corroded coins may have masses different from those of the general population. Discard grossly discrepant masses lying 4 standard deviations from the mean in any one year. (For example, if one column has an average mass of 3.000 g and a standard deviation of 0.030 g, the 4-standard-deviation limit is  $\pm(4 \times 0.030) = \pm 0.120$  g. A mass that is 2.880 or 3.120 g should be discarded.) After rejecting discrepant data, recompute the average and standard deviation for each column.

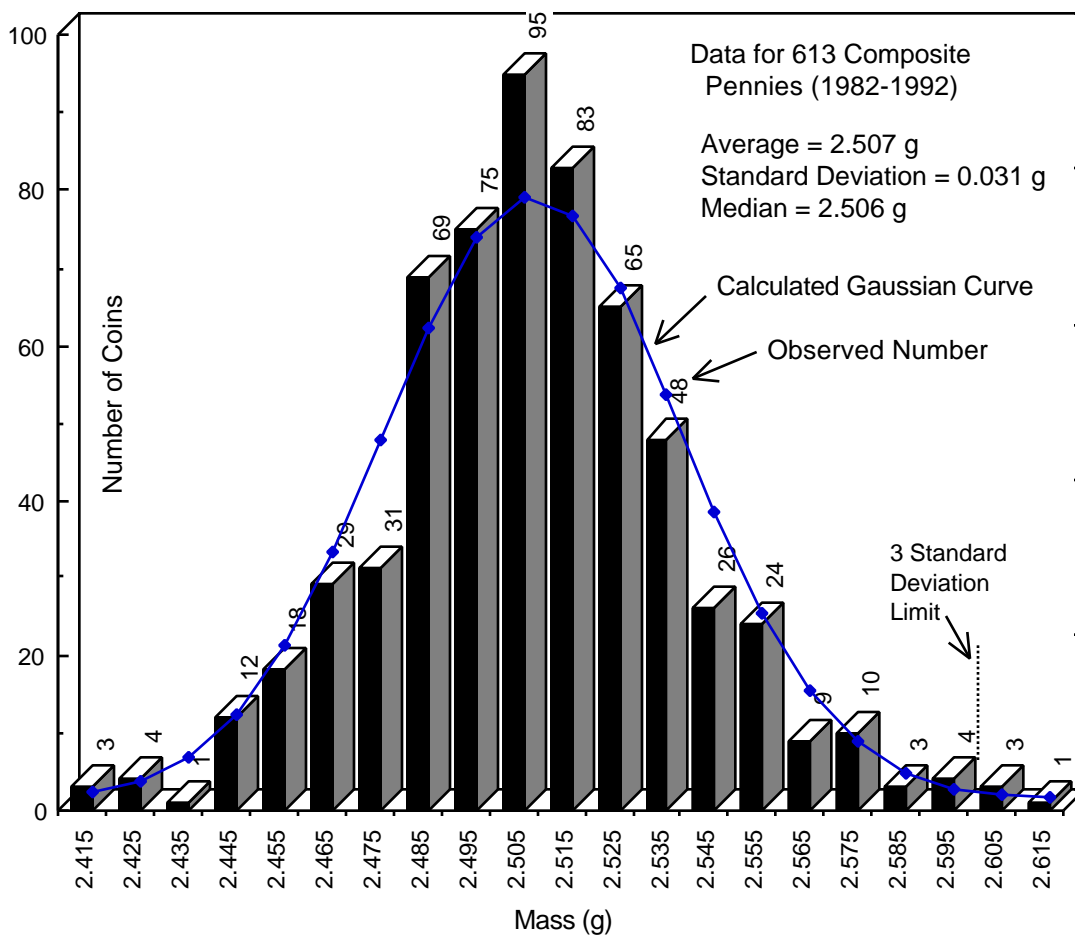
### Confidence Intervals and *t* Test

Select the two years ( 1982) in which the zinc coins have the highest and lowest average masses. For each of the two years, compute the 95% confidence interval. Use the *t* test to compare the two mean values at the 95% confidence level. Are the two average masses significantly different? Try the same for two years of brass coins ( 1982). Try the same for the one year whose coins you segregated into those from Philadelphia and those from Denver. Do the two mints produce coins with the same mass?

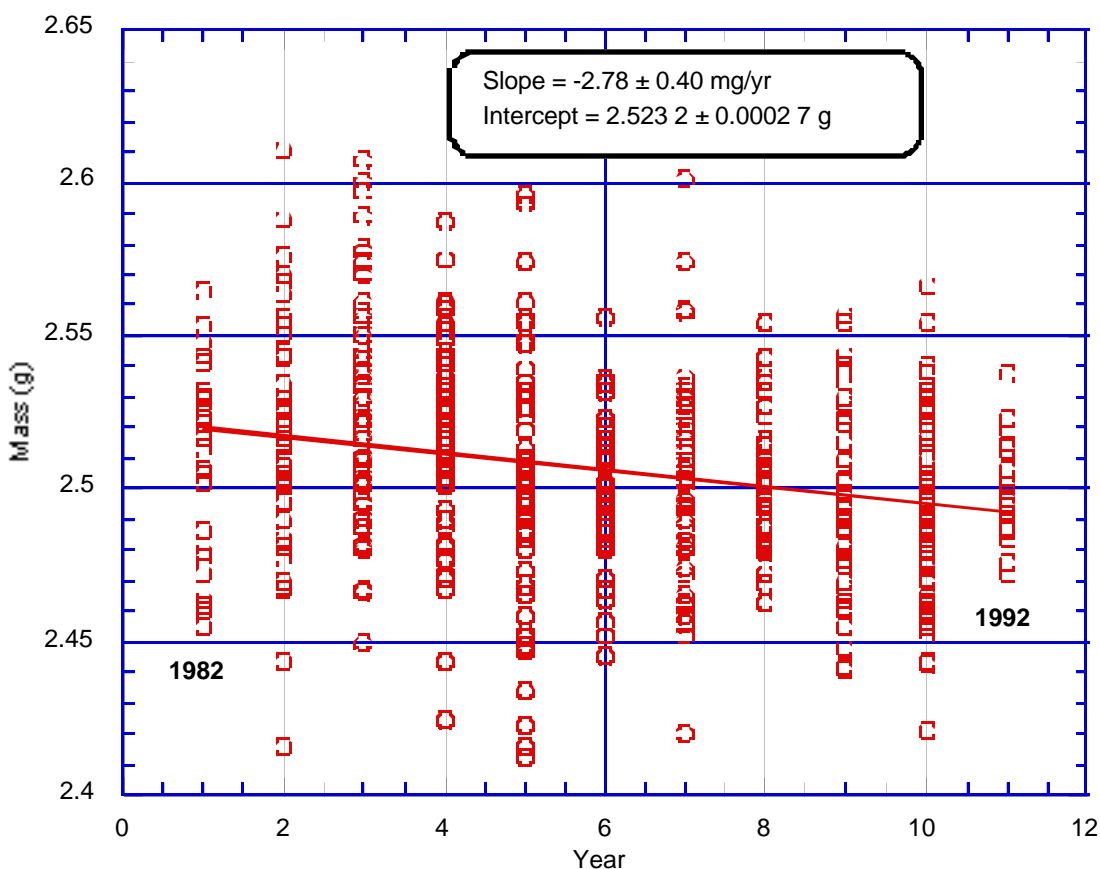
### Distribution of Masses

List the masses of all pennies made in or after 1983 in a single column, sorted from lowest to

highest mass. There should be at least 300 masses listed. Divide the data into 0.01-g intervals (e.g., 2.480 to 2.489 g) and prepare a bar graph, like that shown in Figure 1. Find the mean  $\bar{x}$ , median, and standard deviation ( $s$ ) for all coins in the graph. For random (Gaussian) data, only 3 out of 1 000 measurements should lie outside of  $\bar{x} \pm 3s$ . Indicate which bars (if any) lie beyond  $\pm 3s$ . In Figure 1 two bars at the right are outside of  $\bar{x} \pm 3s$ .



**Figure 1.** Distribution of penny masses from the years 1982 to 1992 measured in Dan's house by Jimmy Kusznir and Doug Harris in December 1992.



**Figure 2.** Penny mass versus year for 612 coins. Because the slope of the least-squares line is *significantly* less than 0, we conclude that the average mass of older pennies is greater than the average mass of newer pennies.

### Least-Squares Analysis: Do Pennies Have the Same Mass Each Year?

Prepare a graph like Figure 2 in which the ordinate ( $y$ -axis) is the mass of zinc pennies minted each year since 1982 and the abscissa ( $x$ -axis) is the year. For simplicity, let 1982 be year 1, 1983 be year 2, and so on. If the mass of a penny increases systematically from year to year, then the least-squares line through the data will have a positive slope. If the mass decreases, the slope will be negative. If the mass is constant, the slope will be 0. Even if the mass is really constant, your selection of coins is random and the slope is not exactly 0.

We want to know whether the slope is *significantly* different from zero. Suppose that you have data for 14 years. Enter all of the data into two columns of the least-squares spreadsheet in Figure 5-9 in the textbook. Column B ( $x_i$ ) is the year (1 to 14) and column C ( $y_i$ ) is the mass of each penny. Your table will have several hundred entries. (If you are not using a spreadsheet, just tabulate the average mass for each year. Your table will have only 14 entries.) Calculate the slope ( $m$ ) and intercept ( $b$ ) of the best straight line through all points and find the uncertainties in slope ( $s_m$ ) and intercept ( $s_b$ ).

Use Student's  $t$  to find the 95% confidence interval for the slope:

$$\text{confidence interval for slope} = m \pm ts_m \quad (1)$$

where Student's  $t$  is for  $n-2$  degrees of freedom. For example, if you have  $n = 300$  pennies,  $n-2 = 298$ , and it would be reasonable to use the value of  $t (= 1.960)$  at the bottom of the table for  $n =$  . If the least-squares slope is  $m \pm s_m = -2.78 \pm 0.40$  mg/year, then the 95% confidence interval is  $m \pm ts_m = -2.78 \pm (1.960)(0.40) = -2.78 \pm 0.78$  mg/year.

The 95% confidence interval is  $-2.78 \pm 0.78 = -3.56$  to  $-2.00$  mg/year. We are 95% confident that the true slope is in this range and is, therefore, *not* 0. We conclude that older zinc pennies are heavier than newer zinc pennies.

### Does the Distribution of Masses Follow a Gaussian Distribution?

The smooth Gaussian curve superimposed on the data in Figure 1 was calculated from the formula

$$y_{\text{calc}} = \frac{\text{number of coins}/100}{s\sqrt{2}} e^{-(x-\bar{x})^2/2s^2} \quad (2)$$

Now we carry out a  $\chi^2$  test (pronounced “ki squared”) to see if the observed distribution (the bars in Figure 1) agrees with the Gaussian curve. The statistic  $\chi^2$  is given by

$$\chi^2 = \frac{(y_{\text{obs}} - y_{\text{calc}})^2}{y_{\text{calc}}} \quad (3)$$

where  $y_{\text{obs}}$  is the height of a bar on the chart,  $y_{\text{calc}}$  is the ordinate of the Gaussian curve (Equation 2), and the sum extends over all bars in the graph. The calculations for the data in Figure 1 are shown in Table 1.

At the bottom of Table 1 we see that  $\chi^2$  for all 21 bars is 43.231. In Table 2 we find a critical value of 31.4 for 20 degrees of freedom (degrees of freedom = one less than number of categories). Because  $\chi^2$  from Equation 2 exceeds the critical value, we conclude that *the distribution is not Gaussian*.

It would be reasonable to omit the smallest bars at the edge of the graph from the calculation of  $\chi^2$  because these bars contain the fewest observations but make large contributions to  $\chi^2$ . Suppose that we reject bars lying  $>3$  standard deviations from the mean. This removes the two bars at the right side of Figure 1 which give the last two entries in Table 1. Omitting these two points gives  $\chi^2 = 30.277$ , which is still greater than the critical value of 28.9 for 18 degrees of freedom in Table 2. Our conclusion is that at the 95% confidence level the observed distribution in Figure 1 is not quite Gaussian. It is possible that exceptionally light coins are nicked and exceptionally heavy coins are dirty or corroded. You would need to inspect these

coins to verify this hypothesis.

**Table 1. Calculation of  $\chi^2$  for Figure 1**

Mass (g)	Observed number of coins	Calculated ordinate of Gaussian curve	$y_{\text{obs}} - y_{\text{calc}}$	$\frac{(y_{\text{obs}} - y_{\text{calc}})^2}{y_{\text{calc}}}$
(x)	( $y_{\text{obs}}$ )	( $y_{\text{calc}}$ )		
2.415	3	1.060	1.940	3.550
2.425	4	2.566	1.434	0.801
2.435	1	5.611	-4.611	3.789
2.445	12	11.083	0.917	0.076
2.455	18	19.776	-1.776	0.159
2.465	29	31.875	-2.875	0.259
2.475	31	46.409	-15.409	5.116
2.485	69	61.039	7.961	1.038
2.495	75	72.519	2.481	0.085
2.505	95	77.829	17.171	3.788
2.515	83	75.453	7.547	0.755
2.525	65	66.077	-1.077	0.176
2.535	48	52.272	-4.272	0.349
2.545	26	37.354	-11.354	3.451
2.555	24	24.112	-0.112	0.001
2.565	9	14.060	-5.060	1.821
2.575	10	7.406	2.594	0.909
2.585	3	3.524	-0.524	0.078
2.595	4	1.515	2.485	4.076
2.605	3	0.588	2.412	9.894
2.615	1	0.206	0.794	3.060
			$\chi^2$ (all 21 points) =	<b>43.231</b>
			$\chi^2$ (19 points — omitting bottom two points) =	<b>30.277</b>

**Table 2. Critical values of  $\chi^2$  that will be exceeded in 5% of experiments\***

Degrees of freedom	Critical value	Degrees of freedom	Critical value	Degrees of freedom	Critical value
1	3.84	11	19.7	21	32.7
2	5.99	12	21.0	22	33.9
3	7.81	13	22.4	23	35.2
4	9.49	14	23.7	24	36.4
5	11.1	15	25.0	25	37.7
6	12.6	16	26.3	26	38.9
7	14.1	17	27.6	27	40.1
8	15.5	18	28.9	28	41.3
9	16.9	19	30.1	29	42.6
10	18.3	20	31.4	30	43.8

\**Example:* The value of  $\chi^2$  from 15 observations is 17.2. This value is less than 23.7 listed for 14 (= 15-1) degrees of freedom. Because  $\chi^2$  does not exceed the critical value, the observed distribution is consistent with the theoretical distribution.

## Reporting Your Results

### Gathering data:

1. Attach a table of masses, with one column sorted by mass for each year.
2. Divide 1982 into two columns, one for light (zinc) and one for heavy (brass) pennies.
3. One year should be divided into one column from Denver and one from Philadelphia.

### Discrepant data:

1. List the mean ( $\bar{x}$ ) and standard deviation ( $s$ ) for each column.
2. Discard data that lie outside of  $\bar{x} \pm 4s$  and recompute  $\bar{x}$  and  $s$ .

### Confidence intervals and $t$ test:

1. For the year 1982 with highest average mass:

$$95\% \text{ confidence interval } (= \bar{x} \pm ts/\sqrt{n}) = \underline{\hspace{4cm}}$$

For the year 1982 with lowest average mass:

$$95\% \text{ confidence interval } = \underline{\hspace{4cm}}$$

Comparison of means with  $t$ -test:

$$t_{\text{calculated}} = \underline{\hspace{2cm}}$$

$$t_{\text{table}} = \underline{\hspace{2cm}}$$

Is the difference significant?  $\underline{\hspace{2cm}}$

2. For the year 1982 with highest average mass:  
 95% confidence interval = \_\_\_\_\_  
 For the year 1982 with lowest average mass:  
 95% confidence interval = \_\_\_\_\_  
 Comparison of means with  $t$  test:  
 $t_{\text{calculated}} =$  \_\_\_\_\_  
 $t_{\text{table}} =$  \_\_\_\_\_  
 Is the difference significant? \_\_\_\_\_
3. For Philadelphia versus Denver coins in one year:  
 Philadelphia 95% confidence interval = \_\_\_\_\_  
 Denver 95% confidence interval = \_\_\_\_\_  
 Comparison of means with  $t$  test:  
 $t_{\text{calculated}} =$  \_\_\_\_\_  
 $t_{\text{table}} =$  \_\_\_\_\_  
 Is the difference significant? \_\_\_\_\_

*Gaussian distribution of masses:*

Prepare a graph analogous to Figure 1 with labels showing the  $\pm 3s$  limits.

*Least-squares Analysis:*

Prepare a graph analogous to Figure 2 and find the least-squares slope and intercept and their standard deviations.

$m \pm s_m =$  \_\_\_\_\_

$t_{95\% \text{ confidence}} =$  \_\_\_\_\_  $t_{99\% \text{ confidence}} =$  \_\_\_\_\_

95% confidence:  $m \pm ts_m =$  \_\_\_\_\_.

Does interval include zero? \_\_\_\_\_

99% confidence:  $m \pm ts_m =$  \_\_\_\_\_.

Does interval include 0? \_\_\_\_\_

Is there a systematic increase or decrease of penny mass with year? \_\_\_\_\_

$\chi^2$  test:

Write Equation 2 for the smooth Gaussian curve that fits your bar graph.

Construct a table analogous to Table 1 to compute  $\chi^2$  for the complete data set.

Computed value of  $\chi^2 =$  \_\_\_\_\_ Degrees of freedom = \_\_\_\_\_

Critical value of  $\chi^2 =$  \_\_\_\_\_

Do the data follow a Gaussian distribution? \_\_\_\_\_

Now omit bars on the graph that are greater than 3 standard deviations away from the mean and calculate a new value of  $\chi^2$  with the reduced data set.

Computed value of  $\chi^2 =$  \_\_\_\_\_ Degrees of freedom = \_\_\_\_\_

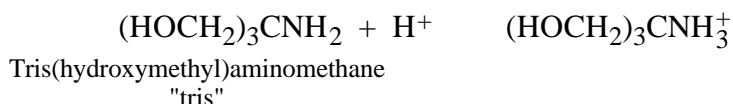
Critical value of  $\chi^2 =$  \_\_\_\_\_

Does the reduced data set follow a Gaussian distribution? \_\_\_\_\_

- 
1. T. H. Richardson, *J. Chem. Ed.* **1991**, *68*, 310. In a related experiment, students measure the mass of copper in the penny as a function of the year of minting: R. J. Stolzberg, *J. Chem. Ed.* **1998**, *75*, 1453.

## 5. Statistical Evaluation of Acid-Base Indicators

This experiment introduces you to the use of indicators and to the statistical concepts of mean, standard deviation,  $Q$  test, and  $t$  test.<sup>1</sup> You will compare the accuracy of different indicators in locating the end point in the titration of the base "tris" with hydrochloric acid:



### REAGENTS

*~0.1 M HCl*: Each student needs ~500 mL of unstandardized solution, all from a single batch that will be analyzed by the whole class.

*Tris*: Solid, primary standard powder should be available (~4 g/student).

*Indicators*: Bromothymol blue (BB), methyl red (MR), bromocresol green (BG), methyl orange (MO), and erythrosine (E) should be available in dropper bottles. See Table 12-4 in the textbook for their preparation.

Color changes to use for the titration of tris with HCl are

BB: blue (pH 7.6)    yellow (pH 6.0) (end point is disappearance of green)

MR: yellow (pH 6.0)    red (pH 4.8) (end point is disappearance of orange)

BG: blue (pH 5.4)    yellow (pH 3.8) (end point is green)

MO: yellow (pH 4.4)    red (pH 3.1) (end point is first appearance of orange)

E: red (pH 3.6)    orange (pH 2.2) (end point is first appearance of orange)

### PROCEDURE

Each student should carry out the following procedure with *one* of the indicators. Different students should be assigned different indicators so that at least four students evaluate each of the indicators.

1. Calculate the molecular mass of tris and the mass required to react with 35 mL of 0.10 M HCl. Weigh this much tris into a 125-mL flask.
2. It is good practice to rinse a buret with a new solution to wash away traces of previous reagents. Wash your 50-mL buret with three 10-mL portions of 0.1 M HCl and discard the washings. Tilt and rotate the buret so that the liquid washes the walls, and drain the liquid through the stopcock. Then fill the buret with 0.1 M HCl to near the 0-mL mark, allow a minute for the liquid to settle, and record the reading to the nearest 0.01 mL.

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1. D. T. Harvey, *J. Chem. Ed.* **1991**, 68, 329.

3. The first titration will be rapid, to allow you to find the approximate end point of the titration. Add ~20 mL of HCl from the buret to the flask and swirl to dissolve the tris. Add 2–4 drops of indicator and titrate with ~1-mL aliquots of HCl to find the end point.
4. From the first titration, calculate how much tris is required to cause each succeeding titration to require 35–40 mL of HCl. Weigh this much tris into a clean flask. Refill your buret to near 0 mL and record the reading. Repeat the titration in Step 3, but use 1 drop at a time near the end point. When you are very near the end point, use less than a drop at a time. To do this, carefully suspend a fraction of a drop from the buret tip and touch it to the inside wall of the flask. Carefully tilt the flask so that the bulk solution overtakes the droplet and then swirl the flask to mix the solution. Record the total volume of HCl required to reach the end point to the nearest 0.01 mL. Calculate the true mass of tris with the buoyancy equation 2-1 in the textbook (density of tris = 1.327 g/mL). Calculate the molality of HCl.
5. Repeat the titration to obtain at least six accurate measurements of the HCl molarity.
6. Use the  $Q$  test in Section 4-6 in the textbook) to decide whether any results should be discarded. Report your retained values, their mean, their standard deviation, and the relative standard deviation ( $s/\bar{x}$ ).

### DATA ANALYSIS

Pool the data from your class to fill in Table 1, which shows two possible results. The quantity  $s_x$  is the standard deviation of all results reported by many students. The pooled standard deviation,  $s_p$ , is derived from the standard deviations reported by each student. If two students see the end point differently, each result might be very reproducible, but their reported molarities will be different. Together, they will generate a large value of  $s_x$  (because their results are so different), but a small value of  $s_p$  (because each one was reproducible).

Select the pair of indicators giving average HCl molarities that are farthest apart. Use the  $t$  test (Equation 4-8 in the textbook) to decide whether the average molarities are significantly different from each other at the 95% confidence level. When you calculate the pooled standard deviation for Equation 4-8, the values of  $s_1$  and  $s_2$  in Equation 4-9 in the textbook are the values of  $s_x$  (not  $s_p$ ) in Table 1. A condition for using Equations 4-8 and 4-9 is that the standard deviations for the two sets of measurements should not be “significantly different” from each other. Use the  $F$  test in Section 4-4 in the textbook to decide whether or not the standard deviations are significantly different. If they are significantly different, use Equations 4-8a and 4-9a for the  $t$  test.

Select the pair of indicators giving the second most different molarities and use the  $t$  test again to see whether or not this second pair of results is significantly different.

**Table 1. Pooled data**

Indicator	Number of measurements	Number of students	Mean HCl molarity (M) <sup>a</sup>		Relative standard deviation (%)	
	( <i>n</i> )	( <i>S</i> )	( $\bar{x}$ )	$s_x$ (M) <sup>b</sup>	$100 s_x / \bar{x}$	$s_p$ (M) <sup>c</sup>
BB	28	5	0.095 65	0.002 25	2.35	0.001 09
MR						
BG	29	4	0.086 41	0.001 13	1.31	0.000 99
MO						
E						

- a. Computed from all values that were not discarded with the  $Q$  test.  
 b.  $s_x$  = standard deviation of all  $n$  measurements (degrees of freedom =  $n - 1$ )  
 c.  $s_p$  = pooled standard deviation for  $S$  students (degrees of freedom =  $n - S$ ). Computed with the equation

$$s_p = \sqrt{\frac{s_1^2(n_1-1) + s_2^2(n_2-1) + s_3^2(n_3-1) + \dots}{n-S}}$$

where there is one term in the numerator for each student using that indicator.

## REPORTING YOUR RESULTS

### *Your Individual Data*

Trial	Mass of tris from balance (g)	True mass corrected for buoyancy (g)	HCl volume (mL)	Calculated HCl molarity (M)
1				
2				
3				
4				
5				
6				

On the basis of the  $Q$  test, should any molarity be discarded? If so, which one? \_\_\_\_\_

Mean value of retained results: \_\_\_\_\_

Standard deviation: \_\_\_\_\_

Relative standard deviation (%): \_\_\_\_\_

### *Pooled Class Data*

1. Attach your copy of Table 1 with all entries filled in.
2. Compare the two most different molarities in Table 1. (Show your  $F$  and  $t$  tests and state your conclusion.)

3. Compare the two second most different molarities in Table 1.

## 6. Preparing Standard Acid and Base

Procedures for preparing standard 0.1 M HCl and standard 0.1 M NaOH are described at the end of Chapter 12 of the textbook. Unknown samples of sodium carbonate or potassium hydrogen phthalate (available from Thorn Smith<sup>1</sup>) can be analyzed by the procedures described in this section.

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1. Thorn Smith Inc., 7755 Narrow Gauge Road, Beulah, MI 49617. Phone: 231-882-4672;  
e-mail: [www.thornsmithlabs.com](http://www.thornsmithlabs.com).

## 7. Using a pH Electrode for an Acid-Base Titration

In this experiment you will use a pH electrode to follow the course of an acid-base titration. You will observe how pH changes slowly during most of the reaction and rapidly near the equivalence point. You will compute the first and second derivatives of the titration curve to locate the end point. From the mass of unknown acid or base and the moles of titrant, you can calculate the molecular mass of the unknown. Section 12-5 of the textbook provides background for this experiment.

### REAGENTS

*Standard 0.1 M NaOH and standard 0.1 M HCl:* From Experiment 6.

*Bromocresol green and phenolphthalein indicators:* See Table 12-4 in the textbook.

*pH calibration buffers:* pH 7 and pH 4. Use commercial standards.

*Unknowns:* Unknowns should be stored in a desiccator by your instructor.

*Suggested acid unknowns:* potassium hydrogen phthalate (Table 12-5, FM 204.23), 2-(*N*-morpholino)ethanesulfonic acid (MES, Table 10-2, FM 195.24), imidazole hydrochloride (Table 10-2, FM 104.54, hygroscopic), potassium hydrogen iodate (Table 12-5, FM 389.91).

*Suggested base unknowns:* tris (Table 12-5, FM 121.14), imidazole (FM 68.08), disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ , FM 141.96), sodium glycinate (may be found in chemical catalogs as glycine, sodium salt hydrate,  $\text{H}_2\text{NCH}_2\text{CO}_2\text{Na}\cdot x\text{H}_2\text{O}$ , FM 97.05 +  $x(18.015)$ ). For sodium glycinate, one objective of the titration is to find the number of waters of hydration from the molecular mass.

### PROCEDURE

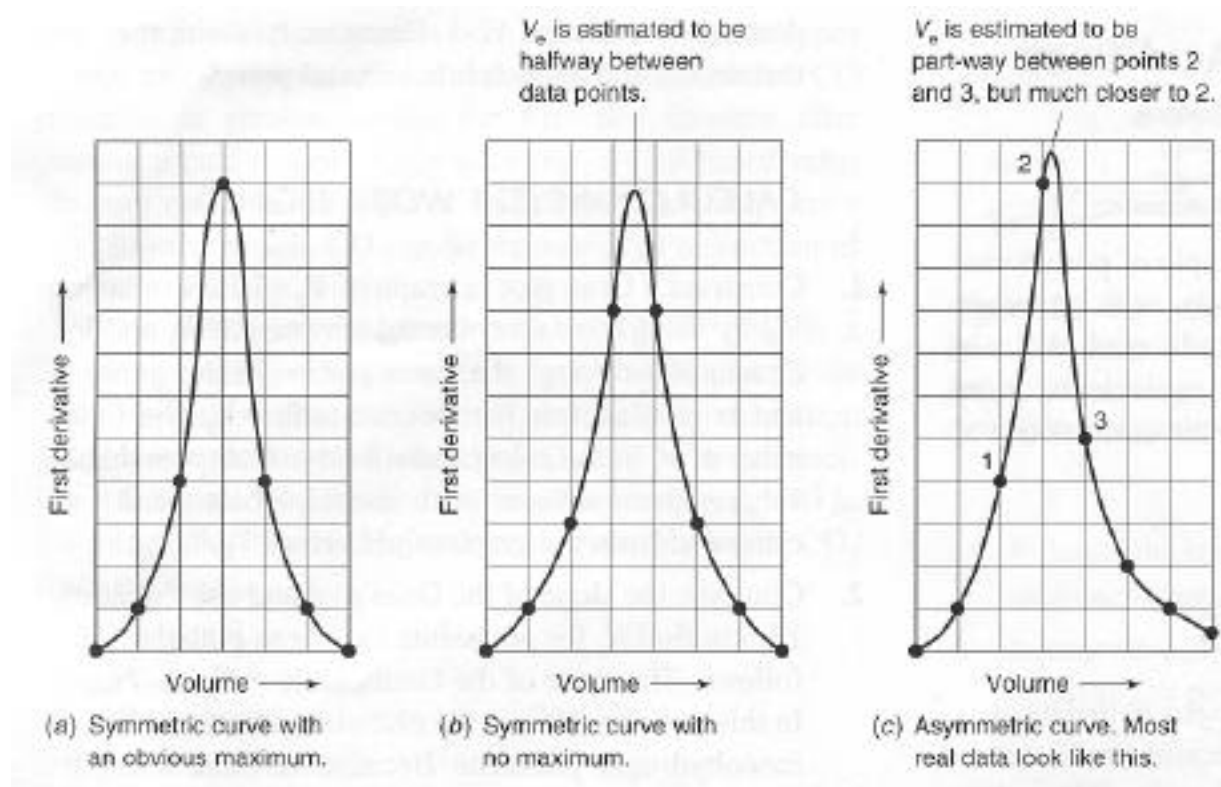
1. Your instructor will recommend a mass of unknown (5-8 mmol) for you to weigh accurately and dissolve in distilled water in a 250-mL volumetric flask. Dilute to the mark and mix well.
2. Following instructions for your particular pH meter, calibrate a meter and glass electrode, using buffers with pH values near 7 and 4. Rinse the electrodes well with distilled water and blot them dry with a tissue before immersing in any new solution.
3. The first titration is intended to be rough, so that you will know the approximate end point in the next titration. For the rough titration, pipet 25.0 mL of unknown into a 125-mL flask. If you are titrating an unknown acid, add 3 drops of phenolphthalein indicator and titrate with standard 0.1 M NaOH to the pink end point, using a 50-mL buret. If you are titrating an unknown base, add 3 drops of bromocresol green

indicator and titrate with standard 0.1 M HCl to the green end point. Add 0.5 mL of titrant at a time so that you can estimate the equivalence volume to within 0.5 mL. Near the end point, the indicator temporarily changes color as titrant is added. If you recognize this, you can slow down the rate of addition and estimate the end point to within a few drops.

4. Now comes the careful titration. Pipet 100.0 mL of unknown solution into a 250-mL beaker containing a magnetic stirring bar. Position the electrode(s) in the liquid so that the stirring bar will not strike the electrode. If a combination electrode is used, the small hole near the bottom on the side must be immersed in the solution. This hole is the salt bridge to the reference electrode. Allow the electrode to equilibrate for 1 min with stirring and record the pH.
5. Add 1 drop of indicator and begin the titration. The equivalence volume will be four times greater than it was in Step 3. Add ~1.5-mL aliquots of titrant and record the exact volume, the pH, and the color 30 s after each addition. When you are within 2 mL of the equivalence point, add titrant in 2-drop increments. When you are within 1 mL, add titrant in 1-drop increments. Continue with 1-drop increments until you are 0.5 mL past the equivalence point. The equivalence point has the most rapid change in pH. Add five more 1.5-mL aliquots of titrant and record the pH after each.

#### **DATA ANALYSIS**

1. Construct a graph of pH versus titrant volume. Mark on your graph where the indicator color change(s) was (were) observed.
2. Following the example in Table 12-3 and Figure 12-6 of the textbook, compute the first derivative (the slope,  $\text{pH}/\text{V}$ ) for each data point within  $\pm 1$  mL of the equivalence volume. From your graph, estimate the equivalence volume as accurately as you can, as shown in Figure 1.

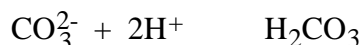


**Figure 1.** Examples of locating the maximum position of the first derivative of a titration curve.

3. Following the example in Table 12-3, compute the second derivative (the slope of the slope,  $(\text{slope})/V$ ). Prepare a graph like Figure 12-7 and locate the equivalence volume as accurately as you can.
4. Go back to your graph from Step 1 and mark where the indicator color changes were observed. Compare the indicator end point to the end point estimated from the first and second derivatives.
5. From the equivalence volume and the mass of unknown, calculate the molecular mass of the unknown.

## 8. Analysis of a Mixture of Carbonate and Bicarbonate

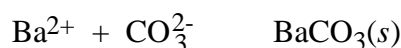
This procedure involves two titrations. First, total alkalinity ( $= [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}]$ ) is measured by titrating the mixture with standard HCl to a bromocresol green end point:



A separate aliquot of unknown is treated with excess standard NaOH to convert  $\text{HCO}_3^-$  to  $\text{CO}_3^{2-}$ :



Then all the carbonate is precipitated with  $\text{BaCl}_2$ :



The excess NaOH is immediately titrated with standard HCl to determine how much  $\text{HCO}_3^-$  was present. A blank titration is required for the  $\text{Ba}^{2+}$  addition because  $\text{Ba}^{2+}$  precipitates some of the NaOH:



From the total alkalinity and bicarbonate concentration, you can calculate the original carbonate concentration.

### REAGENTS

*Standard 0.1 M NaOH and standard 0.1 M HCl:* From Experiment 6.

*CO<sub>2</sub>-free water:* Boil 500 mL of distilled water to expel CO<sub>2</sub> and pour the water into a 500-mL plastic bottle. Screw the cap on tightly and allow the water to cool to room temperature. Keep tightly capped when not in use.

*10 wt % aqueous BaCl<sub>2</sub>:* 35 mL/student.

*Bromocresol green and phenolphthalein indicators:* See Table 12-4 in the textbook.

*Unknowns:* Solid unknowns (2.5 g/student) can be prepared from reagent-grade sodium carbonate or potassium carbonate and bicarbonate. Unknowns should be stored in a desiccator but should not be heated. Heating at 50°–100°C converts NaHCO<sub>3</sub> to Na<sub>2</sub>CO<sub>3</sub>.

## PROCEDURE

1. Accurately weigh 2.0–2.5 g of unknown into a 250-mL volumetric flask by weighing the sample in a capped weighing bottle, delivering some to a funnel in the volumetric flask, and reweighing the bottle. Continue this process until the desired mass of reagent has been transferred to the funnel. Rinse the funnel repeatedly with small portions of CO<sub>2</sub>-free water to dissolve the sample. Remove the funnel, dilute to the mark, and mix well.
2. *Total alkalinity:* Pipet a 25.00-mL aliquot of unknown solution into a 250-mL flask and titrate with standard 0.1 M HCl, using bromocresol green indicator as in Experiment 6 for standardizing HCl. Repeat this procedure with two more 25.00-mL aliquots.
3. *Bicarbonate content:* Pipet 25.00 mL of unknown and 50.00 mL of standard 0.1 M NaOH into a 250-mL flask. Swirl and add 10 mL of 10 wt % BaCl<sub>2</sub>, using a graduated cylinder. Swirl again to precipitate BaCO<sub>3</sub>, add 2 drops of phenolphthalein indicator, and immediately titrate with standard 0.1 M HCl. Repeat this procedure with two more 25.00-mL samples of unknown.
4. *Blank titration:* Titrate with standard 0.1 M HCl a mixture of 25 mL of water, 10 mL of 10 wt % BaCl<sub>2</sub>, and 50.00 mL of standard 0.1 M NaOH. Repeat this procedure twice more. The difference in moles of HCl needed in steps 4 and 3 equals the moles of bicarbonate in the mixture.
5. From the results of step 2, calculate the total alkalinity and its standard deviation. From the results of steps 3 and 4, calculate the bicarbonate concentration and its standard deviation. Using the standard deviations as estimates of uncertainty, calculate the concentration (and uncertainty) of carbonate in the sample. Express the composition of the solid unknown in a form such as 63.4 (±0.5) wt % K<sub>2</sub>CO<sub>3</sub> and 36.6 (±0.2) wt % NaHCO<sub>3</sub>.

## 9. Analysis of an Acid-Base Titration Curve: The Gran Plot

In this experiment, you will titrate a sample of pure potassium hydrogen phthalate (Table 12-5) with standard NaOH. The Gran plot (which you should read about in Section 12-5) will be used to find the equivalence point and  $K_a$ . Activity coefficients are used in the calculations of this experiment.

### PROCEDURE (AN EASY MATTER)

1. Dry about 1.5 g of potassium hydrogen phthalate at 105° C for 1 h and cool it in a desiccator for 20 min. Accurately weigh out ~1.5 g and dissolve it in water in a 250-mL volumetric flask. Dilute to the mark and mix well.
2. Following the instructions for your particular pH meter, calibrate a meter and glass electrode using buffers with pH values near 7 and 4. Rinse the electrode well with distilled water and blot it dry with a tissue before immersing in a solution.
3. Pipet 100.0 mL of phthalate solution into a 250-mL beaker containing a magnetic stirring bar. Position the electrode in the liquid so that the stirring bar will not strike the electrode. The small hole near the bottom on the side of the combination pH electrode must be immersed in the solution. This hole is the reference electrode salt bridge. Allow the electrode to equilibrate for 1 min (with stirring) and record the pH.
4. Add 1 drop of phenolphthalein indicator (Table 12-4) and titrate the solution with standard ~0.1 M NaOH. Until you are within 4 mL of the theoretical equivalence point, add base in ~1.5-mL aliquots, recording the volume and pH 30 s after each addition. Thereafter, use 0.4-mL aliquots until you are within 1 mL of the equivalence point. After that, add base 1 drop at a time until you have passed the pink end point by a few tenths of a milliliter. (Record the volume at which the pink color is observed.) Then add five more 1-mL aliquots.
5. Construct a graph of pH versus  $V_b$  (volume of added base). Locate the equivalence volume ( $V_e$ ) as the point of maximum slope or zero second derivative, as described in Section 12-5. Compare this with the theoretical and phenolphthalein end points.

### CALCULATIONS (THE WORK BEGINS!)

1. Construct a Gran plot (a graph of  $V_b 10^{-\text{pH}}$  versus  $V_b$ ) by using the data collected between  $0.9V_e$  and  $V_e$ . Draw a line through the linear portion of this curve and extrapolate it to the abscissa to find  $V_e$ . Use this value of  $V_e$  in the calculations below. Compare this value with those found with phenolphthalein and estimated from the graph of pH versus  $V_b$ .
2. Compute the slope of the Gran plot and use Equation 12-5 to find  $K_a$  for potassium hydrogen phthalate as follows: The slope of the Gran plot is  $-K_a \text{HP}^- / \text{P}^{2-}$ . In this equation,  $\text{P}^{2-}$  is the phthalate anion and  $\text{HP}^-$  is monohydrogen phthalate. Because the ionic strength

changes slightly as the titration proceeds, so also do the activity coefficients. Calculate the ionic strength at  $0.95V_e$ , and use this "average" ionic strength to find the activity coefficients.

---

**EXAMPLE Calculating Activity Coefficients**

Find  $\alpha_{\text{HP}^-}$  and  $\alpha_{\text{P}^{2-}}$  at  $0.95 V_e$  in the titration of 100.0 mL of 0.020 0 M potassium hydrogen phthalate with 0.100 M NaOH.

**Solution** The equivalence point is 20.0 mL, so  $0.95V_e = 19.0$  mL. The concentrations of  $\text{H}^+$  and  $\text{OH}^-$  are negligible compared with those of  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{HP}^-$ , and  $\text{P}^{2-}$ , whose concentrations are

$$[\text{K}^+] = \frac{100 \text{ mL}}{119 \text{ mL}} (0.020 0 \text{ M}) = 0.016 8 \text{ M}$$

$$[\text{Na}^+] = \frac{19 \text{ mL}}{119 \text{ mL}} (0.100 \text{ M}) = 0.016 0 \text{ M}$$

$$[\text{HP}^-] = (0.050) \frac{100 \text{ mL}}{119 \text{ mL}} (0.020 0 \text{ M}) = 0.000 84 \text{ M}$$

$$[\text{P}^{2-}] = (0.95) \frac{100 \text{ mL}}{119 \text{ mL}} (0.020 0 \text{ M}) = 0.0160 \text{ M}$$

The ionic strength is

$$\begin{aligned} \mu &= \frac{1}{2} \sum c_i z_i^2 \\ &= \frac{1}{2} [(0.016 8) \cdot 1^2 + (0.016 0) \cdot 1^2 + (0.000 84) \cdot 1^2 + (0.016 0) \cdot 2^2] = 0.048 8 \text{ M} \end{aligned}$$

To estimate  $\alpha_{\text{P}^{2-}}$  and  $\alpha_{\text{HP}^-}$  at  $\mu = 0.048 8 \text{ M}$ , interpolate in Table 8-1. In this table, we find that the hydrated radius of  $\text{P}^{2-}$  —phthalate,  $\text{C}_6\text{H}_4(\text{CO}_2^-)_2$ —is 600 pm. The size of  $\text{HP}^-$  is not listed, but we will suppose that it is also 600 pm. An ion with charge  $\pm 2$  and a size of 600 pm has  $\alpha = 0.485$  at  $\mu = 0.05 \text{ M}$  and  $\alpha = 0.675$  at  $\mu = 0.01 \text{ M}$ . Interpolating between these values, we estimate  $\alpha_{\text{P}^{2-}} = 0.49$  when  $\mu = 0.048 8 \text{ M}$ . Similarly, we estimate  $\alpha_{\text{HP}^-} = 0.84$  at this same ionic strength.

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- From the measured slope of the Gran plot and the values of  $\alpha_{\text{HP}^-}$  and  $\alpha_{\text{P}^{2-}}$ , calculate  $\text{p}K_a$ . Then choose an experimental point near  $\frac{1}{3}V_e$  and one near  $\frac{2}{3}V_e$ . Use Equation 10-18 to find  $\text{p}K_a$  with each point. (You will have to calculate  $[\text{P}^{2-}]$ ,  $[\text{HP}^-]$ ,  $\alpha_{\text{P}^{2-}}$ , and  $\alpha_{\text{HP}^-}$  at each point.) Compare the average value of  $\text{p}K_a$  from your experiment with  $\text{p}K_2$  for phthalic acid listed in Appendix G.

## 10. Kjeldahl Nitrogen Analysis

The Kjeldahl nitrogen analysis is widely used to measure the nitrogen content of pure organic compounds and complex substances such as milk, cereal, and flour. Digestion in boiling  $\text{H}_2\text{SO}_4$  with a catalyst converts organic nitrogen into  $\text{NH}_4^+$ . The solution is then made basic, and the liberated  $\text{NH}_3$  is distilled into a known amount of  $\text{HCl}$  (Reactions 7-3 to 7-5 in the textbook). Unreacted  $\text{HCl}$  is titrated with  $\text{NaOH}$  to determine how much  $\text{HCl}$  was consumed by  $\text{NH}_3$ . Because the solution to be titrated contains both  $\text{HCl}$  and  $\text{NH}_4^+$ , we choose an indicator that permits titration of  $\text{HCl}$  without beginning to titrate  $\text{NH}_4^+$ . Bromocresol green, with a transition range of pH 3.8 to 5.4, fulfills this purpose.

The Kjeldahl digestion captures amine ( $-\text{NR}_2$ ) or amide ( $-\text{C}[\text{=O}]\text{NR}_2$ ) nitrogens (where  $\text{R}$  can be  $\text{H}$  or an organic group), but not oxidized nitrogen such as nitro ( $-\text{NO}_2$ ) or azo ( $-\text{N}=\text{N}-$ ) groups, which must be reduced first to amines or amides.

### REAGENTS

*Standard NaOH and standard HCl:* From Experiment 6.

*Bromocresol green and phenolphthalein indicators:* See Table 12-4 in the textbook.

*Potassium sulfate:* 10 g/student.

*Selenium-coated boiling chips:* Hengar selenium-coated granules are convenient. Alternative catalysts are 0.1 g of  $\text{Se}$ , 0.2 g of  $\text{CuSeO}_3$ , or a crystal of  $\text{CuSO}_4$ .

*Concentrated (98 wt %)  $\text{H}_2\text{SO}_4$ :* 25 mL/student

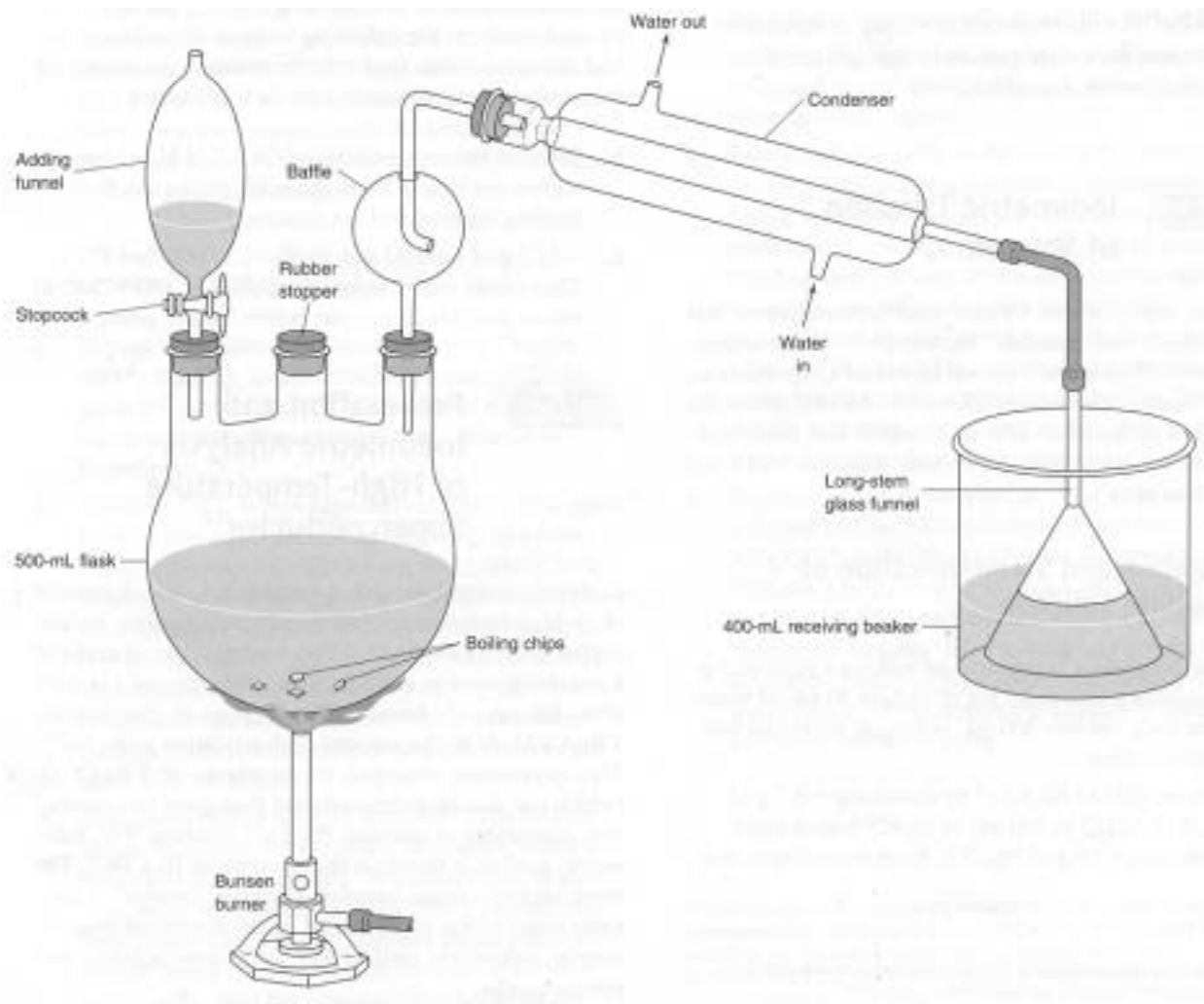
*Unknowns:* Unknowns are pure acetanilide,  $\text{N}$ -2-hydroxyethylpiperazine- $\text{N}'$ -2-ethanesulfonic acid (HEPES buffer, Table 10-2 in the textbook), tris(hydroxymethyl)aminomethane (tris buffer, Table 10-2), or the  $p$ -toluenesulfonic acid salts of ammonia, glycine, or nicotinic acid. Each student needs enough unknown to produce 2-3 mmol of  $\text{NH}_3$ .

### DIGESTION

1. Dry your unknown at  $105^\circ\text{C}$  for 45 min and accurately weigh an amount that will produce 2-3 mmol of  $\text{NH}_3$ . Place the sample in a *dry* 500-mL Kjeldahl flask (Figure 7-2 in the textbook), so that as little as possible sticks to the walls. Add 10 g of  $\text{K}_2\text{SO}_4$  (to raise the boiling temperature) and three selenium-coated boiling chips. Pour in 25 mL of 98 wt %  $\text{H}_2\text{SO}_4$ , washing down any solid from the walls. (**CAUTION:** Concentrated  $\text{H}_2\text{SO}_4$  eats people. If you get any on your skin, flood it immediately with water, followed by soap and water.)
2. In a fume hood, clamp the flask at a  $30^\circ$  angle away from you. Heat gently with a burner until foaming ceases and the solution becomes homogeneous. Continue boiling gently for an

additional 30 min.

3. Cool the flask for 30 min *in the air*, and then in an ice bath for 15 min. Slowly, and with constant stirring, add 50 mL of ice-cold, distilled water. Dissolve any solids that crystallize. Transfer the liquid to the 500-mL 3-neck distillation flask in Figure 1. Wash the Kjeldahl flask five times with 10-mL portions of distilled water, and pour the washings into the distillation flask.



**Figure 1.** Apparatus for Kjeldahl Distillation.

## DISTILLATION

1. Set up the apparatus in Figure 1 and tighten the connections well. Pipet 50.00 mL of

standard 0.1 M HCl into the receiving beaker and clamp the funnel in place below the liquid level.

2. Add 5-10 drops of phenolphthalein indicator to the three-neck flask shown in Figure 23-3 and secure the stoppers. Pour 60 mL of 50 wt % NaOH into the adding funnel and drip this into the distillation flask over a period of 1 min until the indicator turns pink. (**CAUTION:** 50 wt % NaOH eats people. Flood any spills on your skin with water.) Do not let the last 1 mL through the stopcock, so that gas cannot escape from the flask. Close the stopcock and heat the flask gently until two-thirds of the liquid has distilled.
3. Remove the funnel from the receiving beaker *before* removing the burner from the flask (to avoid sucking distillate back into the condenser). Rinse the funnel well with distilled water and catch the rinses in the beaker. Add 6 drops of bromocresol green indicator solution to the beaker and carefully titrate to the blue end point with standard 0.1 M NaOH. You are looking for the first appearance of light blue color. (Practice titrations with HCl and NaOH beforehand to familiarize yourself with the end point.)
4. Calculate the wt % of nitrogen in the unknown.

## 11. EDTA Titration of $\text{Ca}^{2+}$ and $\text{Mg}^{2+}$ in Natural Waters

The most common multivalent metal ions in natural waters are  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ . In this experiment, we will find the total concentration of metal ions that can react with EDTA, and we will assume that this equals the concentration of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ . In a second experiment,  $\text{Ca}^{2+}$  is analyzed separately after precipitating  $\text{Mg}(\text{OH})_2$  with strong base.

### REAGENTS

*Buffer (pH 10):* Add 142 mL of 28 wt % aqueous  $\text{NH}_3$  to 17.5 g of  $\text{NH}_4\text{Cl}$  and dilute to 250 mL with water.

*Eriochrome black T indicator:* Dissolve 0.2 g of the solid indicator in 15 mL of triethanolamine plus 5 mL of absolute ethanol.

*50 wt % NaOH:* Dissolve 100 g of NaOH in 100 g of  $\text{H}_2\text{O}$  in a 250-mL plastic bottle. Store tightly capped. When you remove solution with a pipet, try not to disturb the solid  $\text{Na}_2\text{CO}_3$  precipitate.

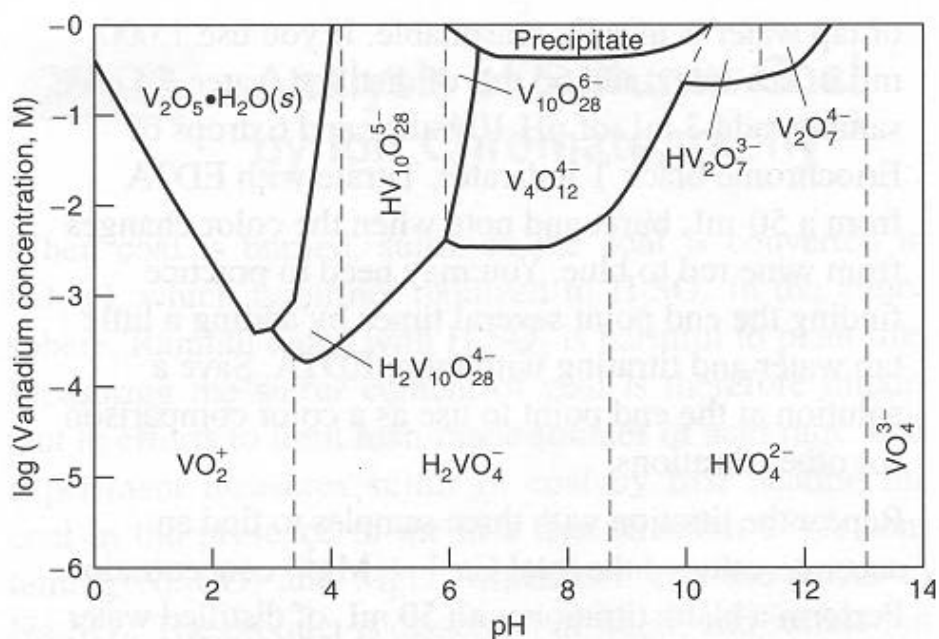
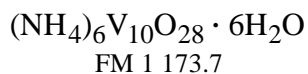
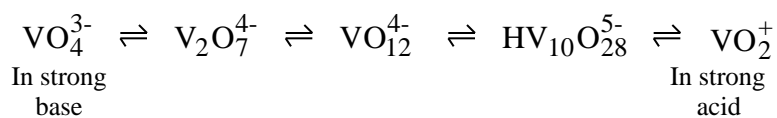
### PROCEDURE

1. Dry  $\text{Na}_2\text{H}_2\text{EDTA}\cdot 2\text{H}_2\text{O}$  (FM 372.24) at  $80^\circ\text{C}$  for 1 h and cool in the desiccator. Accurately weigh out  $\sim 0.6$  g and dissolve it with heating in 400 mL of water in a 500-mL volumetric flask. Cool to room temperature, dilute to the mark, and mix well.
2. Pipet a sample of unknown into a 250-mL flask. A 1.000-mL sample of seawater or a 50.00-mL sample of tap water is usually reasonable. If you use 1.000 mL of seawater, add 50 mL of distilled water. To each sample, add 3 mL of pH 10 buffer and 6 drops of Eriochrome black T indicator. Titrate with EDTA from a 50-mL buret and note when the color changes from wine red to blue. Practice finding the end point several times by adding a little tap water and titrating with more EDTA. Save a solution at the end point to use as a color comparison for other titrations.
3. Repeat the titration with three samples to find an accurate value of the total  $\text{Ca}^{2+} + \text{Mg}^{2+}$  concentration. Perform a blank titration with 50 mL of distilled water and subtract the value of the blank from each result.
4. For the determination  $\text{Ca}^{2+}$ , pipet four samples of unknown into clean flasks (adding 50 mL of distilled water if you use 1.000 mL of seawater). Add 30 drops of 50 wt % NaOH to each solution and swirl for 2 min to precipitate  $\text{Mg}(\text{OH})_2$  (which may not be visible). Add  $\sim 0.1$  g of solid hydroxynaphthol blue to each flask. (This indicator is used because it remains blue at higher pH than does Eriochrome black T.) Titrate one sample rapidly to find the end point; practice finding it several times, if necessary.

5. Titrate the other three samples carefully. After reaching the blue end point, allow each sample to sit for 5 min with occasional swirling so that any  $\text{Ca}(\text{OH})_2$  precipitate may redissolve. Then titrate back to the blue end point. (Repeat this procedure if the blue color turns to red upon standing.) Perform a blank titration with 50 mL of distilled water.
6. Calculate the total concentration of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , as well as the individual concentrations of each ion. Calculate the relative standard deviation of replicate titrations.

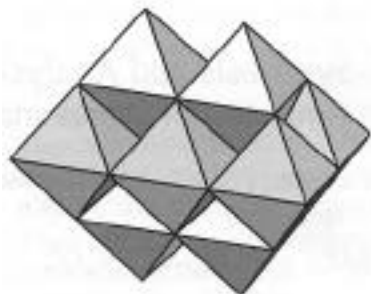
## 12. Synthesis and Analysis of Ammonium Decavanadate<sup>1</sup>

The species in a solution of  $V^{5+}$  depend on both pH and concentration (Figure 1).

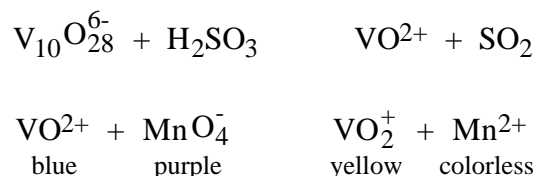


**Figure 1.** Phase diagram for aqueous vanadium(V) as a function of total vanadium concentration and pH. [From J. W. Larson, *J. Chem. Eng. Data* **1995**, *40*, 1276.]

The decavanadate ion ( $V_{10}O_{28}^{6-}$ ), which we will isolate in this experiment as the ammonium salt, consists of 10  $VO_6$  octahedra sharing edges with one another (Figure 2).



After preparing this salt, we will determine the vanadium content by a redox titration and  $\text{NH}_4^+$  by the Kjeldahl method. In the redox titration,  $\text{V}^{5+}$  will first be reduced to  $\text{V}^{4+}$  with sulfurous acid and then titrated with standard permanganate (Color Plate 9 in the textbook).



## REAGENTS

*Ammonium metavanadate* ( $\text{NH}_4\text{VO}_3$ ): 3 g/student.

*50 vol % aqueous acetic acid*: 4 mL/student.

*95 % ethanol*: 200 mL/student.

*KMnO<sub>4</sub>*: 1.6 g/student or prepare 0.02 M  $\text{KMnO}_4$  (~300 mL/student) for use by the class.

*Sodium Oxalate* ( $\text{Na}_2\text{C}_2\text{O}_4$ ): 1 g/student.

*0.9 M H<sub>2</sub>SO<sub>4</sub>*: (1 L/student) Slowly add 50 mL of concentrated (96–98 wt %)  $\text{H}_2\text{SO}_4$  to 900 mL of  $\text{H}_2\text{O}$  and dilute to ~1 L.

*1.5 M H<sub>2</sub>SO<sub>4</sub>*: (100 mL/student) Slowly add 83 mL of concentrated (96–98 wt %)  $\text{H}_2\text{SO}_4$  to 900 mL of  $\text{H}_2\text{O}$  and dilute to ~1 L.

*Sodium bisulfite* ( $\text{NaHSO}_3$ ): 2 g/student.

*Standard 0.1 M HCl*: (75 mL/student) From Experiment 6.

*Standard 0.1 M NaOH*: (75 mL/student) From Experiment 6.

*Phenolphthalein indicator*: See Table 12-4 in the textbook.

*Bromocreson green indicator*: See Table 12-4 in the textbook.

*50 wt % NaOH*: 60 mL/student. Mix 100 g NaOH with 100 mL  $\text{H}_2\text{O}$  and dissolve.

## SYNTHESIS

1. Heat 3.0 g of ammonium metavanadate ( $\text{NH}_4\text{VO}_3$ ) in 100 mL of water with constant stirring (but not boiling) until most or all of the solid has dissolved. Filter the solution and add 4 mL of 50 vol % aqueous acetic acid with stirring.

2. Add 150 mL of 95% ethanol with stirring and then cool the solution in a refrigerator or ice bath.
3. After maintaining a temperature of 0°–10° C for 15 min, filter the orange product with suction and wash with two 15-mL portions of ice-cold 95% ethanol.
4. Dry the product in the air (protected from dust) for 2 days. Typical yield is 2.0–2.5 g.

### **ANALYSIS OF VANADIUM WITH $\text{KMnO}_4$**

#### **Preparation and Standardization of $\text{KMnO}_4$ <sup>2</sup>** (See Section 16-4 in the textbook)

1. Prepare a 0.02 M permanganate solution by dissolving 1.6 g of  $\text{KMnO}_4$  in 500 mL of distilled water. Boil gently for 1 h, cover, and allow the solution to cool overnight. Filter through a clean, fine sintered-glass funnel, discarding the first 20 mL of filtrate. Store the solution in a clean glass amber bottle. Do not let the solution touch the cap.
2. Dry sodium oxalate ( $\text{Na}_2\text{C}_2\text{O}_4$ ) at 105° C for 1 h, cool in a desiccator, and weigh three ~0.25-g samples into 500-mL flasks or 400-mL beakers. To each, add 250 mL of 0.9 M  $\text{H}_2\text{SO}_4$  that has been recently boiled and cooled to room temperature. Stir with a thermometer to dissolve the sample, and add 90-95% of the theoretical amount of  $\text{KMnO}_4$  solution needed for the titration. (This can be calculated from the mass of  $\text{KMnO}_4$  used to prepare the permanganate solution. The chemical reaction is given by Equation 7-1 in the textbook.)
3. Leave the solution at room temperature until it is colorless. Then heat it to 55°–60° C and complete the titration by adding  $\text{KMnO}_4$  until the first pale pink color persists. Proceed slowly near the end, allowing 30 s for each drop to lose its color. As a blank, titrate 250 mL of 0.9 M  $\text{H}_2\text{SO}_4$  to the same pale pink color.

#### **Vanadium Analysis**

1. Accurately weigh two 0.3-g samples of ammonium decavanadate into 250-mL flasks and dissolve each in 40 mL of 1.5 M  $\text{H}_2\text{SO}_4$  (with warming, if necessary).
2. In a fume hood, add 50 mL of water and 1 g of  $\text{NaHSO}_3$  to each and dissolve with swirling. After 5 min, boil the solution gently for 15 min to remove  $\text{SO}_2$ .
3. Titrate the warm solution with standard 0.02 M  $\text{KMnO}_4$  from a 50-mL buret. The end point is taken when the yellow color of  $\text{VO}_2^+$  takes on a dark shade (from excess  $\text{MnO}_4^-$ ) that persists for 15 s.

## ANALYSIS OF AMMONIUM ION BY KJELDAHL DISTILLATION

1. Set up the apparatus in Figure 1 of Experiment 10 and press the stoppers to make airtight connections. Pipet 50.00 mL of standard 0.1 M HCl into the receiving beaker and clamp the funnel in place below the liquid level.
2. Transfer 0.6 g of accurately weighed ammonium decavanadate to the three-neck flask and add 200 mL of water. Add 5–10 drops of phenolphthalein indicator (Table 12-4 in the textbook) and secure the stoppers. Pour 60 mL of 50 wt % NaOH into the adding funnel and drip this into the distillation flask over a period of 1 min until the indicator turns pink. (*Caution:* 50 wt % NaOH eats people. Flood any spills on your skin with water.) Do not let the last 1 mL through the stopcock, so that gas cannot escape from the flask. Close the stopcock and heat the flask gently until two-thirds of the liquid has distilled.
3. Remove the funnel from the receiving beaker *before* removing the burner from the flask (to avoid sucking distillate back into the condenser). Rinse the funnel well with distilled water and catch the rinses in the beaker. Add 6 drops of bromocresol green indicator solution (Table 12-4) to the beaker and carefully titrate to the blue end point with standard 0.1 M NaOH. You are looking for the first appearance of light blue color. (Several practice titrations with HCl and NaOH will familiarize you with the end point.)
4. Calculate the weight percent of nitrogen in the ammonium decavanadate.

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1. G. G. Long, R. L. Stanfield, and F. C. Hentz, Jr., *J. Chem. Ed.* **1979**, 56, 195.
  2. R. M. Fowler and H. A. Bright, *J. Res. National Bureau of Standards* **1935**, 15, 493.

### 13. Iodimetric Titration of Vitamin C<sup>9</sup>

Ascorbic acid (vitamin C) is a mild reducing agent that reacts rapidly with triiodide (See Section 16-7 in the textbook). In this experiment, we will generate a known excess of I<sup>-</sup> by the reaction of iodate with iodide (Reaction 16-18), allow the reaction with ascorbic acid to proceed, and then back titrate the excess I<sup>-</sup> with thiosulfate (Reaction 16-19 and Color Plate 11).

#### Preparation and Standardization of Thiosulfate Solution

1. Prepare starch indicator by making a paste of 5 g of soluble starch and 5 mg of HgI<sub>2</sub> in 50 mL of water. Pour the paste into 500 mL of boiling water and boil until it is clear.
2. Prepare 0.07 M Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub><sup>2</sup> by dissolving ~8.7 g of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O in 500 mL of freshly boiled water containing 0.05 g of Na<sub>2</sub>CO<sub>3</sub>. Store this solution in a tightly capped amber bottle. Prepare ~0.01 M KIO<sub>3</sub> by accurately weighing ~1g of solid reagent and dissolving it in a 500-mL volumetric flask.
3. Standardize the thiosulfate solution as follows: Pipet 50.00 mL of KIO<sub>3</sub> solution into a flask. Add 2 g of solid KI and 10 mL of 0.5 M H<sub>2</sub>SO<sub>4</sub>. Immediately titrate with thiosulfate until the solution has lost almost all its color (pale yellow). Then add 2 mL of starch indicator and complete the titration. Repeat the titration with two additional 50.00-mL volumes of KIO<sub>3</sub> solution.

#### Analysis of Vitamin C

Commercial vitamin C containing 100 mg per tablet can be used. Perform the following analysis three times, and find the mean value (and relative standard deviation) for the number of milligrams of vitamin C per tablet.

1. Dissolve two tablets in 60 mL of 0.3 M H<sub>2</sub>SO<sub>4</sub>, using a glass rod to help break the solid. (Some solid binding material will not dissolve.)
2. Add 2 g of solid KI and 50.00 mL of standard KIO<sub>3</sub>. Then titrate with standard thiosulfate as above. Add 2 mL of starch indicator just before the end point.

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1. D. N. Bailey, *J. Chem. Ed.* **1974**, *51*, 488.

2. An alternative to standardizing Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution is to prepare anhydrous primary standard Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> by refluxing 21 g of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O with 100 mL of methanol for 20 min. Then filter the anhydrous salt, wash with 20 mL of methanol, and dry at 70°C for 30 min. [A. A. Woolf, *Anal. Chem.* **1982**, *54*, 2134.]

## 14. Preparation and Iodometric Analysis of a High-Temperature Superconductor<sup>1</sup>

In this experiment you will determine the oxygen content of yttrium barium copper oxide ( $\text{YBa}_2\text{Cu}_3\text{O}_x$ ). This material is an example of a *nonstoichiometric solid*, in which the value of  $x$  is variable, but near 7. Some of the copper in the formula  $\text{YBa}_2\text{Cu}_3\text{O}_7$  is in the unusual high-oxidation state,  $\text{Cu}^{3+}$ . This experiment describes the synthesis of  $\text{YBa}_2\text{Cu}_3\text{O}_x$ , (which can also be purchased) and then gives two alternative procedures to measure the  $\text{Cu}^{3+}$  content. The *iodometric method* is based on the reactions in Box 16-2 in the textbook. The more elegant *citrate-complexed copper titration*<sup>2</sup> eliminates many experimental errors associated with the simpler iodometric method and gives more accurate and precise results.

### Preparation of $\text{YBa}_2\text{Cu}_3\text{O}_x$

1. Place in a mortar 0.750 g of  $\text{Y}_2\text{O}_3$ , 2.622 g of  $\text{BaCO}_3$ , and 1.581 g of  $\text{CuO}$  (atomic ratio Y:Ba:Cu = 1:2:3). Grind the mixture well with a pestle for 20 min and transfer the powder to a porcelain crucible or boat. Heat in the air in a furnace at  $920^\circ\text{--}930^\circ\text{C}$  for 12 h or longer. Turn off the furnace and allow the sample to cool slowly *in the furnace*. This slow cooling step is critical for achieving an oxygen content in the range  $x = 6.5\text{--}7$  in the formula  $\text{YBa}_2\text{Cu}_3\text{O}_x$ . The crucible may be removed when the temperature is below  $100^\circ\text{C}$ .
2. Dislodge the black solid mass gently from the crucible and grind it to a fine powder with a mortar and pestle. It can now be used for this experiment, but better quality material is produced if the powder is heated again to  $920^\circ\text{--}930^\circ\text{C}$  and cooled slowly as in Step 1. If the powder from Step 1 is green instead of black, raise the temperature of the furnace by  $20^\circ\text{C}$  and repeat Step 1. The final product must be black, or it is not the correct compound.

### Iodometric Analysis

1. *Sodium Thiosulfate and Starch Indicator*. Prepare 0.03 M  $\text{Na}_2\text{S}_2\text{O}_3$  as described in Experiment 13, using 3.7 g of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  instead of 8.7 g. The starch indicator solution is the same one used in Experiment 13.
2. *Standard  $\text{Cu}^{2+}$* . Weigh accurately 0.5–0.6 g of reagent Cu wire into a 100-mL volumetric flask. In a fume hood, add 6 mL of distilled water and 3 mL of 70 wt % nitric acid, and boil gently on a hot plate until the solid has dissolved. Add 10 mL of distilled water and boil gently. Add 1.0 g of urea or 0.5 g of sulfamic acid and boil for 1 min to destroy  $\text{HNO}_2$  and oxides of nitrogen that would interfere with the iodometric titration. Cool to room temperature and dilute to the mark with 1.0 M HCl.
3. *Standardization of  $\text{Na}_2\text{S}_2\text{O}_3$  With  $\text{Cu}^{2+}$* . The titration should be carried out as rapidly as possible under a brisk flow of  $\text{N}_2$ , because  $\text{I}^-$  is oxidized to  $\text{I}_2$  in acid solution by atmospheric oxygen. Use a 180-mL tall-form beaker (or a 150-mL standard beaker) with a loosely fitting two-hole cork at the top. One hole serves as the inert gas inlet and the other is for the buret.

Pipet 10.00 mL of standard  $\text{Cu}^{2+}$  into the beaker and flush with  $\text{N}_2$ . Remove the cork just long enough to pour in 10 mL of distilled water containing 1.0–1.5 g of KI (freshly dissolved) and begin magnetic stirring. In addition to the dark color of iodine in the solution, suspended solid  $\text{CuI}$  will be present. Titrate with  $\text{Na}_2\text{S}_2\text{O}_3$  solution from a 50-mL buret, adding 2 drops of starch solution just before the last trace of  $\text{I}_2$  color disappears. If starch is added too soon, there can be irreversible attachment of  $\text{I}_2$  to the starch and the end point is harder to detect. You may want to practice this titration several times to learn to distinguish the colors of  $\text{I}_2$  and  $\text{I}_2/\text{starch}$  from the color of suspended  $\text{CuI}(s)$ . (Alternatively, Pt and calomel electrodes can be used instead of starch to eliminate subjective judgment of color in finding the end point.<sup>3</sup>) Repeat this standardization two more times and use the average  $\text{Na}_2\text{S}_2\text{O}_3$  molarity from the three determinations.

4. *Superconductor Experiment A.* Dissolve an accurately weighed 150- to 200-mg sample of powdered  $\text{YBa}_2\text{Cu}_3\text{O}_x$  in 10 mL of 1.0 M  $\text{HClO}_4$  in a titration beaker in a fume hood. (Perchloric acid is recommended because it is inert to reaction with superconductor, which might oxidize  $\text{HCl}$  to  $\text{Cl}_2$ . We have used  $\text{HCl}$  instead of  $\text{HClO}_4$  with no significant interference in the analysis. Solutions of  $\text{HClO}_4$  should not be boiled to dryness because of their explosion hazard.) Boil gently for 10 min, so that Reaction 1 in Box 16-2 goes to completion. Cool to room temperature, cap with the two-hole-stopper–buret assembly, and begin  $\text{N}_2$  flow. Dissolve 1.0–1.5 g of KI in 10 mL of distilled water and immediately add the solution to the beaker. Titrate rapidly with magnetic stirring as described in Step 3. Repeat this procedure two more times.
5. *Superconductor Experiment B.* Place an accurately weighed 150- to 200-mg sample of powdered  $\text{YBa}_2\text{Cu}_3\text{O}_x$  in the titration beaker and begin  $\text{N}_2$  flow. Dissolve 1.0–1.5 g of KI in 10 mL of 1.0 M  $\text{HClO}_4$  and immediately add the solution to the titration beaker. Stir magnetically for 1 min, so that the Reactions 3 and 4 of Box 16-2 occur. Add 10 mL of water and rapidly complete the titration. Repeat this procedure two more times.

## CALCULATIONS

1. Suppose that mass,  $m_A$ , is analyzed in Experiment A and the volume,  $V_A$ , of standard thiosulfate is required for titration. Let the corresponding quantities in Experiment B be  $m_B$  and  $V_B$ . Let the average oxidation state of Cu in the superconductor be  $2 + p$ . Show that  $p$  is given by

$$p = \frac{V_B/m_B - V_A/m_A}{V_A/m_A} \quad (1)$$

and  $x$  in the formula  $\text{YBa}_2\text{Cu}_3\text{O}_x$ , is related to  $p$  as follows:

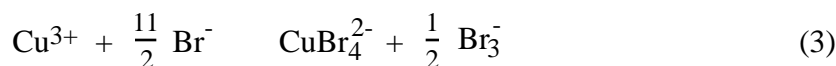
$$x = \frac{7}{2} + \frac{3}{2} (2 + p) \quad (2)$$

For example, if the superconductor contains one  $\text{Cu}^{3+}$  and two  $\text{Cu}^{2+}$ , the average oxidation state of copper is  $7/3$  and the value of  $p$  is  $1/3$ . Setting  $p = 1/3$  in Equation 2 gives  $x = 7$ . Equation 1 does not depend on the metal stoichiometry being exactly Y:Ba:Cu 1:2:3, but Equation 2 does require this exact stoichiometry.

2. Use the average results of Experiments A and B to calculate the values of  $p$  and  $x$  in Equations 1 and 2.
3. Suppose that the uncertainty in mass of superconductor analyzed is 1 in the last decimal place. Calculate the standard deviations for Steps 3, 4, and 5 of the iodometric analysis. Using these standard deviations as uncertainties in volume, calculate the uncertainties in the values of  $p$  and  $x$  in Equations 1 and 2.

### Citrate-Complexed Copper Titration<sup>2</sup>

This procedure directly measures  $\text{Cu}^{3+}$ . The sample is first dissolved in a closed container with 4.4 M HBr, in which  $\text{Cu}^{3+}$  oxidizes  $\text{Br}^-$  to  $\text{Br}_3^-$ :



The solution is transferred to a vessel containing excess  $\text{I}^-$ , excess citrate, and enough  $\text{NH}_3$  to neutralize most of the acid.  $\text{Cu}^{2+}$  is complexed by citrate and is not further reduced to  $\text{CuI}(s)$ . (This eliminates the problem in the iodometric titration of performing a titration in the presence of colored solid.) The  $\text{Br}_3^-$  from Reaction 3 oxidizes  $\text{I}^-$  to  $\text{I}_3^-$ :



and the  $\text{I}_3^-$  is titrated with thiosulfate.

Our experience with the iodometric procedure is that the precision in oxygen content of  $\text{YBa}_2\text{Cu}_3\text{O}_{x}$  is  $\pm 0.04$  in the value of  $x$ . The uncertainty is reduced to  $\pm 0.01$  by the citrate-complexed copper procedure.

### PROCEDURE

1. Prepare and standardize sodium thiosulfate solution as described in Steps 1–3 of the iodometric analysis in the previous section.
2. Place an accurately weighed 20- to 50-mg sample of superconductor in a 4-mL screw-cap vial with a Teflon cap liner and add 2.00 mL of ice-cold 4.4 M HBr by pipet. (The HBr is prepared by diluting 50 mL of 48 wt % HBr to 100 mL.) Cap tightly and gently agitate the vial for 15 min as it warms to room temperature. (We use a motor to rotate the sample slowly for 15 min.)

- Cool the solution back to 0° C and carefully transfer it to the titration beaker (used in the thiosulfate standardization) containing an ice-cold, freshly prepared solution made from 0.7 g of KI, 20 mL of water, 5 mL of 1.0 M trisodium citrate, and approximately 0.5 mL of 28 wt % NH<sub>3</sub>. The exact amount of NH<sub>3</sub> should be enough to neutralize all but 1 mmol of acid present in the sample. When calculating the acid content, remember that each mole of YBa<sub>2</sub>Cu<sub>3</sub>O<sub>x</sub> consumes 2x moles of HBr. (You can estimate that x is close to 7.) Wash the vial with three 1-mL aliquots of 2 M HBr to complete the quantitative transfer to the beaker.
- Add 0.1 mL of 1 wt % starch solution and titrate with 0.1 M standard Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> under a brisk flow of N<sub>2</sub>, using a 250-mL Hamilton syringe to deliver titrant. The end point is marked by a change from dark blue (I<sub>2</sub>-starch) to the light blue-green of the Cu<sup>2+</sup>-citrate complex.
- Run a blank reaction with CuSO<sub>4</sub> in place of superconductor. The moles of Cu in the blank should be the same as the moles of Cu in the superconductor. In a typical experiment, 30 mg of YBa<sub>2</sub>Cu<sub>3</sub>O<sub>6.88</sub> required approximately 350 μL of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> and the blank required 10 μL of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>. If time permits, run two more blanks. Subtract the average blank from the titrant in Step 4.
- Repeat the analysis with two more samples of superconductor.

### CALCULATIONS

- From the thiosulfate required to titrate I<sub>3</sub><sup>-</sup> released in Reaction 4, find the average moles of Cu<sup>3+</sup> per gram of superconductor (1 mol S<sub>2</sub>O<sub>3</sub><sup>2-</sup> = 1 mol Cu<sup>3+</sup>) and the standard deviation for your three samples.
- Defining  $R$  as (mol Cu<sup>3+</sup>)/(g superconductor), show that  $z$  in the formula YBa<sub>2</sub>Cu<sub>3</sub>O<sub>7-x</sub> is

$$z = \frac{1 - 666.20 R}{2 - 15.999 R} \quad (5)$$

where 666.20 is the formula mass of YBa<sub>2</sub>Cu<sub>3</sub>O<sub>7</sub> and 15.999 4 is the atomic mass of O.

- Using your average value of  $R$  and using its standard deviation as an estimate of uncertainty, calculate the average value of  $z$  and its uncertainty. Find the average value of  $x$  and its uncertainty in the formula YBa<sub>2</sub>Cu<sub>3</sub>O<sub>x</sub>. If you are really daring, you should use Equation C-1 in Appendix C of the textbook for propagation of uncertainty in Equation 5.

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1. D. C. Harris, M. E. Hills, and T. A. Hewston, *J. Chem. Ed.* **1987**, 64, 847. Alternative syntheses of YBa<sub>2</sub>Cu<sub>3</sub>O<sub>x</sub> are described by C. D. Cogdell, D. G. Wayment, D. J. Casadonte, Jr., and K. A. Kubat-Martin, *J. Chem. Ed.* **1995**, 72, 840 and P. I. Djurovich and R. J. Watts, *J. Chem. Ed.* **1993**, 70, 497.

2. E. H. Appelman, L. R. Morss, A. M. Kini, U. Geiser, A. Umezawa, G. W. Crabtree, and K. D. Carlson, *Inorg. Chem.* **1987**, *26*, 3237.
3. P. Phinyocheep and I. M. Tang, *J. Chem. Ed.* **1994**, *71*, A115.

## 15. Potentiometric Halide Titration with $\text{Ag}^+$

Mixtures of halides can be titrated with  $\text{AgNO}_3$  solution as described in Section 7-5 in the textbook. In this experiment, you will use the apparatus in Figure 7-9 of the textbook to monitor the activity of  $\text{Ag}^+$  as the titration proceeds. The theory of the potentiometric measurement is described in Section 15-2 of the textbook.

Each student is given a vial containing 0.22–0.44 g of KCl plus 0.50–1.00 g of KI (both weighed accurately). The object is to determine the quantity of each salt in the mixture. A 0.4 M bisulfate buffer (pH 2) should be available in the lab. It is prepared by titrating 1 M  $\text{H}_2\text{SO}_4$  with 1 M NaOH to a pH near 2.0.

### PROCEDURE

1. Pour your unknown carefully into a 50- or 100-mL beaker. Dissolve the solid in ~20 mL of water and pour it into a 100-mL volumetric flask. Rinse the sample vial and beaker many times with small portions of  $\text{H}_2\text{O}$  and transfer the washings to the flask. Dilute to the mark and mix well.
2. Dry 1.2 g of  $\text{AgNO}_3$  at  $105^\circ\text{C}$  for 1 h and cool in a desiccator for 30 min with minimal exposure to light. Some discoloration is normal (and tolerable in this experiment) but should be minimized. Accurately weigh 1.2 g and dissolve it in a 100-mL volumetric flask.
3. Set up the apparatus in Figure 7-9 of the textbook. The silver electrode is simply a 3-cm length of silver wire connected to copper wire. (Fancier electrodes can be prepared by housing the connection in a glass tube sealed with epoxy at the lower end. Only the silver should protrude from the epoxy.) The copper wire is fitted with a jack that goes to the reference socket of a pH meter. The reference electrode for this titration is a glass pH electrode connected to its usual socket on the meter. If a combination pH electrode is employed, the reference jack of the combination electrode is not used. The silver electrode should be taped to the inside of the 100-mL beaker so that the Ag/Cu junction remains dry for the entire titration. The stirring bar should not hit either electrode.
4. Pipet 25.00 mL of unknown into the beaker, add 3 mL of bisulfate buffer, and begin magnetic stirring. Record the initial level of  $\text{AgNO}_3$  in a 50-mL buret and add ~1 mL of titrant to the beaker. Turn the pH meter to the millivolt scale and record the volume and voltage. It is convenient (but is not essential) to set the initial reading to +800 mV by adjusting the meter.
5. Titrate the solution with ~1-mL aliquots until 50 mL of titrant have been added or until you can see two clear potentiometric end points. You need not allow more than 15–30 s for each point. Record the volume and voltage at each point. Make a graph of millivolts versus milliliters to find the approximate positions ( $\pm 1$  mL) of the two end points.
6. Turn the pH meter to standby, remove the beaker, rinse the electrodes well with water, and blot them dry with a tissue. (Silver halide adhering to the glass electrode can be removed by soaking in concentrated sodium thiosulfate solution. This thorough cleaning is not necessary

between Steps 6 and 7 in this experiment. The silver halides in the titration beaker can be saved and converted back to pure  $\text{AgNO}_3$  by using the procedure of E. Thall, *J. Chem. Ed.* **1981**, 58, 561.) Clean the beaker and set up the titration apparatus again. The beaker need not be dry.

7. Now perform an accurate titration, using 1-drop aliquots near the end points (and 1-mL aliquots elsewhere). You need not allow more than 30 s per point for equilibration.
8. Prepare a graph of millivolts versus milliliters and locate the end points as in Figure 7-8 of the textbook. The  $\text{I}^-$  end point is taken as the intersection of the two dashed lines in the inset of Figure 7-8. The  $\text{Cl}^-$  end point is the inflection point at the second break. Calculate milligrams of KI and milligrams of KCl in your solid unknown.

### Analyzing $\text{Cl}^-$ in Streams, Lakes, or Salt Water

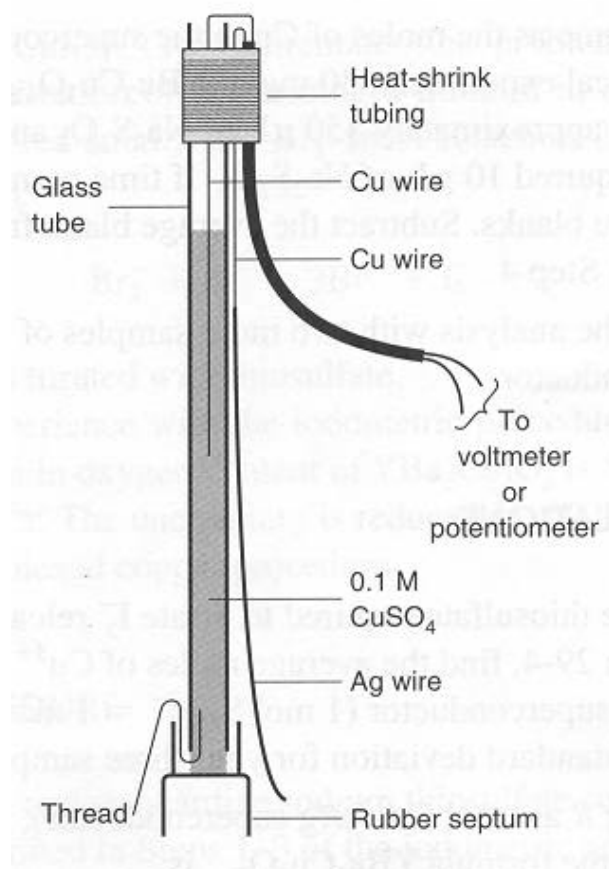
A variation of the preceding procedure could make a class project in environmental analysis. For example, you could study changes in streams as a function of the season or recent rainfall. You could study stratification (layering) of water in lakes. The measurement responds to all ions that precipitate with  $\text{Ag}^+$ , of which  $\text{Cl}^-$  is, by far, the dominant ion in natural waters.

You can use the electrodes in Figure 7-9, or you can construct the rugged combination electrode shown in Figure 1.<sup>1</sup> The indicator electrode in Figure 1 is a bare silver wire in contact with analyte solution. The inner (reference) chamber of the combination electrode contains a copper wire dipped into  $\text{CuSO}_4$  solution. The inner electrode maintains a constant potential because the concentration of  $\text{Cu}^{2+}$  in the solution is constant. The solution makes electrical contact at the septum, where a piece of thread leaves enough space for solution to slowly drain from the electrode into the external sample solution.

### PROCEDURE

1. Collect water from streams or lakes or from the ocean. To minimize bacterial growth, plastic jugs should be filled to the top and tightly sealed. Refrigeration is recommended.
2. Prepare 4 mM  $\text{AgNO}_3$  solution with an accurately known concentration, as in Step 2 at the beginning of this experiment. One student can prepare enough reagent for five people by using 0.68 g of  $\text{AgNO}_3$  in a 1-L volumetric flask.
3. Measure with a graduated cylinder 100 mL of a natural water sample and pour it into a 250-mL beaker. Position the combination electrode from Figure 1 or the pair of electrodes from Figure 7-9 of the textbook in the beaker so that a stirring bar will not hit the electrode.
4. Carry out a rough titration by adding 1.5-mL increments of titrant to the unknown and reading the voltage after 30 s to the nearest millivolt. Prepare a graph of voltage versus volume of titrant to locate the end point, which will not be as abrupt as those in Figure 7-8 of the textbook. If necessary, adjust the volume of unknown in future steps so that the end

point comes at 20–40 mL. If you need less unknown, make up the difference with distilled water.



5. Carry out a more careful titration with fresh unknown. Add three-quarters of the titrant volume required to reach the equivalence point all at once. Then add ~0.4-mL increments (8 drops from a 50-mL buret) of titrant until you are 5 mL past the equivalence point. Allow 30 s (or longer, if necessary) for the voltage to stabilize after each addition. The end point is the steepest part of the curve, which can be estimated by the method shown in Figure 1 of Experiment 7.
6. Calculate the molarity and parts per million ( $\mu\text{g/mL}$ ) of  $\text{Cl}^-$  in the unknown. Use data from several studies with the same sample to find the mean and standard deviation of ppm  $\text{Cl}^-$ .

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1. G. Lisensky and K. Reynolds, *J. Chem. Ed.* **1991**, 68, 334; R. Ramette, *Chemical Equilibrium* (Reading, MA: Addison-Wesley, 1981), p. 649.

## 16. Electrogravimetric Analysis of Copper

Most copper-containing compounds can be electrolyzed in acidic solution, with quantitative deposition of Cu at the cathode. Sections 17-1 to 17-2 of the textbook discuss this technique.

Students may analyze a preparation of their own (such as copper acetylsalicylate<sup>1</sup>) or be given unknowns prepared from CuSO<sub>4</sub>·5H<sub>2</sub>O or metallic Cu. In the latter case, dissolve the metal in 8 M HNO<sub>3</sub>, boil to remove HNO<sub>2</sub>, neutralize with ammonia, and barely acidify the solution with dilute H<sub>2</sub>SO<sub>4</sub> (Using litmus paper to test for acidity). Samples must be free of chloride and nitrous acid.<sup>2</sup> Copper oxide unknowns (soluble in acid) are available from Thorn Smith.<sup>3</sup>

The apparatus shown in Figure 17-5 of the textbook uses any 6–12 V direct-current power supply. A tall-form 150-mL beaker is the reaction vessel.

### PROCEDURE

1. Handle the Pt gauze cathode with a tissue, touching only the thick stem, not the wire gauze. Immerse the electrode in hot 8 M HNO<sub>3</sub> to remove previous deposits, rinse with water and alcohol, dry at 105°C for 5 min, cool for 5 min, and weigh accurately. If the electrode contains any grease, it can be heated to red heat over a burner after the treatment above.<sup>4</sup>
2. The sample should contain 0.2–0.3 g of Cu in 100 mL. Add 3 mL of 98 wt % H<sub>2</sub>SO<sub>4</sub> and 2 mL of freshly boiled 8 M HNO<sub>3</sub>. Position the cathode so that the top 5 mm are above the liquid level after magnetic stirring is begun. Adjust the current to 2 A, which should require 3–4 V. When the blue color of Cu(II) has disappeared, add some distilled water so that new Pt surface is exposed to the solution. If no further deposition of Cu occurs on the fresh surface in 15 min at a current of 0.5 A, the electrolysis is complete. If deposition is observed, continue electrolysis and test the reaction for completeness again.
3. *Without* turning off the power, lower the beaker while washing the electrode with a squirt bottle. Then the current can be turned off. (If current is disconnected before removing the cathode from the liquid and rinsing off the acid, some Cu could redissolve.) Wash the cathode gently with water and alcohol, dry at 105° C for 3 min, cool in a desiccator for 5 min, and weigh.

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1. E. Dudek, *J. Chem. Ed.* **1977**, *54*, 329.

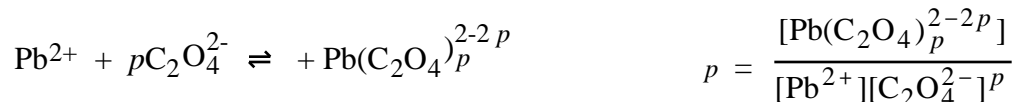
2. J. F. Owen, C. S. Patterson, and G. S. Rice, *Anal. Chem.* **1983**, *55*, 990, describe simple procedures for the removal of chloride from Cu, Ni, and Co samples prior to electrogravimetric analysis.

3. Thorn Smith Inc., 7755 Narrow Gauge Road, Beulah, MI 49617. Phone: 231-882-4672; e-mail: [www.thornsmithlabs.com](http://www.thornsmithlabs.com).

4. Some metals, such as Zn, Ga, and Bi, form alloys with Pt and should not be deposited directly on the Pt surface. The electrode should be coated first with Cu, dried, and then used. Alternatively, Ag can be used in place of Pt for depositing these metals. Platinum anodes are attacked by Cl<sub>2</sub> formed by electrolysis of Cl<sup>-</sup> solutions. To prevent chlorine attack, 1–3 g of a hydrazinium salt (per 100 mL of solution) can be used as an anodic depolarizer, because hydrazine is more readily oxidized than Cl<sup>-</sup>:  $\text{N}_2\text{H}_4 \rightarrow \text{N}_2 + 4\text{H}^+ + 4\text{e}^-$ .

## 17. Polarographic Measurement of an Equilibrium Constant<sup>1</sup>

In this experiment, you will find the overall formation constant ( $K_p$ ) and stoichiometry for the reaction of oxalate with  $\text{Pb}^{2+}$ :



where  $p$  is a stoichiometry coefficient. We will do this by measuring the polarographic half-wave potential for solutions containing  $\text{Pb}^{2+}$  and various amounts of oxalate. The change of half-wave potential,  $E_{1/2}$  [=  $E_{1/2}(\text{observed}) - E_{1/2}(\text{for } \text{Pb}^{2+} \text{ without oxalate})$ ] is expected to obey the equation

$$E_{1/2} = -\frac{RT}{nF} \ln K_p - \frac{pRT}{nF} \ln [\text{C}_2\text{O}_4^{2-}] \quad (1)$$

where  $R$  is the gas constant,  $F$  is the Faraday constant, and  $T$  is temperature in kelvins. You should measure the lab temperature at the time of the experiment or use a thermostatically controlled cell.

An electrode reaction is considered to be *reversible* when it is fast enough to maintain equilibrium at the electrode surface. The shape of a reversible polarographic wave is given by

$$E = E_{1/2} - \frac{RT}{nF} \ln \frac{I}{I_d - I} \quad (2)$$

where  $I$  is current and  $I_d$  is diffusion current.

### PROCEDURE

1. Pipet 1.00 mL of 0.020 M  $\text{Pb}(\text{NO}_3)_2$  into each of five 50-mL volumetric flasks labeled A–E and add 1 drop of 1 wt % Triton X-100 to each. Then add the following solutions and dilute to the mark with water. The  $\text{KNO}_3$  may be delivered carefully with a graduated cylinder. The oxalate should be pipetted.
  - A: Add nothing else. Dilute to the mark with 1.20 M  $\text{KNO}_3$ .
  - B: Add 5.00 mL of 1.00 M  $\text{K}_2\text{C}_2\text{O}_4$ , and 37.5 mL of 1.20 M  $\text{KNO}_3$ .
  - C: Add 10.00 mL of 1.00 M  $\text{K}_2\text{C}_2\text{O}_4$ , and 25.0 mL of 1.20 M  $\text{KNO}_3$ .
  - D: Add 15.00 mL of 1.00 M  $\text{K}_2\text{C}_2\text{O}_4$  and 12.5 mL of 1.20 M  $\text{KNO}_3$ .
  - E: Add 20.00 mL of 1.00 M  $\text{K}_2\text{C}_2\text{O}_4$ .

2. Transfer each solution to a polarographic cell, deoxygenate with bubbling  $N_2$  for 10 min, and record the polarogram from  $-0.20$  to  $-0.95$  V (versus S.C.E.). Measure the residual current, using the same settings and a solution containing just  $1.20$  M  $KNO_3$  (Plus 1 drop of 1 wt % Triton X-100). Record each polarogram on a scale sufficiently expanded to allow accurate measurements.
3. For each polarogram, make a graph of  $E$  versus  $\ln[I/(I_d - I)]$ , using 6–8 points for each graph. Be sure to subtract the residual current at each potential. According to Equation 2,  $E = E_{1/2}$  when  $\ln[I/(I_d - I)] = 0$ . Use this condition to locate  $E_{1/2}$  on each graph.
4. Make a graph of  $E_{1/2}$  versus  $\ln[C_2O_4^{2-}]$ . From the slope, use Equation 1 to find  $p$ , the stoichiometry coefficient. Then use the intercept to find the value of  $p$ . Use the method of least squares to find the standard deviations of the slope and intercept. From the standard deviations, find the uncertainties in  $p$  and  $p$  and express each with the correct number of significant figures.

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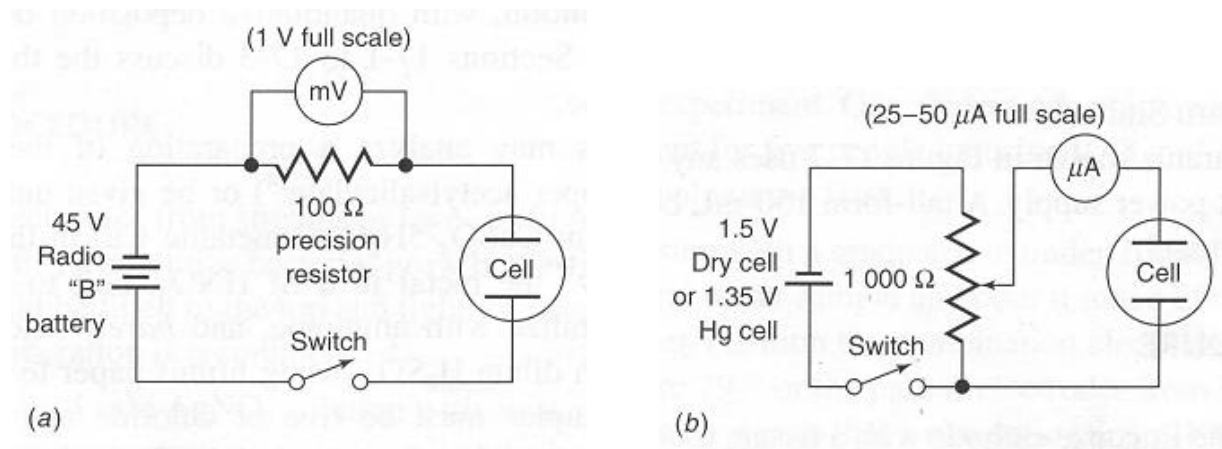
1. W. C. Hoyle and T. M. Thorpe, *J. Chem. Ed.* **1978**, 55, A229.

## 18. Coulometric Titration of Cyclohexene with Bromine<sup>1</sup>

This experiment is described in Section 17-3, and the apparatus is shown in Figure 17-8. You can use a conventional coulometric power supply or the circuits in Figure 1.<sup>2</sup> A stopwatch is manually started as the generator switch is closed. Alternatively, a double-pole, double-throw switch can be used to simultaneously start the generator circuit and an electric clock.

### PROCEDURE

1. The electrolyte is a 60:26:14 (vol/vol/vol) mixture of acetic acid, methanol, and water. The solution contains 0.15 M KBr and 0.1 g of mercuric acetate per 100 mL. (The latter catalyzes the reaction between Br<sub>2</sub> and cyclohexene.) The electrodes should be covered with electrolyte. Begin vigorous magnetic stirring (without spattering) and adjust the voltage of the detector circuit to 0.25 V.



**Figure 1.** Circuits for coulometric titrations. (a) Generator circuit. (b) Detector circuit.

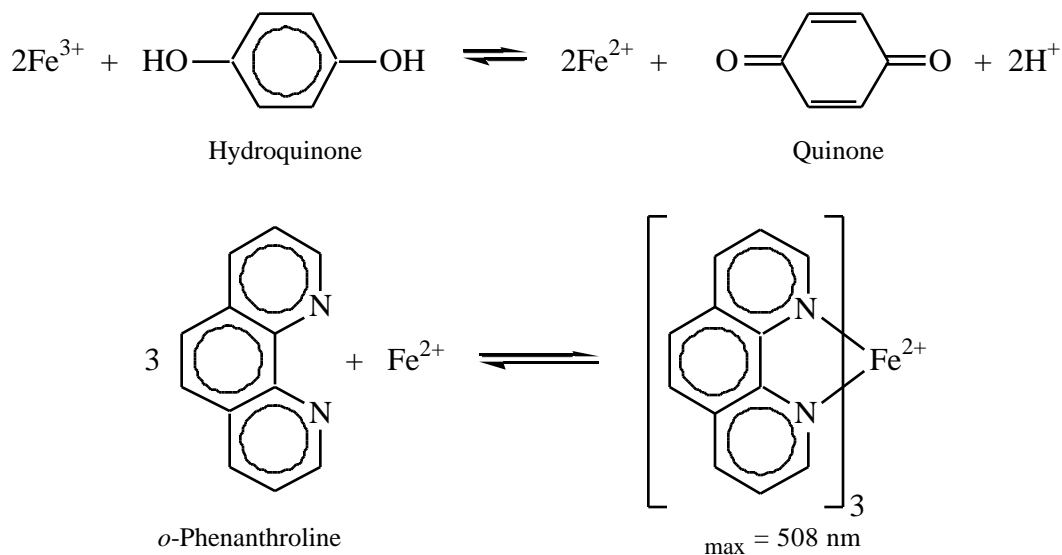
2. Generate Br<sub>2</sub> with the generator circuit until the detector current is 20.0 μA. (The generator current is 5–10 mA.)
3. Pipet 2–5 mL of unknown (containing 1–5 mg of cyclohexene in methanol) into the flask and set the clock or coulometer to 0. The detector current should drop to near 0 because the cyclohexene consumes the Br<sub>2</sub>.
4. Turn the generator circuit on and simultaneously begin timing. While the reaction is in progress, measure the voltage ( $E$ ) across the precision resistor ( $R = 100.0 \pm 0.1 \ \Omega$ ) to find the exact current ( $I$ ) flowing through the cell ( $I = E/R$ ). Continue the electrolysis until the detector current rises to 20.0 μA. Then stop the coulometer and record the time.
5. Repeat the procedure two more times and find the average molarity (and relative standard deviation) of cyclohexene.

6. When you are finished, be sure all switches are off. Soak the generator electrodes in 8 M  $\text{HNO}_3$  to dissolve Hg that is deposited during the electrolysis.

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1. D. H. Evans, *J. Chem. Ed.* **1968**, *45*, 88.
  2. A constant-current circuit for coulometer generator electrodes is given by J. Swim, E. Earps, L. M. Reed, and D. Paul, *J. Chem. Ed.* **1996**, *73*, 679. An operational amplifier circuit for the detector and a circuit for controlled-potential coulometry are given by E. Grimsrud and J. Amend, *J. Chem. Ed.* **1979**, *56*, 131.

## 19. Spectrophotometric Determination of Iron in Vitamin Tablets<sup>1</sup>

In this procedure, iron from a vitamin supplement tablet is dissolved in acid, reduced to Fe<sup>2+</sup> with hydroquinone, and complexed with *o*-phenanthroline to form an intensely colored complex (Color Plate 15 in the textbook).



### REAGENTS

*Hydroquinone*: Freshly prepared solution containing 10 g/L in water. Store in an amber bottle.

*Trisodium citrate*: 25 g/L in water.

*o*-Phenanthroline: Dissolve 2.5 g in 100 mL of ethanol and add 900 mL of water. Store in an amber bottle.

*Standard Fe (0.04 mg Fe/mL)*: Prepare by dissolving 0.281 g of reagent-grade Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O in water in a 1-L volumetric flask containing 1 mL of 98 wt % H<sub>2</sub>SO<sub>4</sub>.

### PROCEDURE

1. Place one tablet of the iron-containing vitamin in a 125-mL flask or 100-mL beaker and boil gently (*in a fume hood*) with 25 mL of 6 M HCl for 15 min. Filter the solution directly into a 100-mL volumetric flask. Wash the beaker and filter several times with small portions of water to complete a quantitative transfer. Allow the solution to cool, dilute to the mark and mix well. Dilute 5.00 mL of this solution to 100.0 mL in a fresh volumetric flask. If the label indicates that the tablet contains <15 mg of Fe, use 10.00 mL instead of 5.00 mL.

2. Pipet 10.00 mL of standard Fe solution into a beaker and measure the pH (with pH paper or a glass electrode). Add sodium citrate solution 1 drop at a time until a pH of ~3.5 is reached. Count the drops needed. (It will require about 30 drops.)
3. Pipet a fresh 10.00-mL aliquot of Fe standard into a 100-mL volumetric flask and add the same number of drops of citrate solution as required in Step 2. Add 2.00 mL of hydroquinone solution and 3.00 mL of *o*-phenanthroline solution, dilute to the mark with water, and mix well.
4. Prepare three more solutions from 5.00, 2.00, and 1.00 mL of Fe standard and prepare a blank containing no Fe. Use sodium citrate solution in proportion to the volume of Fe solution. (If 10 mL of Fe requires 30 drops of citrate solution, 5 mL of Fe requires 15 drops of citrate solution.)
5. Find out how many drops of citrate solution are needed to bring 10.00 mL of the iron supplement tablet solution from Step 1 to pH 3.5. This will require about 3.5 or 7 mL of citrate, depending on whether 5 or 10 mL of unknown was diluted in the second part of Step 1.
6. Transfer 10.00 mL of solution from Step 1 to a 100-mL volumetric flask. Add the required amount of citrate solution determined in Step 5. Then add 2.00 mL of hydroquinone solution and 3.0 mL of *o*-phenanthroline solution; dilute to the mark and mix well.
7. Allow the solutions to stand for at least 10 min. Then measure the absorbance of each solution at 508 nm. (The color is stable, so all solutions may be prepared and all the absorbances measured at once.) Use distilled water in the reference cuvette and subtract the absorbance of the blank from the absorbance of the Fe standards.
8. Make a graph of absorbance versus micrograms of Fe in the standards. Find the slope and intercept (and standard deviations) by the method of least squares. Calculate the molarity of  $\text{Fe}(\text{o-phenanthroline})_3^{2+}$  in each solution and find the average molar absorptivity (in Beer's law) from the four absorbances. (Remember that all the iron has been converted to the phenanthroline complex.)
9. Using the calibration curve (or its least-squares parameters), find the number of milligrams of Fe in the tablet.

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1. R. C. Atkins, *J. Chem. Ed.* **1975**, 52, 550

## 20. Microscale Spectrophotometric Measurement of Iron in Foods by Standard Addition<sup>1</sup>

This microscale experiment uses the same chemistry as that of Experiment 19 to measure iron in foods such as broccoli, peas, cauliflower, spinach, beans, and nuts. A possible project is to compare processed (canned or frozen) vegetables to fresh vegetables. Instructions are provided for small volumes, but the experiment can be scaled up to fit available equipment. Section 5-3 in the textbook describes the method of standard addition.

### REAGENTS

*2.0 M HCl*: 15 mL/student. Dilute 165 mL of concentrated (37 wt %) HCl up to 1 L.

*Hydroquinone*: 4 mL/student; prepared as in Experiment 19.

*Trisodium citrate dehydrate*: 4 g/student.

*o-Phenanthroline*: 4 mL/student; prepared as in Experiment 19.

*Standard Fe (40  $\mu\text{g Fe/mL}$ )*: 4 mL/student; prepared as in Experiment 19.

*6 M HCl*: Dilute 500 mL of 37 wt % HCl up to 1 L with distilled water. Store in a bottle and reuse many times for soaking crucibles.

### PROCEDURE

1. Fill a clean porcelain crucible with 6 M HCl in the hood and allow it to stand for 1 h to remove traces of iron from previous uses. Rinse well with distilled water and dry. After weighing the empty crucible, add 5–6 g of finely chopped food sample and weigh again to obtain the mass of food. (Some foods, like frozen peas, should not be chopped because they will lose their normal liquid content.)
2. This step could require 3 h, during which you can be doing other lab work. Carefully heat the crucible with a Bunsen burner in a hood (Experiment 3, Figure 1). Use a low, flame to *dry* the sample, being careful to avoid spattering. Increase the flame temperature to *char* the sample. Keep the crucible lid and tongs nearby. If the sample bursts into flames, use tongs to place the lid on the crucible to smother the flame. After charring, use the hottest possible flame (bottom of crucible should be red hot) to *ignite* the black solid, converting it to white ash. Continue ignition until all traces of black disappear.
3. After cooling the crucible to room temperature, add 10.00 mL of 2.0 M HCl by pipet and swirl gently for 5 min to dissolve the ash. Filter the mixture through a small filter and collect the filtrate in a vial or small flask. You need to recover >8 mL for the analysis.

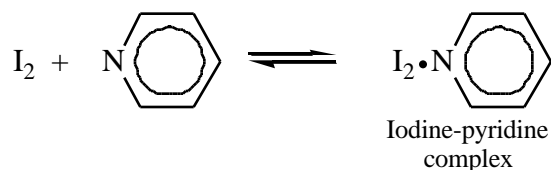
4. Weigh 0.71 g of trisodium citrate dehydrate into each of four 10-mL volumetric flasks. Using a 2-mL volumetric pipet or a 1-mL micropipet, add 2.00 mL of ash solution to each flask. Add 4 mL of distilled water and swirl to dissolve the citrate. The solution will have a pH near 3.6. Using a micropipet, add 0.20 mL of hydroquinone solution and 0.30 mL of phenanthroline solution to each flask.
5. Label the volumetric flasks 0 through 3. Add no Fe standard to flask 0. Using a micropipet, add 0.250 mL of Fe standard to flask 1. Add 0.500 mL of Fe standard to flask 2 and 0.750 mL to flask 3. The four flasks now contain 0, 1, 2, and 3  $\mu\text{g Fe/mL}$ , in addition to Fe from the food. Dilute each to the mark with distilled water, mix well, and allow 15 min to develop full color.
6. Prepare a blank by mixing 0.71 g of trisodium citrate dehydrate, 2.00 mL of 2.0 M HCl, 0.20 mL of hydroquinone solution, 0.30 mL of phenanthroline solution and diluting to 10 mL. The blank does not require a volumetric flask.
7. Measure the absorbance of each solution at 512 nm in a 1-cm cell with distilled water in the reference cell. Before each measurement, remove all liquid from the cuvet with a Pasteur pipet. Then use  $\sim 1$  mL of your next solution (delivered with a clean, dry Pasteur pipet) to wash the cuvet. Remove and discard the washing. Repeat the washing once more with fresh solution and discard the washing. Finally, add your new solution to the cuvet for measuring absorbance.
8. Subtract the absorbance of the blank from each reading and make a graph like that in Figure 5-7 in the textbook to find the Fe content of the unknown solution. Calculate the wt % of Fe in the food. Use Equation 5-17 in the textbook to estimate the uncertainty in the wt % of Fe in the food.

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1. Idea based on P. E. Adams, *J. Chem. Ed.* **1995**, 72, 649.

## 21. Spectrophotometric Measurement of an Equilibrium Constant

In this experiment, we will use the Scatchard plot described in Section 19-2 of the textbook to find the equilibrium constant for the formation of a complex between iodine and pyridine in cyclohexane:<sup>1</sup>



Both  $\text{I}_2$  and  $\text{I}_2$ -pyridine absorb visible radiation, but pyridine is colorless. Analysis of the spectral changes associated with variation of pyridine concentration (with a constant total concentration of iodine) will allow us to evaluate  $K$  for the reaction. The experiment is best performed with a recording spectrophotometer, but single-wavelength measurements can be used.

### PROCEDURE

All operations should be carried out *in a fume hood*, including pouring solutions into and out of the spectrophotometer cell. Only a *capped* cuvette containing the solution whose spectrum is to be measured should be taken from the hood. Do not spill solvent on your hands or breathe the vapors. Used solutions should be discarded in a waste container *in the hood*, not down the drain.

- The following stock solutions should be available in the lab:
  - 0.050–0.055 M pyridine in cyclohexane (40 mL for each student, concentration known accurately).
  - 0.012 0–0.012 5 M  $\text{I}_2$  in cyclohexane (10 mL for each student, concentration known accurately).
- Pipet the following volumes of stock solutions into six 25-mL volumetric flasks labeled A–F, dilute to the mark with cyclohexane, and mix well.

Flask	Pyridine stock solution (mL)	$\text{I}_2$ stock solution (mL)
A	0	1.00
B1.00	1.00	
C2.00	1.00	
D	4.00	1.00
E5.00	1.00	
F	10.00	1.00

- Using glass or quartz cells, record a baseline between 350 and 600 nm with solvent in both the sample and the reference cells. Subtract the absorbance of the baseline from all future absorbances. If possible, record all spectra, including the baseline, on one sheet of chart paper. (If a fixed-wavelength instrument is used, first find the positions of the two absorbance maxima in solution E. Then make all measurements at these two wavelengths.)
- Record the spectrum of each solution A–F or measure the absorbance at each maximum if a fixed-wavelength instrument is used.

### DATA ANALYSIS

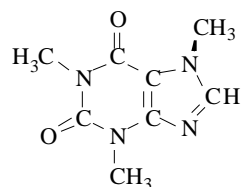
- Measure the absorbance at the wavelengths of the two maxima in each spectrum. Be sure to subtract the absorbance of the blank from each.
- The analysis of this problem follows that of Reaction 19-10 in the textbook, in which P is iodine and X is pyridine. As a first approximation, assume that the concentration of free pyridine equals the total concentration of pyridine in the solution (because  $[\text{pyridine}] \gg [\text{I}_2]$ ). Prepare a graph of  $A/[\text{free pyridine}]$  versus  $A$  (a Scatchard plot), using the absorbance at the  $\text{I}_2$ -pyridine maximum.
- From the slope of the graph, find the equilibrium constant using Equation 19-16 in the textbook. From the intercept, find  $(= p_X - X)$ .
- Now refine the values of  $K$  and  $\epsilon$ . Use  $\epsilon$  to find  $p_X$ . Then use the absorbance at the wavelength of the  $\text{I}_2$ -pyridine maximum to find the concentration of bound and free pyridine in each solution. Make a new graph of  $A/[\text{free pyridine}]$  versus  $A$ , using the new values of  $[\text{free pyridine}]$ . Find a new value of  $K$  and  $\epsilon$ . If justified, perform another cycle of refinement.
- Using the values of free pyridine concentration from your last refinement and the values of absorbance at the  $\text{I}_2$  maximum, prepare another Scatchard plot and see if you get the same value of  $K$ .
- Explain why an isosbestic point is observed in this experiment.

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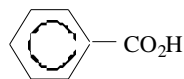
1. For literature values of the equilibrium constant for the reaction between  $\text{I}_2$  and pyridine, see S. S. Barton and R. H. Pottier, *J. Chem. Soc. Perkin Trans. II* **1984**, 731.

## 22. Spectrophotometric Analysis of a Mixture: Caffeine and Benzoic Acid in a Soft Drink<sup>1</sup>

In this experiment we use ultraviolet absorbance (Figure 1) to measure two major species in soft drinks. Caffeine is added as a stimulant and sodium benzoate is a preservative.

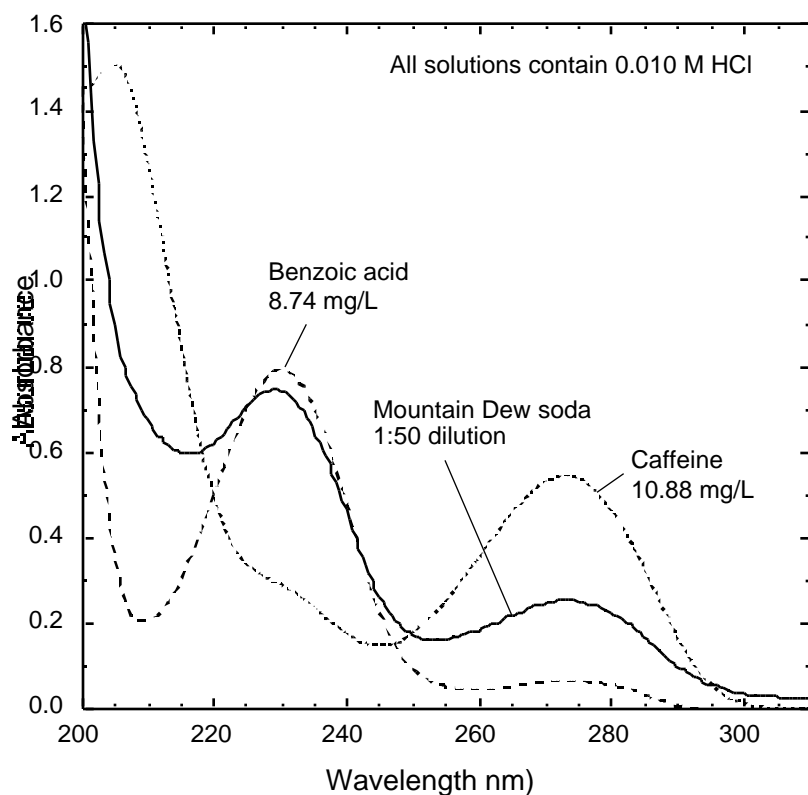


Caffeine  
FM 182.18



Benzoic acid ( $pK_a = 4.20$ )  
FM 122.12

All solutions will contain 0.010 M HCl, so the sodium benzoate is protonated to make benzoic acid. Caffeine has no appreciable basicity, so it is neutral at pH 2.



**Figure 1.** Ultraviolet absorption of benzoic acid, caffeine, and a 1:50 dilution of Mountain Dew soft drink. All solutions contain 0.010 M HCl.

We restrict ourselves to non-diet soft drinks because the sugar substitute aspartame in diet soda has some ultraviolet absorbance that slightly interferes in the present experiment. We also avoid darkly colored drinks because the colorants have ultraviolet absorbance. Mountain Dew, Mello Yello, and, probably, other lightly colored drinks are suitable for this experiment. There is undoubtedly some ultraviolet absorbance from colorants in these beverages that contributes systematic error to this experiment.

The procedure we describe includes the construction of calibration curves. The experiment could be shortened by recording just one spectrum of caffeine (20 mg/L) and one of benzoic acid (10 mg/L) and assuming that Beer's law is obeyed. The experiment could be expanded to use high-performance liquid chromatography (HPLC) and/or capillary electrophoresis to obtain independent measurements of caffeine and benzoic acid (and aspartame in diet drinks).<sup>1</sup>

## Reagents

*Stock solutions:* An accurately known solution containing ~100 mg benzoic acid/L in water and another containing ~200 mg caffeine/L should be available.

*0.10 M HCl:* Dilute 8.2 mL of 37 wt % HCl to 1 L.

## Procedure

- 1. Calibration standards:* Prepare benzoic acid solutions containing 2, 4, 6, 8 and 10 mg/mL in 0.010 M HCl. To prepare a 2 mg/mL solution, mix 2.00 mL of benzoic acid standard plus 10.0 mL of 0.10 M HCl in a 100-mL volumetric flask and dilute to the mark with water. Use 4, 6, 8 and 10 mL of benzoic acid to prepare the other standards. In a similar manner, prepare caffeine standards containing 4, 8, 12, 16 and 20 mg/mL in 0.010 M HCl.
- 2. Soft drink:* Warm ~20 mL of soft drink in a beaker on a hot plate to expel CO<sub>2</sub> and filter the warm liquid through filter paper to remove any particles. After cooling to room temperature, pipet 4.00 mL into a 100-mL volumetric flask. Add 10.0 mL of 0.10 M HCl and dilute to the mark. Prepare a second sample containing 2.00 mL of soft drink instead of 4.00 mL.
- 3. Verifying Beer's law:* Record an ultraviolet baseline from 350 to 210 nm with water in the sample and reference cuvetts (1.000 cm pathlength). Record the ultraviolet spectrum of each of the 10 standards with water in the reference cuvet. Note the wavelength of peak absorbance for benzoic acid (  $\lambda_{\text{max}}$  ) and the wavelength for the peak absorbance of caffeine (  $\lambda_{\text{max}}$  ). Measure the absorbance of each standard at both wavelengths and subtract the baseline absorbance (if your instrument does not do this automatically). Prepare a calibration graph of absorbance versus concentration (M) for each compound at each of the two wavelengths. Each graph should go through 0. The least-squares slope of the graph is the molar absorptivity at that wavelength.
- 4. Unknowns:* Measure the ultraviolet absorption spectrum of the 2:100 and 4:100 dilutions of the soft drink. With the absorbance at the wavelengths  $\lambda_{\text{max}}$  and  $\lambda_{\text{max}}$ , use Equation 19-6 in the textbook to find the concentrations of benzoic acid and caffeine in the original soft drink. Report results from both dilute solutions.

5. *Synthetic unknown*: If your instructor chooses, measure the spectrum of a synthetic, unknown mixture of benzoic acid and caffeine prepared by the instructor. Use Equation 19-6 in the textbook to find the concentration of each component in the synthetic unknown.

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1. V. L. McDevitt, A. Rodriguez and K. R. Williams, *J. Chem. Ed.* **1998**, *75*, 625.

## 23. $\text{Mn}^{2+}$ Standardization by EDTA Titration

Experiments 23–25 illustrate a sequence in which students (1) prepare and standardize a  $\text{Mn}^{2+}$  solution by EDTA titration and then (2) use this standard in the analysis of Mn in steel by two different instrumental techniques.<sup>1</sup>

### Reagents

$\text{MnSO}_4 \cdot \text{H}_2\text{O}$ : (1 g/student) This material is not a primary standard.

EDTA:  $\text{Na}_2\text{H}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$ , 1 g/student.

0.5 M  $\text{NH}_3/\text{NH}_4^+$  buffer (pH 9.3): Mix 6.69 g of  $\text{NH}_4\text{Cl}$  (FM 53.49) plus 7.60 g of 28% aqueous  $\text{NH}_3$  (FM 17.03) with enough water to give a total volume of 250 mL.

Hydroxylamine hydrochloride: ( $\text{NH}_3\text{OH}^+\text{Cl}^-$ , FM 69.49) 1 g/student. (CAUTION: Do not breathe dust from  $\text{NH}_3\text{OH}^+\text{Cl}^-$ ; avoid contact with skin and eyes.)

Pyrocatechol violet indicator: Dissolve 0.1 g in 100 mL  $\text{H}_2\text{O}$ .

### Procedure

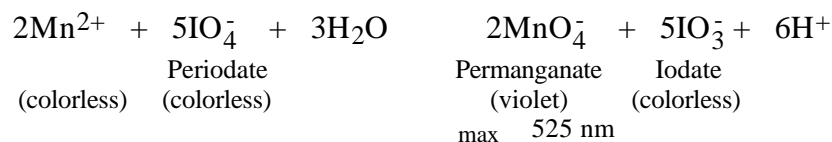
1. *Standard 0.005 M EDTA*: Dry  $\text{Na}_2\text{H}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$  (FM 372.25) at  $80^\circ\text{C}$  for 1 h and cool in a desiccator. Accurately weigh out  $\sim 0.93$  g and dissolve it with heating in 400 mL of distilled water in a 500-mL volumetric flask. Cool to room temperature, dilute to the mark, and mix well.
2.  *$\text{Mn}^{2+}$  stock solution*: Prepare a solution containing  $\sim 1.0$  mg Mn/mL ( $\sim 0.018$  M) by dissolving  $\sim 0.77$  g  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$  (FM 169.01) in a clean plastic screw cap bottle with 250 mL water delivered from a graduated cylinder. Masses and volumes need not be accurate because you will standardize this solution.
3. Rinse a clean 50-mL pipet several times with small volumes of  $\text{Mn}^{2+}$  stock solution and discard the washings into a chemical waste container. Then pipet 50.00 mL of  $\text{Mn}^{2+}$  stock solution into a 250-mL volumetric flask. Add  $\sim 0.8$  g (not accurately weighed) of solid hydroxylamine hydrochloride to the flask, and swirl to dissolve the solid. Add  $\sim 400$  mL of water and swirl to mix the contents. Dilute to the mark with water, place the cap firmly in place, and invert 20 times to mix the solution. This solution contains  $\sim 0.0036$  M  $\text{Mn}^{2+}$ . The reducing agent, hydroxylamine, maintains manganese in the +2 state.
4. Rinse a 50-mL pipet several times with small volumes of the diluted  $\text{Mn}^{2+}$  solution from step 3. Pipet 50 mL of the diluted  $\text{Mn}^{2+}$  solution into a 250-mL Erlenmeyer flask, add 5 mL of pH 9.3 buffer (by graduated cylinder), and add 3–5 drops of pyrocatechol violet indicator. Titrate with standard EDTA from a 50-mL buret and note the end point when the color changes from blue to violet.

5. Repeat step 4 twice more to obtain a total of three replicate titrations. The Erlenmeyer flask must be clean, but it need not be dry for each new titration.
6. From the molarity and volume of standard EDTA required for titration, calculate the molarity and standard deviation of the original ~0.018 M  $\text{MnSO}_4$  stock solution. Express your answer with an appropriate number of significant digits.

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1. Adapted from San Jose State University Laboratory Manual for a curriculum described by S. P. Perone, J. Pesek, C. Stone, and P. Englert, *J. Chem. Ed.* 1998, 75, 1444.

## 24. Measuring Manganese in Steel by Spectrophotometry with Standard Addition

Experiments 23–25 illustrate a sequence in which students (1) prepare and standardize a  $\text{Mn}^{2+}$  solution and then (2) use this standard in the analysis of Mn in steel by two different instrumental techniques.<sup>1</sup> In this experiment, steel is dissolved in acid and its Mn is oxidized to the violet colored permanganate ion, whose absorbance is measured with a spectrophotometer:



Steel is an alloy of iron that typically contains ~0.5 wt % Mn plus numerous other elements. When steel is dissolved in hot nitric acid, the iron is converted to Fe(III). Spectrophotometric interference in the measurement of  $\text{MnO}_4^-$  by Fe(III) is minimized by adding  $\text{H}_3\text{PO}_4$  to form a nearly colorless complex with Fe(III). Interference by most other colored impurities is eliminated by subtracting the absorbance of a reagent blank from that of the unknown. Appreciable Cr in the steel will interfere with the present procedure. Carbon from the steel is eliminated by oxidation with peroxydisulfate ( $\text{S}_2\text{O}_8^{2-}$ ):



### Reagents

*3 M Nitric acid:* (150 mL/student) Dilute 190 mL of 70 wt %  $\text{HNO}_3$  to 1 L with water.

*0.05 M Nitric acid:* (300 mL/student) Dilute 3.2 mL of 70 wt %  $\text{HNO}_3$  to 1 L with water.

*Ammonium hydrogen sulfite:* (0.5 mL/student) 45 wt %  $\text{NH}_4\text{HSO}_3$  in water.

*Potassium periodate ( $\text{KIO}_4$ ):* 1.5 g/student

*Unknowns:* Steel, ~2 g/student. Analyzed samples are available from Thorn Smith.<sup>2</sup>

### Procedure

1. Steel can be used as received or, if it appears to be coated with oil or grease, it should be rinsed with acetone and dried at  $110^\circ\text{C}$  for 5 min, and cooled in a desiccator.
2. Weigh duplicate samples of steel to the nearest 0.1 mg into 250 mL beakers. The mass of steel should be chosen to contain ~2–4 mg of Mn. For example, if the steel contains 0.5 wt % Mn, a 0.6-g sample will contain 3 mg of Mn. Your instructor should give you guidance on how much steel to use.

3. Dissolve each steel sample separately in 50 mL of 3 M HNO<sub>3</sub> by gently boiling in the hood, while covered with a watchglass. If undissolved particles remain, stop boiling after 1 h. Replace the HNO<sub>3</sub> as it evaporates.
4. *Standard Mn<sup>2+</sup> (~0.1 mg Mn/mL):* While the steel is dissolving, pipet 10.00 mL of standard Mn<sup>2+</sup> (~1 mg Mn/mL) from Experiment 23 into a 100-mL volumetric flask, dilute to the mark with water, and mix well. You will use this solution in Experiments 24 and 25. Keep it stoppered, and wrap the stopper with Parafilm or tape to minimize evaporation.
5. Cool the beakers from step 3 for 5 min. Then carefully add ~1.0 g of (NH<sub>4</sub>)<sub>2</sub>S<sub>2</sub>O<sub>8</sub> or K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> and boil for 15 min to oxidize carbon to CO<sub>2</sub>.
6. If traces of pink color (MnO<sub>4</sub><sup>-</sup>) or brown precipitate (MnO<sub>2</sub>) are observed, add 6 drops of 45 wt % NH<sub>4</sub>HSO<sub>3</sub> and boil for 5 min to reduce all manganese to Mn(II):



(The purpose of removing colored species at this time is that the solution from step 6 is eventually going to serve as a colorimetric reagent blank.)

7. After cooling the solutions to near room temperature, filter each solution quantitatively through #41 filter paper into a 250-mL volumetric flask. (If gelatinous precipitate is present, use #42 filter paper.) To complete a "quantitative" transfer, wash the beaker many times with small volumes of hot 0.05 M HNO<sub>3</sub> and pass the washings through the filter to wash liquid from the precipitate into the volumetric flask. Finally, allow the volumetric flasks to cool to room temperature, dilute to the mark with water, and mix well.
8. Transfer ~100 mL of solution from each 250-mL volumetric flask to clean, dry Erlenmeyer flasks and stopper the flasks tightly. Label these solutions A and B and save them for atomic absorption analysis in Experiment 25. To help prevent evaporation, it is a good idea to seal around the stoppers with a few layers of Parafilm or tape.
9. Carry out the following spectrophotometric analysis with one of the unknown steel solutions prepared in step 7:
  - a. Pipet 25.00 mL of liquid from the 250-mL volumetric flask in step 7 into each of three clean, dry 100-mL beakers designated "blank," "unknown," and "standard addition." Add 5 mL of 85 wt % H<sub>3</sub>PO<sub>4</sub> (from a graduated cylinder) into each beaker. Then add standard Mn<sup>2+</sup> (0.1 mg/mL from step 4, delivered by pipet) and solid KIO<sub>4</sub> as follows:

Beaker	Volume of $\text{Mn}^{2+}$ (mL)	Mass of $\text{KIO}_4$ (g)
Blank	0	0
Unknown	0	0.4
Standard addition	5.00	0.4

- b. Boil the unknown and standard addition beakers gently for 5 min to oxidize  $\text{Mn}^{2+}$  to  $\text{MnO}_4^-$ . Continue boiling, if necessary, until the  $\text{KIO}_4$  dissolves.
- c. Quantitatively transfer the contents of each of the three beakers into 50-mL volumetric flasks. Wash each beaker many times with small portions of water and transfer the water to the corresponding volumetric flask. Dilute each flask to the mark with water and mix well.
- d. Fill one 1.000-cm-pathlength cuvet with unknown solution and another cuvet with blank solution. It is always a good idea to rinse the cuvet a few times with small quantities of the solution to be measured and discard the rinses.
- e. Measure the absorbance of the unknown at 525 nm with blank solution in the reference cuvet. For best results, measure the absorbance at several wavelengths to locate the maximum absorbance. Use this wavelength for subsequent measurements.
- f. Measure the absorbance of the standard addition with the blank solution in the reference cuvet. The absorbance of the standard addition will be  $\sim 0.45$  absorbance units greater than the absorbance of the unknown (based on adding  $\sim 0.50$  mg of standard  $\text{Mn}^{2+}$  to the unknown).

10. Repeat step 9 with the other unknown steel solution from step 7.

### Data Analysis

1. From the known concentration of the Mn standard in step 4, calculate the concentration of added Mn in the 50-mL volumetric flask containing the standard addition.
2. All of the  $\text{Mn}^{2+}$  is converted to  $\text{MnO}_4^-$  in step 9. From the difference between the absorbance of the standard addition and the unknown, calculate the molar absorptivity of  $\text{MnO}_4^-$ . Compute the average molar absorptivity from steps 9 and 10.
3. From the absorbance of each unknown and the average molar absorptivity of  $\text{MnO}_4^-$ , calculate the concentration of  $\text{MnO}_4^-$  in each 50-mL unknown solution.

4. Calculate the weight percent of Mn in each unknown steel sample and the percent relative range of your results:

$$\% \text{ relative range} = \frac{100 \times [\text{wt \% in steel 1} - \text{wt \% in steel 2}]}{\text{mean wt \%}}$$

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1. Adapted from San Jose State University Laboratory Manual for a curriculum described by S. P. Perone, J. Pesek, C. Stone, and P. Englert, *J. Chem. Ed.* 1998, 75, 1444.
  2. Thorn Smith Inc., 7755 Narrow Gauge Road, Beulah, MI 49617. Phone: 231-882-4672; e-mail: [www.thornsmithlabs.com](http://www.thornsmithlabs.com).

## 25. Measuring Manganese in Steel by Atomic Absorption Using a Calibration Curve

This experiment complements the results of the spectrophotometric analysis in Experiment 24.<sup>1</sup> In principle, the spectrophotometric analysis and the atomic absorption analysis should give the same value for the weight percent of Mn in the unknown steel. You will use  $\text{Mn}^{2+}$  that you standardized in Experiment 23 as the standard for the atomic absorption analysis.

### Reagents

*0.05 M Nitric acid:* (600 mL/student) Dilute 3.2 mL of 70 wt %  $\text{HNO}_3$  to 1 L with water.

*Unknown steel:* Solutions A and B from step 8 of Experiment 24.

*Standard manganese:*  $\sim 0.1$  mg Mn/mL from step 4 of Experiment 24. This concentration corresponds to  $\sim 100$   $\mu\text{g/mL} = \sim 100$  ppm.

### Calibration Curve

1. Prepare standard solutions containing  $\sim 1, 2, 3, 4$  and  $5$  ppm Mn ( $= \mu\text{g Mn/mL}$ ). Use your standard solution containing  $\sim 100$  ppm Mn from step 4 of Experiment 24. Pipet  $1.00$  mL of the standard into a  $100$ -mL volumetric flask and dilute to  $100$  mL with  $0.05$  M  $\text{HNO}_3$  to prepare a  $1$ -ppm standard. Similarly, pipet  $2.00, 3.00, 4.00,$  and  $5.00$  mL into the other flasks and dilute each to  $100$  mL with  $0.05$  M  $\text{HNO}_3$ . Calculate the concentration of Mn in  $\mu\text{g/mL}$  in each standard. (The purpose of the  $\text{HNO}_3$  is to provide  $\text{H}^+$  ions to compete with  $\text{Mn}^{2+}$  ions for binding sites on the glass surface. Without excess acid, some fraction of metal ions from a dilute solution can be lost to the glass surface. To avoid adding impurity metal ions, we use a dilute solution of the purest available acid.)
2. Measure the atomic absorption signal from each of the five standards in step 1. Use a Mn hollow cathode lamp and a wavelength of  $279.48$  nm. Measure each standard three times.
3. Measure the atomic absorption signal from a blank ( $0.05$  M  $\text{HNO}_3$ ). We will use this signal later to estimate the detection limit from Mn. For this purpose, measure a blank seven separate times and compute the mean and standard deviation of the seven measurements.

### Measuring the Unknown

1. Immediately after measuring the points on the calibration curve, measure the atomic absorption signal from unknown steel solutions A and B from step 8 of Experiment 24. Measure the absorption of each solution three times. (If the signals from A and B do not lie in the calibration range, dilute them as necessary so that they do lie in the calibration range. Dilutions must be done accurately with volumetric pipets and volumetric flasks.)

## Data Analysis

1. Make a calibration graph showing the blank plus 5 standards (7 blank readings and  $3 \times 5 = 15$  standard readings, for a total of  $n = 22$  points). Compute the least-squares slope and intercept and their standard deviations (Section 5-1 of the textbook) and show the least-squares line on the graph. Express the equation of the calibration curve in the form  $y (\pm s_y) = [m (\pm s_m)]x + [b (\pm s_b)]$ , where  $y$  is the atomic absorbance signal and  $x$  is the concentration of Mn in ppm.
2. Use the mean value of the three readings for each unknown to calculate the concentration of Mn solutions A and B.
3. Calculate the uncertainty in Mn concentration in each unknown from Equation 5-14 in the textbook. Because you have measured each unknown three times, the first term in the radical in Equation 5-14 should be  $1/3$ . In Equation 5-14,  $x$  is the mean atomic absorption signal for the unknown and there are 22 values of  $x_i$  for the points on the standard curve.
4. From the Mn concentrations (and uncertainties) in solutions A and B, calculate the wt % Mn (and its uncertainty) in the two replicate steel samples.
5. The uncertainty in wt % Mn is the standard deviation. Find the 95% confidence interval for wt % Mn in each of the two steel samples that you analyzed. For example, suppose that you find the wt % of Mn in steel to be  $0.43_3$ , with a standard deviation of  $0.01_1$ . (The subscripted digits are not significant but are retained to avoid round-off errors.) The standard deviation was derived from three replicate measurements of one solution of dissolved steel. The equation for confidence interval is  $\mu = \bar{x} \pm ts\sqrt{n}$ , where  $\mu$  is the true mean,  $\bar{x}$  is the measured mean,  $s$  is the standard deviation,  $n$  is the number of measurements (3 in this case) and  $t$  is Student's  $t$  for 95% confidence and  $n-1 = 2$  degrees of freedom. In Table 4-2 of the textbook we find  $t = 4.303$ . Therefore the 95% confidence interval is  $0.43_3 \pm ts\sqrt{n} = 0.43_3 \pm (4.303)(0.01_1)\sqrt{3} = 0.43_3 \pm 0.02_7$ .
6. Use the  $t$  test (Equation 4-8 in the textbook) to compare the two atomic absorption results to each other. Are they significantly different at the 95% confidence level?
7. Use the mean wt % Mn for the two samples and the pooled standard deviation (Equation 4-9 in the textbook) to estimate a 95% confidence interval around the mean value. Does the mean spectrophotometric value for wt % Mn from Experiment 24 lie within the 95% confidence interval for the atomic absorption results? (We cannot use the  $t$  test to compare Experiments 24 and 25 because we do not have enough samples in Experiment 24 to find a standard deviation. Otherwise, we would use the  $t$  test.)
8. *Detection limit:* The detection limit of an analytical method is the minimum concentration of analyte that can be "reliably" distinguished from 0. Different statistical criteria for the word "reliably" lead to different definitions of detection limit. If you have measured points on a

calibration curve, one common definition of detection limit is

$$\text{Detection limit (ppm)} = \frac{\bar{y}_B + 3s_B}{b}$$

where  $\bar{y}_B$  is the mean atomic absorbance reading for the blank,  $s_B$  is the standard deviation for the blank, and  $b$  is the least-squares slope of the calibration curve (absorbance/ppm). In this experiment you measured a blank solution seven times. Use the mean and standard deviation from these seven readings to calculate the detection limit. (If you subtracted the mean value of the blank from each absorbance reading when you constructed the standard curve, then  $\bar{y}_B = 0$ .)

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1. Adapted from San Jose State University Laboratory Manual for a curriculum described by S. P. Perone, J. Pesek, C. Stone, and P. Englert, *J. Chem. Ed.* 1998, 75, 1444.

## 26. Properties of an Ion-Exchange Resin<sup>1</sup>

In this experiment, we explore the properties of a cation-exchange resin, which is an organic polymer containing many sulfonic acid groups ( $\text{—SO}_3\text{H}$ ). When a cation, such as  $\text{Cu}^{2+}$ , flows into the resin, the cation is tightly bound by sulfonate groups, and one  $\text{H}^+$  is released for each positive charge bound to the resin. The bound cation can be displaced from the resin by a large excess of  $\text{H}^+$  or by an excess of any other cation for which the resin has some affinity.

In the first part of the experiment, known quantities of  $\text{NaCl}$ ,  $\text{Fe}(\text{NO}_3)_3$ , and  $\text{NaOH}$  will be passed through the resin in the  $\text{H}^+$  form. The  $\text{H}^+$  released by each cation will be measured by titration with  $\text{NaOH}$ .

In the second part, we will analyze a sample of impure vanadyl sulfate ( $\text{VOSO}_4 \cdot 2\text{H}_2\text{O}$ ). As supplied commercially, this salt contains  $\text{VOSO}_4$ ,  $\text{H}_2\text{SO}_4$ , and  $\text{H}_2\text{O}$ . A solution will be prepared from a known mass of reagent. The  $\text{VO}^{2+}$  content can be assayed spectrophotometrically, and the total cation ( $\text{VO}^{2+}$  and  $\text{H}^+$ ) content can be assayed by ion exchange. Together, these measurements enable us to establish the quantities of  $\text{VOSO}_4$ ,  $\text{H}_2\text{SO}_4$ , and  $\text{H}_2\text{O}$  in the sample.

### REAGENTS

*0.3 M NaCl*: A bottle containing 5–10 mL per student, with an accurately known concentration.

*0.1 M Fe(NO<sub>3</sub>)<sub>3</sub> · 6H<sub>2</sub>O*: A bottle containing 5–10 mL per student, with an accurately known concentration.

*VOSO<sub>4</sub>*: The commonly available grade (usually designated "purified") is used for this experiment. Students can make their own solutions and measure the absorbance at 750 nm, or a bottle of stock solution (25 mL per student) can be supplied. The stock should contain 8 g/L (accurately weighed) and be labeled with the absorbance.

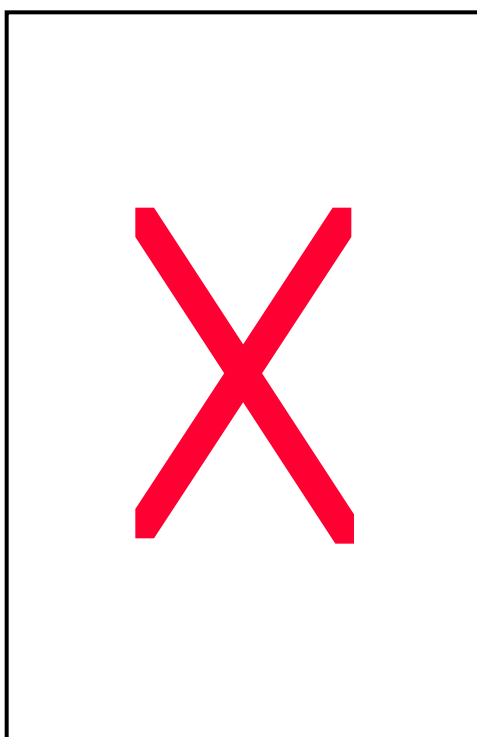
*0.02 M NaOH*: Each student should prepare an accurate 1/5 dilution of standard 0.1 M  $\text{NaOH}$ .

### PROCEDURE

1. Prepare a chromatography column from a 0.7-cm diameter  $\times$  15-cm length of glass tubing, fitted at the bottom with a cork having a small hole that serves as the outlet. Place a small ball of glass wool above the cork to retain the resin. Use a small glass rod to plug the outlet

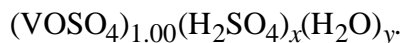
and shut off the column. (Alternatively, an inexpensive column such as  $0.7 \times 15$  cm Econo-Column from Bio-Rad Laboratories<sup>2</sup> works well in this experiment.) Fill the column with water, close it off, and test for leaks. Then drain the water until 2 cm remains and close the column again.

2. Make a slurry of 1.1 g of Bio-Rad Dowex 50W-X2 (100/200 mesh) cation-exchange resin in 5 mL of water and pour it into the column (Figure 1). If the resin cannot be poured all at once, allow some to settle, remove the supernatant liquid with a pipet, and pour in the rest of the resin. If the column is stored between laboratory periods, it should be upright, capped, and contain water above the level of the resin. (When the experiment is finished, the resin can be collected, washed with 1 M HCl and water, and reused.)



3. The general procedure for analysis of a sample is as follows:
  - a. Generate the  $H^+$ -saturated resin by passing  $\sim 10$  mL of 1 M HCl through the column. Apply the liquid sample to the glass wall so as not to disturb the resin.
  - b. Wash the column with  $\sim 15$  mL of water. Use the first few milliliters to wash the glass walls and allow the water to soak into the resin before continuing the washing. (Unlike most other chromatography resins, the one used in this experiment retains water when allowed to run "dry." Ordinarily, you must not let liquid fall below the top of the solid phase in a chromatography column.)

- c. Place a clean 125-mL flask under the outlet and pipet the sample onto the column.
  - d. After the reagent has soaked in, wash it through with 10 mL of H<sub>2</sub>O, collecting all eluate.
  - e. Add 3 drops of phenolphthalein indicator (Table 12-4 in the textbook) to the flask and titrate with standard 0.02 M NaOH.
4. Analyze 2.000-mL aliquots of 0.3 M NaCl and 0.1 M Fe(NO<sub>3</sub>)<sub>3</sub>, following the procedures in Step 3. Calculate the theoretical volume of NaOH needed for each titration. If you do not come within 2% of this volume, repeat the analysis.
  5. Pass 10.0 mL of your 0.02 M NaOH through the column as in Step 3, and analyze the eluate. Explain what you observe.
  6. Analyze 10.00 mL of VOSO<sub>4</sub> solution as described in Step 3.
  7. Using the molar absorptivity of vanadyl ion ( $\epsilon = 18.0 \text{ M}^{-1}\text{-cm}^{-1}$  at 750 nm) and the results of Step 6, express the composition of the vanadyl sulfate in the form



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1. Part of this experiment is taken from M. W. Olson and J. M. Crawford, *J. Chem. Ed.* 1975, 52, 546.
  2. Bio-Rad Laboratories, 2000 Alfred Nobel Drive, Hercules, CA 94547. Phone: 800-424-6723. [www.bio-rad.com](http://www.bio-rad.com)

## 27. Analysis of Sulfur in Coal by Ion Chromatography<sup>1</sup>

When coal is burned, sulfur in the coal is converted to  $\text{SO}_2(g)$ , which is further oxidized to  $\text{H}_2\text{SO}_4$  in the atmosphere. Rainfall laden with  $\text{H}_2\text{SO}_4$  is harmful to plant life. Measuring the sulfur content of coal is therefore important to efforts to limit man-made sources of acid rain. This experiment measures sulfur in coal by first heating the coal in the presence of air in a flux (Section 28-2 in the textbook) containing  $\text{Na}_2\text{CO}_3$  and  $\text{MgO}$ , which converts the sulfur to  $\text{Na}_2\text{SO}_4$ . The product is dissolved in water, and sulfate ion is measured by ion chromatography. Although you will be given a sample of coal to analyze, consider how you would obtain a representative sample from an entire trainload of coal being delivered to a utility company.

### REAGENTS

*Coal:* 1g/student. Coal can be obtained from electric power companies and some heavy industries. Coal is also available as a Standard Reference Material.<sup>2</sup>

*Flux:* 4 g/student. 67 wt %  $\text{MgO}$ /33 wt %  $\text{Na}_2\text{CO}_3$ .

*6 M HCl:* 25 mL/student.

*Phenolphthalein indicator.* See Table 12-4 in the textbook.

*Ammonium sulfate:* Solid reagent for preparing standards.

### PROCEDURE

1. Grind the coal to a fine powder with a mortar and pestle. Mix 1 g of coal (accurately weighed) with ~3 g of flux in a porcelain crucible. Mix thoroughly with a spatula and tap the crucible to pack the powder. Gently cover the mixture with ~1g of additional flux. Cover the crucible and place it in a muffle furnace. Then turn on the furnace and set the temperature to  $800^\circ\text{C}$  and leave the sample in the furnace at  $800^\circ\text{C}$  overnight. The reaction is over when all black particles have disappeared. A burner can be used in place of the furnace, but the burner should not be left unattended overnight.
2. After cooling to room temperature, place the crucible into a 150-mL beaker and add 100 mL of distilled water. Heat the beaker on a hot plate to just below boiling for 20 min to dissolve as much solid as possible. Pour the liquid through filter paper in a conical funnel directly into a 250-mL volumetric flask. Wash the crucible and beaker three times with 25-mL portions of distilled water and pour the washings through the filter. Add 5 drops of phenolphthalein indicator to the flask and neutralize with 6 M HCl until the pink color disappears. Dilute to 250 mL and mix well.

3. Pipet 25.00 mL of sulfate solution from the 250-mL volumetric flask in Step 2 into a 100-mL volumetric flask and dilute to volume to prepare a fourfold dilution for ion chromatography. Inject a sample of this solution into an ion chromatograph<sup>3</sup> to be sure that the concentration is in a reasonable range for analysis. More or less dilution may be necessary. Prepare the correct dilution for your equipment.
4. Assuming that the coal contains 3 wt % sulfur, calculate the concentration of  $\text{SO}_4^{2-}$  in the solution in Step 3. Using ammonium sulfate and appropriate volumetric glassware, prepare five standards containing 0.1, 0.5, 1.0, 1.5, and 2.0 times the calculated concentration of  $\text{SO}_4^{2-}$  in the unknown.
5. Analyze all solutions by ion chromatography. Prepare a calibration curve from the standards, plotting peak area versus  $\text{SO}_4^{2-}$  concentration. Use the least-squares fit to find the concentration of  $\text{SO}_4^{2-}$  in the unknown. Calculate the wt % of S in the coal.

- 
1. E. Koubek and A. E. Stewart, *J. Chem. Ed.* **1992**, *69*, A146.
  2. Standards containing 0.5 - 5 wt % S are available from NIST Standard Reference Materials Program, Room 204, Building 202, Gaithersburg MD 20899-0001 (Phone: 301-975-6776; E-mail: SRMINFO@enh.nist.gov).
  3. For example, chromatography can be done with 25-50  $\mu\text{L}$  of sample on a 4-mm-diameter  $\times$  250-mm-long Dionex Ionpac AS5 analytical column and an AG5 guard column using 2.2 mM  $\text{Na}_2\text{CO}_3$ /2.8 mM  $\text{NaHCO}_3$  eluent at 2.0 mL/min with ion suppression.  $\text{SO}_4^{2-}$  is eluted near 6 min and is detected by its conductivity on a full-scale setting of 30 microsiemens. Many combinations of column and eluent are suitable for this analysis.

## 28. Measuring Carbon Monoxide in Automobile Exhaust by Gas Chromatography<sup>1</sup>

Carbon monoxide is a colorless, odorless, poisonous gas emitted from automobile engines because of incomplete combustion of fuel to CO<sub>2</sub>. A well-tuned car with a catalytic converter might emit 0.01 vol % CO, whereas a poorly maintained car could emit as much as 15 vol % CO! In this experiment you will collect samples of auto exhaust and measure the CO content by gas chromatography. A possible class project is to compare different types of cars and different states of maintenance of vehicles. CO emission is greatest within the first few minutes after starting a cold engine. After warm-up, a well-tuned vehicle may emit too little CO to detect with an inexpensive gas chromatograph. CO can be measured as a function of time after start-up.

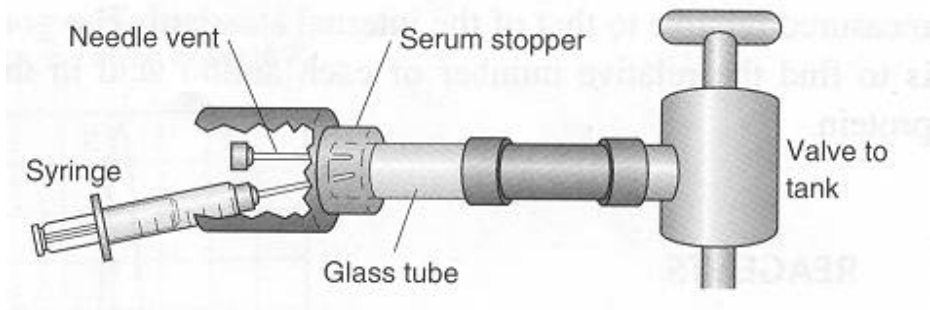
Chromatography is performed at 50°–60° C with a 2-m-long packed column containing 5A molecular sieves with He carrier gas and thermal conductivity detection. The column should be flushed periodically by disconnecting it from the detector and flowing He through for 30 h. Flushing after 50–100 injections desorbs H<sub>2</sub>O and CO<sub>2</sub> from the sieves.

### REAGENTS

*CO gas standard:* Lecture bottle containing 1 vol % CO in N<sub>2</sub>.<sup>2</sup>

### PROCEDURE

1. Attach with heavy tape a heat-resistant hose to the exhaust pipe of a car. (*Caution: Avoid breathing the exhaust.*) Use the free end of the hose to collect exhaust in a heavy-walled, 0.5-L plastic zipper-type bag from the grocery. Flush the bag well with exhaust before sealing it tightly. Allow the contents to come to room temperature for analysis.
2. Inject a 1-mL sample of air into the gas chromatograph by using a gas-tight syringe and adjust the temperature and/or flow rate if necessary so that N<sub>2</sub> is eluted within 2 min. You should see peaks for O<sub>2</sub> and N<sub>2</sub>.
3. Inject 1.00-mL of standard 1 vol % CO in N<sub>2</sub>. One way to obtain gas from a lecture bottle is to attach a hose to the tank (*in a hood*) with a serum stopper on a glass tube at the end of the hose (Figure 1). Place a needle in the serum stopper and slowly bleed gas from the tank to flush the hose. Insert a gas-tight syringe into the serum stopper, remove the vent needle, and slowly withdraw gas into the syringe. Then close the tank to prevent pressure buildup in the tubing. When you inject the standard into the chromatograph, you should see a peak for CO with about three times the retention time of N<sub>2</sub>. Adjust the detector attenuation so the CO peak is near full scale. Reinject the standard twice and measure the peak area each time.



**Figure 1.** Collecting a sample of standard gas mixture from a lecture bottle. Remove the vent needle when withdrawing gas into the syringe. Do not open the tank valve so much that the connections pop open. (**CAUTION:** *Handle CO only in a hood.*)

- Inject two 1.00-mL samples of auto exhaust and measure the peak area each time. Compute the vol % of CO in the unknown from its average peak area:

$$\frac{\text{vol \% CO in the unknown}}{\text{vol \% CO in the standard}} = \frac{\text{peak area of unknown/detector attenuation}}{\text{peak area of standard/detector attenuation}}$$

- 
- D. Jaffe and S. Herndon, *J. Chem. Ed.* **1995**, 72, 364.
  - Available, for example, from Scott Specialty Gases, Route 611, Plumsteadville PA 18949 (Phone: 215-766-8861).

## 29. Amino Acid Analysis by Capillary Electrophoresis<sup>1</sup>

In this experiment you will hydrolyze a protein with HCl to break the protein into its component amino acids. After the addition of an internal standard, the amino acids and the internal standard will be derivatized (chemically converted) to a form that absorbs light strongly at 420 nm.

Naphthalene- 2,3-dicarboxaldehyde (NDA, FM 184.19)	Amino acid	Derivatized product ( $\lambda_{\text{max}} = 420 \text{ nm}$ )
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The mixture will then be separated by capillary electrophoresis, and the quantity of each component will be measured relative to that of the internal standard. The goal is to find the relative number of each amino acid in the protein.

### REAGENTS

*Protein:* 6 mg/student. Use a pure protein such as lysozyme or cytochrome *c*. The amino acid content should be available in the literature for you to compare with your results.<sup>2</sup>

*6 M HCl:* Dilute 124 mL of concentrated (37 wt %) HCl up to 250 mL with distilled water.

*0.05 M NaOH:* Dissolve 0.50 g of NaOH (FM 40.00) in 250 mL of distilled water.

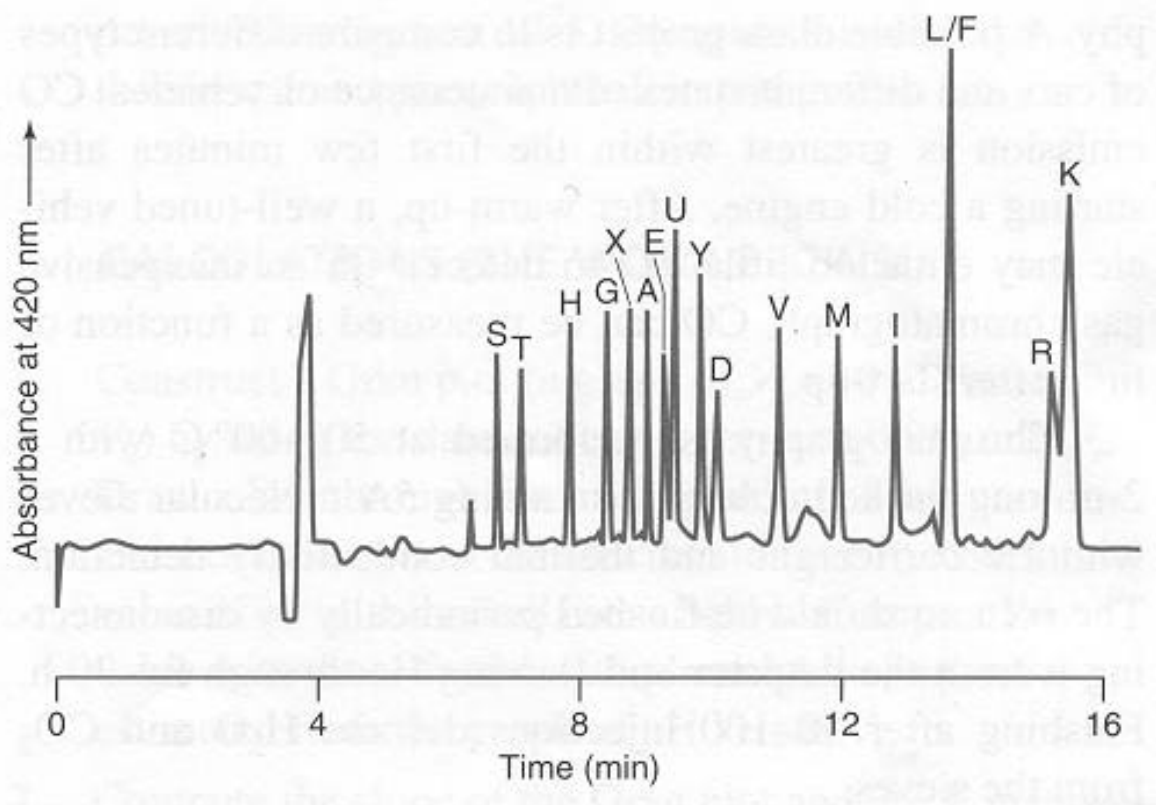
*1.5 M NH<sub>3</sub>:* Dilute 26 mL of 28 wt % NH<sub>3</sub> up to 250 mL with distilled water.

*10 mM KCN:* Dissolve 16 mg of KCN (FM 65.12) in 25 mL of distilled water.

*Borate buffer (pH 9.0):* To prepare 20 mM buffer, dissolve 0.19 g of sodium tetraborate (Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> · 10H<sub>2</sub>O, FM 381.37) in 70 mL of distilled water. Using a pH electrode, adjust the pH to 9.0 with 0.3 M HCl (a 1:20 dilution of 6 M HCl with distilled water) and dilute to 100 mL with distilled water.

*Run buffer (20 mM borate–50 mM sodium dodecyl sulfate, pH 9.0):* Prepare this as you prepared borate buffer but add 1.44 g of CH<sub>3</sub>(CH<sub>2</sub>)<sub>11</sub>OSO<sub>3</sub>Na (FM 288.38) prior to adjusting the pH with HCl.

*Amino acids:* Prepare 100 mL of standard solution containing all 15 of the amino acids in Figure 1, each at a concentration near 2.5 mM in a solvent of 0.05 M NaOH. Table 11-1 in the textbook gives molecular masses of amino acids.



**Figure 1.** Electropherogram of standard mixture of NDA-derivatized amino acids plus internal standard, all at equal concentrations, from P. L. Weber and D. R. Buck, *J. Chem. Ed.* **1994**, *71*, 609. Abbreviations for amino acids are given in Table 11-1 in the textbook. The internal standard, designated X, is  $\gamma$ -aminoadipic acid. U is an unidentified peak. Acid hydrolysis converts Q into E and converts N into D, so Q and N are not observed. The analysis of lysine (K) is not reliable because its NDA derivative is unstable. Cysteine (C) and tryptophan (W) are degraded during acid hydrolysis and are not observed. Proline (P) is not a primary amine, so it does not react with NDA to form a detectable product.

- *Aminoadipic acid internal standard:* Prepare a 5 mM solution by dissolving 40 mg of  $\text{HO}_2\text{C}(\text{CH}_2)_3\text{CH}(\text{NH}_2)\text{CO}_2\text{H}$  (FM 161.16) in 50 mL of distilled water.

*Naphthalene-2,3-dicarboxaldehyde (NDA):* Prepare a 10 mM solution by dissolving 18 mg of NDA in 10 mL of acetonitrile.

### **Hydrolysis of the Protein**

1. Fit a rubber septum onto a glass tube (17 × 55 mm) that is sealed at one end and evacuate it through a needle. Dissolve 6 mg of protein in 0.5 mL of 6 M HCl in a small vial. Purge the solution with  $\text{N}_2$  for 1 min to remove  $\text{O}_2$  and immediately transfer the liquid by syringe into the evacuated tube. Add 0.5 mL of fresh HCl to the vial, purge, and transfer again into the

tube. Heat the part of the glass tube containing the liquid at 100°–110° C in an oil bath for 18–24 h, preferably behind a shield.

2. After cooling, remove the septum and transfer the liquid to a 25-mL round-bottom flask. Evaporate the solution to dryness with gentle heat and suction from a water aspirator. Use a trap like that in Figure 2-15 in the textbook whenever you use an aspirator. Rinse the hydrolysis tube with 1 mL of distilled water, add it to the flask, and evaporate to dryness again. Dissolve the residue in 1.0 mL of 0.05 M NaOH and filter it through a 0.45- $\mu$ m pore size syringe filter. The total concentration of all amino acids in this solution is ~50 mM.

### Derivatization

3. Using a micropipet, place 345  $\mu$ L of 20 mM borate buffer into a small screw-cap vial. Add 10  $\mu$ L of the standard amino acid solution. Then add 10  $\mu$ L of 5 mM  $\alpha$ -aminoadipic acid (the internal standard), 90  $\mu$ L of 10 mM KCN, and 75  $\mu$ L of 10 mM naphthalene-2,3-dicarboxaldehyde. The fluorescent yellow-green color of the amino acid-NDA product should appear within minutes. After 25 min, add 25  $\mu$ L of 1.5 M  $\text{NH}_3$  to react with excess NDA. Wait 15 min before electrophoresis.
4. Place 345  $\mu$ L of 20 mM borate buffer (pH 9.0) into a small screw-cap vial. Add 10  $\mu$ L of the hydrolyzed protein solution in 0.05 M NaOH from Step 2. Then add 10  $\mu$ L of 5 mM  $\alpha$ -aminoadipic acid (the internal standard), 60  $\mu$ L of 10 mM KCN, and 50  $\mu$ L of 10 mM naphthalene-2,3-dicarboxaldehyde. After 25 min, add 25  $\mu$ L of 1.5 M  $\text{NH}_3$  to react with excess NDA. After 15 min, begin electrophoresis. (Precise timing reduces variations between the standard and the unknown.)

### Electrophoresis and Analysis of Results

5. (*Caution: Electrophoresis uses a dangerously high voltage of 20–24 kV. Be sure to follow all safety procedures.*) Electrophoresis is conducted with a 50- $\mu$ m-inner-diameter uncoated silica capillary and spectrophotometric detection at 420 nm. Precondition the column by injecting 30  $\mu$ L of 0.1 M NaOH and flushing 15 min later with 30  $\mu$ L each of distilled water and then run buffer. The column should be reconditioned in the same manner after every 3–4 runs.
6. Inject 3 nL of standard amino acid mixture from Step 3. The electropherogram should look similar to Figure 1. Measure the area of each peak (or the height, if you do not have a computer for measurement of area). Repeat the injection and measure the areas again.
7. Find the quotient

$$\frac{\text{area of amino acid peak}}{\text{area of internal standard peak}}$$

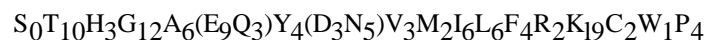
for each amino acid in the standard mixture. (Use peak heights if area is not available.) Prepare a table showing the relative areas for each peak in each injection of standard and find the average quotient for each amino acid from both runs.

8. Inject 3 nL of derivatized, hydrolyzed protein from Step 4 and measure the same quotient as measured in Step 7. Repeat the injection and find the average quotient from both injections.
9. You know the concentration of internal standard in the protein hydrolysate from the volume and concentration of internal standard used in Step 4. Find the concentration of each amino acid in the protein hydrolysate by using Equation 5-19 in the textbook.
10. Find the mole ratio of amino acids in the protein. If there were no experimental error, you could divide all concentrations by the lowest one. Because the lowest concentration has a large relative error, pick an amino acid with two or three times the concentration of the least concentrated amino acid. Define this concentration to be exactly 2 or 3. Then compute the molarities of other amino acids relative to the chosen amino acid. Your result is a formula such as  $S_{10.6}T_{6.6}H_{0.82}G_{12.5}A_{11.2}E_{5.3}Y_3D_{19.8}V_{6.1}M_{2.2}I_{5.7}(L + F)_{11.5}$ .

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1. P. L. Weber and D. R. Buck, *J. Chem. Ed.* **1994**, *71*, 609.
  2. For hen egg white lysozyme, the amino acid content is



(R. E. Canfield and A. K. Liu, *J. Biol. Chem.* **1965**, *240*, 2000; D. C. Philips, *Scientific American*, May 1966.) For horse cytochrome *c*, the composition is

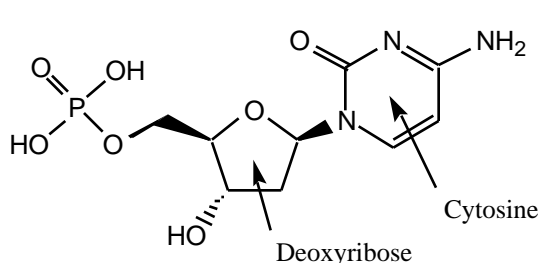


(E. Margoliash and A. Schejter, *Adv. Protein Chem.* **1966**, *21*, 114.) Both proteins are available from Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178 (Phone: 314-771-5750).

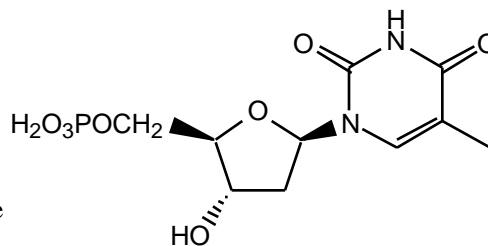
### 30. DNA Composition by High-Performance Liquid Chromatography<sup>1</sup>

This experiment illustrates quantitative analysis by high performance liquid chromatography (HPLC). It uses only aqueous eluent, so there is no hazardous waste to dispose.

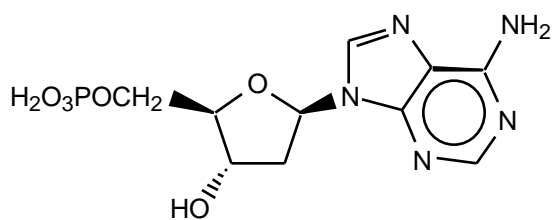
The genetic material deoxyribonucleic acid, is a polymer made of four nucleotides abbreviated C, T, A, and G:



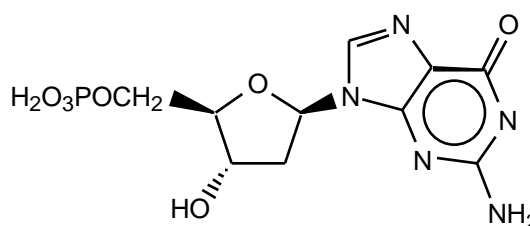
2'-Deoxycytidine 5'-monophosphate (C)



Thymidine 5'-monophosphate (T)



2'-Deoxyadenosine 5'-monophosphate (A)



2'-Deoxyguanosine 5'-monophosphate (G)

In double-stranded DNA, C is hydrogen bonded to G and A is hydrogen bonded to T. Therefore the concentrations of C and G are equal and the concentrations of A and T are equal. DNA from different organisms has different relative amounts of (C + G) and (A + T). When DNA is hydrolyzed by the enzyme nuclease P<sub>1</sub>, it is cleanly broken into the four nucleotides.

#### REAGENTS

*Standard nucleotide solution:* The standard should contain accurately weighed quantities of the nucleotide monophosphates<sup>2</sup> at concentrations of ~20 mM. The molecular masses of the free acids are C - 307.2, T - 322.2, A - 331.2, G - 347.2. Place the required quantities of the solid acids in a 5-mL volumetric flask and add 2 mL of water and 1.6 mL of 0.10 M NaOH (2 mol NaOH per mol of nucleotide). Dissolve the solid, dilute to the mark with water, mix well, and store the standard in a refrigerator. (The volume of the standard changes when it is cooled, but this is not important. Only the relative concentrations of nucleotides in the standard are important in this experiment.)

*Hydrolyzed DNA:* The volumes of DNA and nuclease P<sub>1</sub> solutions should be the minimum required for the number of people doing the experiment. Prepare a solution containing 1 mg/mL of calf thymus (or other) DNA.<sup>2</sup> Dissociate the DNA into single strands by heating at 100°C for 10 min and then cooling immediately on ice. Prepare nuclease P<sub>1</sub><sup>2</sup> at a final concentration of 5 units/mL<sup>3</sup> in 50 mM sodium acetate buffer (pH 5.3) containing 0.6 mM ZnCl<sub>2</sub>. Mix 20 μL of DNA solution with 20 μL of nuclease solution in a small vial with a conical bottom. Heat the vial at 50°C for 1 h and analyze it immediately or store it in the refrigerator.

*HPLC Eluent:* Prepare 0.010 M potassium phosphate buffer by dissolving 0.010 mol K<sub>2</sub>HPO<sub>4</sub> in 800 mL of water, titrating with ~1 M HCl to pH 7.2, and diluting to 1.00 L.

## CHROMATOGRAPHY

1. A variety of C<sub>18</sub>-silica columns should work in this experiment. A 0.46 × 15 cm column with 5 μm particles or a 0.46 × 25 cm column with 10 μm particles are reasonable. Equilibrate the column with 20 empty column volumes of 0.010 M phosphate buffer (pH 7.2) at a flow rate of 1.2 mL/min before beginning chromatography. Establish a flat baseline with an ultraviolet detector at or near 260 nm.
2. Inject 10 μL of the nucleotide standard. You should observe a clean separation of all four peaks (C < T < G < A) with an elution time of 5-10 min. Measure the areas of all four peaks, preferably by computer integration. Alternatively, you can estimate peak area from the formula: area of Gaussian peak = 1.064 × peak height × w<sub>1/2</sub>, where w<sub>1/2</sub> is the width at half-height (Figure 23-9 in the textbook). Express the areas of C, T, and A relative to the area of G, which we will define as 1.000. Repeat the procedure with a second injection and measure the relative areas. List the relative peak areas in each run and the average of the two runs.
3. Inject 10 μL of hydrolyzed DNA and measure the relative areas of the peaks. Repeat the process a second time. List the relative areas in each run and the average of the two runs.

## CALCULATIONS

1. From the average peak areas of the two standard runs, find the response factors for C, T, and A relative to G. For example, the response factor for C is obtained from the equation

$$\frac{\text{area of C}}{\text{concentration of C}} = F \frac{\text{area of G}}{\text{concentration of G}}$$

$$\frac{A_C}{[C]} = F \frac{A_G}{[G]}$$

There will be similar equations for T, and A. We are using G as the internal standard.

- From the average peak areas of the two injections of hydrolyzed DNA, find the relative concentrations  $[C]/[G]$ ,  $[T]/[G]$ , and  $[A]/[G]$  by using the response factors from the standard mixture. What is the theoretical value of  $[C]/[G]$ ? What is the theoretical relationship between  $[T]/[G]$  and  $[A]/[G]$ ?
- Find the fraction of nucleotides that are C + G by evaluating the expression

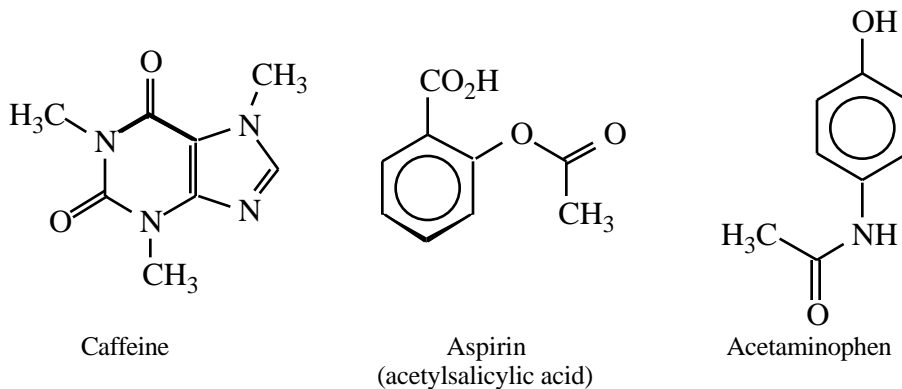
$$\text{Fraction of C + G: } \frac{[C] + [G]}{[C] + [G] + [A] + [T]} = \frac{\frac{[C]}{[G]} + \frac{[G]}{[G]}}{\frac{[C]}{[G]} + \frac{[G]}{[G]} + \frac{[A]}{[G]} + \frac{[T]}{[G]}}$$

For calf thymus DNA, the literature value of the fraction of C + G is 0.42.

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- S. M. Wietstock, *J. Chem. Ed.* **1995**, 72, 950.
  - Sigma, P. O. Box 14508, St. Louis, MO, 63178 Phone: 800-325-3010. [www.sigma-aldrich.com](http://www.sigma-aldrich.com)
  - For nuclease P<sub>1</sub>, one unit is defined as the amount that will liberate 1.0 μmol of acid soluble nucleotides from yeast ribonucleic acid per min at pH 5.3 at 37°C. The commercial preparation has at least 200 units/mg of nuclease P<sub>1</sub>.

### 31. Analysis of Analgesic Tablets by High-Performance Liquid Chromatography<sup>1</sup>

Nonprescription headache medications such as Excedrin or Vanquish contain mixtures of acetaminophen and aspirin for relief and caffeine as a stimulant. This experiment describes conditions for separating and measuring the components by high-performance liquid chromatography (HPLC). Instructions are given for measuring caffeine, but any and all of the components could be measured.



#### REAGENTS

*HPLC solvent:* Organic solvents should be handled in a fume hood. All solvents in this experiment should be HPLC-grade. Mix 110 mL of acetonitrile, 4.0 mL of triethylamine, and 4.0 mL of acetic acid in a 2-L volumetric flask and dilute to the mark with HPLC-grade water. Filter through a 0.45- $\mu$ m filter and store in a tightly capped amber bottle.

*Caffeine stock solution (100  $\mu$ g/mL):* Dissolve 1.000 g of caffeine in 50 mL of HPLC solvent in a 100-mL volumetric flask with gentle heating (in the hood). Cool to room temperature and dilute to the mark with HPLC solvent. Dilute 10.00 mL to 100 mL with HPLC solvent in a volumetric flask to obtain 1 000  $\mu$ g/mL. Dilute once again to obtain 100  $\mu$ g/mL.

*Acetaminophen and aspirin samples:* Prepare two solutions, each containing one of the analytes at a concentration of  $\sim$ 50  $\mu$ g/mL in HPLC solvent. Filter through 0.22  $\mu$ m nylon syringe filters and store in capped amber bottles.

#### PROCEDURE

1. *Caffeine quantitative analysis standards:* Dilute the 100  $\mu$ g/mL stock solution down to 50, 10, and 5  $\mu$ g/mL with HPLC solvent. Filter  $\sim$ 3 mL of each solution through a 0.22- $\mu$ m syringe filter into a capped vial. Filter  $\sim$ 3 mL of the 100  $\mu$ g/mL solution into a fourth vial.

2. *Sample preparation:* Grind the analgesic tablet into a fine powder with a clean mortar and pestle. Dissolve ~0.5 g (weighed accurately) in 50 mL of HPLC solvent with gentle heating. Cool to room temperature and dilute to volume with HPLC solvent. Dilute 10.00 mL of this solution to 100 mL with HPLC solvent in a volumetric flask. Filter ~3 mL of the dilute solution through a 0.22- $\mu$ m syringe filter into a capped vial.
3. *Chromatography conditions:* Use a 2.1-mm-diameter 10-cm-long C<sub>18</sub>-silica column with 5- $\mu$ m particle size and ultraviolet detection at 254 nm. With a flow rate of 1.5 mL/min, each run is complete in 4 min.
4. *Calibration curve:* Inject 10  $\mu$ L of each of the caffeine standards (5, 10, 50, and 100  $\mu$ g/mL) into the HPLC and measure the peak area. Repeat this process twice more and use the average areas from the three runs to construct a calibration curve of area versus concentration. Compute the least-squares slope and intercept for the line through points.
5. *Qualitative analysis:* Record a chromatogram of 10  $\mu$ L of the analgesic tablet solution. Then mix 2 drops of the tablet solution with 2 drops of 50  $\mu$ g/mL caffeine solution in a test tube or vial. Inject 10  $\mu$ L of the mixture into the chromatograph and observe which peak grows. Repeat the process again by adding 50  $\mu$ g/mL acetaminophen and 50  $\mu$ g/mL aspirin and identify which peaks in the analgesic are acetaminophen and aspirin.
6. *Quantitative analysis:* Inject 10  $\mu$ L of the analgesic tablet solution and measure the area of the caffeine peak. Repeat this process twice more and take the average from three injections. Using your calibration graph, determine the concentration of caffeine in the solution and the weight percent of caffeine in the original tablet.

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1. G. K. Ferguson, *J. Chem. Ed.* **1998**, 75, 467.

## 32. Anion Content of Drinking Water by Capillary Electrophoresis<sup>1</sup>

Chloride, sulfate, and nitrate are the major anions in fresh water. Fluoride is a minor species added to some drinking water at a level near 1.6 ppm to help prevent tooth decay. In this experiment, you will measure the three major anions by capillary electrophoresis. Possible class projects are to compare water from different sources (homes, lakes, rivers, ocean) and various bottled drinking waters.

Your equipment should be similar to that in Figure 26-14 in the textbook. Convenient capillary dimensions are a diameter of 75  $\mu\text{m}$  and a length of 40 cm from the inlet to the detector (total length = 50 cm). Because the anions have little ultraviolet absorbance at wavelengths above 200 nm, we add chromate anion ( $\text{CrO}_4^{2-}$ ) to the buffer and use indirect ultraviolet detection at 254 nm. The principle of indirect detection is explained in Figure 26-6.

One other significant condition for a successful separation in this experiment is to reduce the electroosmotic flow rate to permit a better separation of the anions based on their different electrophoretic mobilities. At pH 8, electroosmotic flow is so fast that the anions are swept from the injector to the detector too quickly to be separated well from one another. To reduce the electroosmotic flow, we could lower the pH to protonate some of the  $-\text{O}^-$  groups on the wall. Alternatively, what we do in this experiment is to add the cationic surfactant

tetradecyl(trimethyl)ammonium ion,  $\text{CH}_3(\text{CH}_2)_{13}\text{N}^+(\text{CH}_3)_3$ , which is attracted to the  $-\text{O}^-$  groups on the wall and partially neutralizes the negative charge of the wall. This cationic surfactant is abbreviated OFM<sup>+</sup>, for "osmotic flow modifier."

### REAGENTS

*Run buffer:* 4.6 mM  $\text{CrO}_4^{2-}$  + 2.5 mM OFM<sup>+</sup> at pH 8. Dissolve 1.08 g  $\text{Na}_2\text{CrO}_4 \cdot 4\text{H}_2\text{O}$  (FM 234.02) plus 25.0 mL of 100 mM tetradecyl(trimethyl)ammonium hydroxide<sup>2</sup> in 800 mL of HPLC-grade  $\text{H}_2\text{O}$ . Place a pH electrode in the solution and add solid boric acid ( $\text{H}_3\text{BO}_3$ ) (with magnetic stirring) to reduce the pH to 8.0. Dilute to 1.00 L with HPLC-grade  $\text{H}_2\text{O}$ , mix well, filter through a 0.45- $\mu\text{m}$  filter, and store in the refrigerator in a tightly capped plastic bottle. Degas prior to use.

*Quantitative standards:* Prepare one stock solution containing 1 000 ppm  $\text{Cl}^-$ , 1 000 ppm  $\text{NO}_3^-$ , and 1 000 ppm  $\text{SO}_4^{2-}$  by dissolving the following salts in HPLC-grade  $\text{H}_2\text{O}$ : 2.103 g KCl (FM 74.55), 1.631 g  $\text{KNO}_3$  (FM 101.10), and 1.814 g  $\text{K}_2\text{SO}_4$  (FM 174.26). (Concentration refers to the mass of the anion. For example, 1 000 ppm sulfate means 1 000  $\mu\text{g}$  of  $\text{SO}_4^{2-}$  per mL of solution, not 1 000  $\mu\text{g}$  of  $\text{K}_2\text{SO}_4$ .) Dilute the stock solution with HPLC-grade  $\text{H}_2\text{O}$  to make standards with concentrations of 2, 5, 10, 20, 50, and 100 ppm of the anions. Store the solutions in tightly capped plastic bottles.

*Standards for qualitative analysis:* Prepare four separate 1.00-L solutions, each containing just one anion at a concentration of  $\sim 50$  ppm. To do this, dissolve  $\sim 0.105$  g KCl,  $\sim 0.082$  g  $\text{KNO}_3$ ,  $\sim 0.091$  g  $\text{K}_2\text{SO}_4$ , or  $\sim 0.153$  g KF (FM 58.10) in 1.00 L.

## PROCEDURE

- 0. CAUTION:** *Electrophoresis uses a dangerously high voltage. Be sure to follow all safety procedures for the instrument.*
- 1.** When preparing a capillary for its first use, wash through 1 M NaOH for 15 min, followed by 0.1 M NaOH for 15 min, followed by run buffer for 15 min. In this experiment, wash the column with run buffer for 1 min between sample injections.
- 2. Identify the peaks:** Inject a 50 ppm mixture of  $\text{Cl}^-$ ,  $\text{NO}_3^-$ , and  $\text{SO}_4^{2-}$  by applying a pressure of 0.3 bar for 5 s. Then insert the sample end of the capillary back in run buffer and perform a separation for 5 min at 10 kV with the capillary thermostatted near 25°C. The voltage should be positive at the injector and negative at the detector. The detector should be set at 254 nm. After the run, wash the column with run buffer for 1 min. Mix the 50 ppm anion mixture with an equal volume of 50 ppm  $\text{Cl}^-$  and run an electropherogram of the mixture. The  $\text{Cl}^-$  peak should be twice the size it was in the first run. Repeat the procedure with additions of  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ , and  $\text{F}^-$ . This process tells you which peak belongs to each anion and where to look for  $\text{F}^-$  in drinking water.
- 3. Calibration curves:** Inject each of the standard mixtures from lowest concentration to highest concentration (2, 5, 10, 20, 50, and 100 ppm) and measure the area of each peak in each run. Repeat the sequence twice more and use the average peak area at each concentration to construct a calibration curve for each anion. Find the least-squares straight line to fit the graph of area versus concentration for each anion.
- 4. Unknowns:** Make three replicate injections of each unknown water sample and measure the areas of the peaks. Use the average area of each peak and the calibration curves to find the concentrations of the anions in the water. If you analyze any saltwater samples, they should be diluted by a factor of 100 with HPLC-grade water to bring the anion concentrations down to the range of fresh waters.

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1. S. Demay, A. Martin-Girardeau, and M.-F. Gonnord, *J. Chem. Ed.* **1999**, 76, 812.
  2. 100 mM tetradecyl(trimethyl)ammonium hydroxide (Catalog number WAT049387) is available from Waters Corp., 34 Maple Street, Milford, MA 01757; phone 508-478-2000; www.waters.com. The surfactant is sold under the trade name "Osmotic Flow Modifier," abbreviated OFM<sup>+</sup>OH<sup>-</sup>.