17. Polarographic Measurement of an Equilibrium Constant[1]

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

$$Pb^{2+} + pC_2O_4^{2-} \rightleftharpoons + Pb(C_2O_4)_p^{2-2p} \qquad \beta_p = \frac{[Pb(C_2O_4)_p^{2-2p}]}{[Pb^{2+}][C_2O_4^{2-}]^p}$$

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

$$\Delta E_{1/2} = -\frac{RT}{nF}\ln\beta_p - \frac{pRT}{nF}\ln[C_2O_4^{2-}] \qquad (1)$$

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

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

where I is current and I_d is diffusion current.

PROCEDURE

1. Pipet 1.00 mL of 0.020 M $Pb(NO_3)_2$ 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 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 KNO_3.
B: Add 5.00 mL of 1.00 M $K_2C_2O_4$, and 37.5 mL of 1.20 M KNO_3.
C: Add 10.00 mL of 1.00 M K_2C_2O, and 25.0 mL of 1.20 M KNO_3.
D: Add 15.00 mL of 1.00 M $K_2C_2O_4$ and 12.5 mL of 1.20 M KNO_3.
E: Add 20.00 mL of 1.00 M $K_2C_2O_4$.

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 $\Delta E_{1/2}$ 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 β_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 β_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[1]

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.[2] 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_2 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.

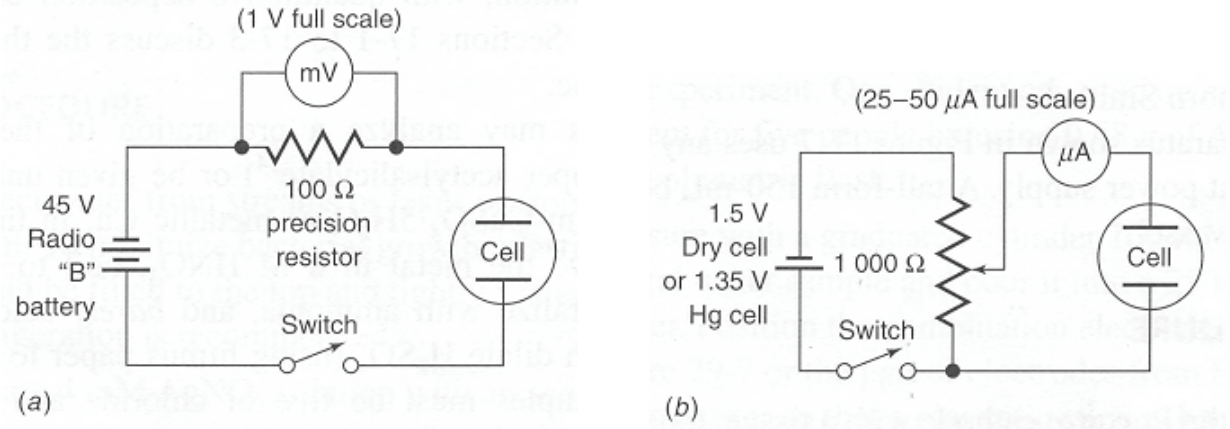

Figure 1. Circuits for coulometric titrations. (*a*) Generator circuit. (*b*) Detector circuit.

2. Generate Br_2 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_2.

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 HNO_3 to dissolve Hg that is deposited during the electrolysis.

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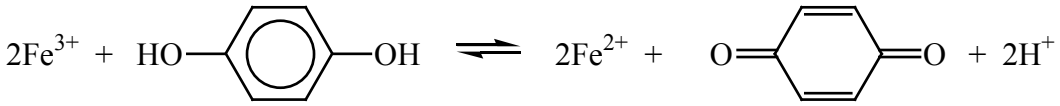
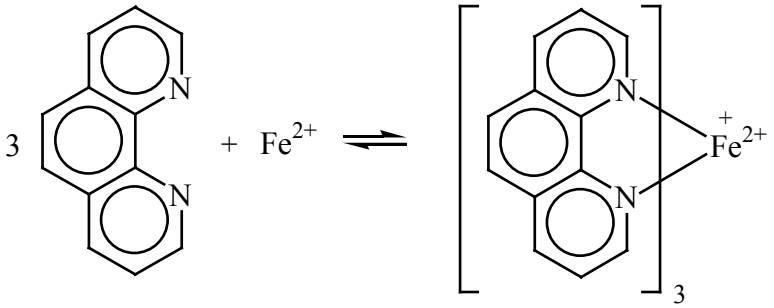
1. D. H. Evans, *J. Chem. Ed*. **1968**, *45*, 88.
2. A constant-current circuit for coulometer generator electrodes is given by J. Swim, E. Earps, L. M. Reed, and D. Paul, *J. Chem. Ed*. **1996**, *73*, 679. An operational amplifier circuit for the detector and a circuit for controlled-potential coulometry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

19. Spectrophotometric Determination of Iron in Vitamin Tablets[1]

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



Hydroquinone Quinone



o-Phenanthroline λ_{max} = 508 nm

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 $Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$ in water in a 1-L volumetric flask containing 1 mL of 98 wt % H_2SO_4.

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 $Fe(o\text{-phenanthroline})_3^{2+}$ in each solution and find the average molar absorptivity (ε 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[1]

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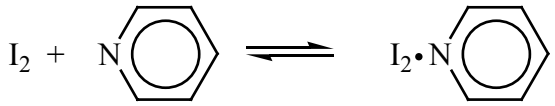
1. Idea based on P. E. Adams, *J. Chem. Ed.* **1995**, *72*, 649.

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:[1]



Iodine-pyridine complex

Both I_2 and 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.

1. The following stock solutions should be available in the lab:

(a) 0.050–0.055 M pyridine in cyclohexane (40 mL for each student, concentration known accurately).

(b) 0.012 0–0.012 5 M I_2 in cyclohexane (10 mL for each student, concentration known accurately).

2. 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)	I_2 stock solution (mL)
A	0	1.00
B	1.00	1.00
C	2.00	1.00
D	4.00	1.00
E	5.00	1.00
F	10.00	1.00

3. 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.)

4. Record the spectrum of each solution A–F or measure the absorbance at each maximum if a fixed-wavelength instrument is used.

DATA ANALYSIS

1. Measure the absorbance at the wavelengths of the two maxima in each spectrum. Be sure to subtract the absorbance of the blank from each.

2. 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 [pyridine] >> [I_2]). Prepare a graph of ΔA/[free pyridine] versus ΔA (a Scatchard plot), using the absorbance at the I_2·pyridine maximum.

3. From the slope of the graph, find the equilibrium constant using Equation 19-16 in the textbook. From the intercept, find $\Delta\varepsilon$ ($= \varepsilon_{PX} - \varepsilon_X$).

4. Now refine the values of K and $\Delta\varepsilon$. Use $\Delta\varepsilon$ to find ε_{PX}. Then use the absorbance at the wavelength of the I_2·pyridine maximum to find the concentration of bound and free pyridine in each solution. Make a new graph of ΔA /[free pyridine] versus ΔA, using the new values of [free pyridine]. Find a new value of K and $\Delta\varepsilon$. If justified, perform another cycle of refinement.

5. Using the values of free pyridine concentration from your last refinement and the values of absorbance at the I_2 maximum, prepare another Scatchard plot and see if you get the same value of K.

6. Explain why an isosbestic point is observed in this experiment.

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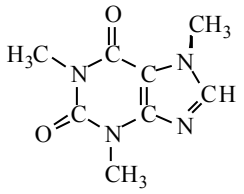
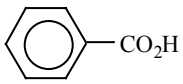
1. For literature values of the equilibrium constant for the reaction between I_2 and pyridine, see S. S. Barton and R. H. Pottier, *J. Chem. Soc. Perkin Trans. II* **1984**, 731.

22. Spectrophotometric Analysis of a Mixture: Caffeine and Benzoic Acid in a Soft Drink[1]

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.

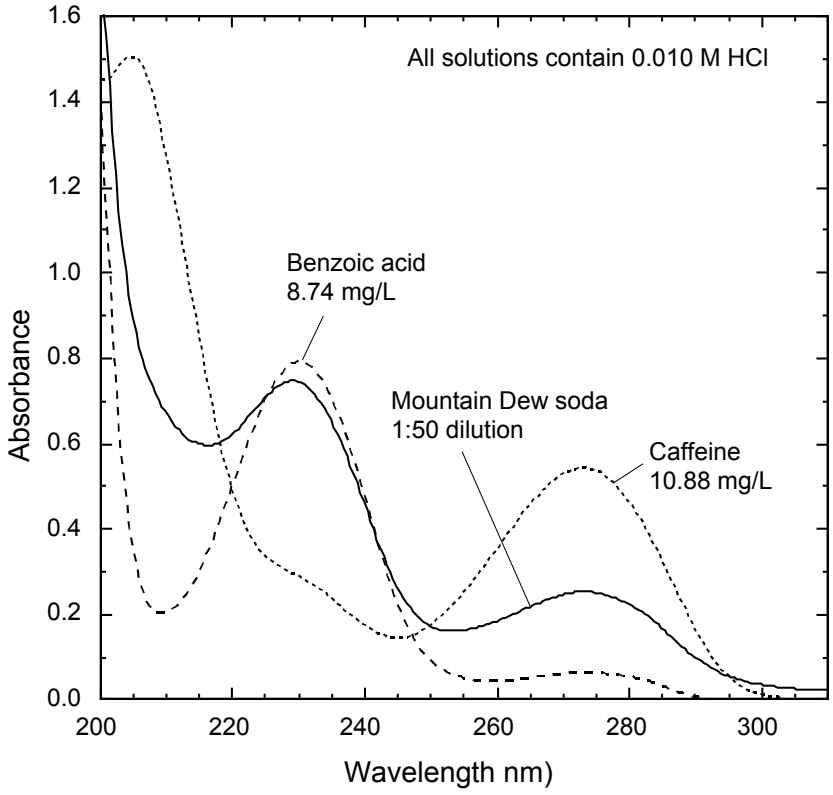


Figure 1. Ultraviolet absorption of benzoic acid, caffeine, and a 1:50 dilution of Mountain Dew soft drink. All solutions contain 0.010 M HCl.

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).[1]

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_2 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 cuvets (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 (λ') and the wavelength for the peak absorbance of caffeine (λ''). 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 λ' and λ'', 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. Rodriquez and K. R. Williams, *J. Chem. Ed.* **1998**, *75*, 625.

23. Mn^{2+} Standardization by EDTA Titration

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

Reagents

$MnSO_4 \cdot H_2O$: (1 g/student) This material is not a primary standard.

EDTA: $Na_2H_2EDTA \cdot 2H_2O$, 1 g/student.

0.5 M NH_3/NH_4^+ buffer (pH 9.3): Mix 6.69 g of NH_4Cl (FM 53.49) plus 7.60 g of 28% aqueous NH_3 (FM 17.03) with enough water to give a total volume of 250 mL.

Hydroxylamine hydrochloride: ($NH_3OH^+Cl^-$, FM 69.49) 1 g/student. (**CAUTION:** Do not breathe dust from $NH_3OH^+Cl^-$; avoid contact with skin and eyes.)

Pyrocatechol violet indicator: Dissolve 0.1 g in 100 mL H_2O.

Procedure

1. *Standard 0.005 M EDTA:* Dry $Na_2H_2EDTA \cdot 2H_2O$ (FM 372.25) at 80˚C for 1 h and cool in a desiccator. Accurately weigh out ~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. *Mn^{2+} stock solution:* Prepare a solution containing ~1.0 mg Mn/mL (~0.018 M) by dissolving ~0.77 g $MnSO_4 \cdot H_2O$ (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 Mn^{2+} stock solution and discard the washings into a chemical waste container. Then pipet 50.00 mL of Mn^{2+} stock solution into a 250-mL volumetric flask. Add ~0.8 g (not accurately weighed) of solid hydroxylamine hydrochloride to the flask, and swirl to dissolve the solid. Add ~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 ~0.0036 M 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 Mn^{2+} solution from step 3. Pipet 50 mL of the diluted 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 $MnSO_4$ 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 Mn^{2+} solution and then (2) use this standard in the analysis of Mn in steel by two different instrumental techniques.[1] 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:

$$\underset{\text{(colorless)}}{2Mn^{2+}} + \underset{\substack{\text{Periodate}\\\text{(colorless)}}}{5IO_4^-} + 3H_2O \rightarrow \underset{\substack{\text{Permanganate}\\\text{(violet)}\\\lambda_{max} \approx 525\text{ nm}}}{2MnO_4^-} + \underset{\substack{\text{Iodate}\\\text{(colorless)}}}{5IO_3^-} + 6H^+$$

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 MnO_4^- by Fe(III) is minimized by adding H_3PO_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 ($S_2O_8^{2-}$):

$$C(s) + 2S_2O_8^{2-} + 2H_2O \rightarrow CO_2(g) + 4SO_4^{2-} + 4H^+$$

Reagents

3 M Nitric acid: (150 mL/student) Dilute 190 mL of 70 wt % HNO_3 to 1 L with water.

0.05 M Nitric acid: (300 mL/student) Dilute 3.2 mL of 70 wt % HNO_3 to 1 L with water.

Ammonium hydrogen sulfite: (0.5 mL/student) 45 wt % NH_4HSO_3 in water.

Potassium periodate (KIO_4): 1.5 g/student

Unknowns: Steel, ~2 g/student. Analyzed samples are available from Thorn Smith.[2]

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°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_3 by gently boiling in the hood,

while covered with a watchglass. If undissolved particles remain, stop boiling after 1 h. Replace the HNO_3 as it evaporates.

4. *Standard Mn^{2+} (~0.1 mg Mn/mL):* While the steel is dissolving, pipet 10.00 mL of standard Mn^{2+} (~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 $(NH_4)_2S_2O_8$ or $K_2S_2O_8$ and boil for 15 min to oxidize carbon to CO_2.

6. If traces of pink color (MnO_4^-) or brown precipitate (MnO_2) are observed, add 6 drops of 45 wt % NH_4HSO_3 and boil for 5 min to reduce all manganese to Mn(II):

$$2MnO_4^- + 5HSO_3^- + H^+ \rightarrow 2Mn^{2+} + 5SO_4^{2-} + 3H_2O$$

$$MnO_2(s) + HSO_3^- + H^+ \rightarrow Mn^{2+} + SO_4^{2-} + H_2O$$

(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_3 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_3PO_4 (from a graduated cylinder) into each beaker. Then add standard Mn^{2+} (0.1 mg/mL from step 4, delivered by pipet) and solid KIO_4 as follows:

Beaker	Volume of Mn^{2+} (mL)	Mass of 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 Mn^{2+} to MnO_4^-. Continue boiling, if necessary, until the 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 ~0.45 absorbance units greater than the absorbance of the unknown (based on adding ~0.50 mg of standard 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 Mn^{2+} is converted to MnO_4^- in step 9. From the difference between the absorbance of the standard addition and the unknown, calculate the molar absorptivity of 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 MnO_4^-, calculate the concentration of 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:

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

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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.

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

This experiment complements the results of the spectrophotometric analysis in Experiment 24.[1] 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 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 % HNO_3 to 1 L with water.

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

Standard manganese: ~0.1 mg Mn/mL from step 4 of Experiment 24. This concentration corresponds to ~100 μg/mL = ~100 ppm.

Calibration Curve

1. Prepare standard solutions containing ~1, 2, 3, 4 and 5 ppm Mn (= μg Mn/mL). Use your standard solution containing ~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 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 HNO_3. Calculate the concentration of Mn in μg/mL in each standard. (The purpose of the HNO_3 is to provide H^+ ions to compete with 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 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 abosroption 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 μ 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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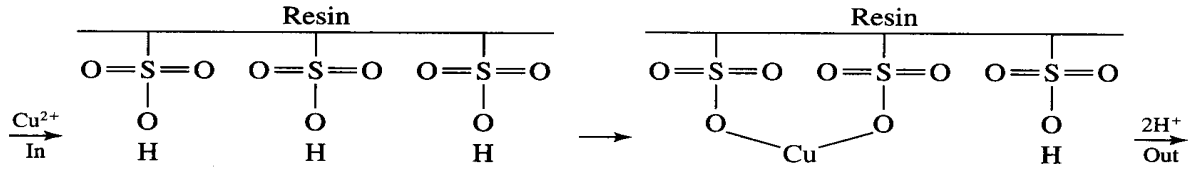
1. Adapted from San Jose State University Laboratory Manual for a curriculum described by S. P. Perone, J. Pesek, C. Stone, and P. Englert, *J. Chem. Ed.* 1998, *75*, 1444.

26. Properties of an Ion-Exchange Resin[1]

In this experiment, we explore the properties of a cation-exchange resin, which is an organic polymer containing many sulfonic acid groups (—SO_3H). When a cation, such as Cu^{2+}, flows into the resin, the cation is tightly bound by sulfonate groups, and one 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 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 NaCl, $Fe(NO_3)_3$, and NaOH will be passed through the resin in the H^+ form. The H^+ released by each cation will be measured by titration with NaOH.

In the second part, we will analyze a sample of impure vanadyl sulfate ($VOSO_4 \cdot 2H_2O$). As supplied commercially, this salt contains $VOSO_4$, H_2SO_4, and H_2O. A solution will be prepared from a known mass of reagent. The VO^{2+} content can be assayed spectrophotometrically, and the total cation (VO^{2+} and H^+) content can be assayed by ion exchange. Together, these measurements enable us to establish the quantities of $VOSO_4$, H_2SO_4, and H_2O in the sample.

REAGENTS

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

0.1 M $Fe(NO_3)_3 \cdot 6H_2O$: A bottle containing 5–10 mL per student, with an accurately known concentration.

$VOSO_4$: 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 NaOH.

PROCEDURE

1. Prepare a chromatography column from a 0.7-cm diameter × 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 × 15 cm Econo-

Column from Bio-Rad Laboratories[2] 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 5OW-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.)

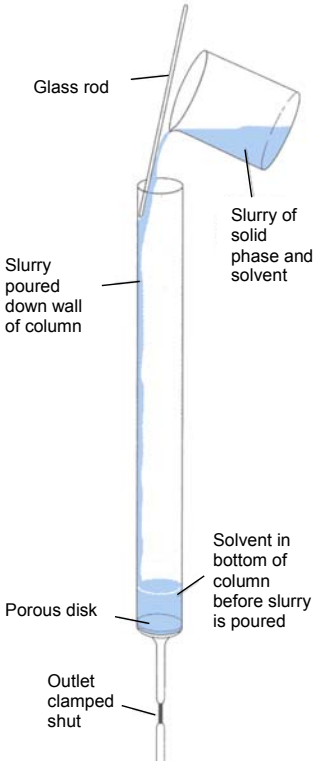


Figure 1. Pouring a slurry of stationary phase into a chromatography column.

3. The general procedure for analysis of a sample is as follows:

a. Generate the H^+-saturated resin by passing ~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 ~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_2O, 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 $Fe(NO_3)_3$, 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 $VOSO_4$ solution as described in Step 3.

7. Using the molar absorptivity of vanadyl ion ($\varepsilon = 18.0\ M^{-1}\cdot cm^{-1}$ at 750 nm) and the results of Step 6, express the composition of the vanadyl sulfate in the form

$$(VOSO_4)_{1.00}(H_2SO_4)_x(H_2O)_y.$$

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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

27. Analysis of Sulfur in Coal by Ion Chromatography[1]

When coal is burned, sulfur in the coal is converted to $SO_2(g)$, which is further oxidized to H_2SO_4 in the atmosphere. Rainfall laden with H_2SO_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 Na_2CO_3 and MgO, which converts the sulfur to Na_2SO_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.[2]

Flux: 4 g/student. 67 wt % MgO/33 wt % Na_2CO3.

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˚ C and leave the sample in the furnace at 800˚ 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[3] 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 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 SO_4^{2-} in the unknown.

5. Analyze all solutions by ion chromatography. Prepare a calibration curve from the standards, plotting peak area versus SO_4^{2-} concentration. Use the least-squares fit to find the concentration of SO_4^{2-} in the unknown. Calculate the wt % of S in the coal.

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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 µL of sample on a 4-mm-diameter × 250-mm-long Dionex Ionpac AS5 analytical column and an AG5 guard column using 2.2 mM Na_2CO_3/2.8 mM $NaHCO_3$ eluent at 2.0 mL/min with ion suppression. 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[1]

Carbon monoxide is a colorless, odorless, poisonous gas emitted from automobile engines because of incomplete combustion of fuel to CO_2. 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_2O and CO_2 from the sieves.

REAGENTS

CO gas standard: Lecture bottle containing 1 vol % CO in N_2.[2]

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_2 is eluted within 2 min. You should see peaks for O_2 and N_2.

3. Inject 1.00-mL of standard 1 vol % CO in N_2. 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_2. Adjust the detector attenuation so the CO peak is near full scale. Reinject the standard twice and measure the peak area each time.

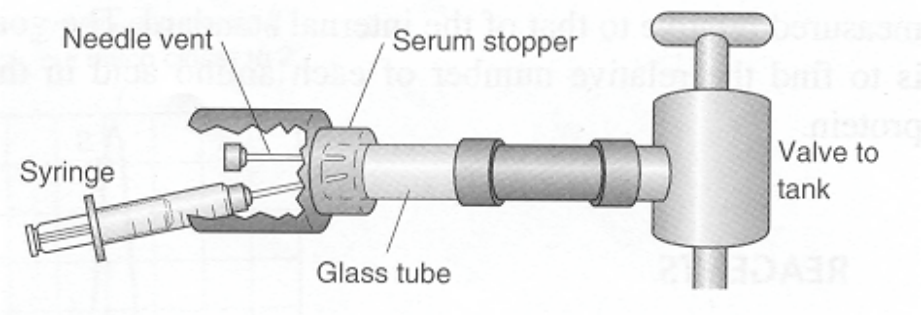


Figure 1. Collecting a sample of standard gas mixture from a lecture bottle. Remove the vent needle when withdrawing gas into the syringe. Do not open the tank valve so much that the connections pop open. **(CAUTION:** *Handle CO only in a hood.)*

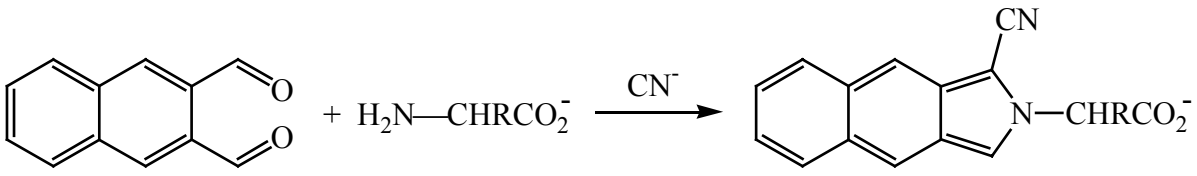
4. Inject two 1.00-mL samples of auto exhaust and measure the peak area each time. Compute the vol % of CO in the unknown from its average peak area:

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

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

29. Amino Acid Analysis by Capillary Electrophoresis[1]

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 (λ_{max} = 420 nm)

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.[2]

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_3: Dilute 26 mL of 28 wt % NH_3 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 0.76 g of sodium tetraborate ($Na_2B_4O_7 \cdot 10H_2O$, 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 $CH_3(CH_2)_{11}OSO_3Na$ (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 11-1in the textbook gives molecular masses of amino acids.

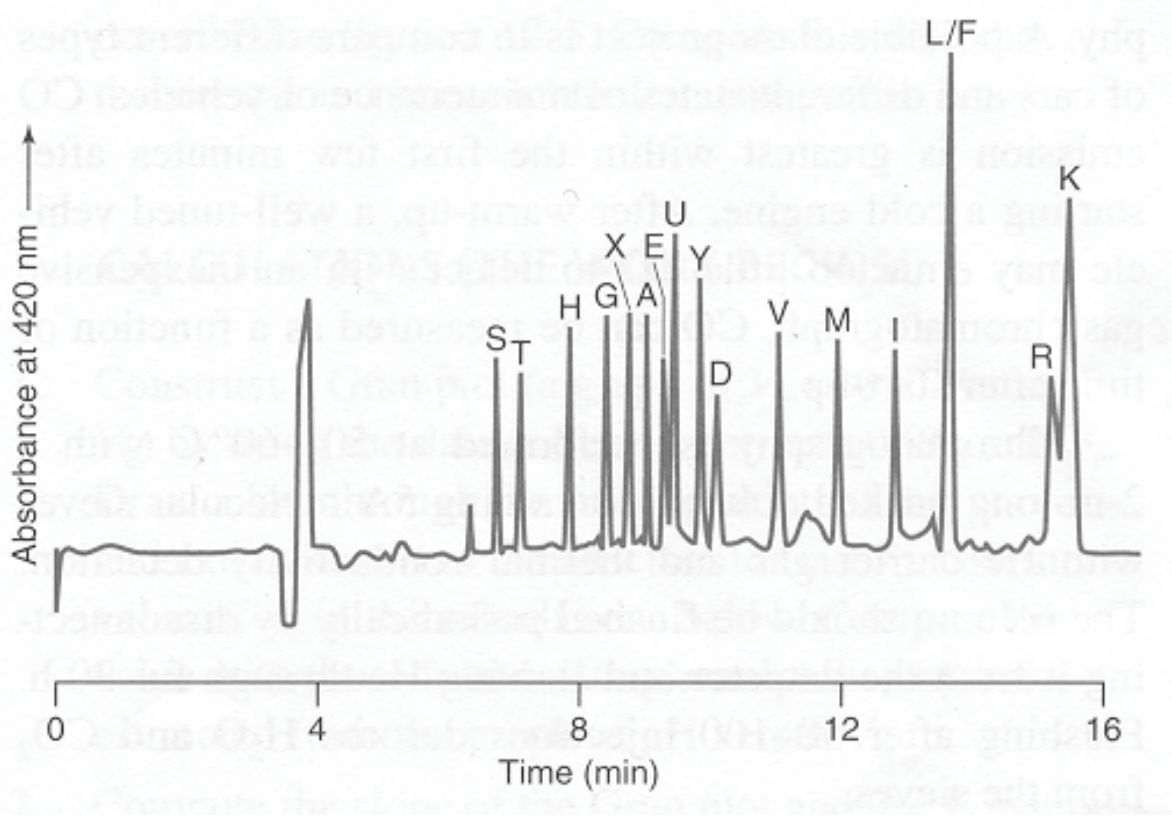


Figure 1. Electropherogram of standard mixture of NDA-derivatized amino acids plus internal standard, all at equal concentrations, from P. L. Weber and D. R. Buck, *J. Chem. Ed.* **1994**, *71*, 609. Abbreviations for amino acids are given in Table 11-1 in the textbook. The internal standard, designated X, is α-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.

α- Aminoadipic acid internal standard: Prepare a 5 mM solution by dissolving 40 mg of $HO_2C(CH_2)_3CH(NH_2)CO_2H$ (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 N_2 for 1 min to remove 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˚–l10˚ 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-μm pore size syringe filter. The total concentration of all amino acids in this solution is ~50 mM.

Derivatization

3. Using a micropipet, place 345 μL of 20 mM borate buffer into a small screw-cap vial. Add 10 μL of the standard amino acid solution. Then add 10 μL of 5 mM α-aminoadipic acid (the internal standard), 90 μL of 10 mM KCN, and 75 μL 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 μL of 1.5 M NH_3 to react with excess NDA. Wait 15 min before electrophoresis.

4. Place 345 μL of 20 mM borate buffer (pH 9.0) into a small screw-cap vial. Add 10 μL of the hydrolyzed protein solution in 0.05 M NaOH from Step 2. Then add 10 μL of 5 mM α-aminoadipic acid (the internal standard), 60 μL of 10 mM KCN, and 50 μL of 10 mM naphthalene-2,3-dicarboxaldehyde. After 25 min, add 25 μL of 1.5 M 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-μm-inner-diameter uncoated silica capillary and spectrophotometric detection at 420 nm. Precondition the column by injecting 30 μL of 0.1 M NaOH and flushing 15 min later with 30 μL 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 $S_{10.6}T_{6.6}H_{0.82}G_{12.5}A_{11.2}E_{5.3}Y_{\equiv 3}D_{19.8}V_{6.1}M_{2.2}I_{5.7}(L + F)_{11.5}$.

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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

$$S_{10}T_7H_1G_{12}A_{12}(E_2Q_3)Y_3(D_8N_{13})V_6M_2I_6L_8F_3R_{11}K_6C_8W_8P_2$$

(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

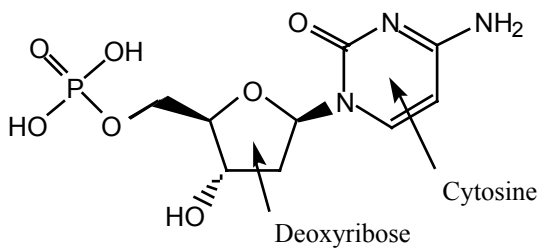
$$S_0T_{10}H_3G_{12}A_6(E_9Q_3)Y_4(D_3N_5)V_3M_2I_6L_6F_4R_2K_{19}C_2W_1P_4$$

(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).

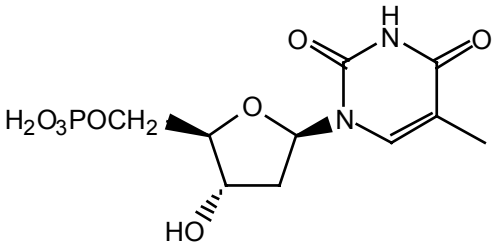
30. DNA Composition by High-Performance Liquid Chromatography[1]

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

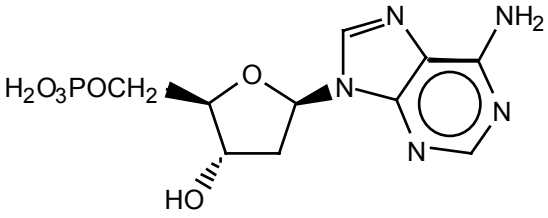
The genetic material deoxyribonucleic acid, is a polymer made of four nucleotides abbreviated C, T, A, and G:



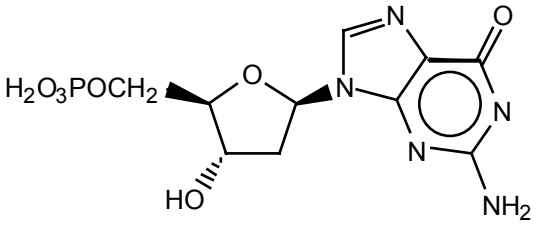
2'-Deoxycytidine 5'-monophosphate (C)



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_1, it is cleanly broken into the four nucleotides.

REAGENTS

Standard nucleotide solution: The standard should contain accurately weighed quantities of the nucleotide monophosphates[2] 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_1 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.[2] Dissociate the DNA into single strands by heating at 100°C for 10 min and then cooling immediately on ice. Prepare nuclease P_1[2] at a final concentration of 5 units/mL[3] in 50 mM sodium acetate buffer (pH 5.3) containing 0.6 mM $ZnCl_2$. 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_2HPO_4 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_{18}-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 × $w_{1/2,\ \text{where}}$ $w_{1/2}$ 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

$$\frac{\text{area of C}}{\text{concentration of C}} = F\left(\frac{\text{area of G}}{\text{concentration of G}}\right)$$

$$\frac{A_C}{[C]} = F\left(\frac{A_G}{[G]}\right)$$

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

2. 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]?

3. Find the fraction of nucleotides that are C + G by evaluating the expression

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

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

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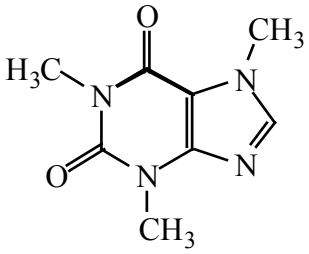
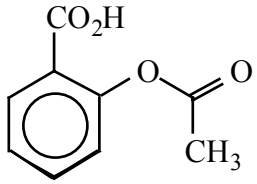
1. S. M. Wietstock, *J. Chem. Ed.* **1995**, *72*, 950.
2. Sigma, P. O. Box 14508, St. Louis, MO, 63178 Phone: 800-325-3010. www.sigma-aldrich.com
3. For nuclease P_1, one unit is defined as the amount that will liberate 1.0 μmol of acid soluble nucleotides from yeast ribonucleic acid per min at pH 5.3 at 37°C. The commercial preparation has at least 200 units/mg of nuclease P_1.

31. Analysis of Analgesic Tablets by High-Performance Liquid Chromatography[1]

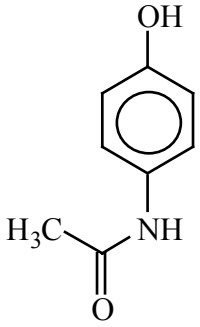
Nonprescription headache medications such as Excedrin or Vanquish contain mixtures of acetaminophen and aspirin for relief and caffeine as a stimulant. This experiment describes conditions for separating and measuring the components by high-performance liquid chromatography (HPLC). Instructions are given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



Caffeine



Aspirin
(acetylsalicylic acid)



Acetaminophen

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-µm filter and store in a tightly capped amber bottle.

Caffeine stock solution (100 µg/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 µg/mL. Dilute once again to obtain 100 µg/mL.

Acetaminophen and aspirin samples: Prepare two solutions, each containing one of the analytes at a concentration of ~50 µg/mL in HPLC solvent. Filter through 0.22 µm nylon syringe filters and store in capped amber bottles.

PROCEDURE

1. *Caffeine quantitative analysis standards:* Dilute the 100 µg/mL stock solution down to 50, 10, and 5 µg/mL with HPLC solvent. Filter ~3 mL of each solution through a 0.22-µm syringe filter into a capped vial. Filter ~3 mL of the 100 µg/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-μm syringe filter into a capped vial.

3. *Chromatography conditions:* Use a 2.1-mm-diameter ∞ 10-cm-long C_{18}-silica column with 5-μm 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 μL of each of the caffeine standards (5, 10, 50, and 100 μg/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 μL of the analgesic tablet solution. Then mix 2 drops of the tablet solution with 2 drops of 50 μg/mL caffeine solution in a test tube or vial. Inject 10 μL of the mixture into the chromatograph and observe which peak grows. Repeat the process again by adding 50 μg/mL acetaminophen and 50 μg/mL aspirin and identify which peaks in the analgesic are acetaminophen and aspirin.

6. *Quantitative analysis:* Inject 10 μL 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[1]

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 μm 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 (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 $-O^-$ groups on the wall. Alternatively, what we do in this experiment is to add the cationic surfactant tetradecyl(trimethyl)ammonium ion, $CH_3(CH_2)_{13}\overset{+}{N}(CH_3)_3$, which is attracted to the $-O^-$ groups on the wall and partially neutralizes the negative charge of the wall. This cationic surfactant is abbreviated OFM^+, for "osmotic flow modifier."

REAGENTS

Run buffer: 4.6 mM CrO_4^{2-} + 2.5 mM OFM^+ at pH 8. Dissolve 1.08 g $Na_2CrO_4 \cdot 4H_2O$ (FM 234.02) plus 25.0 mL of 100 mM tetradecyl(trimethyl)ammonium hydroxide[2] in 800 mL of HPLC-grade H_2O. Place a pH electrode in the solution and add solid boric acid (H_3BO_3) (with magnetic stirring) to reduce the pH to 8.0. Dilute to 1.00 L with HPLC-grade H_2O, mix well, filter through a 0.45-μm 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 Cl^-, 1 000 ppm NO_3^-, and 1 000 ppm SO_4^{2-} by dissolving the following salts in HPLC-grade H_2O: 2.103 g KCl (FM 74.55), 1.631 g KNO_3 (FM 101.10), and 1.814 g K_2SO_4 (FM 174.26). (Concentration refers to the mass of the anion. For example, 1 000 ppm sulfate means 1 000 μg of SO_4^{2-} per mL of solution, not 1 000 μg of K_2SO_4.) Dilute the stock solution with HPLC-grade H_2O 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 ~50 ppm. To do this, dissolve ~0.105 g KCl, ~0.082 g KNO_3, ~0.091 g K_2SO_4, or ~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 Cl^-, NO_3^-, and 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 Cl^- and run an electropherogram of the mixture. The Cl^- peak should be twice the size it was in the first run. Repeat the procedure with additions of NO_3^-, SO_4^{2-}, and F^-. This process tells you which peak belongs to each anion and where to look for 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-Girardequ, 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^+OH^-.