Experiments

To Accompany
Exploring Chemical Analysis, 5th Edition (2012)
Daniel C. Harris

Experiments

The following experiments illustrate major analytical techniques described in the textbook, *Exploring 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. Lists of many additional experiments published in the *Journal of Chemical Education* are also given at the web site for *Exploring Chemical Analysis*. Some experiments refer to standards or unknowns from Thorn Smith.[1]

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.

When equipment permits, it is environmentally friendly and economical to reduce the scale of an experiment. For example, 50-mL burets can be replaced by 10-mL burets at some loss in analytical precision. An even smaller scale is possible with microburets made from 2-mL pipets.[2] Micropipets and small volumetric flasks can replace large glass pipets and large volumetric flasks (again at a sacrifice of precision). With properly chosen electrodes,

---

1 Thorn Smith Inc., 7755 Narrow Gauge Road, Beulah, MI 49617. Phone: 231-882-4672; 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^+), silver alloys (for Ag, Cu, Zn, Ni), soda ash (for Na_2CO_3), soluble antimony (for Sb), soluble choride, soluble oxalate, soluble phosphate, soluble sulfate, steels (for C, Mn, P, S, Si, Ni, Cr, Mo), and Zn ore (for Zn). Primary standards are also available: potassium hydrogen phthalate (to standardize NaOH), As_2O_3 (to standardize I_2), $CaCO_3$ (to standardize EDTA), $Fe(NH_4)_2(SO_4)_2$ (to standardize dichromate or permanganate) , $K_2Cr_2O_7$ (to standardize thiosulfate), $AgNO_3$, Na_2CO_3 (to standardize acid), NaCl (to standardize $AgNO_3$), and $Na_2C_2O_4$ (to standardize $KMnO_4$).

2 M. M. Singh, C. McGowan, Z. Szafran, and R. M. Pike, *J. Chem. Ed.* **1998**, *75*, 371; *ibid.* **2000**, *77*, 625.

potentiometric titrations can be performed on a scale of a few milliliters, instead of tens of milliliters, by using a syringe or a micropipet to deliver titrant. Instructions here were written for the commonly available, large-size lab equipment, but your modification to a smaller scale is encouraged.

0. Green Analytical Chemistry[3]

"Green chemistry" is a set of ideas designed to protect our environment and conserve our resources. These ideas can be traced back to the book *Green Chemistry: Theory and Practice*[4] published in 1998 by Paul Anastas and John Warner. They defined green chemistry as "the use of a set of principles that reduces or eliminates the use or generation of hazardous substances in the design, manufacture, and application of chemical products." Green chemistry is a way of thinking about chemistry. It is not a separate chemical discipline like analytical, organic, or physical. In fact, it is hoped that the current generation of chemistry students taking this course will have this mode of thinking become so natural to them that we will no longer have to think about "green chemistry," but just "chemistry."

How, then, does analytical chemistry fit into this way of thinking? What is green analytical chemistry? To answer these questions, we must first reconsider what is analytical chemistry and what is green chemistry.

During this course, you will learn a series of methods to qualitatively and quantitatively characterize a sample or chemical system. Knowledge of these methods and the chemical principles behind them is an asset valued by many industries. On the other hand, analytical chemists may be viewed as a necessary evil, for example, to analyze samples in order to comply with Food and Drug Administration or other regulatory agency requirements. Analytical chemistry is something that is tolerated, but the analysis costs money, rather than adding to a company's profits. Consider the following example: You work for a detergent manufacturer and a product development chemist comes into your lab with a bottle of detergent, asking you to determine the silicone level in this product. While you could easily do what you are told, upon questioning you find out that the product development chemist really doesn't want to know the silicone concentration. What he *really* wants to know is why the product isn't performing the way it should (maybe it foams too much or doesn't clean effectively) and he *thinks* it's related to the silicone concentration. A good analytical chemist does not merely provide data, but rather supplies information and knowledge upon which educated decisions can be based.

---

3 This section was contributed by Douglas E. Raynie, Department of Chemistry and Biochemistry, South Dakota State University, Brookings SD 57007; douglas.raynie@sdstate.edu.

4 P. T. Anastas and J. C. Warner, *Green Chemistry: Theory and Practice* (New York: Oxford University Press, 1998).

Analytical chemists are problem solvers. We use our knowledge to become partners with our customers to answer their questions. In the detergent analysis example, one analytical chemist might have run the silicone analysis and then several other analyses in order to eventually solve the problem. But the analytical chemist who was a true scientific partner in the process understood the problem and used his knowledge of chemistry to develop a more refined hypothesis and streamline the analyses need to answer the ultimate question more easily. This role of the analytical chemist was summarized by Prof. Herb Laitinin who editorialized, "Analysis of a sample is not the true aim of analytical chemistry…the real purpose of the analysis is to solve a problem."[5] Development of problem-solving skills comes with a combination of analytical knowledge and experience. *In summary, the analytical chemist is a problem solver and solving problems involves a means of thinking about chemistry.*

What is Green Chemistry?

We've defined green chemistry as something that is applied over the entire lifespan of a product. Hidden within this definition is a combination of environmental and economic concerns. That is, a product may have environmental benefits, but if it cannot compete in the marketplace, it is doomed to failure. Green chemistry is guided by the set of 12 principles developed by Anastas and Warner[4] and shown in Table 1.

If green chemistry and concern for the environment are the right things to do, why haven't we been doing this all along? Think about organic chemistry. It isn't always easy to choose the right reagents to put the correct functional groups in the right places for a complex synthesis. Now consider that you are working as a product development chemist in industry. Not only must you synthesize the compound with properties desired by your company, but you must do it in a manner that can be passed on to a process engineer who can develop the process with high yield, speed, safety, and minimal cost. Now add environmental concerns on top of all of that and the lament of a certain amphibian character, "It's not easy being green," becomes more obvious. *In summary, green chemistry involves a way of thinking about chemistry in an environmentally and economically sound manner.*

---

5 H. A. Laitinen, *Anal. Chem.*, **1966**, *38*, 1441.

Table 1. The 12 Principles of Green Chemistry[4]

1. **Prevention**
   It is better to prevent waste than to treat or clean up waste after it has been created.
2. **Atom Economy**
   Synthetic methods should be designed to maximize the incorporation of all materials used in the process into the final product.
3. **Less Hazardous Chemical Syntheses**
   Wherever practicable, synthetic methods should be designed to use and generate substances that possess little or no toxicity to human health and the environment.
4. **Designing Safer Chemicals**
   Chemical products should be designed to effect their desired function while minimizing their toxicity.
5. **Safer Solvents and Auxiliaries**
   The use of auxiliary substances (e.g., solvents, separation agents, etc.) should be made unnecessary wherever possible and innocuous when used.
6. **Design for Energy Efficiency**
   Energy requirements of chemical processes should be recognized for their environmental and economic impacts and should be minimized. If possible, synthetic methods should be conducted at ambient temperature and pressure.
7. **Use of Renewable Feedstocks**
   A raw material or feedstock should be renewable rather than depleting whenever technically and economically practicable.
8 **Reduce Derivatives**
   Unnecessary derivatization (use of blocking groups, protection/ deprotection, temporary modification of physical/chemical processes) should be minimized or avoided if possible, because such steps require additional reagents and can generate waste.
9. **Catalysis**
   Catalytic reagents (as selective as possible) are superior to stoichiometric reagents.
10. **Design for Degradation**
    Chemical products should be designed so that at the end of their function they break down into innocuous degradation products and do not persist in the environment.
11. **Real-time analysis for Pollution Prevention**
    Analytical methodologies need to be further developed to allow for real-time, in-process monitoring and control prior to the formation of hazardous substances.
12. **Inherently Safer Chemistry for Accident Prevention**
    Substances and the form of a substance used in a chemical process should be chosen to minimize the potential for chemical accidents, including releases, explosions, and fires.

What is Green Analytical Chemistry?

Reviewing the previous summary statement, we can correctly guess that green analytical chemistry is a marriage of thought processes — a unique way of thinking about how to do chemistry. Performing chemical analyses in a green manner does not mean that we must make compromises to the requisite accuracy, precision, and other analytical demands. Inserting green values into the laboratory is about several factors related to the 12 green chemistry principles, including waste, energy, toxicity, and others. For example, the hazard associated with a given chemical is related to both our exposure to the chemical and the inherent risk possessed by the chemical. While we can wear personal protective equipment, like safety glasses, lab coats, and latex gloves, or work in a fume hood, we cannot change the inherent risk associated with a compound. So perhaps we can change the reagent. We've been doing this for years. Benzene and carbon tetrachloride were banished from the lab since the mid-1970s. But our practices must continue to evolve as our knowledge about chemicals and their inherent risk progresses.

At least half of the 12 principles of green chemistry apply to the analytical laboratory:

Principle 1: Prevent Waste. While the wastes generated in chemical production overshadow the amount of waste coming from a typical analytical laboratory, individual analytical procedures may involve as much as one liter of organic solvent to extract the analytes from the sample. When used in an analytical procedure, even water, which everyone would agree is an environmentally benign solvent, is converted to wastewater which must be treated appropriately. So the professional analyst must stay abreast of developments that can minimize waste generation.

Principle 5: Safer Solvents and Auxiliaries. The inherent risk associated with a chemical must be considered and alternatives explored. The risk may be toxicological, but may also be flammability, corrosiveness, or other attributes. If alternatives do not exist, appropriate exposure controls are needed.

Principle 6: Energy Efficiency. Several common analytical procedures can be considered energy intensive. For example, not only are large amounts of solvents used in extractions, in most cases the solvent must then be evaporated to concentrate the solutes for analysis. Instrumental procedures can require high temperatures or have high power demands. Sometimes trade-offs must be considered. Does it require less energy to keep a drying oven turned on overnight and maintain a constant temperature or to turn off the oven and require a rapid heat up just prior to use? If water is used as a nontoxic solvent, do we pay a price in the energy needed to

evaporate water compared with many organic solvents? Later, we will present a set of metrics to judge these compromises.

Principle 8: Reduce Derivatives. Many times chemical derivatives are employed to enhance the solubility or detectability of sample components. For example, the determination of fats in foods requires conversion of fatty acids into methyl esters for gas chromatography. However, technological advances can provide similar analytical results while avoiding the need for derivatization. For example, modern high-performance liquid chromatography can provide results equivalent to previous generations of gas chromatography.

Principle 11: Real-time Analysis. This principle *is* analytical chemistry!!! The field of process analytical chemistry, involving in-line, on-line, or at-line procedures, is a valuable tool in chemical processing. By monitoring the creation of a by-product, the poisoning of a catalyst, reaction pH, or other properties, feedback is provided to allow a reaction or process to stay in control, avoiding potential process disruptions.

Principle 12: Safer Chemistry. The flammability of solvents, oxidative capability of reagents, and similar factors should be considered in developing analytical methods.

Several of the experiments in this laboratory manual illustrate green chemistry. For example, Experiment 11 (Kjeldahl Nitrogen Analysis) uses selenium-coated boiling chips, rather than a mercury compound, as a catalyst (Principle 9); Experiment 15 (Preparation and Iodometric Analysis of a High-Temperature Semiconductor) reminds us that high-temperature superconductors could help us toward energy sustainability (Principle 6); and Experiments 31 (Analysis of Sulfur in Coal by Ion Chromatography) and 32 (Measuring Carbon Monoxide in Automobile Exhaust by Gas Chromatography) illustrate the role of analytical chemistry in pollution prevention (Principles 1 and 11). Experiment 37, Green Chemistry: Liquid Carbon Dioxide Extraction of Lemon Peel Oil, demonstrates the potential of supercritical fluid extraction to address solvent alternatives (Principles 1 and 5). We challenge students to discuss your experiments relative to the 12 green chemistry principles as you write your reports.

Now that we've gotten some idea of what green analytical chemistry is, let's discuss some specific green analytical considerations. Some review articles[6,7,8] present more detailed discussions of advances in the field of analytical chemistry that exhibit green chemistry

6 P.T. Anastas, *Crit. Rev. Anal. Chem.* **1999**, *29*, 167.

7 J. Namiesnik, *J. Sep. Sci.* **2001**, *24*, 151.

8 L.H. Keith, L.U. Gron, and J.L. Young, *Chem. Rev.* **2007**, *107*, 2695.

attributes. However, general considerations for the practice of green analytical chemistry can be summarized:

Planning. Proper planning allows the maximum amount of information to be obtained from the minimal number of analyses. A branch of statistics called Design of Experiments can be used to guide planning laboratory procedures. The developing field of chemometrics allows us to uncover relationships between sample sets that might be hidden if conventional thinking is used.

Sampling. Attention to proper sampling is an often overlooked portion of chemical analysis, but perhaps the most important step in the entire analysis. A common misconception is that an analytical method can be made green simply by being performed at the micro-scale. While this is true, one must take care to ensure that the appropriate (minimum) sample size is used. The sample must be collected to be statistically representative of the system under study and homogenized to reduce error.

Direct Analysis. Methods which do not require sample work-up prior to the measurement step have several advantages, including greenness. Techniques employing ion-selective electrodes, reflectance spectroscopy, or surface analysis can often provide chemical information without using additional analytical reagents.

Sample Preparation. Organic extractions and acid digestions generate the greatest amount of waste (while using hazardous solvents or acids) in many procedures. This field has received considerable attention in modern times. Acid digestions can be made safer through the application of microwave irradiation. Alternative solvent processes using water, ionic liquids, or careful manipulation of heat and energy can greatly enhance the sample preparation process.

Chromatography. Separation of analyte from other sample components is necessary in most analytical procedures. Chromatography can involve copious amounts of solvents, sorbents, and other chemicals. Microcolumn chromatography reduces solvent consumption, minimizes the use of chromatographic sorbents, and provides superior performance.

Data Reduction. Statistical manipulations can mine information from analytical results. Many times unambiguous compound identification is not needed; rather knowledge of trends is sufficient.

Field Analysis and Process Analysis. Taking the analysis to the sample generally provides several green advantages. Near instantaneous feedback can minimize the likelihood of a process upset. Similarly, the backhoe operator at an environmental clean-up site would like

instantaneous notification that the site is clean so she can quit digging, rather than waiting for results to come back from the laboratory. One contribution of analytical chemistry to the environmental movement has been the identification of hazards within the environment. Careful execution of field and process analysis may advance analytical chemistry from an identification and compliance model to a hazard prevention model.

How Do We Decide if a Method is Green?

A good place to start is to compare an analytical method against the 12 principles of green chemistry. However, keep in mind that compromises are made along the way. One method might use less solvent, while another is less energy intensive. No procedure is truly green; rather, one method may be considered greener than another.

One set of metrics used to assess the green profile of a method is associated with the National Environmental Monitoring Index (NEMI) database (www.nemi.gov). The NEMI database was compiled by researchers from several federal agencies, the American Chemical Society Green Chemistry Institute, and others. This free, on-line database lists regulatory and nonregulatory methods and procedures for analysis of water, sediment, air, and tissues. It presents information on cost and performance of each method. Presented with each method is a green profile, developed in collaboration with 25 environmental methods experts from five regulatory agencies and private labs. An example of this profile is shown in Figure 1 for the Environmental Protection Agency method for the determination of total water hardness by EDTA titration (similar to Experiment 12).

Figure 1. Green Profile for EPA Method 130.2: Total Hardness of Water by Titrimetry

The symbol for the green profile is simple, visual, and allows an individual to make a value judgment against the profile criteria. Here's a description of the symbol criteria:

Hazardous: A method fails this criterion if it uses chemicals in either of two important databases, the Resource Conservation and Recovery Act D, F, P, or U lists[9] or the Environmental

9 http://www.epa.gov/epawaste/hazard/wastetypes/pdfs/listing-ref.pdf

Protection Agency Toxic Release Inventory.[10] About half of the methods in the NEMI database fail this criterion.

Waste Amount: A method fails this criterion if the amount of waste generated is greater than 50 grams. This 50 grams includes the mass of sample, as well as all chemicals used in the procedure, but does not include calibration standards, cleaning (unless significant amounts of chemicals are stipulated in cleaning steps), and related factors. The waste criterion is perhaps the most rigorous. When applied to 560 methods on the NEMI database, approximately 2/3 failed this criterion. Failures generally were due to solvent use for organic analysis and mineral acid use for inorganics.

Corrosive: A method fails this criterion if the pH during the analysis is less than 2 or greater than 12. The failure rate for this criterion was about 20% for the NEMI methods.

PBT (Persistent, Bioaccumulative, or Toxic): A method fails this criterion if it uses chemicals considered to be persistent in the environment, bioaccumulative, or toxic as defined by the EPA Toxic Release Inventory. Only about 5% of the NEMI methods did not meet this criterion and each of the methods that failed the PBT criterion also failed for hazard. Most commonly, these methods included lead or mercury compounds.

For the EPA water hardness method (EPA Method 130.2) illustrated in Figure 1, we see that the method is considered green by hazard and PBT criteria, but not by waste and corrosion criteria. During the analysis, the pH is less than 2 and 66 grams of waste are generated.

Summary

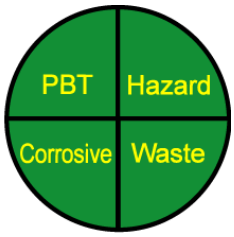
Both analytical chemistry and green chemistry are based on a way of thinking about how you perform experiments. Green analytical chemistry is a convergence of these thought processes. *Good analytical chemistry is inherently green chemistry*.

For each experiment in this laboratory manual, a green profile is displayed. A suggested component of your report should be a discussion of why a particular experiment met or failed the individual green profile criteria. Suggest ways that the experiment may be made greener. If you follow through with this exercise, these thought processes should become increasingly natural to you. If you develop this mode of thinking so that it becomes second nature, you will be poised to take on leadership responsibilities in your generation of professional chemists.

---

[10] http://www.epa.gov/tri/trichemicals/pbt%20chemicals/pbt_chem_list.htm

1. Calibration of Volumetric Glassware



Green Profile
See Section 0

An important trait of a good analyst is the ability to extract the best possible data from his or her 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.

Calibrating a 50-mL Buret

This procedure tells how to construct a graph such as Figure 3-2 in the textbook to convert the measured volume delivered by a buret to the true volume delivered at 20˚C.

1. Fill the buret with distilled water and force any air bubbles out the tip. See whether the buret drains without leaving drops on its walls. If drops are left, clean the buret with soap and water or soak it with cleaning solution.[11] Adjust the meniscus to be at or slightly below 0.00 mL, and touch the buret tip to a beaker to remove the suspended drop of water. Allow the buret to stand for 5 min while you weigh a 125-mL flask fitted with a rubber stopper. (Hold the flask with a paper towel to prevent fingerprints from changing its mass.) If the level of the liquid in the buret has changed, tighten the stopcock and repeat the procedure. Record the level of the liquid.

2. Drain approximately 10 mL of water at a rate < 20 mL/min into the weighed flask, and cap it tightly to prevent evaporation. Allow 30 s for the film of liquid on the walls to descend before you read the buret. Estimate all readings to the nearest 0.01 mL. Weigh the flask again to determine the mass of water delivered.

3. Drain the buret from 10 to 20 mL, and measure the mass of water delivered. Repeat the procedure for 30, 40, and 50 mL. Then do the entire procedure (10, 20, 30, 40, 50 mL) a second time.

4. Use Table 2-5 in the textbook to convert the mass of water to the volume delivered. Repeat any set of duplicate buret corrections that do not agree to within 0.04 mL. Prepare a

[11] Prepare cleaning solution by dissolving 36 g of ammonium peroxydisulfate, $(NH_4)_2S_2O_8$, in a *loosely stoppered* 2.2-L ("one gallon") bottle of 98 wt% sulfuric acid. Add ammonium peroxydisulfate every few weeks to maintain the oxidizing strength.

calibration graph like Figure 3-2 in the textbook, showing the correction factor at each 10-mL interval.

EXAMPLE Buret Calibration

When draining the buret at 24˚C, you observe the following values:

Final reading	10.01		10.08 mL
Initial reading	0.03		0.04
Difference	9.98		10.04 mL
Mass	9.984		10.056 g
Actual volume delivered	10.02		10.09 mL
Correction	+0.04		+0.05 mL
Average correction		+0.045 mL	

To calculate the actual volume delivered when 9.984 g of water is delivered at 24˚C, use the conversion factor 1.003 8 mL/g in Table 2-5 of the textbook. We find that 9.984 g occupies (9.984 g)(1.003 8 mL/g) = 10.02 mL. The average correction for both sets of data is +0.045 mL.

To obtain the correction for a volume greater than 10 mL, add successive masses of water collected in the flask. Suppose that the following masses were measured:

Volume interval (mL)	Mass delivered (g)
0.03–10.01	9.984
10.01–19.90	9.835
19.90–30.06	10.071
Sum 30.03 mL	29.890 g

The total volume of water delivered is (29.890 g)(1.003 8 mL/g) = 30.00 mL. Because the indicated volume is 30.03 mL, the buret correction at 30 mL is –0.03 mL.

What does this mean? Suppose that Figure 3-2 in the textbook applies to your buret. If you begin a titration at 0.04 mL and end at 29.00 mL, you would deliver 28.96 mL if the buret

were perfect. Figure 3-2 tells you that the buret delivers 0.03 mL less than the indicated amount; so only 28.93 mL was actually delivered. To use the calibration curve, either begin all titrations near 0.00 mL or correct both the initial and the final readings. Use the calibration curve whenever you use your buret.

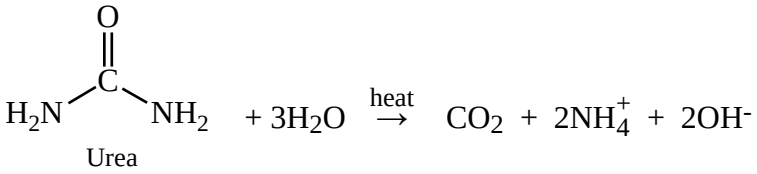
Calibrating Other Glassware

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.

2. Gravimetric Determination of Calcium as $CaC_2O_4 \cdot H_2O$[12]

Green Profile
See Section 0

Calcium ion can be analyzed by precipitation with oxalate in basic solution to form $CaC_2O_4 \cdot H_2O$. 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. Slow precipitation is achieved by dissolving Ca^{2+} and $C_2O_4^{2-}$ in acidic solution and gradually raising the pH by thermal decomposition of urea:



Urea

Reagents

Ammonium oxalate solution: Make 1 L of solution containing 40 g of $(NH_4)_2C_2O_4$ plus 25 mL of 12 M HCl. Each student will need 80 mL of this solution.

Methyl red indicator: Dissolve 20 mg of the indicator in 60 mL of ethanol and add 40 mL H_2O.

0.1 M HCl: (225 mL/student) Dilute 8.3 mL of 37% HCl up to 1 L.

Urea: 45 g/student.

Unknowns: Prepare 1 L of solution containing 15–18 g of $CaCO_3$ plus 38 mL of 12 M HCl. Each student will need 100 mL of this solution. Alternatively, solid unknowns are available from Thorn Smith.[1]

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. Alternatively, 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

[12] C. H. Hendrickson and P. R. Robinson, *J. Chem. Ed.* **1979,** *56,* 341.

cooldown after each cycle).[13] 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 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 with small portions of distilled water. 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 2-12 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 to the funnel. 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. This drying procedure does not remove the water of crystallization.

6. Calculate the average molarity of Ca^{2+} in the unknown solution or the average weight percent of Ca in the unknown solid. Report the standard deviation and relative standard deviation ($s / \overline{x}$ = standard deviation/average).

[13] R. Q. Thompson and M. Ghadiali, *J. Chem. Ed.* **1993**, *70*, 170.

3. Gravimetric Determination of Iron as Fe_2O_3[14]

Green Profile
See Section 0

A sample containing iron can be analyzed by precipitation of the hydrous oxide from basic solution, followed by ignition to produce Fe_2O_3:

$$Fe^{3+} + (2 + x)H_2O \xrightarrow{\text{Base}} FeOOH \cdot xH_2O(s) + 3H^+$$

Hydrous ferric oxide

$$FeOOH \cdot xH_2O \xrightarrow{900^oC} Fe_2O_3(s)$$

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.[1]

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.

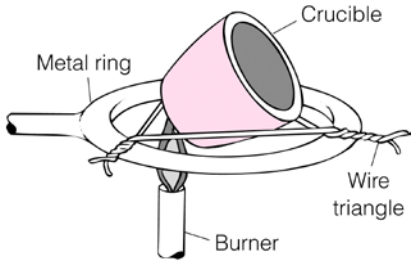


Figure 1. Positioning a crucible above a burner.

14 D. A. Skoog and D. M. West, *Fundamentals of Analytical Chemistry*, 3d ed. (New York: Holt, Rinehart and Winston, 1976).

2. Accurately weigh three samples of unknown containing enough Fe to produce ~0.3 g of Fe_2O_3. 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 well with distilled water. Add 5 mL of 6 M HNO_3 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[15] 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.

4. Decant the supernatant liquid through coarse, ashless filter paper (Whatman 41 or Schleicher and Schuell Black Ribbon, as in Figures 2-13 and 2-14 in the textbook). Do not pour liquid higher than 1 cm from the top of the funnel. Proceed to step 5 if a reprecipitation is desired. Wash the precipitate repeatedly with hot 1 wt% NH_4NO_3 until little or no Cl^- is detected in the filtered supernate. (Test for Cl^- by acidifying a few milliliters of filtrate with 1 mL of dilute HNO_3 and adding a few drops of 0.1 M $AgNO_3$. If precipitate is observed, Cl^- is present.) Finally, transfer the solid to the filter with the aid of a rubber policeman and more hot liquid. Proceed to step 6 if a reprecipitation is not used.

5. Wash the gelatinous mass twice with 30 mL of boiling 1 wt% aqueous NH_4NO_3, decanting the supernate through the filter. Then put the filter paper back into the beaker with the precipitate, add 5 mL of 12 M HCl to dissolve the iron, and tear the filter paper into small pieces with a glass rod. Add ammonia with stirring and reprecipitate the iron. Decant through a funnel fitted with a fresh sheet of ashless filter paper. Wash the solid repeatedly with hot 1 wt% NH_4NO_3 until little or no Cl^- is detected in the filtered supernate. Then transfer all the solid to the filter with the aid of a rubber policeman and more hot liquid.

6. Allow the filter to drain overnight, if possible, protected from dust. Carefully lift the paper out of the funnel, fold it (Figure 2), and transfer it to a porcelain crucible that has been brought to constant mass.

---

[15] Basic reagents should not be stored in glass bottles because they slowly dissolve glass. If ammonia from a glass bottle is used, it may contain silica particles and should be freshly filtered.

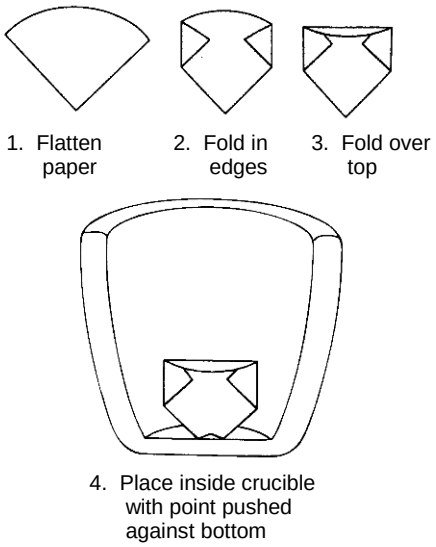


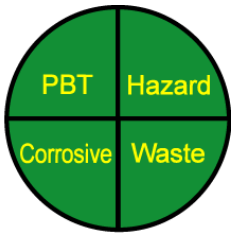
Figure 2. Folding filter paper and placing it inside a crucible for ignition. Continue folding paper so entire package fits at the bottom of the crucible. Be careful not to puncture the paper.

7. Dry the crucible cautiously with a small flame, as shown in Figure 1. The flame should be directed at the top of the container, and the lid should be off. Avoid spattering. After the filter paper and precipitate are dry, *char* the filter paper by increasing the flame temperature. The crucible should have free access to air to avoid reduction of iron by carbon. (The lid should be kept handy to smother the crucible if the paper inflames.) Any carbon left on the crucible or lid should be burned away by directing the burner flame at it. Use tongs to manipulate the crucible. Finally, *ignite* the product for 15 min with the full heat of the burner directed at the base of the crucible where the Fe_2O_3 is located.

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 ($s/\overline{x}$).

4. Penny Statistics[16]



Green Profile
See Section 0

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 data set for the entire class containing 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

[16] 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.

of 0.030 g, the 4-standard-deviation limit is ±(4 × 0.030) = ±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 $\bar{x} \pm 3s$.

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 4-9 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)

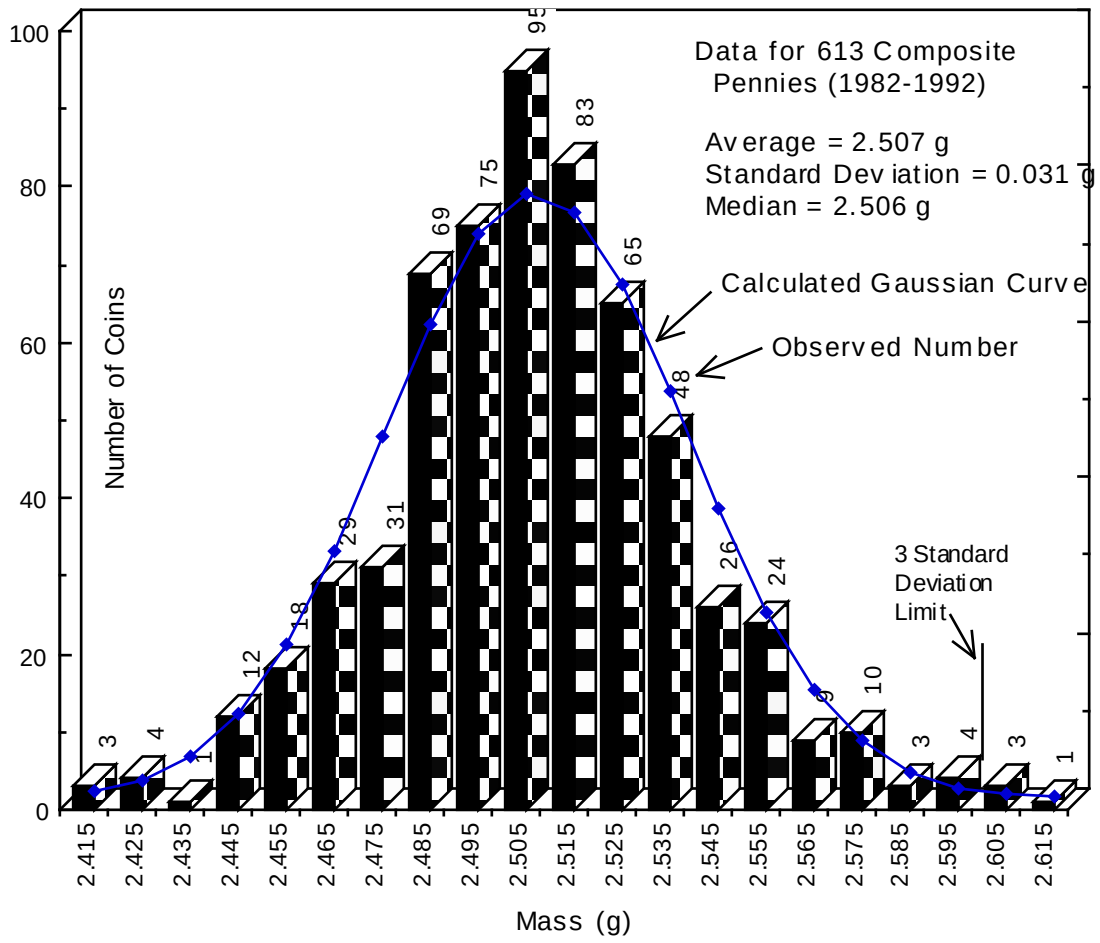


Figure 1. Distribution of penny masses from the years 1982 to 1992 measured in Dan's house by Jimmy Kusznir and Doug Harris in December 1992.

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:

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

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 $n = \infty$. 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.

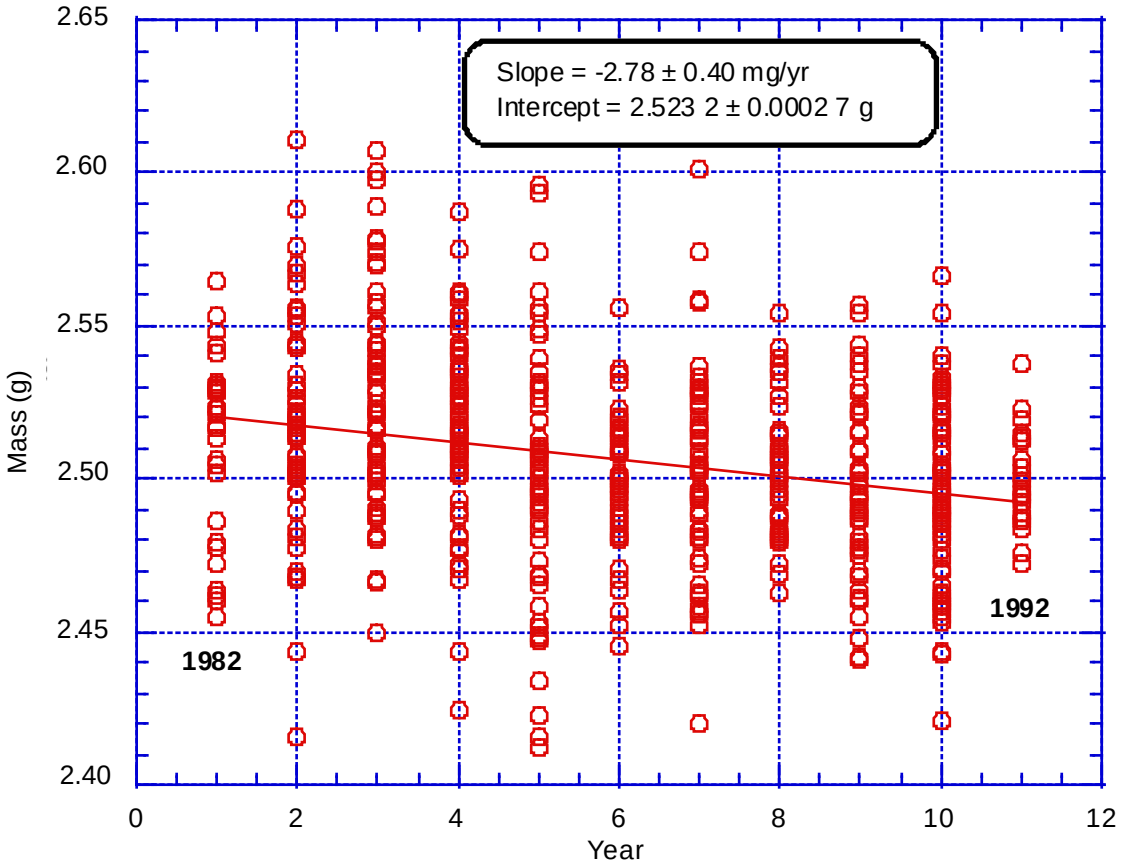


Figure 2. Penny mass versus year for 612 coins. Because the slope of the least-squares line is *significantly* less than 0, we conclude that the average mass of older pennies is greater than the average mass of newer pennies.

The 95% confidence interval is –2.78 ± 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

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

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

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

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

Table 1. Calculation of χ^2 for Figure 1

Mass (g) (x)	Observed number of coins (y_{obs})	Calculated ordinate of Gaussian curve (y_{calc})	$y_{obs} - y_{calc}$	$\frac{(y_{obs} - y_{calc})^2}{y_{calc}}$
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
			χ^2 (all 21 points) =	**43.231**
			χ^2 (19 points — omitting bottom two points) =	**30.277**

Table 2. Critical values of χ^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 χ^2 from 15 observations is 17.2. This value is less than 23.7 listed for 14 (= 15 – 1) degrees of freedom. Because χ^2 does not exceed the critical value, the observed distribution is consistent with the theoretical distribution.

At the bottom of Table 1 we see that χ^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 χ^2 from Equation 3 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 χ^2 because these bars contain the fewest observations but make large contributions to χ^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.

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% confidence interval (= $\bar{x} \pm ts/\sqrt{n}$) = _____

   For the year ≥ 1982 with lowest average mass:

   95% confidence interval = _____

   Comparison of means with *t*-test:

   $t_{calculated}$ = _____

   t_{table} = _____

   Is the difference significant? _____

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:

   $t_{calculated}$ = _____

   t_{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:

   $t_{calculated}$ = _____

   t_{table} = _____

   Is the difference significant? _____

Gaussian distribution of masses:

Prepare a graph analogous to Figure 1 with labels showing the ±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? _____

χ^2 test:

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

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

Computed value of χ^2 = _____ Degrees of freedom = _____

Critical value of χ^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 χ^2 with the reduced data set.

Computed value of χ^2 = _____ Degrees of freedom = _____

Critical value of χ^2 = _____

Does the reduced data set follow a Gaussian distribution? _____

5. Statistical Evaluation of Acid-Base Indicators[17]

Green Profile
See Section 0

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

$$(HOCH_2)_3CNH_2 + H^+ \rightarrow (HOCH_2)_3CNH_3^+$$

Tris(hydroxymethyl)aminomethane
"tris"

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 should be available in dropper bottles:

Bromothymol blue (BB): Dissolve 0.100 g in 16.0 mL 0.010 0 M NaOH and add 225 mL H_2O

Methyl red (MR): Dissolve 20 mg in 60 mL of ethanol and add 40 mL H_2O

Bromocresol green (BG): Dissolve 0.100 g in 14.3 mL 0.010 0 M NaOH and add 225 mL H_2O

Methyl orange (MO): Dissolve 10 mg in 100 mL H_2O

Erythrosine (E): Dissolve 100 mg disodium erythrosine in 100 mL H_2O

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 perform the following procedure with *one* indicator. Different students should be assigned different indicators so that at least four students evaluate each indicator.

[17] D. T. Harvey, *J. Chem. Ed.* **1991**, *68*, 329.

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.
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 molarity of HCl.
5. Repeat the titration to obtain at least six accurate measurements of the HCl molarity.
6. Use the Grubbs test in Section 4-5 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 ($s/\overline{x}$).

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 the 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).

Table 1. Pooled data

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

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

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

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

Select the pair of indicators giving average HCl molarities that are farthest apart. Use the *t* test (Equation 4-5 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 4-5, the values of s_1 and s_2 in Equation 4-6 in the textbook are the values of s_x (not s_p) in Table 1.

A condition for using Equations 4-5 and 4-6 is that the standard deviations for the two sets of measurements should not be "significantly different" from each other. The *F* test tells us whether two standard deviations are "significantly" different from each other. *F* is the quotient of the squares of the standard deviations:

$$F_{\text{calculated}} = \frac{s_1^2}{s_2^2} \qquad (1)$$

We always put the larger standard deviation in the numerator so that $F \geq 1$. If $F_{\text{calculated}} > F_{\text{table}}$ in Table 2, then the difference is significant.

Use the *F* test in Equation 1 to decide whether or not the standard deviations for the two indicators giving the largest difference in mean HCl molarity are significantly different.

Table 2. Critical values of $F = s_1^2 / s_2^2$ at 95% confidence level

Degrees of freedom for s_2	Degrees of freedom for s_1													
	2	3	4	5	6	7	8	9	10	12	15	20	30	∞
2	19.0	19.2	19.2	19.3	19.3	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.5	19.5
3	9.55	9.28	9.12	9.01	8.94	8.89	8.84	8.81	8.79	8.74	8.70	8.66	8.62	8.53
4	6.94	6.59	6.39	6.26	6.16	6.09	6.04	6.00	5.96	5.91	5.86	5.80	5.75	5.63
5	5.79	5.41	5.19	5.05	4.95	4.88	4.82	4.77	4.74	4.68	4.62	4.56	4.50	4.36
6	5.14	4.76	4.53	4.39	4.28	4.21	4.15	4.10	4.06	4.00	3.94	3.87	3.81	3.67
7	4.74	4.35	4.12	3.97	3.87	3.79	3.73	3.68	3.64	3.58	3.51	3.44	3.38	3.23
8	4.46	4.07	3.84	3.69	3.58	3.50	3.44	3.39	3.35	3.28	3.22	3.15	3.08	2.93
9	4.26	3.86	3.63	3.48	3.37	3.29	3.23	3.18	3.14	3.07	3.01	2.94	2.86	2.71
10	4.10	3.71	3.48	3.33	3.22	3.14	3.07	3.02	2.98	2.91	2.84	2.77	2.70	2.54
11	3.98	3.59	3.36	3.20	3.10	3.01	2.95	2.90	2.85	2.79	2.72	2.65	2.57	2.40
12	3.88	3.49	3.26	3.11	3.00	2.91	2.85	2.80	2.75	2.69	2.62	2.54	2.47	2.30
13	3.81	3.41	3.18	3.02	2.92	2.83	2.77	2.71	2.67	2.60	2.53	2.46	2.38	2.21
14	3.74	3.34	3.11	2.96	2.85	2.76	2.70	2.65	2.60	2.53	2.46	2.39	2.31	2.13
15	3.68	3.29	3.06	2.90	2.79	2.71	2.64	2.59	2.54	2.48	2.40	2.33	2.25	2.07
16	3.63	3.24	3.01	2.85	2.74	2.66	2.59	2.54	2.49	2.42	2.35	2.28	2.19	2.01
17	3.59	3.20	2.96	2.81	2.70	2.61	2.55	2.49	2.45	2.38	2.31	2.23	2.15	1.96
18	3.56	3.16	2.93	2.77	2.66	2.58	2.51	2.46	2.41	2.34	2.27	2.19	2.11	1.92
19	3.52	3.13	2.90	2.74	2.63	2.54	2.48	2.42	2.38	2.31	2.23	2.16	2.07	1.88
20	3.49	3.10	2.87	2.71	2.60	2.51	2.45	2.39	2.35	2.28	2.20	2.12	2.04	1.84
30	3.32	2.92	2.69	2.53	2.42	2.33	2.27	2.21	2.16	2.09	2.01	1.93	1.84	1.62
∞	3.00	2.60	2.37	2.21	2.10	2.01	1.94	1.88	1.83	1.75	1.67	1.57	1.46	1.00

If they are significantly different, use Equations 2 and 3 below in place of Equations 4-5 and 4-6 of the textbook for the *t* test.

$$t_{\text{calculated}} = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{s_1^2/n_1 + s_1^2/n_2}} \qquad (2)$$

$$\text{Degrees of freedom} = \left\{ \frac{(s_1^2/n_1 + s_2^2/n_2)^2}{\left(\frac{(s_1^2/n_1)^2}{n_1 + 1} + \frac{(s_2^2/n_2)^2}{n_2 + 1} \right)} \right\} - 2 \qquad (3)$$

Equation 3 usually gives a non-integer result, which you can round to the nearest integer

Select the pair of indicators giving the second most different molarities in Table 1 and use the *t* test again to see whether or not this second pair of results is significantly different.

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

Green Profile
See Section 0

Hydrochloric acid and sodium hydroxide are the most common strong acids and bases used in the laboratory. Both reagents need to be standardized to learn their exact concentrations. Section 10-5 in the textbook provides background information for the procedures described below. Unknown samples of sodium carbonate or potassium hydrogen phthalate (available from Thorn Smith[1]) can be analyzed by the procedures described in this section.

Reagents

50 wt% NaOH: (3 mL/student) Dissolve 50 g of reagent-grade NaOH in 50 mL of distilled water and allow the suspension to settle overnight. Na_2CO_3 is insoluble in the solution and precipitates. Store the solution in a tightly sealed polyethylene bottle and handle it gently to avoid stirring the precipitate when liquid is withdrawn.

Phenolphthalein indicator: Dissolve 50 mg in 50 mL of ethanol and add 50 mL of distilled water.

Bromocresol green indicator: Dissolve 100 mg in 14.3 mL of 0.01 M NaOH and add 225 mL distilled water.

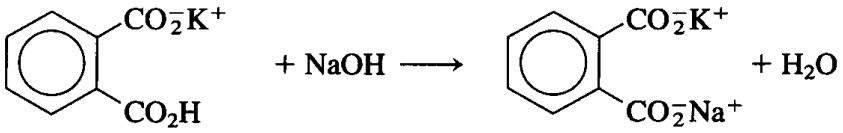
Concentrated (37 wt%) HCl: 10 mL/student.

Primary standards: Potassium hydrogen phthalate (~2.5 g/student) and sodium carbonate (~1.0 g/student).

0.05 M NaCl: 50 mL/student.

Standardizing NaOH

1. Dry primary standard grade potassium hydrogen phthalate for 1 h at 105˚C and store it in a capped bottle in a desiccator.



Potassium hydrogen phthalate
FM 204.22

2. Boil 1 L of distilled water for 5 min to expel CO_2. Pour the water into a polyethylene bottle, which should be tightly capped whenever possible. Calculate the volume of 50 wt% NaOH needed to prepare 1 L of 0.1 M NaOH. (The density of 50 wt% NaOH is 1.50 g per milliliter of solution.) Use a graduated cylinder to transfer this much NaOH to the bottle of water. (**CAUTION:** 50 wt% NaOH eats people. Flood any spills on your skin with water.) Mix well and cool the solution to room temperature (preferably overnight).
3. Weigh four samples of solid potassium hydrogen phthalate and dissolve each in ~25 mL of distilled water in a 125-mL flask. Each sample should contain enough solid to react with ~25 mL of 0.1 M NaOH. Add 3 drops of phenolphthalein to each flask and titrate one rapidly to find the end point. The buret should have a loosely fitted cap to minimize entry of CO_2 from the air.
4. Calculate the volume of NaOH required for each of the other three samples and titrate them carefully. During each titration, periodically tilt and rotate the flask to wash all liquid from the walls into the bulk solution. Near the end, deliver less than 1 drop of titrant at a time. To do so, carefully suspend a fraction of a drop from the buret tip, touch it to the inside wall of the flask, wash it into the bulk solution by careful tilting, and swirl the solution. The end point is the first appearance of faint pink color that persists for 15 s. (The color will slowly fade as CO_2 from the air dissolves in the solution.)
5. Calculate the average molarity ($\overline{x}$), the standard deviation (s), and the percent relative standard deviation ($= 100 \times s/\overline{x}$). If you were careful, the relative standard deviation should be < 0.2%.

Standardizing HCl

1. Use the table inside the cover of the textbook to calculate the volume of ~37 wt% HCl that should be added to 1 L of distilled water to produce 0.1 M HCl and prepare this solution.
2. Dry primary standard grade sodium carbonate for 1 h at 105˚C and cool it in a desiccator.

3. Weigh four samples, each containing enough Na_2CO_3 to react with ~25 mL of 0.1 M HCl and place each in a 125-mL flask. When you are ready to titrate each one, dissolve it in ~25 mL of distilled water. Add 3 drops of bromocresol green indicator and titrate one rapidly to a green color to find the approximate end point.

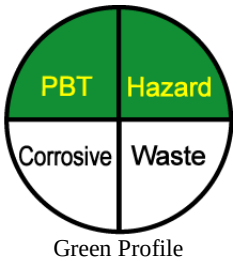
$$2HCl + \underset{\text{FM 105.99}}{Na_2CO_3} \rightarrow CO_2 + 2NaCl + H_2O$$

4. Carefully titrate each sample until it turns from blue into green. Then boil the solution to expel CO_2. The color should return to blue. Carefully add HCl from the buret until the solution turns green again and report the volume of acid at this point.

5. Perform one blank titration of 50 mL of 0.05 M NaCl containing 3 drops of indicator. Subtract the volume of HCl needed for the blank from that required to titrate Na_2CO_3.

6. Calculate the mean HCl molarity, standard deviation, and percent relative standard deviation.

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



Green Profile
See Section 0

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 10-4 of the textbook provides background for this experiment. Experiment 10 teaches you how to use Excel SOLVER to fit a theoretical titration curve to the data obtained in Experiment 7.

Reagents

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

Bromocresol green indicator: Dissolve 0.100 g of the indicator in 14.3 mL 0.010 0 M NaOH and add 225 mL H_2O.

Phenolphthalein indicator: Dissolve 50 mg of the indicator in 50 mL of ethanol and add 50 mL H_2O.

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 10-3, FM 204.22), 2-(*N*-morpholino)ethanesulfonic acid (MES, Table 9-2, FM 195.24), imidazole hydrochloride (Table 9-2, FM 104.54, hygroscopic), potassium hydrogen iodate (Table 10-3, FM 389.91).

Suggested base unknowns: tris (Table 10-3, FM 121.14), imidazole (FM 68.08), disodium hydrogen phosphate (Na_2HPO_4, FM 141.96), sodium glycinate (may be found in chemical catalogs as glycine, sodium salt hydrate, $H_2NCH_2CO_2Na{\cdot}xH_2O$, 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 electrode well with distilled water and blot it 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 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.

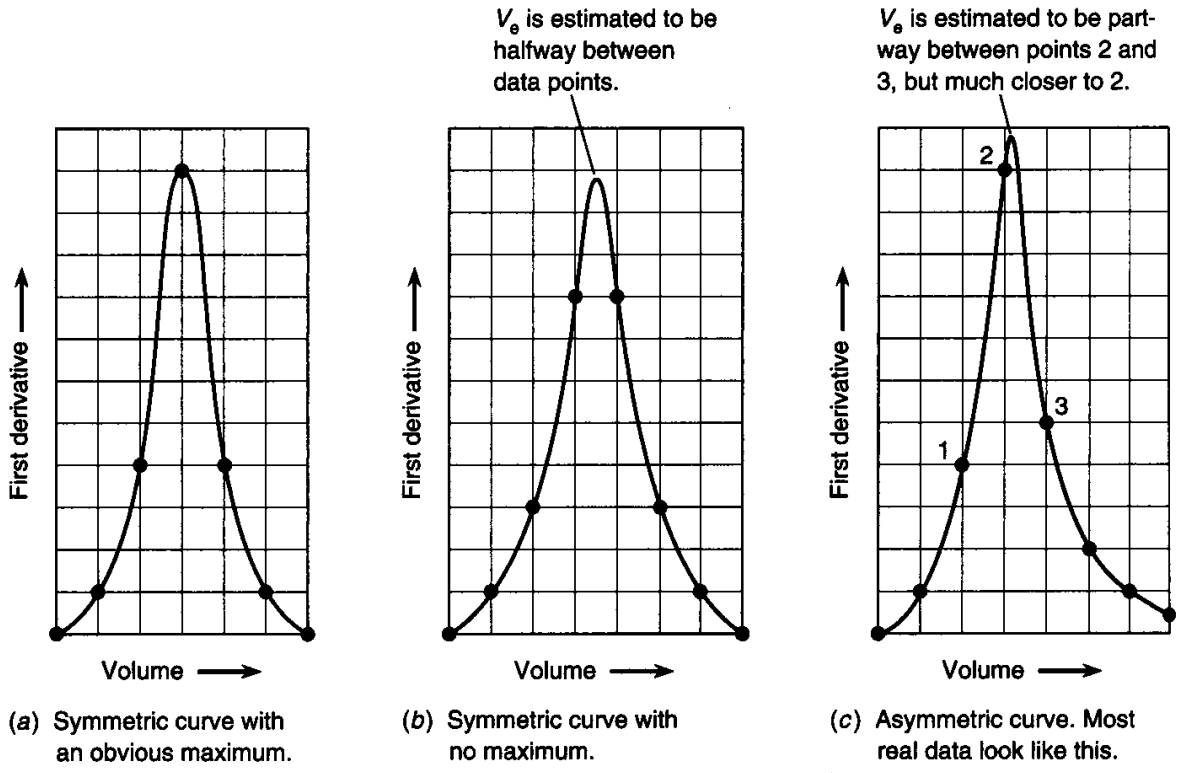


Figure 1. Locating the maximum position of the first derivative of a titration curve.

Data Analysis

1. Construct a graph of pH versus titrant volume. Mark on your graph where the indicator color change(s) was observed.
2. Following the example in Figures 10-4 and 10-5 of the textbook, compute the first derivative (the slope, $\Delta pH/\Delta V$) for each data point within ±1 mL of the equivalence volume. From your graph, estimate the equivalence volume as accurately as you can, as shown in Figure 1.
3. Following the example in Figure 10-5, compute the second derivative (the slope of the slope, $\Delta(\text{slope})/\Delta V$). Prepare a graph like Figure 10-6 in the textbook 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

Green Profile
See Section 0

This procedure involves two titrations. First, total alkalinity (= $[HCO_3^-]$ + 2$[CO_3^{2-}]$) is measured by titrating the mixture with standard HCl to a bromocresol green end point:

$$HCO_3^- + H^+ \rightarrow H_2CO_3$$
$$CO_3^{2-} + 2H^+ \rightarrow H_2CO_3$$

A separate aliquot of unknown is treated with excess standard NaOH to convert HCO_3^- to CO_3^{2-}:

$$HCO_3^- + OH^- \rightarrow CO_3^{2-} + H_2O$$

Then all the carbonate is precipitated with $BaCl_2$:

$$Ba^{2+} + CO_3^{2-} \rightarrow BaCO_3(s)$$

The excess NaOH is immediately titrated with standard HCl to determine how much HCO_3^- was present. 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_2-free water: Boil 500 mL of distilled water to expel CO_2 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 $BaCl_2$: 35 mL/student.

Bromocresol green and phenolphthalein indicators: See Experiment 7 for recipes.

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 and should not be heated. Heating at 50˚–100˚C converts $NaHCO_3$ to Na_2CO_3.

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_2-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% $BaCl_2$, using a graduated cylinder. Swirl again to precipitate $BaCO_3$, 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. From the results of step 2, calculate the total alkalinity and its standard deviation. From the results of step 3, 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_2CO_3 and 36.6 (±0.2) wt% $NaHCO_3$.

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

Green Profile
See Section 0

In this experiment, you will titrate a sample of pure potassium hydrogen phthalate (Table 10-3) with standard NaOH. A Gran plot will be used to find the equivalence point and K_a. Activity coefficients are used in the calculations of this experiment.

Using a Gran Plot to Find the End Point of a Titration

A problem with using derivatives to find the end point of a titration is that titration data are least accurate right near the end point because buffering is minimal and electrode response is sluggish. A Gran plot uses data from before the end point (typically from 0.8 V_e or 0.9 V_e up to V_e) to locate the end point.

Consider the titration of a weak acid, HA:

$$HA \rightleftharpoons H^+ + A^- \qquad K_a = \frac{[H^+]\gamma_{H^+}[A^-]\gamma_{A^-}}{[HA]\,\gamma_{HA}} \qquad (1)$$

It will be necessary to include activity coefficients in this discussion because a pH electrode responds to hydrogen ion *activity*, not concentration.

At any point between the initial point and the end point of the titration, it is usually a good approximation to say that each mole of NaOH converts 1 mol of HA into 1 mol of A^-. If we have titrated V_a mL of HA (whose formal concentration is F_a) with V_b mL of NaOH (whose formal concentration is F_b), we can write

$$[A^-] = \frac{\text{moles of } OH^- \text{ delivered}}{\text{total volume}} = \frac{V_b F_b}{V_b + V_a}$$

$$[HA] = \frac{\text{original moles of HA} - \text{moles of } OH^-}{\text{total volume}} = \frac{V_a F_a - V_b F_b}{V_a + V_b}$$

Substitution of these values of $[A^-]$ and [HA] into the equilibrium constant gives

$$K_a = \frac{[H^+]\gamma_{H^+} + V_b F_b \gamma_{A^-}}{(V_a F_a - V_b F_b)\,\gamma_{HA}}$$

which can be rearranged to

$$\underbrace{V_b[H^+]\gamma_{H^+}}_{10^{-pH}} = \frac{\gamma_{HA}}{\gamma_{A^-}} K_a \left(\frac{V_a F_a - V_b F_b}{F_b} \right) \qquad (2)$$

The term on the left is $V_b \cdot 10^{-pH}$, because $[H^+]\gamma_{H^+} = 10^{-pH}$. The term in parentheses on the right is

$$\frac{V_a F_a - V_b F_b}{F_b} = \frac{V_a F_a}{F_b} - V_b = V_e - V_b$$

Equation 2 can, therefore, be written in the form

Gran plot equation: $$V_b \cdot 10^{-pH} = \frac{\gamma_{HA}}{\gamma_{A^-}} K_a (V_e - V_b) \qquad (3)$$

A graph of $V_b \cdot 10^{-pH}$ versus V_b is called a *Gran plot*. If γ_{HA}/γ_{A^-} is constant, the graph is a straight line with a slope of $-K_a\gamma_{HA}/\gamma_{A^-}$ and an x-intercept of V_e. Figure 1 shows a Gran plot for the titration in Figure 10-4 of the textbook. Any units can be used for V_b, but the same units should be used on both axes. In Figure 1, V_b is expressed in microliters on both axes.

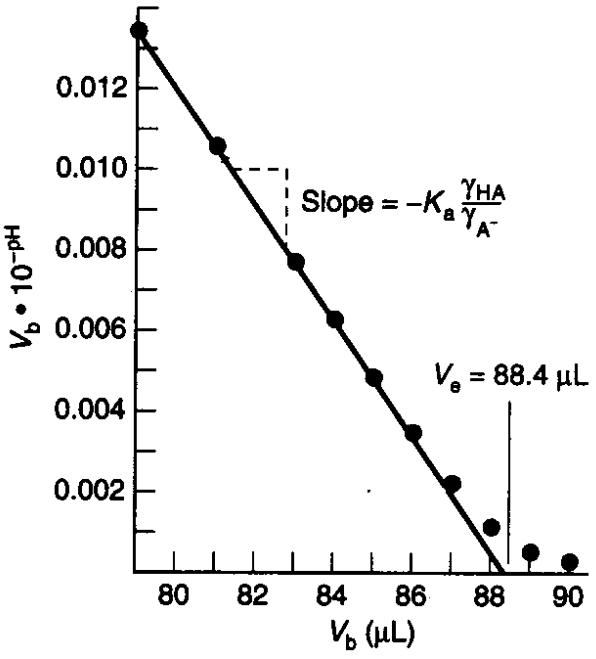


Figure 1. Gran plot for the first equivalence point of Figure 10-4 of the textbook. The last 10–20% of volume prior to V_e is normally used for a Gran plot.

The beauty of a Gran plot is that it enables us to use data taken *before* the end point to find the end point. The slope of the Gran plot enables us to find K_a. Although we derived the Gran function for a monoprotic acid, the same plot ($V_b \cdot 10^{-pH}$ versus V_b) applies to polyprotic acids (such as H_6A in Figure 10-4 of the textbook).

The Gran function, $V_b \cdot 10^{-pH}$, does not actually go to 0, because 10^{-pH} is never 0. The curve must be extrapolated to find V_e. The reason the function does not reach 0 is that we have used the approximation that every mole of OH^- generates 1 mol of A^-, which is not true as V_b approaches V_e. Only the linear portion of the Gran plot is used.

Another source of curvature in the Gran plot is changing ionic strength, which causes γ_{HA}/γ_{A^-} to vary. In Figure 10-4 of the textbook, this variation was avoided by maintaining nearly constant ionic strength with $NaNO_3$. Even without added salt, the last 10–20% of data before V_e gives a fairly straight line because the quotient γ_{HA}/γ_{A^-} does not change very much. The Gran plot in the acidic region gives accurate results even if there is CO_2 dissolved in the strong base titrant. The Gran plot in the basic region can be used to measure CO_2 in the strong base.

For completeness, we note that if weak base, B, is titrated with a strong acid, the Gran function is

$$V_a \cdot 10^{+pH} = \left(\frac{1}{K_a} \cdot \frac{\gamma_B}{\gamma_{BH^+}} \right) (V_e - V_a) \qquad (4)$$

where V_a is the volume of strong acid and K_a is the acid dissociation constant of BH^+. A graph of $V_a \cdot 10^{+pH}$ versus V_a should be a straight line with a slope of $-\gamma_B / (\gamma_{BH^+} K_a)$ and an x-intercept of V_e.

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 (recipe in Experiment 7) 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 10-4. Compare this with the theoretical and phenolphthalein end points.

Calculations (The Work Begins!)

1. Construct a Gran plot (a graph of $V_b 10^{-pH}$ 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 3 to find K_a for potassium hydrogen phthalate as follows: The slope of the Gran plot is $-K_a\gamma_{HA}/\gamma_{A^-} = -K_a\gamma_{HP^-}/\gamma_{P^{2-}}$. In this equation, HP^- is monohydrogen phthalate (the weak acid) and P^{2-} is phthalate anion (the weak base). 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 γ_{HP^-} and $\gamma_{P^{2-}}$ 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 H^+ and OH^- are negligible compared with those of K^+, Na^+, HP^-, and P^{2-}, whose concentrations are

$$[K^+] = \underbrace{\left(\frac{100\text{ mL}}{119\text{ mL}}\right)}_{\text{Dilution factor}} \underbrace{(0.020\ 0\text{ M})}_{\text{Initial concentration}} = 0.016\ 8\text{ M}$$

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

$$[HP^-] = \underbrace{(0.050)}_{\text{Fraction remaining}} \underbrace{\left(\frac{100\text{ mL}}{119\text{ mL}}\right)}_{\text{Dilution factor}} \underbrace{(0.020\ 0\text{ M})}_{\text{Initial concentration}} = 0.000\ 84\text{ M}$$

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

The ionic strength is

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

To estimate $\gamma_{P^{2-}}$ and γ_{HP^-} at $\mu = 0.048\ 8$ M, interpolate in Table 12-1. In this table, we find that the hydrated radius of P^{2-} [phthalate, $C_6H_4(CO_2^-)_2$] is 600 pm. The size of HP^- is not listed, but we will suppose that it is also 600 pm. An ion with charge ±2 and a size of 600 pm has $\gamma = 0.485$ at $\mu = 0.05$ M and $\gamma = 0.675$ at $\mu = 0.01$ M. Interpolating between these values, we estimate $\gamma_{P^{2-}} = 0.49$ when $\mu = 0.048\ 8$ M. Similarly, we estimate $\gamma_{HP^-} = 0.84$ at this same ionic strength.

---

3. From the measured slope of the Gran plot and the values of γ_{HP^-} and $\gamma_{P^{2-}}$, calculate pK_a. Then choose an experimental point near $\frac{1}{3}V_e$ and one near $\frac{2}{3}V_e$. Use Equation 1 to find pK_a with each point. (You will have to calculate $[P^{2-}]$, $[HP^-]$, $\gamma_{P^{2-}}$, and γ_{HP^-} at each point.) Compare the average value of pK_a from your experiment with pK_2 for phthalic acid listed in the Appendix of the textbook.

10. Fitting a Titration Curve with Excel Solver®[18]

Green Profile
See Section 0

Excel Solver provides tremendous power for curve fitting and solving equations. Practicing scientists and engineers make frequent use of this capability, which you will never regret making the effort to learn. Study Section 10-7 of the textbook before working on this experiment.

We will fit the theoretical titration curve to the potentiometric data (pH versus volume of titrant) that you obtained in Experiments 7 or 9. The results of your titration can be plotted in a graph such as Figure 1.

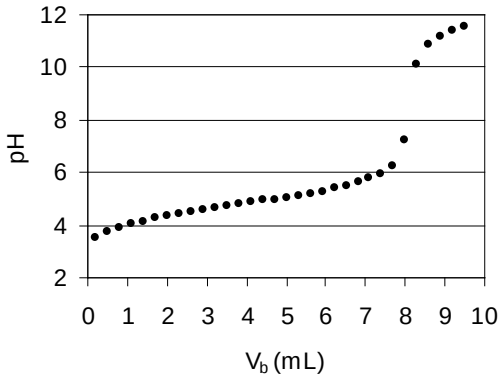


Figure 1. pH measured during the titration of acetic acid (HA) with standard NaOH. The objective of Experiment 10 is to fit a theoretical titration curve to the measured points and extract the concentration of HA and its acid dissociation constant, K_a, from the data.

For the titration of a weak acid with standard NaOH

$$HA + NaOH \rightarrow Na^+ + A^- + H_2O$$

the theoretical titration curve is described by Equation 10-14 in the textbook:

Fraction of titration for weak acid by strong base:

$$\phi = \frac{C_b V_b}{C_a V_a} = \frac{\alpha_{A^-} - \dfrac{[H^+] - [OH^-]}{C_a}}{1 + \dfrac{[H^+] - [OH^-]}{C_b}} \qquad (10\text{-}14)$$

where ϕ is the fraction of the way to the end point, C_b is the concentration of standard base, V_b is the volume of base added at a given point in the titration, C_a is the (unknown) initial concentration of weak acid, V_a is the initial volume of acid being titrated, α_{A^-} is the fraction of weak acid in the form A^-, $[H^+]$ is the concentration of H^+ at a point in the titration, and $[OH^-]$ is

[18] J. Burnett and W. A. Burns, "Using a Spreadsheet to Fit Experimental pH Titration Data to a Theoretical Expression: Estimation of Analyte Concentration and K_a," *J. Chem. Ed.* **2006**, *83*, 1190.

the concentration of OH^- at that same point. Equation 10-14 neglects activity coefficients. The expression for α_{A^-} is

Fraction of weak acid in the form A^-:

$$\alpha_{A^-} = \frac{K_a}{[H^+] + K_a} \qquad (10\text{-}12)$$

where K_a is the acid dissociation constant for HA. By finding the curve that best fits the titration data, we will find the best values of C_a and K_a. You could analyze the titration of a weak base with standard HCl by using Equations 10-15 and 10-16 in the textbook.

Procedure

1. Create a spreadsheet like Figure 2. Type the constants (C_b, V_a, etc.) in cells A3:A8. Enter the known concentration of standard base in cell B3 and the initial volume of HA in cell B4. Enter 1e-14 for K_w in cell B8. Leave cells B5:B7 blank for the moment

2. Enter experimental volumes of base ($V_{b,obs}$) and pH in columns A and B (beginning in cells A12 and B12 in Figure 2). Prepare a graph of the experimental points, as in Figure 1.

3. *Estimate* the equivalence volume, V_e. In Figure 1, the steep part of the titration curve is near V_b = 8 mL. In the spreadsheet, we find the steepest rise in pH between 8.01 and 8.31 mL. So we can estimate $V_e \approx 8.1$ mL for this example. (The equivalence point in your titration will be at some other volume of base.) From the initial volume of acid (V_a = 200 mL) and the concentration of standard base (C_b = 0.4905 M), we can estimate the concentration of acid:

$$V_a C_a = V_e C_b \quad \Rightarrow \quad C_a = \frac{V_e C_b}{V_a} \approx \frac{(8.1\text{ mL})(0.4905\text{ M})}{(200\text{ mL})} = 0.196\text{ M}$$

In Figure 2, we enter the estimated value C_a = 0.196 M in cell B5. You will enter the estimated value of C_a for your titration.

4. For any titration, pH = pK_a when $V_b = \frac{1}{2}V_e$. If $V_e \approx 8.1$ mL, then $\frac{1}{2}V_e \approx 4.05$ mL. Inspecting the data in the spreadsheet, we see that pH ≈ 4.85 near $V_b \approx 4.05$ mL. In Figure 2, the estimated value of 4.85 for pK_a is entered in cell B6. You will enter the estimated pK_a for your titration. The spreadsheet calculates $K_a = 10^{-pK_a}$ in cell B7. Eventually, we will use Excel SOLVER to find the best values for C_a and pK_a to fit the titration data.

5. Compute $[H^+] = 10^{-pH_{obs}}$ in column C and $[OH^-] = K_w/[H^+]$ in column D. Compute α_A- with Equation 10-12 in column E. Compute ϕ in column F with the ugly expression at the right side of Equation 10-14. Spreadsheet formulas are given in the upper part of Figure 2.

6. From ϕ in column F, calculate the volume of base, $V_{b,calc}$, in column G.

$$\phi = \frac{C_b V_{b,calc}}{C_a V_a} \quad \Rightarrow \quad V_{b,calc} = \frac{\phi C_a V_a}{C_b}$$

7. The calculated titration curve is a graph of observed pH versus $V_{b,calc}$. We have not yet optimized the values of C_a and pK_a, but we have pretty good estimates that should give a reasonable fit to the experimental data. To superimpose the calculated titration curve on the data in Figure 1, click on the graph in your spreadsheet. In Excel 2007, in the Chart Tools ribbon go to Design and then Select Data. Click Add. Name the new data "Calc". The x values are $V_{b,calc}$ in column G. The y values are pH in column B. Click OK and the calculated points appear on the graph. To change the calculated points to a smooth curve, click on one of the calculated points so they are all highlighted. Select Chart Tools, Format, Format Selection. For Marker Options, click None to remove the markers. For Line Color, select Solid Line and give it a color. For Line Style, select a Width of 0.75 points. Click Close and you should see the smooth calculated curve.

For earlier versions of Excel, go to the Chart menu, select Source Data. Select the Series tab and click Add. Name the new data "Calc". The x values are $V_{b,calc}$ in column G. The y values are pH in column B. Click OK and the calculated points appear on the graph. To change the calculated points to a smooth curve, select the chart symbol of Calc in the legend of the graph. Double click the chart symbol and the Format Legend Key window appears. For Line, select Automatic. For Marker, select None. Click OK and you should see the smooth calculated curve.

8. Before finding the best values of C_a and pK_a to fit the data, we are going to give each experimental point a weight in column H. The greater the weight, the more importance we attach to an experimental point in the least-squares fitting procedure. We could have weighted all points equally with a weight of 1. An empirical weight that attaches more

	A	B	C	D	E	F	G	H	I
1	Titration of Acetic Acid with NaOH								Σ(wt*residual^2) =
2					$[H^+]$: C11 = 10^-B11				0.265
3	C_b =	0.4905	M		$[OH^-]$: D11 = $B$8/C11				= sum(I11:I42)
4	V_a =	200	mL		Alpha(A^-): E11 = $B$7/(C11+$B$7)				
5	C_a =	0.01990	M		Phi: F11 = (E11-(C11-D11)/$B$5)/(1+(C11-D11)/$B$3)				
6	pK_a =	4.850			$V_{b,calc}$: G11 = F11*$B$5*$B$4/$B$3				
7	K_a =	1.41E-05	= 10^-B6		Weight: H11 = (B12-B11)/(A12-A11)				
8	K_w =	1.00E-14			wt*($V_{b,obs}$ - $V_{b,calc}$)^2: I11 = H11*(A11-G11)^2				
9									
10	$V_{b,obs}$								wt*residual^2 =
11	(mL)	pH_{obs}	$[H^+]$	$[OH^-]$	Alpha(A^-)	Phi	$V_{b,calc}$	Weight	wt*($V_{b,obs}$ - $V_{b,calc}$)^2
12	0.21	3.48	3.3E-04	3.0E-11	0.041	0.024	0.20	0.80	1.39E-04
13	0.51	3.72	1.9E-04	5.2E-11	0.069	0.059	0.48	0.50	3.89E-04
14	0.81	3.87	1.3E-04	7.4E-11	0.095	0.088	0.71	0.47	4.31E-03
15	1.11	4.01	9.8E-05	1.0E-10	0.126	0.121	0.98	0.47	7.33E-03
16	1.41	4.15	7.1E-05	1.4E-10	0.166	0.163	1.32	0.33	2.66E-03
17	1.71	4.25	5.6E-05	1.8E-10	0.201	0.198	1.61	0.33	3.61E-03
18	2.01	4.35	4.5E-05	2.2E-10	0.240	0.238	1.93	0.23	1.45E-03
19	2.31	4.42	3.8E-05	2.6E-10	0.271	0.269	2.18	0.27	4.34E-03
20	2.61	4.50	3.2E-05	3.2E-10	0.309	0.307	2.49	0.27	3.69E-03
21	2.91	4.58	2.6E-05	3.8E-10	0.349	0.348	2.82	0.30	2.21E-03
22	3.21	4.67	2.1E-05	4.7E-10	0.398	0.397	3.22	0.17	1.44E-05
23	3.51	4.72	1.9E-05	5.2E-10	0.426	0.425	3.45	0.20	8.07E-04
24	3.81	4.78	1.7E-05	6.0E-10	0.460	0.459	3.72	0.23	1.73E-03
25	4.11	4.85	1.4E-05	7.1E-10	0.500	0.499	4.05	0.23	8.06E-04
26	4.41	4.92	1.2E-05	8.3E-10	0.540	0.540	4.38	0.20	2.01E-04
27	4.71	4.98	1.0E-05	9.5E-10	0.574	0.574	4.66	0.23	6.95E-04
28	5.01	5.05	8.9E-06	1.1E-09	0.613	0.613	4.97	0.23	3.48E-04
29	5.31	5.12	7.6E-06	1.3E-09	0.651	0.650	5.28	0.30	3.48E-04
30	5.61	5.21	6.2E-06	1.6E-09	0.696	0.696	5.65	0.27	3.44E-04
31	5.91	5.29	5.1E-06	1.9E-09	0.734	0.733	5.95	0.30	4.96E-04
32	6.21	5.38	4.2E-06	2.4E-09	0.772	0.772	6.26	0.37	1.05E-03
33	6.51	5.49	3.2E-06	3.1E-09	0.814	0.813	6.60	0.40	3.27E-03
34	6.81	5.61	2.5E-06	4.1E-09	0.852	0.852	6.91	0.50	5.18E-03
35	7.11	5.76	1.7E-06	5.8E-09	0.890	0.890	7.22	0.70	9.18E-03
36	7.41	5.97	1.1E-06	9.3E-09	0.929	0.929	7.54	1.03	1.79E-02
37	7.71	6.28	5.2E-07	1.9E-08	0.964	0.964	7.82	3.17	4.06E-02
38	8.01	7.23	5.9E-08	1.7E-07	0.996	0.996	8.08	9.70	4.83E-02
39	8.31	10.14	7.2E-11	1.4E-04	1.000	1.007	8.17	2.37	4.46E-02
40	8.61	10.85	1.4E-11	7.1E-04	1.000	1.037	8.41	1.17	4.44E-02
41	8.91	11.20	6.3E-12	1.6E-03	1.000	1.083	8.79	0.63	9.30E-03
42	9.21	11.39	4.1E-12	2.5E-03	1.000	1.129	9.16	0.50	1.20E-03
43	9.51	11.54	2.9E-12	3.5E-03	1.000	1.183	9.60	0.50	3.68E-03

Figure 2. Initial spreadsheet for fitting Equation 10-14 to experimental points for titration of HA with standard NaOH.

	A	B	C	D	E	F	G	H	I
1	Titration of Acetic Acid with NaOH								Σ(wt*residual^2) =
2					[H^+]: C11 = 10^-B11				0.240
3	C_b =	0.4905	M		[OH^-]: D11 = $B$8/C11				= sum(I11:I42)
4	V_a =	200	mL		Alpha(A^-): E11 = $B$7/(C11+$B$7)				
5	C_a =	0.01981	M		Phi: F11 = (E11-(C11-D11)/$B$5)/(1+(C11-D11)/$B$3)				
6	pK_a =	4.840			$V_{b,calc}$: G11 = F11*$B$5*$B$4/$B$3				
7	K_a =	1.44E-05	= 10^-B6		Weight: H11 = (B12-B11)/(A12-A11)				
8	K_w =	1.00E-14			wt*($V_{b,obs}$ - $V_{b,calc}$)^2: I11 = H11*(A11-G11)^2				
9									
10	$V_{b,obs}$								wt*residual^2 =
11	(mL)	pH_{obs}	[H^+]	[OH^-]	Alpha(A^-)	Phi	$V_{b,calc}$	Weight	wt*($V_{b,obs}$ - $V_{b,calc}$)^2
12	0.21	3.48	3.3E-04	3.0E-11	0.042	0.025	0.20	0.80	4.57E-05
13	0.51	3.72	1.9E-04	5.2E-11	0.070	0.061	0.49	0.50	1.76E-04
14	0.81	3.87	1.3E-04	7.4E-11	0.097	0.090	0.73	0.47	3.29E-03
15	1.11	4.01	9.8E-05	1.0E-10	0.129	0.124	1.00	0.47	5.64E-03
16	1.41	4.15	7.1E-05	1.4E-10	0.169	0.166	1.34	0.33	1.65E-03
17	1.71	4.25	5.6E-05	1.8E-10	0.204	0.201	1.63	0.33	2.27E-03
18	2.01	4.35	4.5E-05	2.2E-10	0.244	0.242	1.96	0.23	6.99E-04
19	2.31	4.42	3.8E-05	2.6E-10	0.275	0.273	2.21	0.27	2.77E-03
20	2.61	4.50	3.2E-05	3.2E-10	0.314	0.312	2.52	0.27	2.18E-03
21	2.91	4.58	2.6E-05	3.8E-10	0.354	0.353	2.85	0.30	9.96E-04
22	3.21	4.67	2.1E-05	4.7E-10	0.403	0.402	3.25	0.17	2.39E-04
23	3.51	4.72	1.9E-05	5.2E-10	0.431	0.430	3.47	0.20	2.47E-04
24	3.81	4.78	1.7E-05	6.0E-10	0.465	0.464	3.75	0.23	7.91E-04
25	4.11	4.85	1.4E-05	7.1E-10	0.506	0.505	4.08	0.23	2.41E-04
26	4.41	4.92	1.2E-05	8.3E-10	0.546	0.545	4.40	0.20	9.31E-06
27	4.71	4.98	1.0E-05	9.5E-10	0.580	0.579	4.68	0.23	2.34E-04
28	5.01	5.05	8.9E-06	1.1E-09	0.618	0.618	4.99	0.23	8.00E-05
29	5.31	5.12	7.6E-06	1.3E-09	0.656	0.655	5.29	0.30	8.78E-05
30	5.61	5.21	6.2E-06	1.6E-09	0.701	0.700	5.66	0.27	6.23E-04
31	5.91	5.29	5.1E-06	1.9E-09	0.738	0.738	5.96	0.30	7.15E-04
32	6.21	5.38	4.2E-06	2.4E-09	0.776	0.776	6.27	0.37	1.18E-03
33	6.51	5.49	3.2E-06	3.1E-09	0.817	0.817	6.60	0.40	3.08E-03
34	6.81	5.61	2.5E-06	4.1E-09	0.855	0.855	6.90	0.50	4.35E-03
35	7.11	5.76	1.7E-06	5.8E-09	0.893	0.893	7.21	0.70	6.92E-03
36	7.41	5.97	1.1E-06	9.3E-09	0.931	0.931	7.52	1.03	1.24E-02
37	7.71	6.28	5.2E-07	1.9E-08	0.965	0.965	7.79	3.17	2.25E-02
38	8.01	7.23	5.9E-08	1.7E-07	0.996	0.996	8.04	9.70	1.19E-02
39	8.31	10.14	7.2E-11	1.4E-04	1.000	1.007	8.14	2.37	7.14E-02
40	8.61	10.85	1.4E-11	7.1E-04	1.000	1.037	8.38	1.17	6.25E-02
41	8.91	11.20	6.3E-12	1.6E-03	1.000	1.084	8.75	0.63	1.58E-02
42	9.21	11.39	4.1E-12	2.5E-03	1.000	1.130	9.12	0.50	3.67E-03
43	9.51	11.54	2.9E-12	3.5E-03	1.000	1.183	9.56	0.50	1.21E-03

Figure 3. Spreadsheet after executing SOLVER to optimize the values of C_a and pK_a to minimize the weighted sum of the squares of the residuals in cell I2.

significance to points near V_e is the derivative $\Delta pH / \Delta V_{b,obs}$.[19] For the first weight in cell H12, the formula is

$$\text{weight} = \frac{\Delta pH}{\Delta V_b} = \frac{B13 - B12}{A13 - A12} = \frac{3.72 - 3.48}{0.51 - 0.21} = 0.80$$

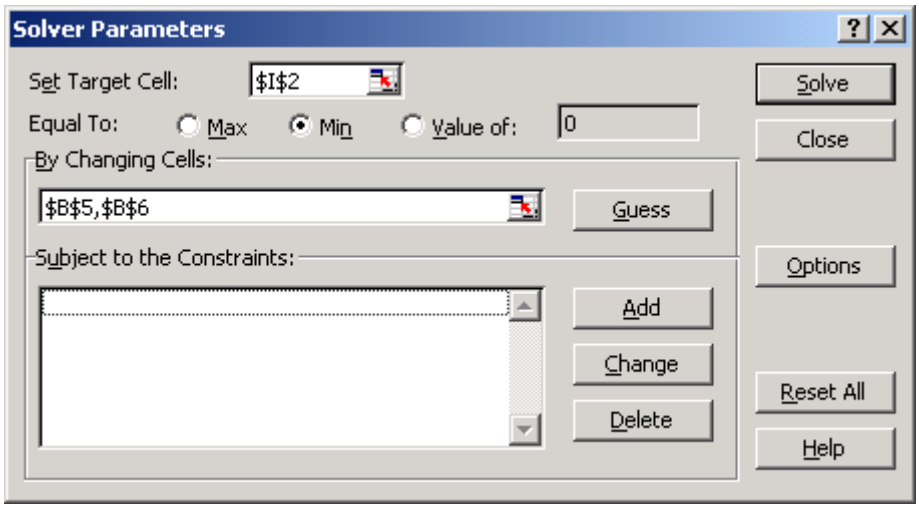
This procedure finds a weight for every point except the last one in cell H43, because there is no data in cells A44 and B44 with which to compute a derivative. For simplicity, assign the last weight in cell H43 equal to the weight computed in cell H42.

9. In least squares curve fitting, we seek to minimize the sum of squares of the difference between an observed and a calculated quantity. In your experiment, you measured $V_{b,obs}$ and calculated $V_{b,calc}$. We want to minimize the sum $(V_{b,obs} - V_{b,calc})^2$. To find V_e (and, therefore C_a) more accurately, we attach higher weights to points near V_e. Therefore, we will minimize the sum $\Sigma[\text{weight}*(V_{b,obs} - V_{b,calc})^2]$. In column I of the spreadsheet compute the product $\text{weight}*(V_{b,obs} - V_{b,calc})^2$ for each row. In cell I2, compute the sum of weighted squares of the residuals:

$$\text{sum} = \Sigma[\text{weight}*(V_{b,obs} - V_{b,calc})^2] \quad \Rightarrow \quad \text{I2} = \text{SUM(I12:I43)}$$

10. Now we are ready for a least-squares optimization of C_a and pK_a to find the theoretical titration curve that best fits the experimental points. In Excel 2007, in the Data ribbon, select Solver. (If you don't see Solver in the Analysis section of the ribbon, click the Microsoft Office button at the top left of the spreadsheet. Click Excel Options and select Add-Ins. Highlight Solver Add-In, click OK, and Solver is loaded.) In the Data ribbon, click on Solver. In the Solver window, Set Target Cell $I$2 Equal To Min By Changing Cells $B$5,$B$6.

[19] The weighting function $\Delta pH / \Delta V_{b,obs}$ is recommended by Burnett and Burns[18] to improve the accuracy of finding V_e and, hence, C_a. In general, the accuracy of measuring pH is poorest near V_e, so it could be argued that these points should be given the least weight, not the most weight. However, points near V_e are most important for finding V_e accurately, so we weight them the highest. The Gran plot in the preceding experiment is the best way to find V_e without having to rely on less accurate data near V_e.



Click the Solve button at the upper right. Solver takes a few seconds to vary the quantities C_a and pK_a in cells B5 and B6 to minimize the sum in cell I2. The result is shown in Figure 3. The sum in cell I2 is reduced from 0.428 in Figure 2 to 0.240. The optimum values C_a = 0.01981 M and pK_a = 4.840 appear in cells B5 and B6 in Figure 3.

To use Solver in earlier versions of Excel, go to the Tools menu, select Solver, and follow the same instructions given above. (If you don't see Solver in the Tools menu, select Add-Ins and click on Solver in the Add-Ins window. Click OK and Solver appears in the Tools menu.)

11. Superimpose the optimum computed curve on your experimental points to obtain a graph like Figure 4.

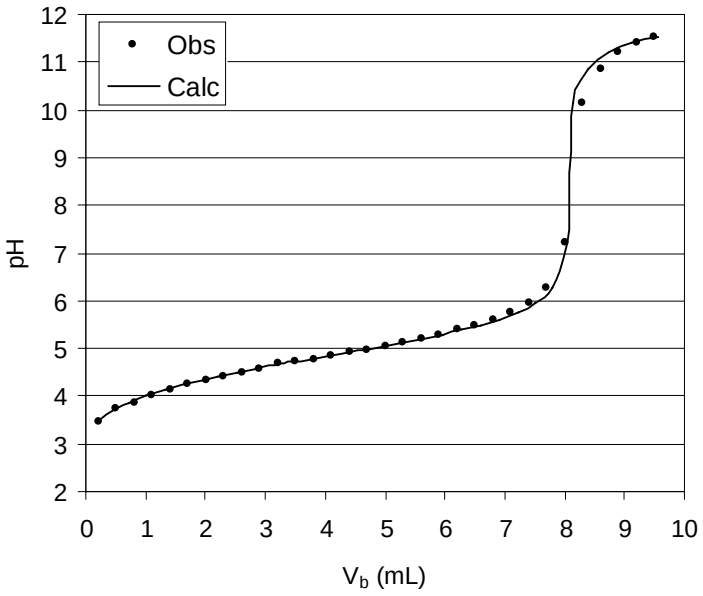


Figure 4. Calculated titration curve (smooth line) superimposed on measured points after optimizing the fit with SOLVER.

Try different starting values of C_a and pK_a in cells B5 and B6 to see how well SOLVER can optimize two parameters at once. You will find that if the initial values of C_a and pK_a are not close to the correct values, SOLVER might not be able to find an answer. In this experiment, it was easy to find values of C_a and pK_a that are close to the true values by inspection of the experimental titration data.

In case you could not find good starting estimates for C_a and pK_a, try to optimize one variable at a time. In the Solver window, Set Target Cell $I$2 Equal To Min By Changing Cells $B$5. Once Solver has found the best value in cell B5, Set Target Cell $I$2 Equal To Min By Changing Cells $B$6. With these two individually optimized values in cells B5 and B6, you can optimize them together by Set Target Cell $I$2 Equal To Min By Changing Cells $B$5,$B$6.

From this exercise, turn in a graph such as Figure 4 and a spreadsheet such as Figure 3. Report the optimum values of C_a and pK_a that fit your experimental data.

11. Kjeldahl Nitrogen Analysis

Green Profile
See Section 0

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 H_2SO_4 with a catalyst converts organic nitrogen into NH_4^+. The solution is then made basic, and the liberated NH_3 is distilled into a known amount of HCl (Reactions 10-5 to 10-7 in the textbook). Unreacted HCl is titrated with NaOH to determine how much HCl was consumed by NH_3. Because the solution to be titrated contains both HCl and NH_4^+, we choose an indicator that permits titration of HCl without beginning to titrate NH_4^+. Bromocresol green, with a transition range of pH 3.8 to 5.4, fulfills this purpose.

The Kjeldahl digestion captures amine ($–NR_2$) or amide ($–C[=O]NR_2$) nitrogens (where R can be H or an organic group), but not oxidized nitrogen such as nitro ($–NO_2$) or azo (–N=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: Recipes in Experiment 7.

Potassium sulfate: 10 g/student.

Selenium-coated boiling chips: Hengar selenium-coated granules are convenient. Alternative catalysts are 0.1 g of Se, 0.2 g of $CuSeO_3$, or a crystal of $CuSO_4$.

Concentrated (98 wt%) H_2SO_4: 25 mL/student

Unknowns: Unknowns are pure acetanilide, *N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid (HEPES buffer, Table 9-2 in the textbook), tris(hydroxymethyl)aminomethane (tris buffer, Table 9-2), or the *p*-toluenesulfonic acid salts of ammonia, glycine, or nicotinic acid. Each student needs enough unknown to produce 2–3 mmol of NH_3.

Digestion

1. Dry your unknown at 105˚C for 45 min and accurately weigh an amount that will produce 2–3 mmol of NH_3. Place the sample in a *dry* 500-mL Kjeldahl flask (Figure 10-8 in the textbook), so that as little as possible sticks to the walls. Add 10 g of K_2SO_4 (to raise the

boiling temperature) and three selenium-coated boiling chips. Pour in 25 mL of 98 wt% H_2SO_4, washing down any solid from the walls. (**CAUTION:** Concentrated H_2SO_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˚ 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.

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 in Figure 1 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.

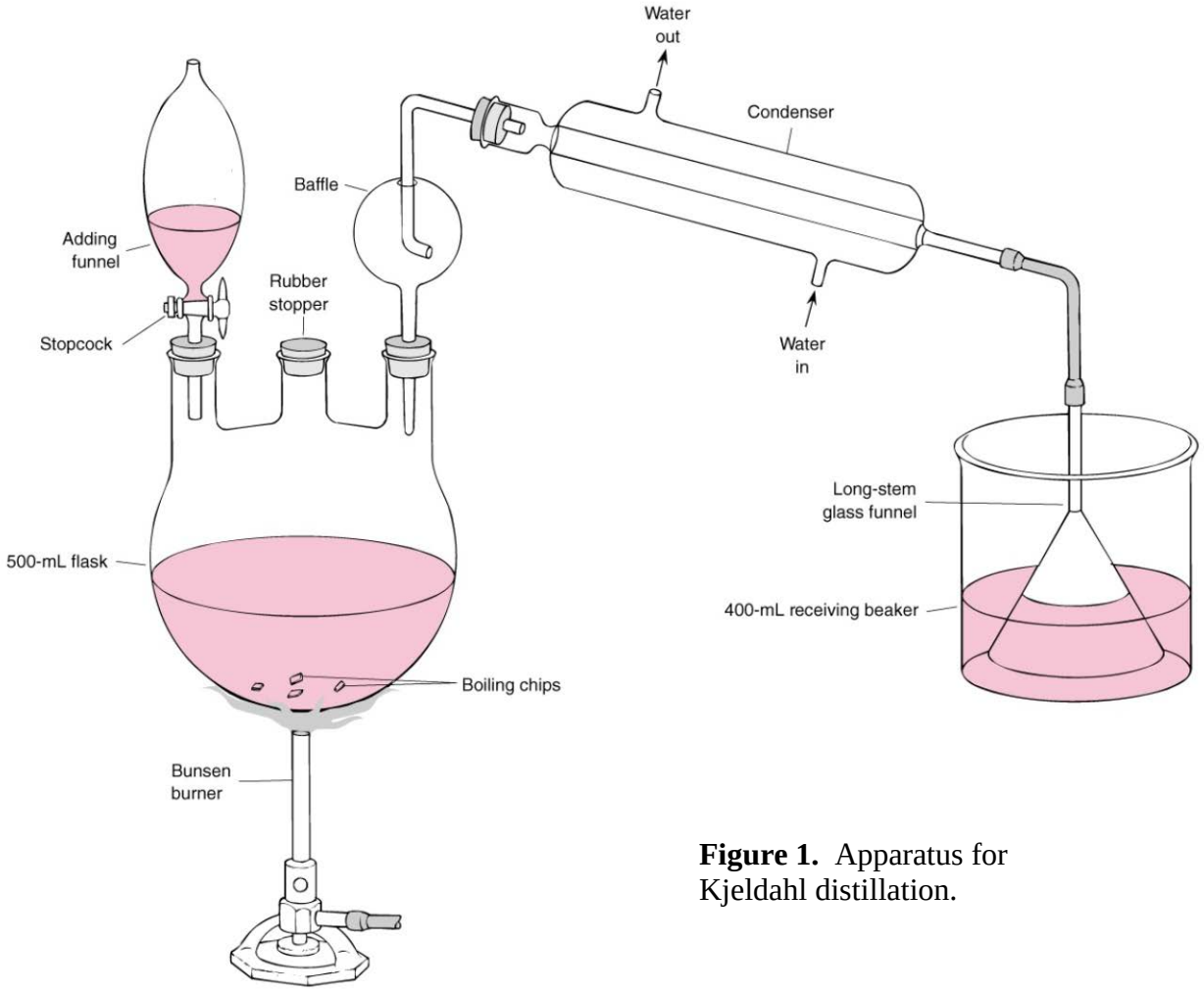


Figure 1. Apparatus for Kjeldahl distillation.

12. EDTA Titration of Ca^{2+} and Mg^{2+} in Natural Waters

Green Profile
See Section 0

The most common multivalent metal ions in natural waters are Ca^{2+} and 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 Ca^{2+} and Mg^{2+}. In a second experiment, Ca^{2+} is analyzed separately after precipitating $Mg(OH)_2$ with strong base.

Reagents

EDTA: $Na_2H_2EDTA \cdot 2H_2O$ (FM 374.24), 0.6 g/student.

Buffer (pH 10): Add 142 mL of 28 wt% aqueous NH_3 to 17.5 g of NH_4Cl and dilute to 250 mL with distilled water.

Eriochrome black T indicator: Dissolve 0.2 g of the solid indicator in 15 mL of triethanolamine plus 5 mL of absolute ethanol. (Alternatively, Calmagite could be used by dissolving 0.05 g in 100 mL of water. The color changes are the same for both indicators.)

Hydroxynaphthol blue indicator: 0.5 g/student.

Unknowns: 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.

50 wt% NaOH: Dissolve 100 g of NaOH in 100 g of H_2O in a 250-mL plastic bottle. Store tightly capped. When you remove solution with a pipet, try not to disturb the solid Na_2CO_3 precipitate.

Procedure

1. Dry $Na_2H_2EDTA \cdot 2H_2O$ (FM 372.24) at 80˚C for 1 h and cool in the desiccator. Accurately weigh out ~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 Ca^{2+} + 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 of 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 $Mg(OH)_2$ (which may not be visible). Add ~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 $Ca(OH)_2$ 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 Ca^{2+} and Mg^{2+}, as well as the individual concentrations of each ion. Calculate the relative standard deviation of replicate titrations.

13. Synthesis and Analysis of Ammonium Decavanadate[20]

Green Profile
See Section 0

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

$$VO_4^{3-} \rightleftharpoons V_2O_7^{4-} \rightleftharpoons V_4O_{12}^{4-} \rightleftharpoons HV_{10}O_{28}^{5-} \rightleftharpoons VO_2^{+}$$

In strong base

In strong acid

NH_4^+, acetic acid, alcohol

$$(NH_4)_6V_{10}O_{28} \cdot 6H_2O$$

FM 1 173.7

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).

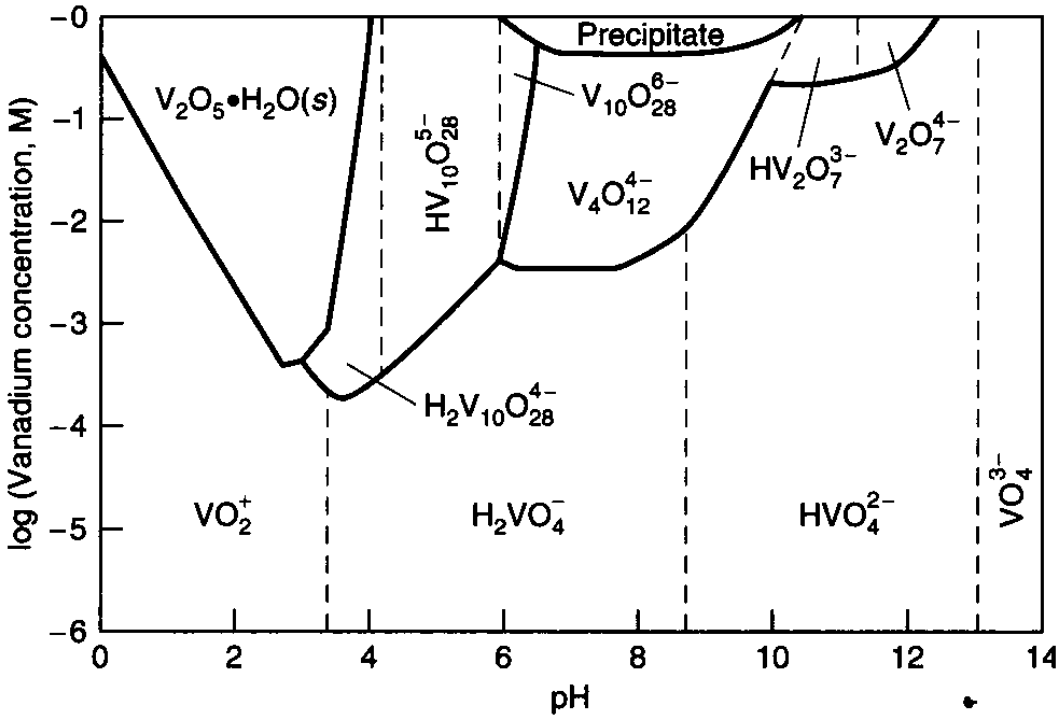


Figure 1. Phase diagram for aqueous vanadium(V) as a function of total vanadium concentration and pH. [From J. W. Larson, *J. Chem. Eng. Data* **1995**, *40*, 1276.] The region marked "precipitate" probably refers to a vanadium hydroxide.

[20] G. G. Long, R. L. Stanfield, and F. C. Hentz, Jr., *J. Chem. Ed.* **1979**, *56*, 195.

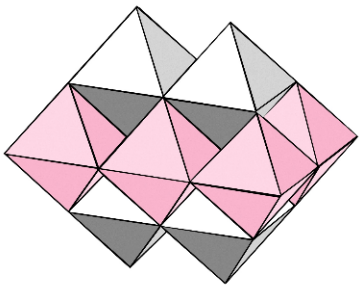


Figure 2. Structure of $V_{10}O_{28}^{6-}$ anion, consisting of VO_6 octahedra sharing edges with one another. There is a vanadium atom at the center of each octahedron.

After preparing this salt, we will determine the vanadium content by a redox titration and NH_4^+ by the Kjeldahl method. In the redox titration, V^{5+} will first be reduced to V^{4+} with sulfurous acid and then titrated with standard permanganate.

$$V_{10}O_{28}^{6-} + H_2SO_3 \rightarrow VO^{2+} + SO_2$$

$$\underset{\text{blue}}{VO^{2+}} + \underset{\text{purple}}{MnO_4^-} \rightarrow \underset{\text{yellow}}{VO_2^+} + \underset{\text{colorless}}{Mn^{2+}}$$

Reagents

Ammonium metavanadate (NH_4VO_3): 3 g/student.

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

95 % ethanol: 200 mL/student.

$KMnO_4$: 1.6 g/student or prepare 0.02 M $KMnO_4$ (~300 mL/student) for use by the class.

Sodium Oxalate ($Na_2C_2O_4$): 1 g/student.

0.9 M H_2SO_4: (1 L/student) Slowly add 50 mL of concentrated (96–98 wt%) H_2SO_4 to 900 mL of H_2O and dilute to ~1 L.

1.5 M H_2SO_4: (100 mL/student) Slowly add 83 mL of concentrated (96–98 wt%) H_2SO_4 to 900 mL of H_2O and dilute to ~1 L.

Sodium hydrogen sulfite ($NaHSO_3$, also called sodium bisulfite): 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 and bromocresol green indicators: Recipes in Experiment 7.

50 wt% NaOH: 60 mL/student. Mix 100 g NaOH with 100 mL H_2O and dissolve.

Synthesis

1. Heat 3.0 g of ammonium metavanadate (NH_4VO_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˚–l0˚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 $KMnO_4$

Preparation and Standardization of $KMnO_4$[21] (See Section 6-3 in the textbook)

1. Prepare a 0.02 M permanganate solution by dissolving 1.6 g of $KMnO_4$ 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 ($Na_2C_2O_4$) 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 H_2SO_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 $KMnO_4$ solution needed for the titration. (This can be calculated from the mass of $KMnO_4$ used to prepare the permanganate solution. The chemical reaction is given by Equation 6-1 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 $KMnO_4$ until the first pale pink color persists. Proceed slowly near the end, allowing 30 s for each drop to lose its color.
4. As a blank, titrate 250 mL of 0.9 M H_2SO_4 to the same pale pink color. Subtract the blank volume from each titration volume. Compute the average molarity of $KMnO_4$.

---

[21] R. M. Fowler and H. A. Bright, *J. Res. National Bureau of Standards* **1935**, *15*, 493.

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 H_2SO_4 (with warming, if necessary).
2. In a fume hood, add 50 mL of water and 1 g of $NaHSO_3$ to each and dissolve with swirling. After 5 min, boil the solution gently for 15 min to remove SO_2.
3. Titrate the warm solution with standard 0.02 M $KMnO_4$ from a 50-mL buret. The end point is taken when the yellow color of VO_2^+ takes on a dark shade (from excess MnO_4^-) that persists for 15 s.
4. Calculate the average wt% of vanadium in the ammonium decavanadate and compare your result to the theoretical value.

Analysis of Ammonium Ion by Kjeldahl Distillation

1. Set up the apparatus in Figure 1 of Experiment 11 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 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. (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.

14. Iodimetric Titration of Vitamin C[22]

Green Profile
See Section 0

Ascorbic acid (vitamin C) is a mild reducing agent that reacts rapidly with triiodide (See Section 16-3 and Box 16-2 in the textbook). In this experiment, we will generate a known excess of I_3^- by the reaction of iodate with iodide (Reaction 16-20), allow the reaction with ascorbic acid to proceed, and then back titrate the excess I_3^- with thiosulfate (Reaction 16-21 and Color Plate 12).

Reagents

Starch indicator: Make a paste of 5 g of soluble starch and 5 mg of Hg_2I_2 in 50 mL of distilled water. Pour the paste into 500 mL of boiling distilled water and boil until it is clear.

Sodium thiosulfate: 9 g $Na_2S_2O_3 \cdot 5H_2O$/student.

Sodium carbonate: 50 mg Na_2CO_3/student.

Potassium iodate: 1 g KIO_3/student.

Potassium iodide: 12 g KI/student.

0.5 M H_2SO_4: 30 mL/student.

Vitamin C: Dietary supplement containing 100 mg of vitamin C per tablet is suitable. Each student needs six tablets.

0.3 M H_2SO_4: 180 mL/student.

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_2 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 $Na_2S_2O_3$[23] by dissolving ~8.7 g of $Na_2S_2O_3 \cdot 5H_2O$ in 500 mL of freshly boiled water containing 0.05 g of Na_2CO_3. Store this solution in a tightly capped amber bottle. Prepare ~0.01 M KIO_3 by accurately weighing ~1g of solid reagent and dissolving it

---

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

23 An alternative to standardizing $Na_2S_2O_3$ solution is to prepare anhydrous primary standard $Na_2S_2O_3$ by refluxing 21 g of $Na_2S_2O_3 \cdot 5H_2O$ 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.]

in a 500-mL volumetric flask. From the mass of KIO_3 (FM 214.00), compute the molarity of the solution.

3. Standardize the thiosulfate solution as follows: Pipet 50.00 mL of KIO_3 solution into a flask. Add 2 g of solid KI and 10 mL of 0.5 M H_2SO_4. 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_3 solution. From the stoichiometries of Reactions 16-20 and 16-21, compute the average molarity of thiosulfate and the relative standard deviation.

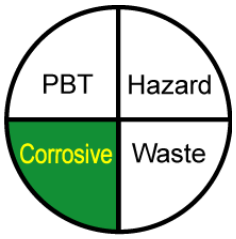
Analysis of Vitamin C

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_2SO_4, 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_3. Then titrate with standard thiosulfate as above. Add 2 mL of starch indicator just before the end point.

15. Preparation and Iodometric Analysis of a High-Temperature Superconductor[24]



Green Profile
See Section 0

In this experiment you will determine the oxygen content of yttrium barium copper oxide ($YBa_2Cu_3O_x$). 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 $YBa_2Cu_3O_7$ is in the unusual high-oxidation state, Cu^{3+}. This experiment describes the synthesis of $YBa_2Cu_3O_x$, (which can also be purchased) and then gives two alternative procedures to measure the Cu^{3+} content. The *iodometric method* is based on the reactions in Problem 16-17 in the textbook. The more elegant *citrate-complexed copper titration*[25] eliminates many experimental errors associated with the simpler iodometric method and gives more accurate and precise results.

Preparation of $YBa_2Cu_3O_x$

1. Place in a mortar 0.750 g of Y_2O_3, 2.622 g of $BaCO_3$, and 1.581 g of 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˚–930˚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 = 6.5–7 in the formula $YBa_2Cu_3O_x$. The crucible may be removed when the temperature is below 100˚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˚–930˚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˚C and repeat step 1. The final product must be black, or it is not the correct compound.

---

[24] D. C. Harris, M. E. Hills, and T. A. Hewston, *J. Chem. Ed.* **1987**, *64*, 847. Alternative syntheses of $YBa_2Cu_3O_x$ 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.

[25] 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.

Iodometric Analysis

1. *Sodium Thiosulfate and Starch Indicator*. Prepare 0.03 M $Na_2S_2O_3$ as described in Experiment 14, using 3.7 g of $Na_2S_2O_3 \cdot 5H_2O$ instead of 8.7 g. The starch indicator solution is the same one used in Experiment 14.
2. *Standard 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 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 $Na_2S_2O_3$ With Cu^{2+}* . The titration should be carried out as rapidly as possible under a brisk flow of N_2, because I^- is oxidized to 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 Cu^{2+} into the beaker and flush with 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 CuI will be present. Titrate with $Na_2S_2O_3$ solution from a 50-mL buret, adding 2 drops of starch solution just before the last trace of I_2 color disappears. If starch is added too soon, there can be irreversible attachment of 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 I_2 and I_2/starch from the color of suspended CuI(*s*). (Alternatively, Pt and calomel electrodes can be used instead of starch to eliminate subjective judgment of color in finding the end point.[26]) Repeat this standardization two more times and use the average $Na_2S_2O_3$ molarity from the three determinations.
4. *Superconductor Experiment A*. Dissolve an accurately weighed 150- to 200-mg sample of powdered $YBa_2Cu_3O_x$ in 10 mL of 1.0 M $HClO_4$ in a titration beaker in a fume hood. (Perchloric acid is recommended because it is inert to reaction with superconductor, which might oxidize HCl to Cl_2. We have used HCl instead of $HClO_4$ with no significant interference in the analysis. Solutions of $HClO_4$ should not be boiled to dryness because of

[26] P. Phinyocheep and I. M. Tang, *J. Chem. Ed.* **1994**, *71*, A115.

their explosion hazard.) Boil gently for 10 min, so that Reaction A in Problem 16-17 goes to completion. Cool to room temperature, cap with the two-hole-stopper–buret assembly, and begin 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 $YBa_2Cu_3O_x$ in the titration beaker and begin N_2 flow. Dissolve 1.0–1.5 g of KI in 10 mL of 1.0 M $HClO_4$ and immediately add the solution to the titration beaker. Stir magnetically for 1 min, so that the Reactions B and C of Problem 16-17 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

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

and x in the formula $YBa_2Cu_3O_x$, is related to p as follows:

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

For example, if the superconductor contains one Cu^{3+} and two 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[25]

This procedure directly measures Cu^{3+}. The sample is first dissolved in a closed container with 4.4 M HBr, in which Cu^{3+} oxidizes Br^- to Br_3^-:

$$Cu^{3+} + \frac{11}{2}Br^- \rightarrow CuBr_4^{2-} + \frac{1}{2}Br_3^- \qquad (3)$$

The solution is transferred to a vessel containing excess I^-, excess citrate, and enough NH_3 to neutralize most of the acid. Cu^{2+} is complexed by citrate and is not further reduced to CuI*(s)*. (This eliminates the problem in the iodometric titration of performing a titration in the presence of colored solid.) Br_3^- from Reaction 3 oxidizes I^- to I_3^-:

$$Br_3^- + 3I^- \rightarrow 3Br^- + I_3^- \qquad (4)$$

and the I_3^- produced in Reaction 4 is titrated with thiosulfate.

Our experience with the iodometric procedure is that the precision in oxygen content of $YBa_2Cu_3O_x$, is ±0.04 in the value of *x*. The uncertainty is reduced to ±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.)
3. 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_3. The exact amount of NH_3 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 $YBa_2Cu_3O_x$ consumes 2*x* 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.

4. Add 0.1 mL of 1 wt% starch solution and titrate with 0.1 M standard $Na_2S_2O_3$ under a brisk flow of N_2, using a 250-mL Hamilton syringe to deliver titrant. The end point is marked by a change from dark blue (I_2-starch) to the light blue-green of the Cu^{2+}-citrate complex.

5. Run a blank reaction with $CuSO_4$ 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 $YBa_2Cu_3O_{6.88}$ required approximately 350 μL of $Na_2S_2O_3$ and the blank required 10 μL of $Na_2S_2O_3$ If time permits, run two more blanks. Subtract the average blank from the titrant in step 4.

6. Repeat the analysis with two more samples of superconductor.

Calculations

1. From the thiosulfate required to titrate I_3^- released in Reaction 4, find the average moles of Cu^{3+} per gram of superconductor (1 mol $S_2O_3^{2-}$ = 1 mol Cu^{3+}) and the standard deviation for your three samples.

2. Defining *R* as (mol Cu^{3+})/(g superconductor), show that *z* in the formula $YBa_2Cu_3O_{7-z}$ is

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

where 666.20 is the formula mass of $YBa_2Cu_3O_7$ and 15.999 4 is the atomic mass of O.

3. 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 $YBa_2Cu_3O_x$. If you are really daring, use Equation C-1 in Appendix C of the textbook D. C. Harris, *Quantitative Chemical Analysis*, 7th ed. (New York: Freeman, 2007) for propagation of uncertainty in Equation 5.

16. Potentiometric Halide Titration with Ag^+

Green Profile
See Section 0

Mixtures of halides can be titrated with $AgNO_3$ solution as described in Section 6-5 in the textbook. In this experiment, you will use the apparatus in Figure 6-4 of the textbook to monitor the activity of Ag^+ as the titration proceeds. The theory of the potentiometric measurement is described in Section 15-1 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 H_2SO_4 with 1 M NaOH to a pH near 2.0.

Reagents

Unknowns: Each student receives a vial containing 0.22–0.44 g of KCl plus 0.50–1.00 g of KI (weighed accurately). The object is to determine the quantity of each salt in the mixture.

Buffer (pH 2): (6 mL/student) Titrate 1 M H_2SO_4 with 1 M NaOH to a pH near 2.0.

Silver nitrate: 1.2 g $AgNO_3$/student.

Procedure

1. Pour your unknown carefully into a 50- or 100-mL beaker. Dissolve the solid in ~20 mL of water and pour the solution into a 100-mL volumetric flask. Rinse the sample vial and beaker five times with small portions of H_2O and transfer the washings to the flask. Dilute to the mark and mix well.

2. Dry 1.2 g of $AgNO_3$ (FM 169.87) at 105˚C for 1 h and cool in a dessicator 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 6-4 of the textbook. The silver electrode is simply a 3-cm length of silver wire soldered 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 pH 2 buffer, and begin magnetic stirring. Record the initial level of $AgNO_3$ 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 abrupt voltage changes. 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 (±1 mL) of the two end points.
6. Turn the pH meter to standby, remove the beaker, rinse the electrodes well with distilled 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 $AgNO_3$.[27]) Clean the beaker and set up the titration apparatus again. The beaker need not be dry.
7. 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 6-5 of the textbook. The I^- end point is taken as the intersection of the two dashed lines in the inset of Figure 6-5. The Cl^- end point is the inflection point (the steepest point) at the second break. Calculate milligrams of KI and milligrams of KCl in your solid unknown.

Analyzing 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

[27] E. Thall, *J. Chem. Ed*. **1981**, *58*, 561.

could study stratification (layering) of water in lakes. The measurement responds to all ions that precipitate with Ag^+, of which Cl^- is, by far, the dominant ion in natural waters.

You can use the electrodes in Figure 6-4 of the textbook, or you can construct the rugged combination electrode shown in Figure 1.[28] 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 $CuSO_4$ solution. The inner electrode maintains a

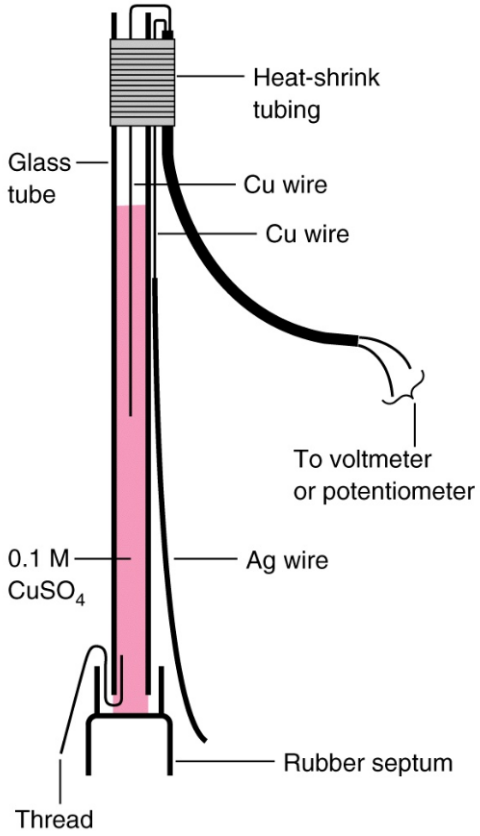


Figure 1. Combination electrode constructed from 8-mm-outer-diameter glass tubing. Strip the insulation off the ends of a two-wire copper cable. One bare Cu wire extends into the glass tube. A silver wire soldered to the other Cu wire is left outside the glass tube. The assembly is held together at the top with heat-shrink tubing. When ready for use, insert a cotton thread into the bottom of the tube and fit a 14/20 serum cap moistened with 0.1 M $CuSO_4$ over the end so that part of the thread is inside and part is outside. After adding 0.1 M $CuSO_4$ to the inside of the tube, the electrode is ready for use.

constant potential because the concentration of 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.

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

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 $AgNO_3$ 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 $AgNO_3$ 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 6-4 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 6-5 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 (μg/mL) of Cl^- in the unknown. Use data from several students with the same sample to find the mean and standard deviation of ppm Cl^-.

17. Measuring Ammonia in an Aquarium with an Ion-Selective Electrode

Green Profile
See Section 0

Box 6-1 in the textbook provides background for this experiment.

Reagents

Standard ammonia solution: Dissolve ~0.382 g of NH_4Cl (FM 53.49) in a 1-L volumetric flask to obtain a solution containing ~100 µg of nitrogen per milliliter. From the mass of NH_4Cl that you weighed, calculate the nitrogen concentration in µg/mL.

Procedure[29,30]

1. Using appropriate dilutions of your standard ammonia solution, prepare standards containing 3, 1, 0.3, and 0.1 µg N/mL in 100-mL volumetric flasks.
2. Pour the 100-mL standard solution containing 0.1 µg N/mL into a clean, dry 150-mL beaker. Immerse an ammonia ion-selective electrode and a reference electrode in the solution and begin magnetic stirring. Do not stir the solution so rapidly that air bubbles are drawn into the liquid. Add 1.0 mL of 10 M NaOH (to convert NH_4^+ into NH_3) and record the voltage to the nearest millivolt when the reading stabilizes. Do not allow more than 5 min before reading the voltage, because the temperature of the solution will increase from the stirring and the reading will change.
3. Repeat the same procedure for the other three standards. Prepare a graph of mV versus log [N], where [N] is the concentration of nitrogen in µg/mL. You should observe a reasonable straight line.
4. Measure 100 mL of freshly collected aquarium water in a graduated cylinder. Pour it into a clean, dry 150-mL beaker and repeat the process in step 2.
5. From the least-squares slope and intercept of the calibration curve from step 3, and the potential measured in step 4, compute the concentration of ammonia nitrogen in the aquarium water.
6. Repeat steps 4 and 5 once more with a fresh sample to obtain a duplicate measurement.

---

29 K. D. Hughes, *Anal. Chem.* **1993**, *65*, 883A.

30 *Standard Methods for the Examination of Water and Wastewater*, 20th ed. (Washington, DC: American Public Health Association, 1998).

18. Electrogravimetric Analysis of Copper

Green Profile
See Section 0

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

Students may analyze a preparation of their own (such as copper acetylsalicylate[31]) or be given unknowns prepared from $CuSO_4 \cdot 5H_2O$ or metallic Cu. In the latter case, dissolve the metal in 8 M HNO_3, boil to remove HNO_2, neutralize with ammonia, and barely acidify the solution with dilute H_2SO_4 (using litmus paper to test for acidity). Samples must be free of chloride and nitrous acid.[32] Copper oxide unknowns (soluble in acid) are available from Thorn Smith.[1]

The apparatus in Figure 17-1 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_3 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.[33]

2. The sample should contain 0.2–0.3 g of Cu in 100 mL. Add 3 mL of 98 wt% H_2SO_4 and 2 mL of freshly boiled 8 M HNO_3. 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

---

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

32 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.

33 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_2 formed by electrolysis of Cl^- 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^-: $N_2H_4 \rightarrow N_2 + 4H^+ + 4e^-$.

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.

4. Report the mass of Cu in the unknown.

19. Measuring Vitamin C in Fruit Juice by Voltammetry with Standard Addition

Green Profile
See Section 0

This experiment is described in Section 17-3 of the textbook and standard addition is discussed in Section 5-3. The three-electrode cell in Figure 17-9 of the textbook employs a wax-impregnated graphite working electrode, an Ag | AgCl reference electrode,[34] and a graphite auxiliary electrode. The electrochemistry at the working electrode is the oxidation of ascorbic acid (vitamin C) in Reaction 17-9.

Reagents

Nitric acid: ~3 M HNO_3.

Standard ascorbic acid: This standard should be freshly prepared each lab period. Follow these directions if standard additions are to be made in 1-mL aliquots: Accurately weigh ~0.5 g of ascorbic acid (FM 176.12) into a 100-mL volumetric flask. Add ~50 mL of water and swirl to dissolve. Add ~1 mL of ~3 M HNO_3, swirl, and dilute to 100 mL with water to obtain ~0.03 ascorbic acid standard. Nitric acid slows the reaction of ascorbic acid with oxygen. If standard additions are to be made in ~100 µL aliquots, prepare the ~0.3 M standard in a 10-mL volumetric flask from 0.5 g ascorbic acid (accurately weighed) and ~100 µL of ~3 M HNO_3.

Unknowns: ~100 mL of fruit juice for each student or group of students working together. Possible unknowns include powdered drink mixes, canned drinks, different brands of orange juice, or vegetable juices.

Preparation of Working Electrode

Many instructors will want to prepare the inexpensive auxiliary and working electrodes in advance and provide them to students. The auxiliary electrode is made of very pure graphite,

[34] A recipe to prepare your own Ag | AgCl reference electrode is given by G. A. East and M. A. del Valle, *J. Chem. Ed.* **2000**, *77*, 97.

which is a form of carbon.[35] It is used as received. The working electrode, called a *wax-impregnated graphite electrode*, is made of the same pure graphite sharpened to a point in a pencil sharpener. Soak the sharpened rod for 3 h in molten paraffin at 100°C to fill up pores with the electrically inert wax. Then use forceps to withdraw the rod slowly from the wax bath and allow an electrically insulating layer of paraffin to form on the outside of the rod. Slice the sharp tip with a razor blade to expose a ~1/2-mm-diameter section of graphite and gently grind the tip with fine sand paper to leave a small, flat exposed graphite surface, as shown in the inset of Figure 17-9 of the textbook. Prior to beginning the experiment, clean the graphite tip by rubbing it on filter paper with the electrode perpendicular to the paper. (If the electrode becomes fouled, cut off the tip with a razor to expose fresh surface.)

Procedure

1. Use small quantities of unknown fruit juice to rinse the inside of a pipet and discard the washings. Then pipet 50 mL of unknown juice into a clean, dry 100-mL beaker containing a stirring bar.

2. Clean the tip of the working electrode by rubbing it perpendicular to a piece of filter paper on a flat surface.

3. Set up the apparatus in Figure 17-9 of the textbook on top of a magnetic stirrer. Use clamps with insulated claws to hold the electrodes in place. (If the claws are bare metal, use tubing or tape to insulate them.) The potentiostat varies the voltage between the working and reference electrodes and measures the current between the working and auxiliary electrodes. The voltammogram (a graph of current versus potential) is recorded on a computer or strip chart.

4. Following instructions for your potentiostat, apply the following sequence of voltages:
   a. Condition the working electrode at –1.5 V (versus Ag | AgCl) for 2 min with stirring. This voltage reduces and removes organic material from the tip of the electrode.
   b. Change the potential to –0.4 V and continue stirring for 30 s. Dislodge any bubbles of gas from the tip of the electrode by gentle tapping. Discontinue stirring and let the solution become calm for an additional 30 s.

[35] Graphite rods (6.15 mm diameter × 102 mm long) can be purchased from Alpha/Aesar, catalog number 40766, 99.9995% carbon. http://www.alfa.com.

c. Scan the potential from –0.4 V to + 1.2 V at a rate of +33 mV/s while measuring current to record a voltammogram such as the lowest trace in Figure 17-10 of the textbook.

5. *Standard addition*. Using a transfer pipet or a microliter pipet, add 1.00 mL of ~0.03 M standard ascorbic acid (or 100 μL of ~0.3 M standard) into the beaker and turn on the stirrer. Repeat the sequence in step 4 to condition the electrode and record a new voltammogram.

6. Add another increment of standard and record another voltammogram by repeating the sequence in step 4. The objective is to increase the current from that of the unknown by a factor of 1.5 to 3 by using at least four standard additions. The total volume of added standard should not exceed ~10 mL. Depending on your unknown, it may be necessary to adjust the concentration of standard or volume of each increment to achieve this goal.

Data Analysis

1. Following the example in Figure 17-10 of the textbook, extrapolate the baseline from the region where there is no reaction (–0.4 to 0 V) into the region of the current plateau (~0.8 V). Measure the peak current on each curve with respect to the baseline. The very shallow peak shifts to higher potential as more acid is added.

2. Prepare a graph of Equation 5-8 of the textbook, such as Figure 5-6. The spreadsheet in Figure 5-5 is handy for this purpose.

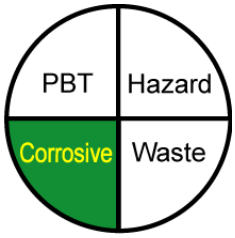
3. Use the method of least squares to find the equation of the straight line and compute the x-intercept (where $y = 0$), which is the concentration of ascorbic acid in the original unknown.

4. Estimate the uncertainty in the x-intercept with the following equation:

$$\text{Standard deviation of } x\text{-intercept} = \frac{s_y}{m}\sqrt{\frac{1}{n} + \frac{\bar{y}^2}{m^2 \sum(x_i - \bar{x})^2}}$$

where s_y is the standard deviation of y (Equation 4-15), m is the slope of the least-squares line (Equation 4-12), n is the number of data points (including the unknown plus the standard additions), $\bar{y}$ is the average value of y for all data points, and $\bar{x}$ is the average value of x for all data points. The sum inside the square root extends over all data points. If you measured the unknown plus five standard additions, $n = 6$ and the sum includes six terms.

20. Polarographic Measurement of an Equilibrium Constant[36]



Green Profile
See Section 0

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. Polarography is described in Section 17-4 of the textbook. 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 (the current at the plateau of the wave).

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.

---

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

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.

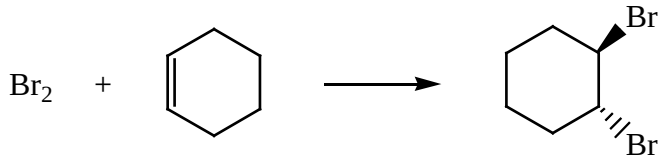
4. Make a graph of $\Delta E_{1/2}$ versus $\ln[C_2O_4^{2-}]$. From the slope, use Equation 1 to find p, the stoichiometry coefficient. Then use the intercept to find the value of β_p. Use the method of least squares to find the standard deviations of the slope and intercept. From the standard deviations, find the uncertainties in p and β_p and express each with the correct number of significant figures.

21. Coulometric Titration of Cyclohexene with Bromine[37]

Green Profile
See Section 0

Coulometry is described in Section 17-1 of the textbook. In this experiment, cyclohexene will be titrated with Br_2 generated by electrolytic oxidation of Br^-:

$$2Br^- \rightarrow Br_2 + 2e^- \quad (1)$$



(2)

Cyclohexene *trans*-1,2-Dibromocyclohexane

The initial solution contains an unknown quantity of cyclohexene and a large amount of Br^-. When Reaction 1 has generated just enough Br_2 to react with all the cyclohexene, the moles of electrons liberated in Reaction 1 are equal to twice the moles of Br_2 and therefore twice the moles of cyclohexene.

The reaction is carried out at a constant current with the apparatus in Figure 1. Br_2 generated at the Pt anode at the left immediately reacts with cyclohexene. When cyclohexene is consumed, the concentration of Br_2 suddenly rises, signaling the end of the reaction.

The equation relating moles of electrons to the electric current and the time is

Relation between electrons, current, and time:

$$\text{Moles of } e^- = \frac{I \cdot t}{F} \quad (3)$$

where I is the current in amperes (= coulombs/s = C/s), t is the time in seconds, and F is the Faraday constant (96 485 C/mol).

The rise in Br_2 concentration is detected by measuring the current between the two detector electrodes at the right in Figure 1. A voltage of 0.25 V applied between these two electrodes is not enough to electrolyze any solute, so only a tiny current of <1 μA flows through the microammeter. At the equivalence point, cyclohexene is consumed, $[Br_2]$ suddenly increases, and detector current flows by virtue of the reactions:

Detector anode: $2Br^- \rightarrow Br_2 + 2e^-$

Detector cathode: $Br_2 + 2e^- \rightarrow 2Br^-$

[37] D. H. Evans, *J. Chem. Ed*. **1968**, *45*, 88.

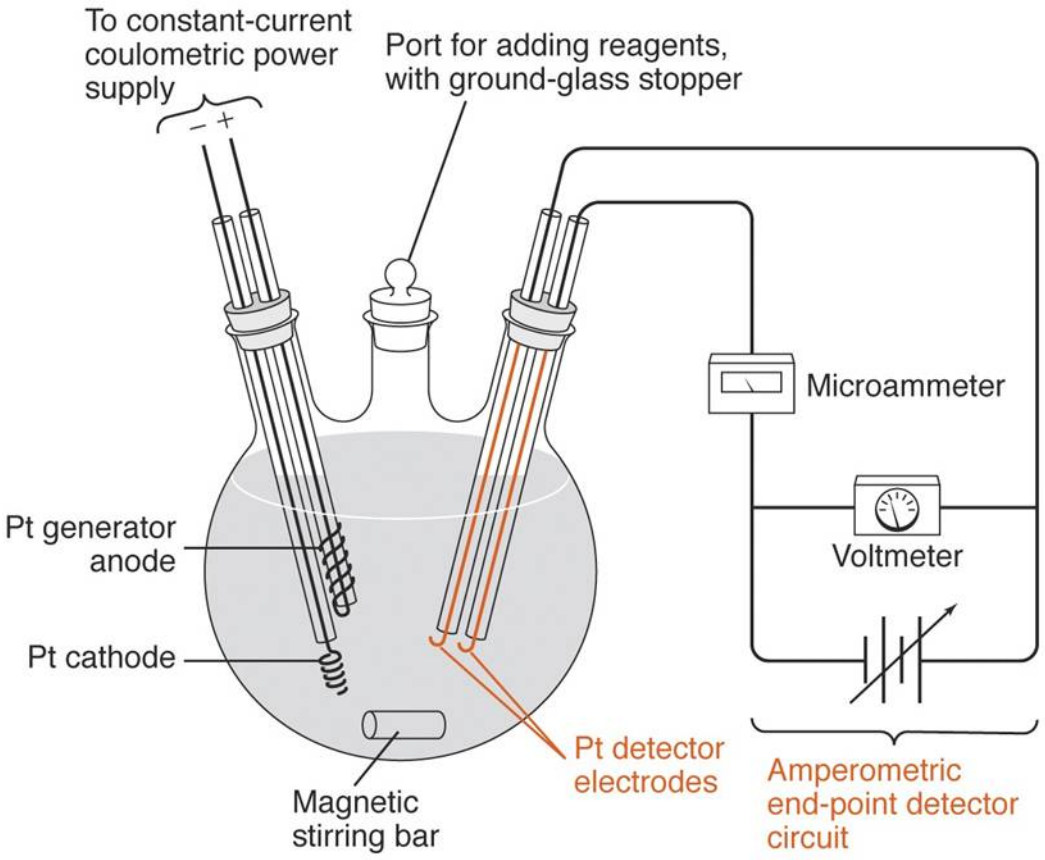


Figure 1. Apparatus for coulometric titration of cyclohexene with Br_2.

In our procedure, enough Br_2 is first generated in the absence of cyclohexene to give a detector current of 20.0 μA. When cyclohexene is added, the current decreases to a tiny value because Br_2 is consumed. Br_2 is then generated by the coulometric circuit, and the end point is taken when the detector again reaches 20.0 μA. Because the reaction is begun with Br_2 present, impurities that can react with Br_2 before analyte is added are eliminated.

The electrolysis current (not to be confused with the detector current) for the Br_2-generating electrodes can be controlled by a hand-operated switch. As the detector current approaches 20.0 μA, you close the switch for shorter and shorter intervals. This practice is analogous to adding titrant dropwise from a buret near the end of a titration. The switch in the coulometer circuit serves as a "stopcock" for addition of Br_2 to the reaction.

EXAMPLE Coulometric Titration

A 2.000-mL volume containing 0.611 3 mg of cyclohexene/mL is to be titrated with a constant current of 4.825 mA. How much time will required for complete titration?

Solution The quantity of cyclohexene is

$$\frac{(2.000\text{ mL})(0.611\,3\text{ mg/mL})}{(82.146\text{ mg/mmol})} = 0.014\,88\text{ mmol}$$

In Reactions 1 and 2, each mole of cyclohexene requires 1 mol of Br_2, which requires 2 mol of electrons. For 0.014 88 mmol of cyclohexene to react, 0.029 76 mmol of electrons must flow. From Equation 3,

$$\text{Moles of } e^- = \frac{I \cdot t}{F} \Rightarrow t = \frac{(\text{moles of } e^-)F}{I} = \frac{(0.029\,76 \times 10^{-3}\text{ mol})(96\,485\text{ C/mol})}{(4.825 \times 10^{-3}\text{ C/s})} = 595.1\text{ s}$$

It will require just under 10 min to complete the reaction.

For this experiment, you can use a commercial coulometer or the circuits in Figure 2.[38] Start timing with a stopwatch when the generator switch is closed.

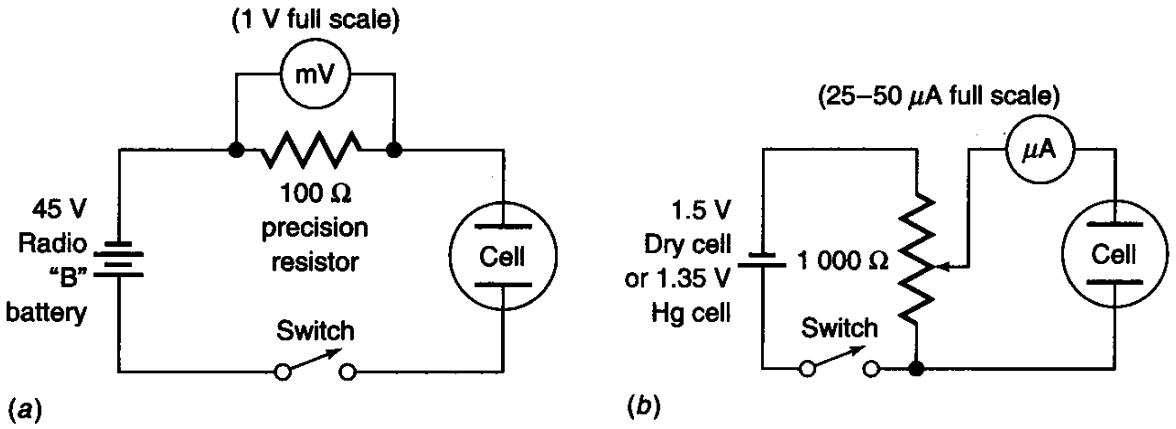


Figure 2. Circuits for coulometric titrations. (*a*) Generator circuit. (*b*) Detector circuit.

[38] 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.

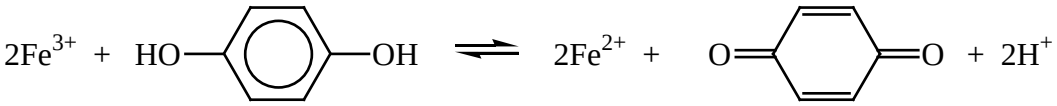
Procedure

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.
2. Generate Br_2 with the generator circuit until the detector current is 20.0 µA. The generator current should be ~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 begin timing. While the reaction is in progress, measure the voltage (E) across the precision resistor (R = 100.0 ± 0.1 Ω) 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.

22. Spectrophotometric Determination of Iron in Vitamin Tablets[39]

Green Profile
See Section 0

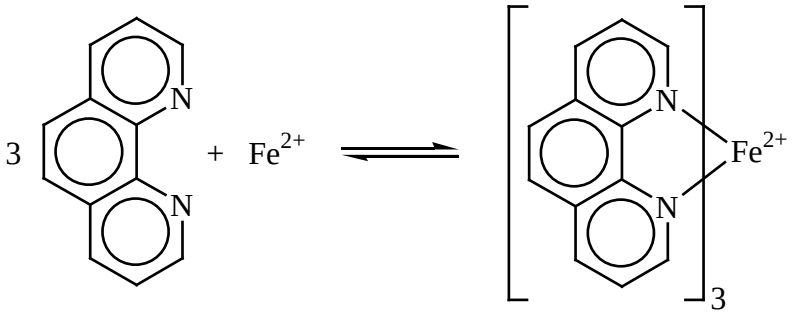
In this procedure, iron from a vitamin supplement tablet is dissolved in acid, reduced to Fe^{2+} with hydroquinone, and complexed with *o*-phenanthroline to form an intensely colored complex (Color Plate 13 in the textbook).



Hydroquinone
FM 110.11

Quinone

``



o-Phenanthroline
FM 180.21

λ_{max} = 510 nm
Red complex

Reagents

Hydroquinone: (20 mL/student) Freshly prepared solution containing 10 g/L in distilled water. Store in an amber bottle.

Trisodium citrate: (20 mL/student) 25 g/L $Na_2citrate \cdot 2H_2O$ (FM 294.10) in distilled water.

o-Phenanthroline: (25 mL/student) Dissolve 2.5 g in 100 mL of ethanol and add 900 mL of distilled water. Store in an amber bottle.

6 M HCl: (25 mL/student) Dilute 124 mL of 37 wt% HCl up to 250 mL with distilled water.

Standard Fe (0.04 mg Fe/mL): (35 mL/student) Dissolve 0.281 g of reagent-grade $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ (FM 392.14) in distilled 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

[39] R. C. Atkins, *J. Chem. Ed.* **1975**, *52*, 550

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. Determine how many drops of citrate solution are needed to bring 10.00 mL of the iron 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 510 nm in a 1-cm cell. (The color is stable, so all solutions may be prepared and all the absorbances measured at once.) Use distilled water in the reference cuvet 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, find the number of milligrams of Fe in the tablet. Use Equation 4-16 in the textbook to find the uncertainty in the number of milligrams of Fe.

23. Microscale Spectrophotometric Measurement of Iron in Foods by Standard Addition[40]

Green Profile
See Section 0

This microscale experiment uses the same chemistry as that of Experiment 22 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 22.

Trisodium citrate dehydrate: 4 g/student.

o-Phenanthroline: 4 mL/student; prepared as in Experiment 22.

Standard Fe (40 µg Fe/mL): 4 mL/student; prepared as in Experiment 22.

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 use. 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

[40] Idea based on P. E. Adams, *J. Chem. Ed.* **1995**, *72*, 649.

flame to *ignite* the black solid, converting it to white ash. The bottom of crucible should be red hot during ignition. 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, and 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 5-5 in the textbook to find the Fe content of the unknown solution. Note that when all solutions have the same final volume (as they do in this experiment), the functions to plot in the standard addition graph are the absorbance on the *y*-axis and the final concentration of added standard Fe on the *x*-axis. Calculate the wt% of Fe in the food.

9. Estimate the uncertainty in wt% Fe from the uncertainty in the *x*-intercept of the least-squares line in the standard addition graph. If you fit four points (unknown plus three standards), the uncertainty in the *x*-intercept (the standard deviation of $[X]_f$) is

$$\text{Standard deviation of intercept} = \frac{s_y}{m\sqrt{D}}\sqrt{n(\text{intercept})^2 - 2(\text{intercept})\Sigma x_i + \Sigma(x_i^2)} \qquad (1)$$

where s_y is the standard deviation of y (Equation 4-15 in the textbook), m is the slope of the least-squares line (Equation 4-12), D is given by Equation 4-14, n is the number of points on the graph, including the unknown (4 in this experiment), intercept is the x-intercept, and x_i are the values of x for the 4 points.

24. Spectrophotometric Determination of Nitrite in Aquarium Water

Green Profile
See Section 0

Background for this experiment[29,30] is found in Box 6-1 and Section 18-4.

Reagents

Sodium nitrite: 1 g $NaNO_2$ (FM 69.00)/student.

Sodium oxalate: 4 g $Na_2C_2O_4$ (FM 134.00)/student.

Potassium permanganate: 1 g $KMnO_4$ (FM 158.03)/student.

0.9 M H_2SO_4: 1 L/student.

Concentrated (96 wt%) H_2SO_4: 20 mL/student.

Color-forming reagent: (Reaction 18-7; 15 mL/student) Mix 1.0 g of sulfanilamide, 0.10 g of *N*-(1-naphthyl)ethylenediamine dihydrochloride, and 10 mL of 85 wt% H_3PO_4 and dilute with distilled water to 100 mL. Store in a dark bottle in the refrigerator.

Procedure

1. Prepare and standardize 0.01 M $KMnO_4$ solution as described in Experiment 13. Reduce the quantities of $KMnO_4$ and $Na_2C_2O_4$ by a factor of 2.
2. Prepare 0.018 M $NaNO_2$ by dissolving 0.62 g of $NaNO_2$ in 500 mL of distilled water.
3. Standardize the $NaNO_2$ as described in Section 6-3 of the textbook.
4. Withdraw ~30 mL of aquarium water and filter it to remove suspended solids. Analyze duplicate 10.00-mL aliquots of freshly drawn aquarium water by the procedure in Section 18-4 of the textbook. Use three or four standard points for the calibration curve such that the unknown lies within the range of the standards (Figure 18-11 of the textbook).

25. Spectrophotometric Measurement of an Equilibrium Constant: The Scatchard Plot

Green Profile
See Section 0

In this experiment, we will use the Scatchard plot described below to find the equilibrium constant for the formation of a complex between iodine and pyridine in cyclohexane:[41]

Iodine-pyridine complex

Both I_2 and $I_2 \cdot$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) allows us to evaluate *K* for the reaction. The experiment is best performed with a recording spectrophotometer, but single-wavelength measurements can be used.

Scatchard Plot

Consider the equilibrium in which the species P and X react to form PX.

$$P + X \rightleftharpoons PX \qquad (1)$$

Neglecting activity coefficients, we can write

$$K = \frac{[PX]}{[P][X]} \qquad (2)$$

Consider a series of solutions in which increments of X are added to a constant amount of P. Letting P_o be the total concentration of P (in the forms P and PX), we can write

$$[P] = P_o - [PX]$$

Now the equilibrium expression, Equation 2, can be rearranged as follows:

---

41 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.

$$\frac{[PX]}{[X]} = K[P] = K(P_o - [PX]) \qquad (3)$$

A graph of [PX]/[X] versus [PX] will have a slope of $-K$ and is called a *Scatchard plot*. It is widely used in biochemistry to measure equilibrium constants.

If we know [PX], we can find [X] with the mass balance

$$X_o = [\text{total X}] = [PX] + [X]$$

To measure [PX], we might use spectrophotometric absorbance. Suppose that P and PX each have some absorbance at wavelength λ, but X has no absorbance at this wavelength. For simplicity, let all measurements be made in a cell of pathlength 1.000 cm so that we can omit b (= 1.000 cm) when writing Beer's law.

The absorbance at some wavelength is the sum of absorbances of PX and P:

$$A = \varepsilon_{PX}[PX] + \varepsilon_P[P]$$

Substituting $[P] = P_o - [PX]$, we can write

$$A = \varepsilon_{PX}[PX] + \underbrace{\varepsilon_P P_o}_{A_o} - \varepsilon_P[PX]$$

But $\varepsilon_P P_o$ is A_o, the initial absorbance before any X is added. Therefore,

$$A = [PX](\varepsilon_{PX} - \varepsilon_P) + A_o \quad \Rightarrow \quad [PX] = \frac{\Delta A}{\Delta \varepsilon} \qquad (4)$$

where $\Delta\varepsilon = \varepsilon_{PX} - \varepsilon_P$ and ΔA ($= A - A_o$) is the observed absorbance after each addition of X minus the initial absorbance.

Substituting [PX] from Equation 4 into Equation 3 gives

Scatchard equation:

$$\frac{\Delta A}{[X]} = K\Delta\varepsilon P_o - K\Delta A \qquad (5)$$

A graph of $\Delta A/[X]$ versus ΔA should be a straight line with a slope of $-K$. Absorbance measured while P is titrated with X can be used to find K for the reaction of X with P.

Procedure

All operations should be carried out *in a fume hood*, including pouring solutions into and out of the spectrophotometer cell. Only a *capped* cuvet 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 1, 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, Equation 5), using the absorbance at the $I_2 \cdot$pyridine maximum.

3. From the slope of the graph, find the equilibrium constant by using Equation 5. 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 $I_2 \cdot$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.

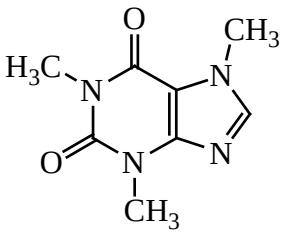
5. Using the values of free pyridine concentration from your last refinement and the values of absorbance at the I_2 maximum, prepare another Scatchard plot and see if you get the same value of K.

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

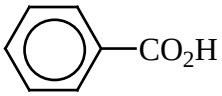
26. Spectrophotometric Analysis of a Mixture: Caffeine and Benzoic Acid in a Soft Drink[42]

Green Profile
See Section 0

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.

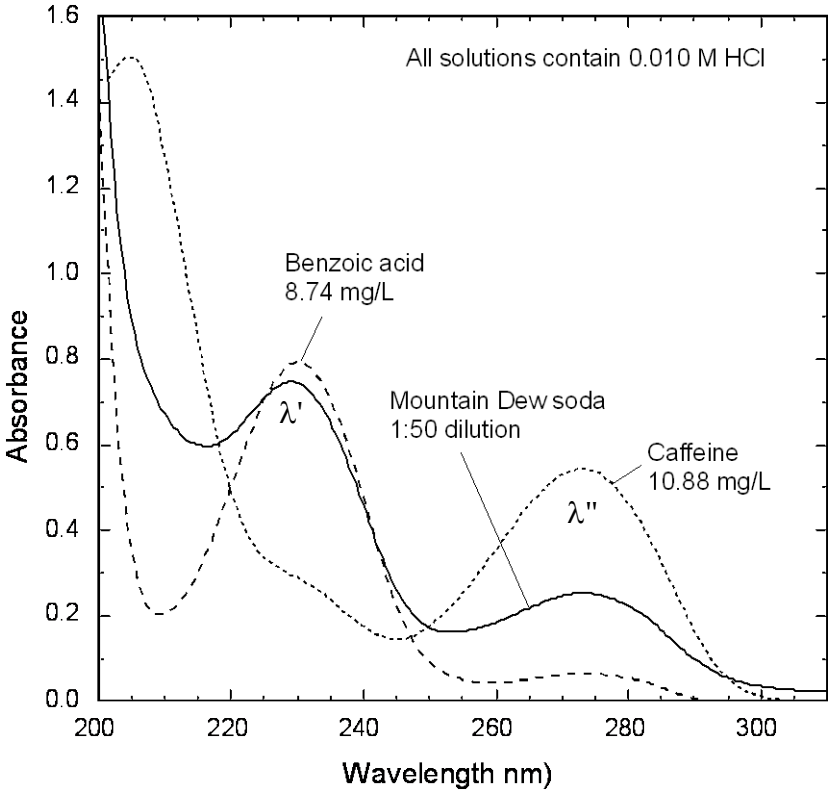


Figure 1. Ultraviolet absorption of benzoic acid, caffeine, and a 1:50 dilution of Mountain Dew soft drink. All solutions contain 0.010 M HCl.

[42] V. L. McDevitt, A. Rodriquez and K. R. Williams, *J. Chem. Ed.* **1998**, *75*, 625.

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 to 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).[42]

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

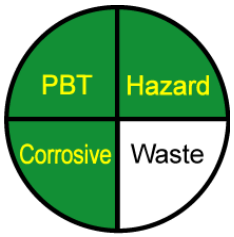
1. *Calibration standards:* Prepare benzoic acid solutions containing 2, 4, 6, 8 and 10 mg/L in 0.010 M HCl. To prepare a 2 mg/L solution, mix 2.00 mL of benzoic acid standard plus 10.0 mL of 0.10 M HCl in a 100-mL volumetric flask and dilute to the mark with water. Use 4, 6, 8 and 10 mL of benzoic acid to prepare the other standards. In a similar manner, prepare caffeine standards containing 4, 8, 12, 16 and 20 mg/L 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 Equations 19-5 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 Equations 19-5 in the textbook to find the concentration of each component in the synthetic unknown.

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



Green Profile
See Section 0

Experiments 27-29 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.[43]

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.

0.50 M NH_3/1.1 M NH_4^+ buffer (pH 9.7): Mix 6.69 g of NH_4Cl (FM 53.49) plus 16.86 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.)

Calmagite indicator: Dissolve 0.05 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 0.80 g of solid hydroxylamine hydrochloride

[43] 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.

to the flask, and swirl to dissolve the solid. Add ~150 mL of water and swirl to mix the contents. Dilute to the mark with water, set 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 10 mL of pH 9.7 buffer (by graduated cylinder), and add 3-5 drops of calmagite indicator. The pH of the solution will be near 9.2. Titrate with standard EDTA from a 50-mL buret and note the end point when the color changes from wine red to blue.

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.

28. Measuring Manganese in Steel by Spectrophotometry with Standard Addition

Green Profile
See Section 0

Experiments 27-29 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.[43] 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.[1]

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 27 into a 100-mL volumetric flask, dilute to the mark with water, and mix well. You will use this solution in Experiments 28 and 29. 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 ($MnO_2(s)$) 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 29. 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:

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

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

Green Profile
See Section 0

This experiment complements the results of the spectrophotometric analysis in Experiment 28.[43] 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 27 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 28.

Standard manganese: ~0.1 mg Mn/mL from step 4 of Experiment 28. 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 28. 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 28. 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 4-6 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 4-19 in the textbook. Because you have measured each unknown three times, the first term in the radical in Equation 4-19 should be 1/3. In Equation 4-19, 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 4-4 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 4-4 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 4-6 in the textbook) to estimate a 95% confidence interval around the mean value. Does the mean spectrophotometric value for wt% Mn from Experiment 28 lie within the 95% confidence interval for the atomic absorption results? (We cannot use the *t* test to compare Experiments 28 and 29 because we do not have enough samples in Experiment 28 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. If you have measured points on a calibration curve, one common definition of detection limit is

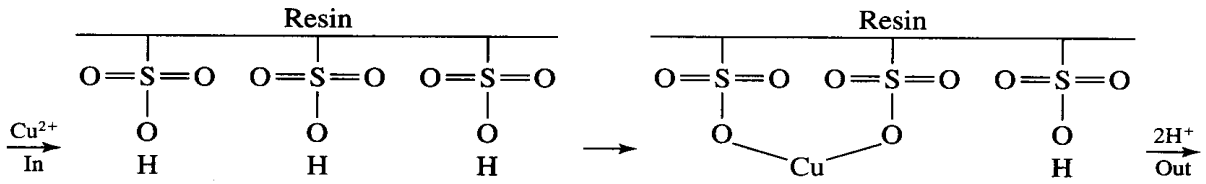
$$\text{Detection limit (ppm)} = \frac{\bar{y}_B + 3s_B}{m}$$

where $\bar{y}_B$ is the mean atomic absorbance reading for the blank, s_B is the standard deviation for the blank, and m 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 $\bar{y}_B = 0$.)

30. Properties of an Ion-Exchange Resin[44]

Green Profile
See Section 0

This experiment explores 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, releasing one H^+ for each positive charge bound to the resin. Cu^{2+} 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.



First, 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 of the experiment, we analyze 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

Bio-Rad Dowex 50W-X2 (100/200 mesh) cation-exchange resin: 1.1 g/student.

0.3 M NaCl: (5–10 mL/student) Accurately weigh ~17.5 g of NaCl (FM 58.44) and dissolve it in a 1-L volumetric flask.

0.1 M $Fe(NO_3)_3 \cdot 6H_2O$: (5–10 mL/student) Accurately weigh ~35 g of $Fe(NO_3)_3 \cdot 6H_2O$ (FM 349.95) and dissolve it in a 1-L volumetric flask.

[44] Part of this experiment is taken from M. W. Olson and J. M. Crawford, *J. Chem. Ed.* 1975, *52*, 546.

$VOSO_4$: 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. For example, 50.0 mL of 0.1 M NaOH can be pipetted into a 250-mL volumetric flask and diluted to volume with distilled water.

0.1 M HCl: 50 mL/student.

Phenolphthalein indicator. Recipe in Experiment 7.

Procedure

1. Prepare a chromatography column from glass tubing that has a 0.7-cm diameter and is 15-cm long by fitting the tubing at the bottom with a cork that has a small hole to serve as the outlet. Place a small ball of glass wool above the cork to retain the resin. Use a 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[45] works well.) Fill the column with water, close it off, and test for leaks. 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 ~10 mL of 1 M HCl through the column. Apply the liquid sample to the glass wall so as not to disturb the resin.

[45] Bio-Rad Laboratories, www.bio-rad.com.

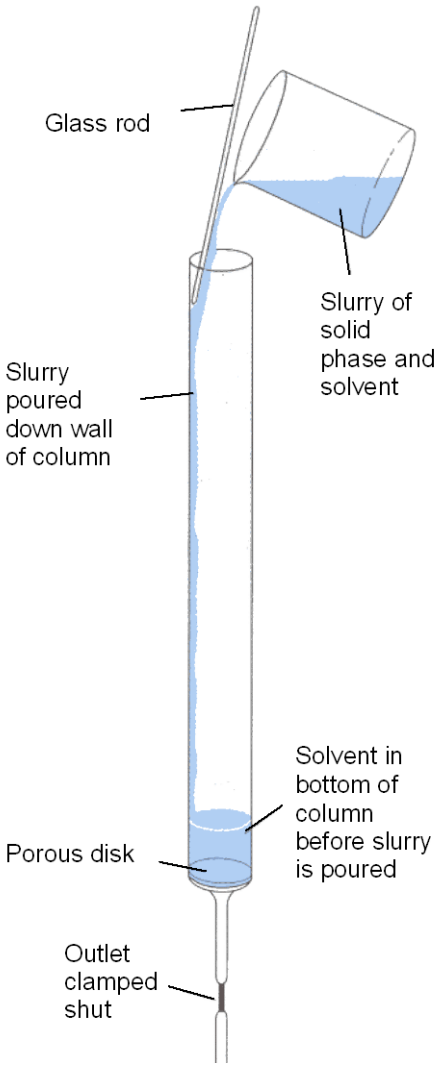


Figure 1. Pouring a slurry of stationary phase into a chromatography column.

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 chromatography resins, the one 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 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 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 procedure 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 directed in step 3, and titrate the eluate. Explain what you observe.

6. Analyze 10.00 mL of $VOSO_4$ solution as described in step 3.

7. Step 6 gives the total cation content (= VO^{2+} + $2H_2SO_4$) of 10.00 mL of $VOSO_4$ solution. From the absorbance at 750 nm and the molar absorptivity of VO^{2+} (ε = 18.0 $M^{-1}{\cdot}cm^{-1}$ at 750 nm), calculate the concentration of VO^{2+} in the solution. By difference, calculate the concentration of H_2SO_4 in $VOSO_4$ reagent. From the difference between the mass of $VOSO_4$ reagent that was used and the content of $VOSO_4$ and H_2SO_4, calculate the mass of H_2O in the $VOSO_4$ reagent. Express the composition of the vanadyl sulfate in the form $(VOSO_4)_{1.00}(H_2SO_4)_x(H_2O)_y$.

31. Analysis of Sulfur in Coal by Ion Chromatography[46]

Green Profile
See Section 0

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 2-10 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.[47]

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

6 M HCl: 25 mL/student.

Phenolphthalein indicator: Recipe in Experiment 7.

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

46 E. Koubek and A. E. Stewart, *J. Chem. Ed.* **1992**, *69*, A146.

47 Standards containing 0.5–5 wt% S are available from NIST Standard Reference Materials Program, Room 204, Building 202, Gaithersburg MD 20899-0001 (E-mail: SRMINFO@enh.nist.gov).

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[48] 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.

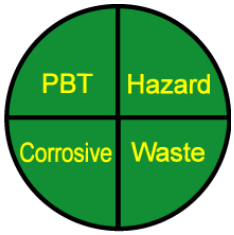
4. Assuming that the coal contains 3 wt% sulfur, calculate the concentration of SO_4^{2-} in the solution in step 3. Using ammonium sulfate and appropriate volumetric glassware, prepare five standards containing 0.1, 0.5, 1.0, 1.5, and 2.0 times the calculated concentration of SO_4^{2-} in the unknown.

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.

---

[48] 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.

32. Measuring Carbon Monoxide in Automobile Exhaust by Gas Chromatography[49]



Green Profile
See Section 0

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 an old "clunker" 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.[50]

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

---

[49] D. Jaffe and S. Herndon, *J. Chem. Ed.* **1995**, *72*, 364.

[50] Available, for example, from Scott Specialty Gases, Route 611, Plumsteadville PA 18949 (Phone: 215-766-8861; http://www.scottgas.com/).

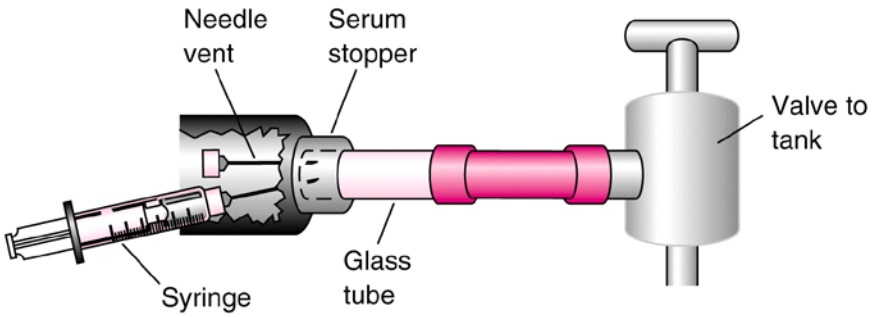


Figure 1. Collecting a sample of standard gas mixture from a lecture bottle. Remove the vent needle when withdrawing gas into the syringe. Do not open the tank valve so much that the connections pop open. **(CAUTION:** *Handle CO only in a hood.)*

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.

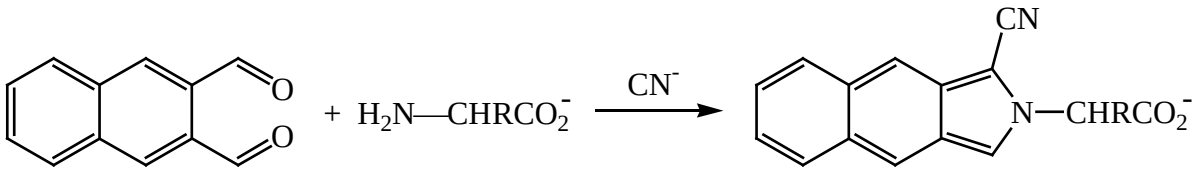
4. Inject two 1.00-mL samples of auto exhaust and measure the peak area each time. Compute the vol% of CO in the unknown from its average peak area:

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

33. Amino Acid Analysis by Capillary Electrophoresis[51]

Green Profile
See Section 0

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.[52]

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.

---

51 P. L. Weber and D. R. Buck, *J. Chem. Ed.* **1994**, *71*, 609.

52 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-Aldrich; http://www.sigmaaldrich.com/chemistry/aldrich-chemistry/aldrich-handbook.html).

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 ($Na_2B_4O_7 \cdot 10H_2O$, 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.

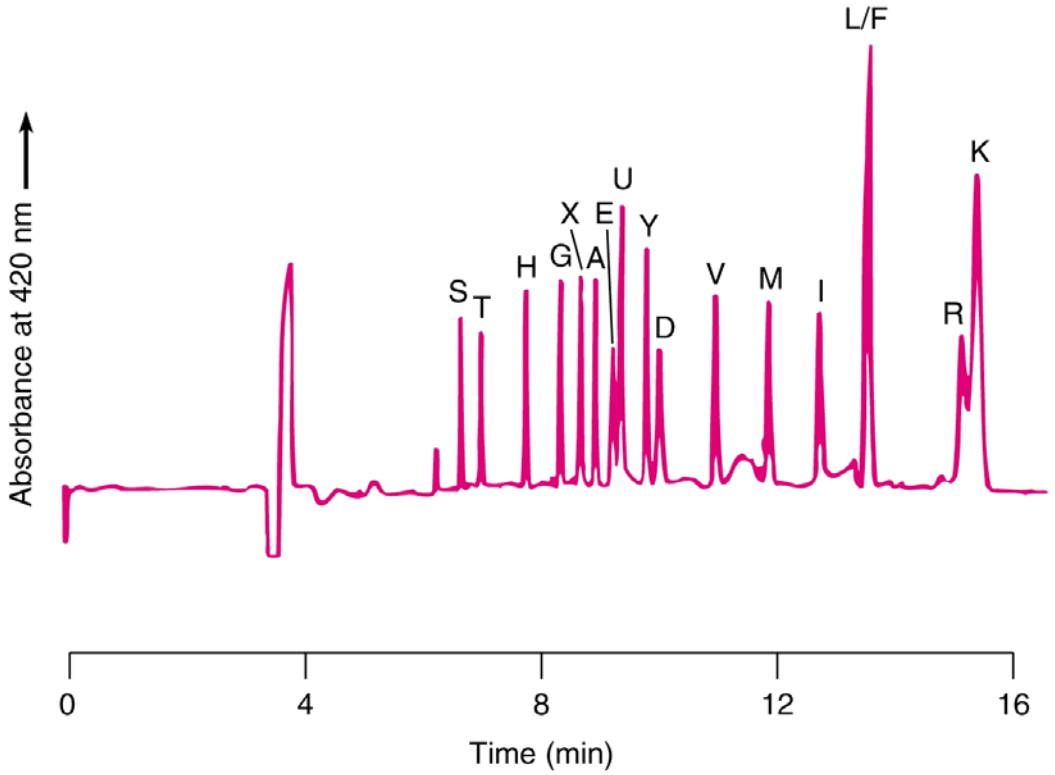


Figure 1. Electropherogram of standard mixture of NDA-derivatized amino acids plus internal standard, all at equal concentrations. Abbreviations for amino acids are given in Table 11-1 in the textbook. The internal standard, designated X, is α-aminoadipic acid. U is an unidentified peak. Acid hydrolysis 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. [From P. L. Weber and D. R. Buck, *J. Chem. Ed.* **1994**, *71*, 609.]

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.

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. Use a glass tube (17 × 55 mm) sealed at one end. Fit the open end with a rubber septum 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 house vacuum or a water aspirator. Use a trap (Figure 2-12 of the textbook) between the vacuum source and the sample 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.)* Conduct the electrophoresis in a 50-µm-inner-diameter uncoated silica capillary with 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 the derivatized 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. Find the concentration of each amino acid in the protein hydrolysate by using the following equation:

$$\frac{\text{concentration ratio (X/S) in unknown}}{\text{concentration ratio in standard mixture}} = \frac{\text{area ratio (X/S) in unknown}}{\text{area ratio in standard mixture}}$$

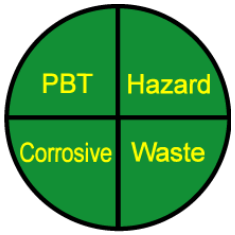
in which X refers to an amino acid and S is the internal standard. The numerator on the right side of the equation was found in step 8 and the denominator on the right side was found in step 7. (Use the average values from the two sets of runs.) The denominator on the left side is known from the masses of amino acids and internal standard weighed into the standard solution. For each amino acid, you can now solve the equation to find the numerator on the left side, which is the quotient

$$\frac{\text{concentration of amino acid in protein hyrolysate}}{\text{concentration internal standard in protein hydrolysate}}$$

But you know the concentration of internal standard in the protein hydrolysate from the volume and concentration of internal standard used in step 4. Therefore, you can calculate the concentration of each amino acid in the hydrolysate.

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 $S_{10.6}T_{6.6}H_{0.82}G_{12.5}A_{11.2}E_{5.3}Y_{\equiv 3}D_{19.8}V_{6.1}M_{2.2}I_{5.7}(L + F)_{11.5}$.

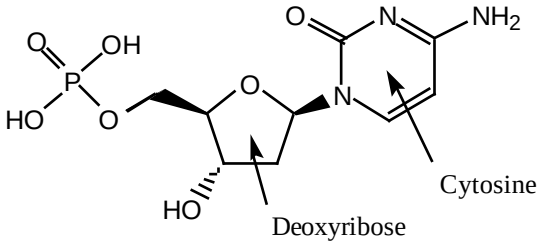
34. DNA Composition by High-Performance Liquid Chromatography[53]



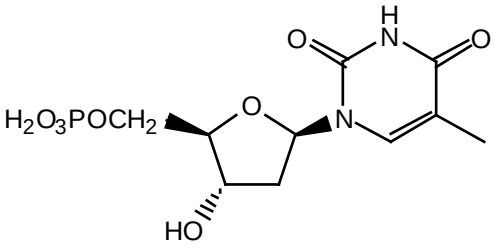
Green Profile
See Section 0

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

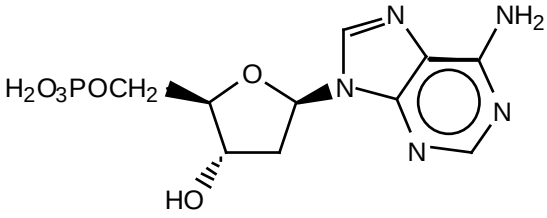
The genetic material deoxyribonucleic acid, is a polymer made of four nucleotides abbreviated C, T, A, and G:



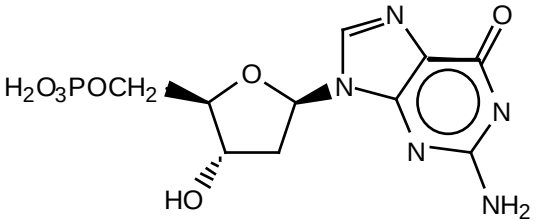
2'-Deoxycytidine 5'-monophosphate (C)



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.

53 S. M. Wietstock, *J. Chem. Ed.* **1995**, *72*, 950.

Reagents

Standard nucleotide solution: The standard should contain accurately weighed quantities of the nucleotide monophosphates[54] 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.[54] Dissociate the DNA into single strands by heating at 100°C for 10 min and then cooling immediately on ice. Prepare nuclease P_1[54] at a final concentration of 5 units/mL[55] 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.

---

54 Available from Sigma-Aldrich; http://www.sigmaaldrich.com/chemistry/aldrich-chemistry/aldrich-handbook.html.

55 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.

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 × $w_{1/2}$, where $w_{1/2}$ is the width at half-height (Figure 21-3 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]?

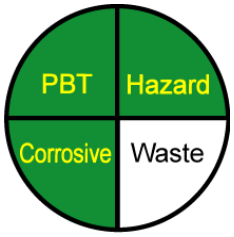
3. Find the fraction of nucleotides that are C + G by evaluating the expression

Fraction of C + G:

$$\frac{[C] + [G]}{[C] + [G] + [A] + [T]} = \frac{\frac{[C]}{[G]} + \frac{[G]}{[G]}}{\frac{[C]}{[G]} + \frac{[G]}{[G]} + \frac{[A]}{[G]} + \frac{[T]}{[G]}}$$

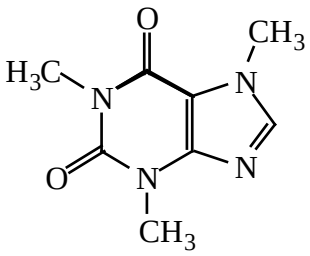
For calf thymus DNA, the literature value of the fraction of C + G is 0.42.

35. Analysis of Analgesic Tablets by High-Performance Liquid Chromatography[56]

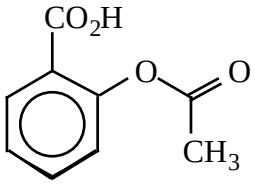


Green Profile
See Section 0

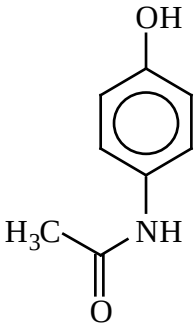
Nonprescription headache medications such as Excedrin or Vanquish contain mixtures of acetaminophen and aspirin for relief and caffeine as a stimulant. This experiment describes conditions for separating and measuring the components by high-performance liquid chromatography (HPLC). Instructions are given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

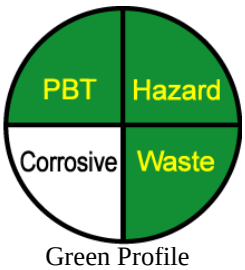
---

[56] G. K. Ferguson, *J. Chem. Ed*. **1998**, *75*, 467.

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.

36. Anion Content of Drinking Water by Capillary Electrophoresis[57]



Green Profile
See Section 0

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, which is discussed in Sections 23-5 through 23-7 in the textbook. 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 23-14 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 23-17 of the textbook.

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[58] in 800 mL of

[57] S. Demay, A. Martin-Girardequ, and M.-F. Gonnord, *J. Chem. Ed.* **1999**, *76*, 812.

[58] 100 mM tetradecyl(trimethyl)ammonium hydroxide (Catalog number WAT049387) is available from Waters Corp., 34 Maple Street, Milford, MA 01757; Phone: 1-800-478-2000; www.waters.com. The surfactant is sold under the trade name "Osmotic Flow Modifier," abbreviated OFM^+OH^-.

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 1.000 L of 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.*

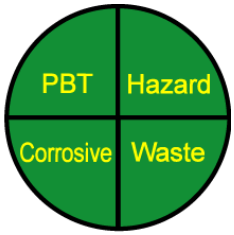
1. When preparing a capillary for its first use, wash through 1 M NaOH for 15 min, followed by 0.1 M NaOH for 15 min, followed by run buffer for 15 min. In this experiment, wash the column with run buffer for 1 min between sample injections.

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.

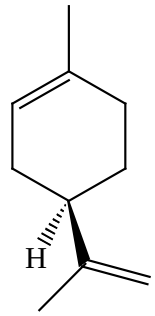
37. Green Chemistry: Liquid Carbon Dioxide Extraction of Lemon Peel Oil[59,60]



Green Profile
See Section 0

In analytical procedures, solvent extraction can provide the initial separation of analyte from bulk sample. In this experiment, we extract a natural product from a renewable feedstock using liquid carbon dioxide as a nontoxic solvent under ambient conditions while generating minimal waste. If you really want to calculate the waste produced in the procedure, you would need to account for waste created in generating the energy required to make Dry Ice (solid CO_2) used in the experiment, including the amount of Dry Ice lost between the location where it was made and your laboratory. You would also need to account for transportation of Dry Ice from the manufacturer to your laboratory. Even "green" procedures have hidden costs.

"Essential oils" used for flavors and fragrances are volatile organic compounds, including monoterpenes such as limonene, commonly found in plants such as citrus. Essential oils are often isolated by steam distillation in which citrus is boiled in water and the oil co-distills with the water. Liquid condensed from the steam contains both water and oil, which separate from each other. Steam distillation could hydrolyze or decompose some oils. Alternatively, organic solvents used to extract oils may be flammable, toxic, or otherwise undesirable. How can we isolate essential oils without using water or organic solvents?



D-Limonene

Supercritical fluid extraction uses carbon dioxide at elevated temperature and pressure to achieve a liquid or *supercritical fluid* state suitable for chemical extractions. In the phase diagram for CO_2 in Figure 1, the liquid-vapor boundary line ends at the *critical point*. At higher temperature and pressure than the critical point, separate liquid and gaseous phases no longer exist. Rather, there is a single supercritical phase whose properties are between those of the liquid and gas.

---

59 Adapted from L. C. McKenzie, J. E. Thompson, R. Sullivan, and J. E. Hutchison, "Green Chemical Processing in the Teaching Laboratory: A Convenient Liquid CO_2 Extraction of Natural Products," *Green Chem.* **2004**, *6*, 355-358.

60 Experiment contributed by Douglas E. Raynie, Department of Chemistry and Biochemistry, South Dakota State University, Brookings SD 57007; douglas.raynie@sdstate.edu.

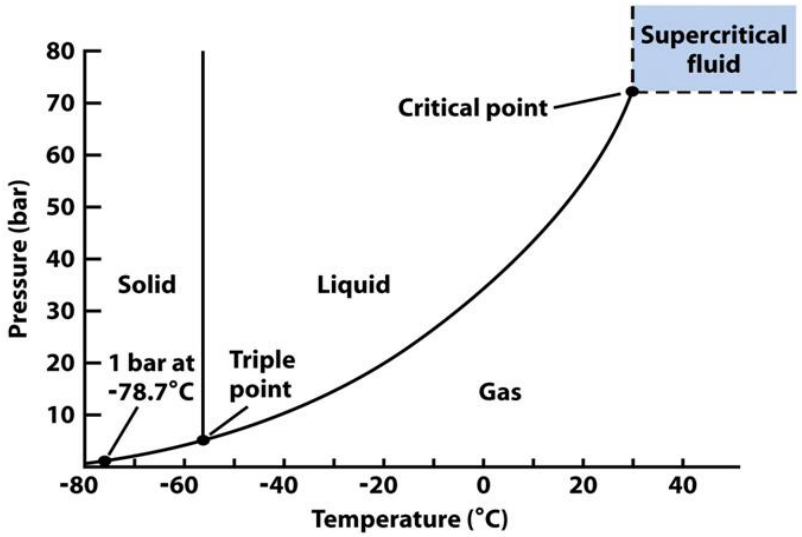


Figure 1. Phase diagram for CO_2.

A supercritical fluid might act as a liquid solvent to dissolve solutes, yet maintains viscosity and diffusion properties similar to those of a gas. Supercritical CO_2 has advantages for extracting essential oils from flowers, fruit rind, and other products. Supercritical CO_2 is nonflammable, relatively nontoxic, and readily available. To perform a supercritical extraction, an instrument is needed to heat and pressurize CO_2 above its critical point (31°C and 74 bar).

To mimic a true supercritical fluid extraction, we will use liquid CO_2, which has properties similar to the supercritical fluid. Dry Ice sublimes at atmospheric pressure and temperatures above -78°C. If CO_2 is sealed in a vessel during sublimation, the pressure in the vessel increases. After the temperature and pressure have increased sufficiently, liquid CO_2 forms. We can exploit the ease of CO_2 liquefaction to extract essential oils from citrus rind.

Reagents and Supplies

Crushed (powdered) Dry Ice: 25 g/student

Grated lemon peel (zest): 2.5 g/student. Grate only the colored part of the lemon peel with the smallest grating surface of a cheese grater.[61]

Methanol: 5 mL/student

15-mL polypropylene centrifuge tube: one/student (Corning #430052 recommended)

Copper wire: 20-cm length of 18-22 gauge wire for each student

[61] Instead of lemon peel, you may chose to use orange peel. Orange oil will be about 95% D-limonene, which is sufficiently pure to characterize by infrared spectroscoy and nuclear magnetic resonance.

500-mL plastic graduated cylinder or wide-mouth polycarbonate jar: one per student. Alternatively, construct a watertight cylinder from a 30-cm-long clear plastic tube with a 25-mm diameter and 3-mm wall thickness cemented to a flat plastic base with silicone calk.

Filter paper: one 1.5-cm-diameter circle of Whatman #1 filter paper per student

Thermometer: Several students can share a single thermometer to measure water at 40-50ºC.

Forceps: Several students can share a single pair of forceps capable of reaching into the cylinder to remove the centrifuge tube.

Tweezers: Several students can share a single pair.

Pasteur pipet: One per student.

Safety Precautions

0. Pressure generated during this experiment creates a ***risk of flying plastic centrifuge caps and rupture of plastic vessels***. The source for this procedure[59] reports that caps blew off in 4% of all experiments, so you can *EXPECT* this to happen in your laboratory. Under no circumstance should vessels other than the recommended ones be used..

1. ***Do not use any glass*** in this experiment. Substitution of glass centrifuge tubes or graduated cylinders could result in serious injuries if shattering should occur.

2. ***Always wear eye protection*.**

3. ***Use gloves when handling dry ic*e**. Contact with dry ice can damage your skin.

4. ***Do not liquefy CO_2 more than 5 times in the same centrifuge tube***. After repeated liquefaction, the tube may become brittle and rupture.

Procedure

0. Read safety precautions and the entire procedure before beginning the experiment.

1. Verify that the cap fits tightly on the centrifuge tube. If the cap does not stop turning when you screw it down, try a different cap or different tube. Unscrew the cap and put it aside.

2. Following Figure 2, coil the copper wire into 3 circles on one end and leave the other end straight. The coils should fit into the centrifuge tube and rest above the tapered section of the tube. The straight section of wire should be contained within the tube when the cap is screwed on. Place a small circle of filter paper between the coils so that the filter paper will support the solid to be extracted. Insert the coil with its filter paper into the centrifuge tube.

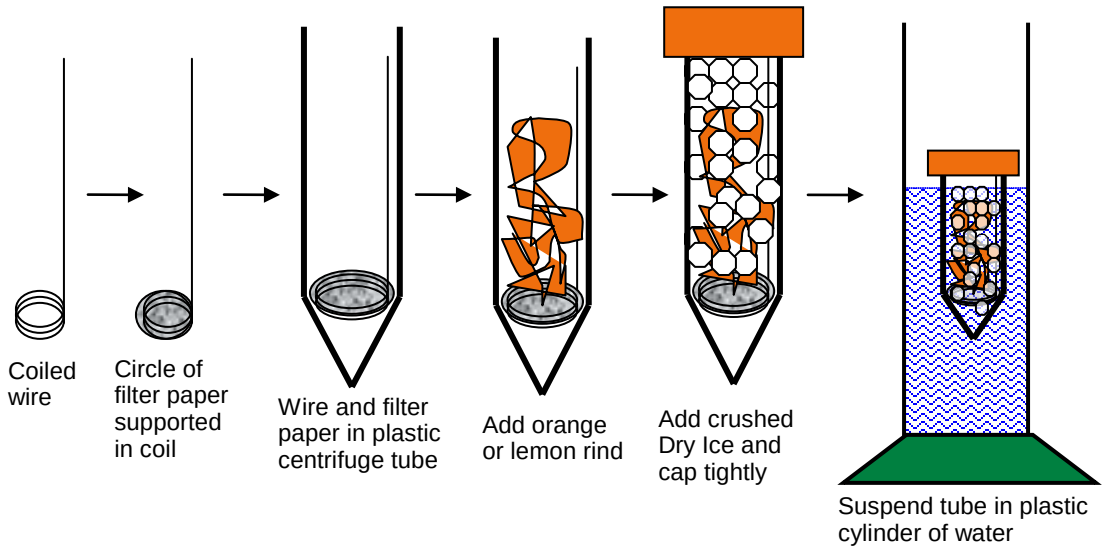


Figure 2. Extraction procedure.[59]

3. Add ~2.5 g of grated lemon peel to the tube, but *do not pack tightly*. The peel should rest on the filter paper. During extraction, liquid CO_2 must penetrate the loosely packed peel and reach the bottom tip of the centrifuge tube.

4. Fill the 500-mL plastic cylinder or wide-mouth polycarbonate water bottle one-half to two-thirds full of warm tap water at 40-50°C. *Do not heat the cylinder or add hot water at any time later in the experiment*, or the cap can blow off the centrifuge tube during the extraction.

5. Wearing gloves and using a scoop or small beaker, fill the centrifuge tube with crushed Dry Ice. Tap the tube on the lab bench and add more Dry Ice to fill the tube. Take the cap that you have already tested for a tight fit and screw it tight. If the seal is not tight, try a different cap or, if necessary, start again with a different tube and cap.

6. Place the centrifuge tube with the tapered end down into the cylinder or plastic jar of warm water. Sublimation of Dry Ice will build high pressure inside the tube and liquid CO_2 will appear in about 15 seconds. Even though the cap is tight, gas will escape through the threads.

7. View the boiling CO_2 in the centrifuge tube from the side of the cylinder. *Do not look into the top of the cylinder.* If the cap blows, it will fly straight up and hit you in the face.

8. If liquid CO_2 does not appear within a minute, the seal is not tight enough to maintain sufficient pressure in the centrifuge tube. Take the tube out of the cylinder with forceps,

tighten the cap, and place it back in the warm water. If liquid CO_2 does not appear after several attempts to tighten the cap, try a different cap. If necessary, begin again with a fresh combination of tube and cap. [62]

9. When the cap is tight, liquid will appear in the tube and gas will slowly escape through the threads. Slowly rotate the cylinder so that the cold centrifuge tube does not freeze to the cylinder wall. *Do not remove the centrifuge tube from the cylinder while liquid CO_2 is present.* The cylinder protects you in case the pressurized centrifuge tube explodes.

10. Liquid CO_2 in the centrifuge tube boils vigorously and extracts essential oil from the lemon peel. After a few minutes, CO_2 will have escaped from the tube and no liquid will be left. Approximately 0.1 mL of pale yellow oil should be visible at the bottom of the centrifuge tube. If the tube is packed too tightly with lemon peel, liquid CO_2 cannot reach the tip of the tube and extracted oil will not collect at the tip.

11. When no liquid CO_2 is left in the centrifuge tube, remove the tube from the cylinder with forceps and unscrew the cap slightly to allow the remaining pressure to escape. After all pressure has been released, it is safe to remove the cap. Using tweezers, carefully pull the wire from the tube to remove the lemon peel.

12. Transfer the extracted oil by Pasteur pipet into a gas chromatography autosampler vial and dilute with methanol. Use gas chromatography—mass spectrometry to compare your extract to a commercial sample of lemon oil. We recommend a DB-5 or other 5% phenyl, nonpolar column with a 10°C/min temperature gradient. Extracted lemon oil will have about six major components.

13. As part of your lab report, identify the major peaks in the chromatogram from their mass spectra. Perform a literature search on supercritical fluid extraction. (a) Find one reference to the use of supercritical fluid extraction in the flavor and fragrance industry. (b) Aside from essential oils, what types of compounds are commonly extracted via supercritical fluid extraction? (c) Identify a vendor of supercritical fluid extraction equipment.[63]

---

[62] In some cases, CO_2 might not liquefy or no extract is visible. If the centrifuge tube is rinsed with a small amount of methanol, enough extract to be observed via gas chromatography–mass spectrometry is usually obtained.

[63] *Instructors:* The University of Oregon Greener Education Materials for Chemists database (http://greenchem.uoregon.edu/Pages/Overview.php?CategoryIDString=&FullTextSearchKeywords=orange&CategoriesToSearch=&NumberOfMainCategories=7&AnyAll=Any&ID=85) contains comments from people who have used this experiment and allows you to add comments from your experience.