Laboratory Manual

to Accompany

Quantitative Chemistry and Instrumental Analysis

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Introduction to the Analytical Laboratory

- **With few exceptions, ANSI approved safety goggles are required at all times in the laboratory when chemical reactions in the area are underway.** Prescription glasses are not adequate eye protection.

- **Contact lenses are not recommended** in the laboratory unless they must be worn for proper eyesight where prescription glasses would be unsatisfactory, very inconvenient, or unsafe.

- **A lab coat is required** and should be worn buttoned.

- Acids, bases, and other liquid reagents are used frequently. **Closed-toe shoes are recommended** to protect feet from splashes.

- Some analyses may require more than the normal lab time to complete (especially when oven drying and some other steps are required). The laboratory is open during many times outside of the scheduled lab time to complete analyses. Goggles and a lab coat must be worn when working outside of normal lab times. **Failure to wear personal protection will result in the loss of this privilege. This requirement is not negotiable and no warnings will be given.**

- University Public Safety may ask you to leave a laboratory area if working after normal university hours. This request must be complied with immediately and without argument.

- Radios and tape or CD players are acceptable in this laboratory if the volume is low enough that a quiet conversation can be heard by everyone in the area. The volume must never be so loud that it can be heard in other laboratory areas, classrooms, etc.

- Food and drink have no place in the laboratory when chemical reactions, preparations, or transfers are being performed.

- It is **your** responsibility to get information about chemicals in use and their safe handling. Much of this aspect is covered in prelaboratory lecture. The Material Safety Data Sheets (MSDS) are available and should be referenced for important chemical information. If in doubt about a safety procedure, find out the appropriate information prior to starting the analysis.

- **Properly dispose of waste materials**. Some solids may not be disposed in the trash; some liquids may not be disposed in the sink.

- **Maintain a neat and orderly work area.** This is especially important since the laboratory is shared by others. Used glassware must be cleaned and dried on the drying rack over the sink and replaced in the appropriate storage as soon as is practical.

- Unscheduled and unapproved experiments are not to be performed.

- It is **your** responsibility to know the locations of fire extinguishers, eyewash stations, safety showers, fume hoods, and fire blankets and their proper use.
- You must report conditions which could lead to an unsafe situation (*e.g.* frayed electrical cords, burned electrical insulation, leaky gas valves, unsafe conduct of others, etc.).

The Laboratory Notebook

An important part of scientific study and chemical analysis is bookkeeping. Active scientists will often spend as much or more time recording observations, procedures, and data than actually performing an experiment. Consider the possibility of discovering a cure for cancer only to find you cannot repeat the experiment leading to the cure because you did not write down the procedure you used. Or, perhaps, you perform a chemical analysis which may have taken hours to complete all the time recording data on scraps of paper. First of all, scraps of paper may appear to be trash and get inadvertently discarded or, as is often the case, since the data is recorded quickly it is usually not neatly organized so is difficult later to decipher. As you will discover (if you haven't already), losing large blocks of time is frustrating and inefficient.

To prevent frustration, make analysis report writing easier, and generally practice good laboratory habits, a bound laboratory notebook is required for Quantitative Chemistry and Instrumental Analysis. The laboratory notebook will be collected several times during the course for inspection and suggestions. The following outline gives a general format that the laboratory notebook should follow. Exact form is not nearly as important as overall organization and being up-to-date at all times.

The laboratory notebook should be written in such a way that a scientist with your level of training could pick up the notebook and repeat exactly what you did. This means that sufficient detail is important but not so much detail as to be distracting or unnecessary to repeat the procedure.

The properly prepared laboratory notebook is considered a legally binding document. In the research laboratory, the laboratory notebook simplifies the final step in a project: the research article or conference presentation. In the analytical laboratory, the notebook contains all of the data necessary to complete the analysis. Because of these and other important roles that the lab book plays, it should be protected from loss and damage as any important document.

Outline of a Practical Laboratory Notebook

Use only pen in the notebook. There are exceptions in some scientific disciplines, especially those in which data may be collected in inclement weather where a pen may bleed. Mistakes should be crossed out with a single line through the mistake, not obliterated. There is no shame in misrecorded numbers. If a section of a page or even a whole page is to be disregarded, a line or X through the section or page is sufficient and acceptable. Never remove a page from the notebook.

Every odd numbered page (right-hand pages) should be numbered. Every page is actually numbered; however, only the right hand pages should be used. The rationale for this is that the paper used for most laboratory notebooks is thin and writing can be seen through the paper.

Table of Contents

Each entry should have the title of the analysis, date, and page number. Not all analyses must necessarily be completed and recorded on sequential pages like a chapter in a book. For example, if data were recorded on one day and another analysis started and then the data reduction of the first completed later, there would be two different entries in the table of contents for the first analysis.

Literature References

If pertinent literature references are available, they should be recorded in the book for quick retrieval.

Proposed Method

The proposed procedure is that which you intend to follow. It can be in outline or paragraph form (or both). Especially important is indicating what data is important, which measurements can be approximate and which must be analytical, etc.

Actual Procedure Followed

If the actual procedure followed does not differ from that proposed, this section is omitted. If the actual procedure differs only in minor ways, these may be simply noted. Major changes to the proposed procedure must be carefully written out.

Data Tables, Observations, Apparatus

Data *must* be taken directly into the laboratory notebook (some exceptions apply). Data such as mass, temperature, volume, etc. must be recorded in the notebook as it is collected. Do not record data on pieces of paper with the intention of going back and recording it "properly" later.

Visual observations are very important and may prove useful during report writing. Always take time to record expected, unexpected, and unusual observations. Often these lead to interesting alternative studies.

Unusual or uncommon apparatus or systems which are difficult to describe should be drawn neatly. Remember, "a visual representation = 10^3 words[1]."

Calculations/Results

With some exception, calculations should be performed in the laboratory notebook and results neatly tabulated. This makes report writing much easier later.

Conclusions/Discussion

In outline or paragraph form, writing out conclusions help to write the laboratory report later.

As pointed out above, the left hand pages are not normally used for recording data; however, they are good for recording ideas and short reminder notes. Recently, computers have revolutionized how data is collected and recorded. For this course,

[1]"A picture is worth a thousand words".

data acquired directly to computer may be printed and permanently mounted in the laboratory notebook with tape or rubber cement.

A good reference on writing the laboratory notebook is Kanare, H.M., *Writing the Laboratory Notebook*, American Chemical Society, Washington, D.C., 1985. Much of this book is devoted to writing the laboratory notebook in a non-academic laboratory environment but is still applicable.

The Analysis Report

The dissemination of scientific information is as important as the collection and analysis of the data. Every scientific discipline has its own style of writing and analytical chemistry is no different. For this course, the style used by the American Chemical Society (ACS) journals will be used as a guide. For more information, consult the ACS Style Guide[1] and other scientific writing guides[2]

The following is a very short list of journals published by the ACS:

Analytical Chemistry
Journal of the American Chemical Society
Journal of Organic Chemistry
Journal of Physical Chemistry
Journal of Inorganic Chemistry
Journal of Chemical Education

After every analysis or experiment, a report will be prepared and turned in for grading. The grade will be determined by several factors among which are adherence to consistent style (even if it deviates from ACS style), quality of writing, use of references and proper citations, and accuracy and precision of results. An article submitted for publication in a journal undergoes a similar "grading" process known as peer review which is the criteria that will be used although somewhat less stringent.

Outline of a Report

Every written report will share several common attributes. You may wish to add sections as necessary to write a complete report. Refer to any of the journals listed above as a guide to style and organization.

Abstract

The abstract should be short, describing only what the analysis was to determine, the method used, and the results with appropriate statistical analysis.

Introduction

The Introduction should include a brief outline of the method, perhaps a history of the method, what the method measures, alternatives, etc. Additionally, this section lends itself to theory development.

Experimental

This section should be a concise review of the procedure used in the analysis or experiment. If a published procedure is used without modification, only a quick review and citation is necessary. If a published procedure is used with

[1]Dodd, Janet S., Ed., *The ACS Style Guide*, American Chemical Society, Washington, D.C., 1986.
[2]For example: Day, Robert A., How to Write and Publish a Scientific Paper, 3rd Ed., Oryx Press, Phoenix, 1988.

modifications, a more complete description is necessary (and remember to give appropriate citations). If major modifications are made or the method is personally developed, a very complete description of the experiment or analysis must be made. What is important is that someone else could read the experimental and repeat the experiment or analysis exactly. Measurements and values reported should be those personally acquired, not those proposed in the lab manual or journal or book reference. The use of figures, line drawings, etc. is recommended when appropriate but must be numbered and referenced by number in the text. Figures can be interspersed in the text or appended at the end of the report.

Data

Where appropriate, data which lends itself to arranged in tables should be tabulated as such. Many pieces of data may already be in the text of the **Experimental** and need not be repeated except where clarity would be improved. Every table must be numbered and referenced in the text.

Calculations

Unlike a published journal article which does not normally show common calculations, a **Calculations** section should be incorporated into the analysis reports. Only examples of nonroutine calculations need be shown. In the event of a calculation error, it is possible to determine quickly where the error was made. This nonstandard section can be informal, even handwritten, although the use of a computer-based equation editor (such as that available for Microsoft Word™ (for Windows® and Macintosh®) gives a more polished look.

Results/Discussion

The results obtained should be described in text and/or tabular form. The use of graphs, tables, and other figures where appropriate is highly recommended. In fact, it is much easier to write about pictures and tables. Also, hidden relationships can often be elucidated when graphs and tables are used discriminately.

Acknowledgment

If other people are especially helpful in acquiring and/or analyzing data or in researching and/or writing the report, they should be thanked for their assistance as a courtesy.

Bibliography or Literature Cited

Use ACS bibliographic style. The ACS style can be found in the ACS Style Guide or copied from any ACS journal. Literature references must be numbered and referenced in the order cited; *i.e.* the first reference in the report is the first citation. It is not the custom of chemical journals to list references in alphabetical order of author's last name.

Quoting Text and Plagiarism

Many times, other authors illustrate a concept in such a way that it is difficult to improve upon. If used judicially, quoting can amplify and improve the report text. It goes without saying that copying text without appropriate quoting and citation is considered plagiarism[3].

[3]Plagiarism at any level is an offense of considerable magnitude and has severe consequences in the both the academic and professional environment.

Statistical Analysis in Chemistry

The Lab You Can Eat

The first portion of this experiment generates the data necessary to perform a complete statistical analysis similar to that seen by chemists. After completing the data collection phase of the lab, data will be tabulated and treated by different statistical functions and tests.

Procedure

1. Obtain 10-20 kernels of popcorn.
2. Weigh a single kernel to the best precision possible in a weighing boat or on weighing paper and record the weight in your laboratory notebook.
3. Place the kernel in a small beaker and, while holding the beaker with tongs, heat the kernel over a Bunsen or Tirrel burner until the kernel pops. Take care to not point the beaker at anyone since the popcorn might pop out and burn them.
4. As soon as the kernel pops, transfer it to a small tared weighing boat and immediately weigh the popped kernel. Do not let the popped kernel burn. A kernel is considered "popped" if the hull explodes to release steam; it does not necessarily have to look like the typical fluffy white popped kernel. Try to touch as little as possible the weighing boat and kernel with your hands. Record the weight of the popped kernel next to the corresponding "unpopped" weight.

   Why must the kernel be weighed immediately?

   Why should the analyst not touch the apparatus and materials being weighed?

5. Repeat steps 2-4 until you have at least 10 successfully popped kernels.

Analysis

Determine the average mass, variance, and standard deviation of the mass of the unpopped and popped kernels.

Why are the averages different? Why are the standard deviations different or the same?

Report the mass of a single kernel with 95% CL before popping. Report the mass of a single kernel with 95% CL after popping.

Explain what a 95% CL means.

Correctly report the average mass of a kernel from the popcorn bag indicating, first, a ± of 1 standard error of the mean and, second, 2 standard errors of the mean.

Why are S.E. intervals different than CL intervals?

Collect the data from other analysts. Treat the data two ways...

1. Treat all of the data as if there is no variation from operator to operator, method to method, balance to balance, etc. Thus, you may compile all of the data into one large data set. Graph the histogram of masses with 5-9 bars.

   Repeat the average, variance, standard deviation, and CL (95%) calculations. Explain any differences in the averages and precisions.

2. Treat all of the data as if there are potentially differences between data sets that are not obvious. That is, treat each data set individually and calculate a pooled standard deviation and determine the CL intervals from s_{pooled}.

   Explain differences between these numbers and those calculated just previously.

Use appropriate tests to determine if there are statistical differences in mean and variance between your data set and one or two others. What can you conclude? What tests can be used to determine statistical differences between all of the data sets simultaneously?

Discussion

Where might these mathematical techniques be used, other than in popcorn analysis, by chemists, physicians, engineers, etc.?

Optional Lab Experience

Pop 30-50 g of popcorn by the appropriate means in a container suitable for food products. Eat the popped popcorn with optional salt, congealed and salted fat from cream[1], or other condiment.

[1]Butter or margarine

Volumetric Analysis

Acid-Base Titrations

Introduction

Titrimetric analyses are among the most precise analytical methods in use. Relative precisions of 0.2% or better are not uncommon. While an individual analysis is somewhat slow, time is saved over other methods in that there is very little apparatus to set up and calibrations need not be performed.

In this analysis, you will prepare and standardize dilute acid and base solutions. Using the standard solutions you will perform the typical quality control analyses of a variety of acidic and basic substances.

Procedure

Preparation of Base (~0.1 *M* NaOH)

Being careful to avoid pipetting any solid, transfer about 5 mL[1] (graduated pipette) of 50% NaOH solution to about 1 L of boiled deionized water in a plastic container. Mix thoroughly and keep the bottle closed except when transferring liquid[2].

Preparation of Acid (~0.1 *M* HCl)

Measure into a clean, glass stoppered bottle approximately 1 L of water. With a graduated pipette or cylinder, transfer between 10 and 20 mL of concentrated HCl (use extreme caution). Stopper, mix, and label the bottle. Calculate the approximate concentration of the diluted acid.

Standardization of NaOH solution

Dry primary standard grade potassium hydrogen phthalate ($KHC_8H_4O_4$, KHP, 204.23 g mol^{-1}) 1-2 hours at 110°C and cool in a desiccator[3]. Accurately weigh 0.2-0.4 g samples into 125 or 250 mL Erlenmeyer flasks and dissolve in 25 - 50 mL boiled deionized water. Add 2 or 3 drops of phenolphthalein indicator and titrate with base to end point (faint pink color persisting for 20-30 s). Calculate the concentration of the NaOH and 95% confidence limits by Student's-t. Perform *at least* 3 replicates. If the precision of the replicates is poor, perform additional standardizations[4]. Typically, the range of concentrations should differ by not more than 1-2%.

For the analyses to be performed the acceptable range of base concentration is ~0.09-0.15 M. If the concentration is determined to be outside of this range, adjust the concentration of the stock solution to bring it into this range.

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[1]If the 50% NaOH is not a freshly opened bottle, 8-10 mL may be necessary.

[2]Basic aqueous solutions tend to absorb CO_2 from the air. The hydrolysis of CO_2 to hydrogen carbonate alters the concentration of the NaOH solution appreciably and makes the end points in the titration broad. Never store NaOH solutions in bottles with ground glass stoppers - the stoppers tend to become tightly stuck.

[3]To avoid decomposition, the primary standard KHP should not be dried over 120°C. The lid of the drying vial must not be in place while drying or cooling.

[4] The author considers the precision "good" if the range of concentrations differ by not more than 1-2%.

Time saving hint:
You can speed titrations by titrating the first sample quickly and calculating a close approximate to the base concentration. From the approximate base concentration, calculate the NaOH needed for the replicate analysis. Titrate replicates quickly to within 1-2 mL, then slowly titrate to endpoint. Allow about a minute for the solution on the walls of the burette to drain down.

Standardization of HCl

Solutions of HCl may be standardized against primary standard Na_2CO_3 or against standard base. Since standard base is available, it is much faster to use that solution.

From the burette containing the standard NaOH, dispense 20-40 mL into a clean (not necessarily dry) flask. Add 2-3 drops of phenolphthalein indicator. From the second burette, deliver the HCl solution until the pink color disappears then add about 1 mL extra. Record the volume of HCl dispensed. Finally, back-titrate with standard base to endpoint. From the total volume of NaOH and HCl delivered, calculate the concentration of the HCl and 95% confidence limit.

Analysis of Unknowns

Analysis of an Impure Solid

Dry solid sample 1-2 hours at 110°C and cool in a desiccator. Weigh 0.3 - 0.6 g samples into Erlenmeyer flasks and dissolve in 25-50 mL boiled distilled water. Solvent should not be added to the samples until just prior to titration. (Alternatively, the samples can be dissolved in unboiled deionized water, boiled for about 1 minute, and cooled just prior to titration.) Add indicator and titrate as above. Perform at least 3 replicate analyses, more if necessary[5].

The unknown solid is impure KHP. Determine from the titration data the %KHP in your sample. Report the results with 95% confidence limits obtained from Student's-t. You may wish to compare different statistical methods of reporting your results in your discussion. If necessary, perform a Q- or T-test to discard poor data. Do not use the Q-test on less than 4 replicate analyses.

Analysis of Antacids

Antacids typically contain bases such as calcium carbonate, magnesium carbonate, and magnesium hydroxide. Though not very soluble in water, these compounds can be titrated with acid if sufficient time is allowed for reaction of the acid with the solid. The endpoints are also often very broad because of the slow reaction. The pH of the solution made by dissolving the antacid in water is in the range of 8 to 9, indicating the presence of HCO_3^- ion so methyl orange is the indicator of choice since it changes from orange to red in slightly acid solution) .

[5]It is good practice to set up one analysis for a given sample size to determine if a sufficient amount of titrant will be required (>10 mL) or if excess titrant will be needed (>40 mL). After sample size has been explored, replicate samples may be prepared and analyzed.

The indirect procedure used here is a titration of the acid remaining after the addition of excess standard HCl to the antacid and the formed CO_2 removed by heating. Phenolphthalein may be use in place of methyl orange since HCl and not HCO_3^- will be titrated.

Cut or break an antacid tablet in thirds or quarters. Transfer the sample to a tared Erlenmeyer flask, weigh, and add 25 mL of standard HCl by pipette or burette. Add 2 or 3 drops of phenolphthalein indicator. Heat on a hot plate to a *gentle* boil for several minutes to remove CO_2. If the pink color of the indicator persists, add 10 mL more HCl and continue to boil. Any remaining solids are due to the presence of binders and fillers used in manufacture and may be ignored. Allow to cool and titrate to endpoint with standard base. If less than 10 mL of base are required to reach endpoint, add an additional 10 mL of standard acid and continue titration to endpoint. Record total amount of acid and base added and calculate the number of grams of HCl neutralized per gram of antacid. Perform at least 2 replicate analyses.

Analysis of Commercial Vinegar

Determine the density of the vinegar solution to the best possible precision by pipetting a 10 or 15 mL aliquot into a small pre-weighed Erlenmeyer flask and stoppering. Determine the mass of the vinegar by difference. Perform replicate determinations of the density.

Assuming a concentration of 5%$^{w}/_{w}$ acetic acid, determine the amount of vinegar necessary to require 10-40 mL of the standard base. If the volume is less than 10 mL, calculate and prepare a standard dilution. Pipette an aliquot of the vinegar solution (or dilution) into an Erlenmeyer flask. Add 2-3 drops of phenolphthalein indicator to the solution and titrate with standard base to end point. Perform *at least* 3 replicate titrations that agree within 0.1-0.5 %

From density and volume titrated, determine the weight percentage (%$^{w}/_{w}$) of acetic acid in the solution. Report the precision and use the Q- or T-test to discard poor data if necessary. Compare the weight % to that recorded on the bottle.

Discussion Items

Calculate the propagated error from the indeterminate errors in the apparatus and solutions. Assume the glassware meets ASTM standards. Balances are ±1 in the last decimal place. Which is the best statistical value to report (*i.e.* the propagated error or confidence limits)? Why?

Assuming a density of 1.0 g/mL for 1 *M* HCl (stomach acid), calculate the number of grams of 1 *M* HCl neutralized per tablet of antacid. Comment on the manufacturer's claim, "neutralizes many times its own weight in stomach acid."

If you used a data rejection test in any of the results, give your justification.

Determination of %Fe in Iron Ore

Introduction

The analysis of iron in a sample is conveniently performed by the reaction of Fe^{2+} with permanganate. Permanganate undergoes several different chemical reactions depending on, for one, pH and is summarized below:

$$MnO_4^- + 8\,H^+ + 5\,e^- \rightarrow Mn^{2+} + 4\,H_2O$$

$$MnO_4^- + 4\,H^+ + 3\,e^- \rightarrow MnO_2 + 2\,H_2O$$

$$MnO_4^- + 8\,H^+ + 4\,e^- \rightarrow Mn^{3+} + 4\,H_2O$$

Mn^{3+} must be stabilized with a complexing agent such as pyrophosphate or fluoride.

$$MnO_4^- + e^- \rightarrow MnO_4^{2-} \text{ in strongly basic solution.}$$

The reaction of permanganate with Fe^{2+} proceeds as follows:

$$MnO_4^- + 8\,H^+ + 5\,Fe^{2+} \rightarrow Mn^{2+} + 4\,H_2O + Fe^{3+}$$

purple colorless pink yellow

Permanganate solutions are intensely purple while solutions containing iron (II) ion are nearly colorless. Solutions of Mn^{2+} and Fe^{3+} are pale pink and yellow, respectively. Thus, it is possible to use the persistence of the color due to MnO_4^- as the endpoint indicator. Since the reaction with permanganate is a 5 electron process, the equivalent mass of $KMnO_4$ is one-fifth of the molar mass.

Iron to be analyzed must first be converted to the +2 oxidation state (the preferred state is, of course, +3). This can be accomplished by the addition of $SnCl_2$,

$$2\,Fe^{3+} + Sn^{2+} \rightarrow 2\,Fe^{2+} + Sn^{4+}$$

and noting the disappearance of the yellow color of the iron (III) ion. A slight excess of tin (II) chloride is used to ensure complete reduction of the iron (III) to iron (II). Excess Sn^{2+} is removed by reacting it with $HgCl_2$.

$$SnCl_2 + 2\,HgCl_2 \rightarrow Hg_2Cl_{2(s)} + SnCl_4$$

Fe^{2+} is not oxidized by Hg^{2+} and the small amount of precipitate formed does not interfere with the titration. Care must be taken to not add a large excess of $SnCl_2$ because the Hg_2Cl_2 formed could be further reduced to free mercury.

$$Hg_2Cl_2 + Sn^{2+}\ 2\,Hg + 2\,Cl^- + Sn^{4+}$$

Hg is an interference since it would be oxidized during the titration by MnO_4^-.

When HCl is used to dissolve samples, Zimmermann-Reinhardt[1] preventative solution is employed to minimize the catalytic oxidation of Cl^- by MnO_4^-.

[1]Apparently, Fe(III) ion catalyzes the oxidation of Cl^- to Cl_2 by MnO_4^-. The Zimmermann-Reinhardt preventative solution complexes the Fe(III) ion thereby reducing its effect on chloride. Furthermore, the complex of iron formed is colorless making the endpoint easier to detect. Zimmermann-Reinhardt solution is prepared by dissolving 70 g $MnSO_4$ in 500 mL of water and

Procedure

Preparation and Standardization of ~0.1 *N* $KMnO_4$

Carefully transfer 300-500 mL of the prepared $KMnO_4$ to a clean amber reagent bottle. Take care to transfer solution from the top third of the stock bottle[2] to avoid any contaminating MnO_2.

Pure iron wire is used as a primary standard form permanganate. Weigh accurately at least 3 samples of clean wire of about 0.2 g each into 500 mL Erlenmeyer flasks. Add 20 mL of 6 *M* HCl to the first sample, cover with a watch glass, and warm gently without boiling (hood) until the iron is completely dissolved. While still hot, add $SnCl_2$ dropwise with swirling until the solution becomes colorless (or very light green). Add 1 or 2 drops excess to complete the reduction. Cool the solution under the tap to nearly room temperature and pour in 20 mL of saturated $HgCl_2$ solution quickly. If the $HgCl_2$ solution is added too slowly, there is a risk of forming free mercury due to a local high concentration of Sn^{2+}. Allow the solution to stand for about 3 minutes to complete the formation of Hg_2Cl_2, dilute to about 300-400 mL, and add 25 mL of Zimmermann-Reinhardt solution. With stirring or swirling, titrate to endpoint with permanganate. Endpoint is marked by appearance of a persistent faint pink tinge.

Reduce and titrate remaining standards as above one at a time. (Why not prepare all samples at the same time?) Calculate the average normality of the $KMnO_4$.

Recommended option:
Prepare a sample for titration exactly as above but containing no iron and titrate to endpoint (typically much less than 0.1 mL). Perform the blank determination at least three times. For best possible accuracy, the blank titrant volume should be subtracted from the sample titrant volume before calculation.

Analysis of an Ore Sample

Weigh into beakers at least three 0.5-1.2 g samples of the ore and dissolve the first with 20 mL of 6 *M* HCl, cover, and warm gently until completely dissolved. (Any solid remaining after 30 min of heating is due to silica.) Treat the sample as before to form the iron (II) ion. Titrate to the first persistent pink color. Repeat on the other samples. If a given sample size fails to require at least 10 mL of titrant, discard that titration and repeat with a sufficiently larger sample. Calculate the %Fe in the sample in the usual manner.

Discussion Items

Comment on why the reduction step should be performed just prior to titration. Would the %Fe result be high or low if all samples were prepared then titrated?

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adding slowly with stirring 110 mL concentrated H_2SO_4 and 200 mL concentrated H_3PO_4 (85%). Finally the solution is diluted to 1.0 L.

[2]The stock solution is prepared by dissolving 3.2 g of $KMnO_4$ in approximately 1 L of water followed by heating to nearly boiling to oxidize any reducing compounds. Since MnO_2 catalyzes the decomposition of $KMnO_4$ solutions, long-term storage of permanganate solutions is not recommended. To maintain a good concentration on standardized solutions, care should be taken to avoid pipetting or decanting any solid from the stock solution.

Is iron metal the best choice for a primary standard in this case? What would be better? (You may need to research this in the library.)

How would the method be modified to assay the purity of sodium oxalate? What other analyses can be performed titrimetrically with permanganate?

Determination of %Cl in a Soluble Chloride by the Mohr Method

Introduction[1]

The Mohr method is an example of a precipitation titration for the determination of silver ion or halide ions. The titration utilizes the differential K_{sp}'s for AgCl (or AgBr) and Ag_2CrO_4 for the titration and end point detection. The silver halides are white to pale yellow while the Ag_2CrO_4 is red. The following equilibria are present during the titration:

$$AgCl \rightarrow Ag^+ + Cl^- \qquad K_{sp} = 1.8 \times 10^{-10}$$

$$Ag_2CrO_4 \rightarrow 2\,Ag^+ + CrO_4^- \qquad K_{sp} = 1.1 \times 10^{-12}$$

In principle, if the concentration of the chromate is kept low, the formation of the silver chromate can be used to signal the appearance of the first excess of silver ion over its equivalence point concentration. In practice, however, end point detection is difficult if not impossible in solutions with chromate ion concentrations as large as 6×10^{-3} *M* (concentration required to bring about precipitation of Ag_2CrO_4 at the solubility of AgCl) because the yellow color of the indicator masks the color of the silver chromate. As a result, indicator concentrations of around 2.5×10^{-3} *M* must be employed. This decrease in chromate concentration requires a silver concentration greater then 1.4×10^{-5} *M* (solubility of AgCl) to produce a red precipitate. In addition, a finite amount of silver nitrate must be added to produce a detectable quantity of the precipitate. Both factors cause an over-consumption of $AgNO_3$ reagent. The problem is especially serious in dilute silver nitrate solutions but small with 0.1 *M* solutions. A correction may be made by determining an *indicator blank* (*i.e.* the amount of silver consumed by the indicator in a chloride-free solution). The best method to reduce the indicator error is to standardize the titrant against pure sodium or potassium chloride. The "working molarity" obtained for the solution will not only compensate for indicator error but also the ability of the analyst to detect the often difficult end point.

Attention must be paid to the pH of the medium when using chromate as the indicator because the equilibrium

$$2\,CrO_4^{2-} + 2\,H^+ \; Cr_2O_7^{2-} + H_2O$$

is displaced to the right at low pH. Silver dichromate is much more soluble than silver chromate so detection of the end point (if possible) becomes difficult. If the solution is made to basic the possibility of precipitating silver hydroxide and further decomposing the hydroxide to insoluble silver oxide exists. Thus, maintaining a neutral pH is highly desirable. Buffering the solution with sodium hydrogen carbonate is a suitable and convenient method for maintaining pH.

Procedure

[1]Similar reference: *Fundamentals of Analytical Chemistry, 4th ed.*, Skoog, D.A. & West, D.M.; Holt, Rinehart and Winston Publishing (1982).

Preparation of Approximately 0.1 *M* $AgNO_3$

Silver nitrate is obtainable in primary standard purity so carefully prepared solutions need not be standardized. Solid $AgNO_3$ and its solutions must not be exposed to sunlight and should be stored in the dark when not actually in use. **The reagent is expensive.** Excess titrant solution and products of the titration will be collected in waste jars. Do not waste reagents.

Transfer approx. 3.0-4.5 g of $AgNO_3$ to a clean, dry weighing bottle (use top-loading balance). Dry at 110°C for no more than 1 h and cool in a desiccator[2]. Weigh the bottle and its contents to the nearest 0.1 mg. Transfer the $AgNO_3$ to a 250 mL volumetric flask and reweigh the bottle and any remaining solid. Dissolve the $AgNO_3$, dilute to the mark and mix well. Calculate the concentration of the solution.

Analysis of a Soluble Chloride

Dry the unknown chloride at 110°C for at least 1 hr and cool in a desiccator. Weigh by difference 0.25 g samples into 250 mL Erlenmeyer flasks. Add 50-100 mL of water to dissolve[3]. Adjust the pH by adding $NaHCO_3$ a spatula-tip full at a time until effervescence ceases. Accurately pipette 1-2 mL of 5% K_2CrO_4 indicator and titrate to the first permanent appearance of the color change due to Ag_2CrO_4. Perform *at least* 3 replicate titrations or until calculated percentages agree to within 0.5%.

Determine an indicator blank by suspending a sample of calcium carbonate (Cl^--free) the size of 2 peas in 50-100 mL of water. Pipette the same amount of indicator you used above and titrate to the same color change you used above. Be careful, *it may not require much titrant.* If you use the same amount of indicator in the unknown and blank you may correct your titration data for indicator error by simply subtracting blank volume for each titrant volume for the unknown. Perform the blank at least three times to check your precision.

Alternate $AgNO_3$ Standardization Procedure

Use this procedure if you suspect your $AgNO_3$ solution has changed concentration or, if during preparation, you diluted past the mark.

Obtain 2-4 g NaCl and dry at 110°C for 1-2 hours. Cool in a desiccator. Weigh by difference 0.1-0.2 g samples into 250 mL Erlenmeyer flasks. Add indicator and titrate as above. If this standardization is used, no blank determination is necessary as the indicator error will be included in the standardization.

Analysis

Calculate the %Cl in each sample and the mean percentage. Determine the precision of your value by the t-test.

[2] $AgNO_3$ is decomposed with prolonged heating. Some discoloration may occur but the effect on purity is ordinarily negligible.

[3] Unlike acid/base titrations, the water need not be boiled to remove CO_2.

Determination of Silver in an Alloy or Soluble Salt

Introduction

Silver can be determined by direct titration with standard potassium thiocyanate (KSCN) using iron(III) ion as the indicator. Solutions of thiocyanate must be standardized against standard silver. Except in the most exacting work, primary standard silver nitrate may be used to make standard silver solutions. Alternatively, solutions of very accurately known concentration may be produced by dissolving a mass of pure silver metal in nitric acid followed by dilution to a known volume.

Procedure

Preparation of 0.1 *M* Ag^+ Solution

Clean the tarnish from a length of 99.99+% silver metal[1]. If necessary, etch the surface clean by dipping the wire into dilute nitric acid momentarily then wash with water and dry. Measure into a tared beaker the amount of wire necessary to prepare 200 mL of 0.1 *M* Ag^+ and add 25-30 mL of 6 *M* HNO_3. Place a watch glass on the beaker and heat the contents to nearly boiling to dissolve the wire. When the wire has completely reacted, boil the solution until no more brown NO_2 is evolved. Rinse the watch glass and quantitatively transfer the solution into a 200 mL volumetric flask. Dilute to the mark with water. Store away from light.

Preparation of ~0.1 *M* KSCN Solution

Prepare about 1 L of 0.1 *M* KSCN from the salt.

Preparation of Fe^{3+} Indicator Solution

The iron(III) ion indicator solution is saturated iron(III) ammonium sulfate in 1 *M* HNO_3. Place about 200 mL of 1 *M* HNO_3 in a beaker and continue to add iron(III) ammonium sulfate with stirring until no more salt dissolves.

Standardization of KSCN Solution with Standard Silver

Pipette 15-25 mL of the standard silver solution into a 250 mL Erlenmeyer flask. Add ~5 mL of 6 *M* HNO_3[2] and 1 mL of iron(III) ion indicator[3] solution. Titrate with thiocyanate to the formation of the red iron-thiocyanate complex. Perform replicates as necessary. Also, perform blank determinations to measure the indicator error. Calculate the concentration of the KSCN solution.

---

[1]Often pronounced "4-nines purity silver". Preparing a metal ion solution from the pure metal is the method preferred for solutions where high accuracy is necessary.

[2]If the nitric acid has a yellow tint due to the presence of dissolved NO_2, boil the acid to expel the oxides.

[3]Quantity is not important. It is important to use nearly the same amount for each titration to avoid a variable indicator error.

Analysis of Silver in Mixture or Salt

Analysis of Alloy

Accurately weigh samples of alloy of appropriate size[4] into 250 mL Erlenmeyer flasks. Add 15-25 mL of 6 *M* nitric acid, cover with a watch glass, and heat the solution to nearly boiling. When the alloy has completely reacted, boil the solution to expel the oxides of nitrogen. Dilute the solution to about 50 mL, add ~1 mL of iron(III) ion indicator solution, and titrate with standard thiocyanate to the iron-thiocyanate end point. Account for indicator error in titrant volume. Perform replicates as necessary.

Report the %Ag in the alloy with appropriate confidence intervals.

Analysis of Silver Salt

Accurately weigh samples of the soluble silver salt of appropriate size[4] into 250 mL Erlenmeyer flasks. Add 15-25 mL of water, 5 mL of 6 *M* nitric acid, and ~1 mL of iron(III) ion indicator solution. Titrate with standard thiocyanate to the iron-thiocyanate end point. Account for indicator error in titrant volume. Perform replicates as necessary.

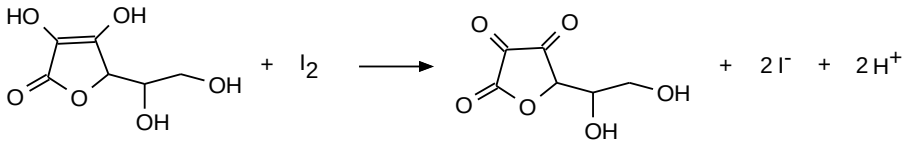
Report the %Ag in the salt (or %silver salt in the mixture) with appropriate confidence intervals.

[4]If the approximate composition of the sample is known, weigh samples such that 15-25 mL of titrant is necessary.

Iodimetric Determination of Ascorbic Acid in Vitamin-C Tablets

Introduction

Vitamin C (ascorbic acid) is a mild reducing agent and can be determined iodimetrically by titration with standard iodine solution. Ascorbic acid is oxidized to dehydroascorbic acid by I_2...



There are a very large number of other species which may be determined directly or indirectly by iodimetric techniques (Tables 1 and 2).

Table 1. Analytes and reactions in several analyses by direct iodimetric titration

Analyte	Analytical Reaction
Antimony(III) ion	$HSbOC_4H_6O_6 + I_2 + H_2O \leftrightarrows HSbO_2C_2H_4O_6 + 2H^+ + 2I^-$
Arsenic(III) ion	$HAsO_2 + I_2 + 2\,H_2O \leftrightarrows H_3AsO_4 + 2H^+ + 2\,I^-$
hexacyanoferrate(II) ion (Ferrocyanide ion)	$2\,Fe(CN)_6^{4-} + I_2 \leftrightarrows 2\,Fe(CN)_6^{3-} + 2\,I^-$
Hydrogen cyanide	$HCN + I_2 \leftrightarrows ICN + H^+ + I^-$
Hydrazine	$N_2H_4 + 2\,I_2 \leftrightarrows N_2 + 4\,H^+ + 4\,I^-$
Sulfide	$H_2S + I_2 \leftrightarrows 2\,H^+ + 2\,I^- + S$
Sulfite	$H_2SO_3 + I_2 + H_2O \leftrightarrows H_2SO_4 + 2\,H^+ + 2\,I^-$
Thiosulfate ion	$2\,S_2O_3^{2-} + I_2 \leftrightarrows S_4O_6^{2-} + 2\,I^-$
Tin(II) ion	$Sn^{2+} + I_2 \leftrightarrows Sn^{4+} + 2\,I^-$

Table 2. Analytes and reactions in several analyses by indirect iodimetric titration

Analyte	Analytical Reaction
Bromine (or chlorine)	$Br_2 + 2\,I^- \leftrightarrows 2\,Br^- + I_2$
Bromate (or chlorate)	$BrO_3^- + 6\,H^+ + 6\,I^- \leftrightarrows Br^- + 3\,I_2 + 3\,H_2O$
Copper(II) ion	$2\,Cu^{2+} + 4\,I^- \leftrightarrows 2\,CuI_{(s)} + I_2$
Dichromate (and chromate in acid solution)	$Cr_2O_7^{2-} + 6\,I^- + 14\,H^+ \leftrightarrows 2\,Cr^{3+} + 3\,I_2 + 7\,H_2O$
Hydrogen peroxide	$H_2O_2 + 2\,H^+ + 2\,I^- \leftrightarrows I_2 + 2\,H_2O$
Nitrite	$2\,HNO_2 + 2\,I^- + 2\,H^+ \leftrightarrows 2\,NO + I_2 + 2\,H_2O$
Oxygen	$O_2 + Mn(OH)_2 + 2\,H_2O \leftrightarrows 4\,Mn(OH)_3$
	$2\,Mn(OH)_3 + 2\,I^- + 6\,H^+ \leftrightarrows 2\,Mn^{2+} + I_2 + 6\,H_2O$
Periodate	$IO_4^- + 7\,I^- + 8\,H^+ \leftrightarrows 4\,I_2 + 4\,H_2O$
Permanganate	$2\,MnO_4^- + 10\,I^- + 16\,H^+ \leftrightarrows 2\,Mn^{2+} + 5\,I_2 + 8\,H_2O$

The presence of molecular iodine, either prior to or past end point, is most often detected using soluble starch as the indicator. Starch forms a dark blue complex with I_2. The exact mechanism of the formation of the colored complex between starch and iodine is not completely understood. The intense blue color imparted to the starch due to this reversible interaction is apparently a *surface* phenomenon with β-amylose[1]; the interaction of iodine with α-amylose (amylopectin) forms a red complex and is essentially irreversible. The so-called "soluble starch" is principally β-amylose.

Procedure

Preparation of 0.05 *M* Iodine Solution

Molecular iodine is only sparingly soluble in water; however, the triiodide ion, I_3^-, which can be easily prepared by the reaction

$$I_2 + I^- \leftrightarrows I_3^-$$

is very soluble and reacts much in the same way as molecular iodine. Because iodine can be purchased in high purity and it has a large molar mass it is possible to prepare standard solutions of iodine gravimetrically. For exacting work, the concentration of the iodine solution should be standardized against a suitable primary or secondary standard. Arsenic(III) oxide is a long-favored primary standard for iodine solutions. It has fallen out of favor recently due to its extremely high toxicity and elaborate regulations for safe storage, handling, and disposal. For this analysis, the iodine solution will be standardized against secondary-standard sodium thiosulfate.

Weigh about 12.7 g of reagent-grade iodine into a 250 mL beaker. Add 40 g of iodate-free potassium iodide[2] to the beaker. Add small portions (~25 mL) of water until all (or most) of the iodine dissolves (do not heat). Transfer the solution to an amber bottle and dilute to about 1 L. If any solid iodine remains it must *not* be transferred to the stock bottle or the iodine solution will increase in concentration over time. Protect the solution from light and air as much as possible. Light will decompose the I_2 and oxygen in the air will oxidize I^- to I_2.

Preparation of 0.1 *M* Sodium Thiosulfate

Add about 25 g of $Na_2S_2O_3 \cdot 5\ H_2O$ to about 1 L of boiled and cooled deionized water. Add about 0.1 g Na_2CO_3 to raise the pH and stir until all of the solids have dissolved. Transfer to a clean container and protect from air and light when not in use.

Preparation of Starch Indicator Solution

Mix about 1-2 g of soluble starch with 15 mL of water in a small beaker. Pour or transfer with a Pasteur pipette the suspension gradually into 500 mL of *boiling* water. Continue boiling until the solution clarifies (up to about 5 min). Add 1 g of boric acid as a preservative and allow the solution to cool. Transfer to a glass-stoppered bottle.

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[1]Rundle, R.E., Foster, J.F., Baldwin, R.R., *J. Amer. Chem. Soc.*, **66**, 2116, (1944).

[2]Test the salt for iodate by dissolving 1 g in 20 mL of water. Add 1 mL of 6 M H_2SO_4 and 2 mL of starch indicator solution. Absence of blue color for 30 s indicates the absence of iodate.

Preparation of Standard 0.01 *M* Potassium Iodate

Dry about 1.2 g of primary-standard KIO_3 at 110°C for 1 h and cool in a desiccator. Weigh 1.1 g of the solid into a 500 mL volumetric flask. If a weighing boat is used, rinse the boat thoroughly to effect a quantitative transfer of the solid. Dissolve the solid and dilute to the mark, and mix thoroughly.

Standardization of the Sodium Thiosulfate

The sodium thiosulfate solution may be standardized against a standard solution of I_2 produced when a known quantity of IO_3^- reacts with excess iodide in acid solution.

$$IO_3^- + 5\,I^- + 6\,H^+ \leftrightarrows 3\,I_2 + 3\,H_2O$$

Note that 3 moles of iodine are produced for every mole of iodate. The thiosulfate reacts with iodine according to the reaction

$$I_2 + 2\,S_2O_3^{2-} \leftrightarrows 2\,I^- + S_4O_6^{2-}$$

Standardization will be performed by first treating an aliquot of the standard iodate solution treated with excess KI and then titrating with thiosulfate to the disappearance of the yellow-brown color of iodine. After the color of the iodine is gone starch indicator is added and the titration completed by adding titrant to the absence of the blue color of the starch-iodine complex. The starch indicator should not be added too soon during the titration or else the dissociation of the starch-iodine complex will be too slow.

Pipette a 50.00 mL aliquot of standard iodate solution into a 125 or 250 mL Erlenmeyer flask. Several flasks may be set up simultaneously but KI should be added only just prior to the analysis so as to avoid air oxidation of the iodide ion. Add about 2 g of iodate-free KI and swirl the flask to dissolve the solid. Acidify the solution with 2 mL of 6 *M* HCl and immediately titrate with thiosulfate until the solution is nearly colorless. Add 3-5 mL[3] of the starch indicator and titrate with stirring to just colorless. Avoid aerating the solution inordinately by stirring gently. The solution may turn blue upon sitting due to air oxidation of the I^-.

From the molarity of the iodate solution, calculate the molarity of the thiosulfate solution. Perform at least three replicates.

Standardization of the Iodine Solution

Transfer a 25.00 mL aliquot of the iodine solution to a 125 or 250 mL Erlenmeyer flask and dilute to about 50 mL. Add about 1 mL of 3 *M* HCl to acidify the solution[4] and titrate immediately with standard thiosulfate as before. Add the starch indicator when the solution is almost colorless and complete the titration to the disappearance of the blue color.

---

[3]The quantity is not critical - a squirt from a pasteur pipette is adequate - but the amount should be about the same each titration to avoid introducing a determinate error.

[4]Acidifying the solution prevents the formation of hypoiodite. Iodimetric titrations cannot be performed in very basic solutions.

Calculate the concentration of the standard iodine. Perform at least three replicates.

Analysis of Vitamin-C Tablets

Accurately weigh two or three 100 mg vitamin-C tablets and place them in a 125 or 250 mL Erlenmeyer flask. Add about 50 mL of water to dissolve the tablets. If necessary, a glass stirring rod can be used to break up the tablets (do not forget to rinse the stirring rod before removing it from the flask). Add 3-5 mL of starch indicator and immediately titrate to the appearance of the blue color which persists for 1 minute. Do not delay in performing the titration because solutions of ascorbic acid are quickly oxidized by oxygen in the air. Do not stir the solution too vigorously.

Perform at least 3 replicates. Calculate the number of milligrams of vitamin-C per tablet. Compare your result to the amount printed on the bottle.

Alternative Method

After dissolving the tablets, pipette a 50.00 mL aliquot of the standard iodine solution into the flask. Back-titrate the excess iodine with standard sodium thiosulfate until the solution is just about colorless. Add 3-5 mL of starch indicator and continue the titration to the disappearance of the color of the starch-I_2 complex. If less than 10 mL of titrant were necessary, repeat the analysis with a larger quantity of standard iodine solution.

From the volumes of the standard solutions, calculate the number of milligrams of vitamin-C per tablet. Compare your result to the printed label.

Discussion

Discuss the role of vitamin-C in mammalian health paying particular attention, of course, to humans.

Gravimetric Analysis

Gravimetric Determination of Chloride in a Soluble Sample

Introduction[1]

The chloride content of a soluble salt can be determined by precipitation as the silver chloride. The precipitate is collected in a filtering crucible, washed, and brought to constant weight by drying at 110°C. Precipitation is carried out in slightly acidic solution to eliminate potential interference from anions of weak acids (*e.g.* CO_3^{2-} or PO_4^{3-}) that form precipitates with silver in neutral solutions. A small excess of silver ion is required to drive the equilibrium towards the solid but a large excess will cause errors in the analysis due to coprecipitation and the formation of soluble complexes.

Silver chloride forms first as a colloid and is coagulated by gently heating. A small quantity of nitric acid is added to the wash liquid to maintain the electrolyte concentration and prevent peptization (breaking down large particles into small, hard to filter particles) during washing. The acid is volatilized during the drying of the precipitate.

Silver halides are susceptible to photodecomposition. The precipitate often acquires a violet color due to the accumulation of finely divided silver. The analytical result, however, will be negligibly affected as long as the quantity of excess silver ion is small and the solid is kept from direct sunlight.

Procedure

Clean 3 or 4 medium (M) or fine (F) fritted glass filtering crucibles by filling each with about 5 mL of conc. HNO_3 and letting them stand for a few minutes. Attach to the suction filtering apparatus and draw the acid through the crucible. Rinse with tap water at least three times. Break the vacuum and turn off the suction. Add 5 mL of 6 *M* NH_3 solution and let stand for a few minutes. Draw off the ammonia solution and rinse with deionized water six to eight times. Place a unique identifying mark on each crucible and dry to constant weight[2] at 110°C.

Dry the unknown at 110°C for at least 1 h and cool in a desiccator. Weigh by difference 0.1-0.2 g samples into 250 mL beakers. To each add 75-125 mL of water and 2-3 mL[3] 6 *M* HNO_3. Slowly and with good swirling add 0.1-0.2 *M* $AgNO_3$ until the precipitate is observed to coagulate[4,5]. Add an additional 3-5 mL of $AgNO_3$. Heat to almost boiling (*but not boiling*) on a hot plate to further

[1]Similar reference: *Fundamentals of Analytical Chemistry, 4th ed.*, Skoog, D.A. & West, D.M.; Holt, Rinehart and Winston Publishing (1982).

[2]Consider constant weight to be achieved when subsequent weighings after drying cycles agree to within ±0.2 mg.

[3]This approximate volume can be quickly dispensed with a couple of normal transfers from a Pasteur pipette.

[4]To determine the amount of $AgNO_3$ needed, calculate the volume that would be required if the sample were pure NaCl.

[5]Use a separate stirring rod for each sample and leave it in the solution throughout the determination.

coagulate and digest the precipitate for 10-15 min. Check for completeness of precipitation by adding 1 drop of $AgNO_3$. If any further precipitation occurs, add an additional 3 mL of $AgNO_3$, digest, and recheck for completeness of precipitation. Cover beakers with a watch glass and age the precipitate for at least 1 h.

Prepare a wash solution by adding 2-5 mL 6 *M* HNO_3 to ~1 L water. Place a crucible on the filtering apparatus. Decant the supernatant through the filtering crucible. Wash the precipitate several times in the beaker with the wash solution. Decant these washings through the crucible also. Finally, transfer the bulk of the precipitate to the crucible, using a rubber "policeman" to dislodge any particles that adhere to the walls of the beaker. Continue washing until the filtrate is found to be free of $AgNO_3$[6]. Place all washings, filtrates and excess $AgNO_3$ in the silver-residue container.

Dry the solids to constant mass at 110°C. Cool in a desiccator. Report the %Cl for each sample and the mean %Cl with appropriate statistical analysis. Dispose of the AgCl in the silver-residue container. Wash the filtering crucibles with 6-12 *M* NH_3.

Optional Additional Analysis

Recover the silver (as the metal or a soluble salt) from the silver chloride by any method. Determine the purity of the metal or salt recovered by titration with standard potassium thiocyanate using iron(III) as the indicator. Discuss the method of recovery in terms of financial economics for silver recovery, time, etc. and the discuss the analytical method. Explore and discuss other methods available for silver analysis.

Ask the instructor for details of the analysis.

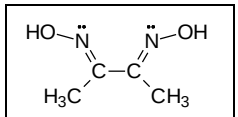
Discussion Items

Discuss gravimetric analysis in general and this analysis specifically. Comment on interferences and applicability of this particular method in other types of samples, *e.g.* sea and brackish water samples.

[6]Washings are readily tested for the presence of Ag^+ by collecting a few milliliters in a test tube and adding a few drops of HCl. Washing is judged complete when little or no turbidity is observed with this test.

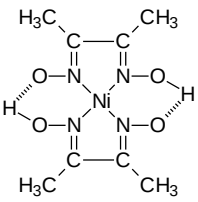
Gravimetric Analysis of Nickel

Introduction



Dimethylglyoxime

One of the most common applications of organic precipitants is the quantitative precipitation of nickel with dimethylglyoxime (DMG). Although no known reagent is completely specific for a single element, DMG is nearly so for nickel(II) ion in ammoniacal solution. The only interfering metals are palladium (in acidic solution), copper(II) ion in high concentration, and mixtures of cobalt(II) and iron(III) ions. Tartrate is added to complex any iron(III) ion that would otherwise precipitate in the basic solution. The tartrate complex also reduces the coprecipitation of iron(III) and aluminum ions with the nickel dimethylglyoximate ($NiDMG_2$)[1].



Nickel dimethylglyoximate

Procedure

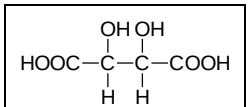
Filtering Crucibles

Prepare 3 or 4 fine (F) or medium (M) fritted-glass filtering crucibles by drawing a small quantity of water followed by a few milliliters of 0.1 to 1 M HNO_3 through the filter pad to remove any water- or acid-soluble substances. Rinse the crucibles with water and draw a few milliliters of 0.1 to 1 M NH_3 through the filter to remove base-soluble substances. Finally, draw a few milliliters of water through the fritted glass, mark to identify, and place in a oven at 110°C to dry. Proceed with the sample preparation while the crucibles dry. Heat to constant mass. The crucibles can be considered to be at constant weight if the mass after the first and second heating are within 0.2 mg.

Sample Preparation

Accurately weigh 3 or 4 samples of the metal. If you are performing an analysis of Monel metal, use 50 - 150 mg of sample. If the sample is steel or other alloy, use 0.6-1 g. Transfer the samples to separate 300 or 400 mL beakers. Add 30 mL of 6 *M* HCl and warm on a hotplate in the hood for 5-10 minutes. Do not boil.

Add 10 mL of 6 *M* HNO_3, cover with a watch glass, and boil *gently* in the hood until the yellow-brown nitrogen oxides are no longer evolved. The nitric acid serves to assure complete oxidation and dissolution of the sample. If the sample is not dissolved after 10-15 min of boiling, add 10 mL of concentrated nitric acid and continue to boil for an additional 10-15. Repeat as necessary but avoid adding excess HNO_3 since it increases the time necessary to remove the nitrogen oxides. Finally, dilute with ~150 mL of water and heat to near boiling.



Tartaric acid

While the solution is hot, add ~1 g of tartaric acid (why?). After the tartaric acid has dissolved, add concentrated ammonia until the odor of ammonia persists in the vapor. Insoluble material should be removed by gravity filtration and washed with a hot solution of ammonia and ammonium

[1]The IUPAC nomenclature for the chelate is bis-dimethylglyoximatonickel(II).

chloride. Make the solution slightly acidic with conc. HCl.

Maintaining the solution at about 70°C, slowly add 20 mL of the 2% dimethylglyoxime reagent[2]. With stirring, add 3 *M* ammonium acetate until a permanent red precipitate just appears[3]. Again, with stirring, slowly add an additional 20 mL of the ammonium acetate solution. Leave the stirring rod in the beaker.

After the precipitate settles, add 1 mL of the DMG reagent to test for complete precipitation. If additional precipitate forms, add an additional 5 mL of the DMG reagent.

Digest the sample at 60-70°C for 1 h. Cool to room temperature and filter through the weighed crucibles. Use rubber policeman to loosen particles sticking to the walls of the beaker. Wash any remaining precipitate out of the beaker with a wash solution prepared by dilution 1 mL of 6 *M* NH_3 in 500 mL of water. Using the same wash solution, wash the precipitate in the crucible with 5-10 successive 5 mL portions.

Dry to constant weight at 110°C and report %Ni with appropriate confidence limits in your sample.

Discussion Items

Research and discuss the specificity of DMG for nickel. Why must the reaction be performed in ammonia? Using lecture notes and library research, discuss the Ni-DMG chelate.

Additional Considerations

The analysis of Monel metal as performed, is incomplete since only the nickel in the sample has been determined. The remaining metal in the sample is predominantly copper which can be determined gravimetrically with potassium ferricyanide or spectroscopically by atomic absorption spectroscopy. However, the sample has been significantly diluted during the preparation of the $Ni(DMG)_2$ so the copper ion must be reconcentrated prior to analysis.

If another alloy (*e.g.* steel) or compound was used, there may be additional metals present which should be analyzed for a complete analytical characterization. Treat the solution accordingly for the further analysis of other metals.

Procedure for Further Analysis or Disposal

Collect all of the filtrates from which the dimethylglyoximatonickel(II) came and acidify carefully with nitric acid. It is reasonable to assume that the solution is acidic when the red color of the colloidal $Ni(DMG)_2$ disappears. Cover the beaker, and, on a hot plate, heat the solution to boiling to reduce the volume to about 25 mL.

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[2]The dimethylglyoxime solution is prepared in ethanol. If percentage Ni in the sample is known, the approximate amount of DMG solution needed plus 50-100% excess should be calculated.

[3]If the precipitate formed during the addition of the DMG, add 20 mL of the ammonium acetate and proceed to the next paragraph.

Save this solution for further analysis (*e.g.* **Atomic Absorption Spectroscopy**) in a acid-washed plastic bottle or prepare it for proper disposal.

Determination of the Thermodynamic K_{sp} of a Sparingly Soluble Salt[1]

Introduction

Lead iodide, PbI_2, is considered to be a sparingly soluble salt, the solubility in pure water being less than 0.002 M at 20°C. The quantitative analysis of the ions in solution leads to the possibility of calculating K_{sp}. A simple analytical method for the determination of species concentration is the absorption of visible light by the solution containing the species of interest, in this case lead (II) ion and iodide ion. Unfortunately, neither Pb^{2+} nor I^- absorb light in the visible portion of the spectrum.

It is possible, however, to convert the I^- to I_2 with NO_2^-, a mild oxidizing agent in slightly acidic solution. The characteristically brown I_2 solution formed in the oxidation-reduction reaction has a reasonably strong absorption band at around 525 nm. The reaction of I^- with NO_2^- is fast and quantitative. Permanganate and dichromate could be used to oxidize the iodide except that they could further oxidize the iodine to iodate.

You are by now aware that the solubility of a precipitate is dependent on the ions coexisting in solution. Common-ions affect the equilibrium by lowering the solubility of the precipitate while non-common ions tend to cause the precipitate to be more soluble.

For the system under study,

$$K_{sp} = a_{Pb^{2+}} \times a_{I^-}^2 = [Pb^{2+}]\,[I^-]^2 \times f_{Pb^{2+}} \times f_{I^-}^2$$

In dilute solutions ($\mu < 0.001$ M), the activity coefficients, *f*, are approximately unity. At higher ionic strengths, the activity coefficients can be calculated with the Debye-Hückel equation and are somewhat less than unity.

In this experiment you will determine thermodynamic K_{sp} of PbI_2 and the effect ionic strength has on the solubility of the precipitate.

Procedure

Preparation of Solutions

From the 0.1 *M* stock bottle of KNO_3, make 4 dilutions to give concentrations ranging from 0.05 *M* to 0.001 *M*. Prepare at least 5 dilutions of the KI stock solution ranging from 10^{-2}-10^{-4} *M*. The KI solutions must be made to the best possible precision since they are standard solutions to be used in constructing the Beer's law plot. You may need to make successive dilutions.

Prepare a quantity of solid PbI_2 in 6 labeled test tubes by mixing ~1 mL of 0.1 *M* $Pb(NO_3)_2$ with ~1 mL of 0.1 *M* KI and centrifuging at maximum velocity in a hanging bucket centrifuge. Decant and dispose of the remaining liquid. To thoroughly clean the precipitate, add about 1 to 3 mL of deionized water and shake the pellet loose. Centrifuge, decant, and repeat twice more. Remove as much of the remaining water as possible from the tubes.

[1]Green, D.B.; Rechtsteiner, G.; Honodel, A. *J. Chem. Educ.* **1996**, *73*, 789-792.

Establishment of Equilibria

To test tube *1*, add sufficient water to fill the test tube two-thirds full. To tube *2*, add about the same amount of the least concentrated KNO_3 dilution. Add similar quantities of the other KNO_3 dilutions to the remaining tubes, using the 0.1 M KNO_3 stock solution for tube *6*. Shake the pellets loose, place in a hot water bath and heat the liquids in the tubes to 30-40°C. Do not allow the solutions in the tubes to boil. Shake gently occasionally while the tubes are warming to establish equilibrium. After 10 min at the slightly elevated temperature, allow the solutions to cool to room temperature on the bench. Shake the tubes gently every minute or two to prevent supersaturation.

When the solutions have cooled to room temperature, centrifuge at maximum rate for 3-5 min to clarify the solution.

Beer's Law Plot and Analysis of Equilibrium Mixtures

Obtain 2 clean, matched cuvettes and standardize the Spectronic 20 at 525 nm with pure water as the reference in one cuvette Into the other cuvette, pipette 1.00 mL of the most concentrated dilution of KI. With another pipette, transfer 1.00 mL of 0.1 *M* HNO_3 to the cuvette. Finally, transfer 1.00 mL of 0.05 *M* KNO_2 to the cuvette and shake gently to mix. Record the %transmittance and convert to absorbance for plotting. Clean and dry the cuvette. Repeat the on the other standards. Construct a Beer's Law plot of absorbance at 525 nm *versus* $[I_2]$.[2] Determine the equation of the line from linear least-squares fitting.

Clean and dry the cuvette. Pipette 1.00 mL of the supernatant solution from test tube *1* into the cuvette. Take care to avoid pipetting any solid. Add 1.00 mL each of the nitric acid and potassium nitrite solutions and shake gently as above. Determine the absorbance of the solution and $[I_2]$ from the equation of the line of the Beer's Law plot. Repeat on the remaining test tubes.

Calculations

From the equilibrium iodide concentrations, determine the $[I^-]$ and $[Pb^{2+}]$ in the equilibrium mixtures. Calculate and tabulate the apparent K_{sp} values of PbI_2 at each ionic strength. Reporting the temperature at which the K_{sp} was determined is important.

Using the concentrations of potassium nitrate, lead(II) ion and iodide ion, calculate the ionic strength, μ, for each equilibrium solution. Calculate the activity coefficients of the Pb^{2+} and I^- from the Debye-Hückel equation at each of the ionic strengths. Using species concentrations and activity coefficients calculate the thermodynamic solubility products, $K°_{sp}$, from each tube. What comparisons and conclusions can you make?

Plot solubility PbI_2 (s= $[Pb^{2+}]$) vs. ionic strength. Calculate and plot the theoretical curve for the solubility of lead iodide using the average $K°_{sp}$ obtained. Discuss the "goodness of fit" and explain any deviations.

[2]Since all analytical mixtures are prepared and treated identically, it is possible to save time by plotting absorbance against $[I^-]$ instead of $[I_2]$.

Construct a plot of $\log_{10}s$ *versus* $\sqrt{\mu}$. Use least-squares fitting to extrapolate the data to zero ionic strength. From the solubility at zero ionic strength calculate the thermodynamic solubility product.

Discussion

Report a percentage difference between your K_{sp} and the literature value at approximately the same temperature. Discuss and compare the different methods used for determining thermodynamic solubility product. Make judgments as to which method might be better and why. Discuss any deviations of the experimental data and the calculated curve in the plot of *s* vs. μ. Also discuss in some detail the theoretical and practical significance of activity and the activity coefficients.

Electrochemical Methods of Analysis

The pH Meter: Determination of Apparent p*Ka* and the Molar Mass of a Compound

Introduction

Every acid has a characteristic pK_a value and it is possible to identify (or partially identify) the acid by measuring it pK_a. A solution of a weak acid or base and its conjugate makes a buffer solution. Titrating the buffer with an acid or base will indicate any basic or acidic sites on the molecule further identifying the nature of the acid.

In this experiment, the basic operation of the pH meter and its use to analyze a weak acid or base will be studied. The optimal buffer pH will be determined and reported and the identity of the acid or base determined from this data and the molar mass. For this analysis assume solution ideality.

Procedure

Care of pH Electrodes

The glass electrode is a fragile and easily broken instrument. Great care must be taken while moving, stirring near, and rinsing the electrode. Rinse the electrode with a gentle stream from a wash bottle and pat it dry with a tissue. Always store the electrode in deionized water or storage buffer when not in use.

Standardizing the pH Meter

1. Plug the instrument into the outlet. Make sure the function switch is in the **STAND BY** position. Set the **TEMPERATURE** control to room temperature.
2. Prepare three 50 mL beakers. Into the first beaker place approximately 30 mL of pH 4 buffer. In the other two, place pH 7 and pH 10 buffer.
3. Rinse and place the electrode in the pH 7 buffer. Adjust the **STANDARDIZATION** control until the display reads **7.00**. Rinse the electrode and place in the pH 4 buffer. Adjust the **SLOPE** control until the display reads **4.00**. Repeat this step until the meter reads the correct pH in the appropriate buffer.
4. Rinse and place the electrode in the pH 10 buffer. If display is off more than 0.2 pH units, adjust the **SLOPE** to correct pH. Check the pH 4 buffer; correct the reading to pH 4 with the **STANDARDIZATION** control. Repeat step 4 until the display reads within 0.2 pH units in all buffers.

Important: During rinsing or any time electrode is out of solution, set **FUNCTION** switch to **STAND BY**. Recheck standardization frequently and readjust as necessary.

Standardization of Acid and Base

Prepare 1 L of 0.1 *M* NaOH by pipetting the appropriate amount of 50% NaOH (CO_2-free) into a clean plastic bottle and diluting with boiled CO_2-free water.

Prepare 1 L of 0.1 *M* HCl by transferring a suitable amount from the concentrated stock solution (12 M) and diluting to about 1 L.

Prepare 250 mL of standard 0.1 *M* potassium hydrogen phthalate solution in a volumetric flask.

Place a 100 mL beaker on a magnetic stirrer and add a stir bar. Pipette a 25 mL aliquot of the standard KHP into the beaker. Carefully dip the pH electrode in the solution and gently stir. If necessary, add enough boiled deionized water to cover the pH electrode and reference junction. Titrate the acid with base, recording volume and pH. On either side of end point add 1-2 mL of titrant for each addition. As end point is approached and 1-2 mL past end point, take a measurement every 0.2-0.5 mL. When very close to end point, titrate even slower. Titrate at least 10 mL past end point. Always allow several seconds for the display to stabilize. Perform at least three replicates.

Plot pH vs. volume of base added. Compare the p*K* obtained the literature value for KHP (pK_{a2}). Also plot the first and second derivative curves for the data. From the titration curve(s), calculate the concentration of the base.

Rinse the electrode well and clean the beaker. Pipette 15 mL of the prepared hydrochloric acid into the beaker. Dip the electrode into the acid and record the pH. Titrate with the freshly standardized base as above. End point in this titration will be sharper since the titration is one of a strong acid with a strong base. For one of the replicates, record pH and mv (mv do not need calibration).

Plot pH vs. volume of base added and the first and second derivative curves. From the end point inflection calculate the concentration of the acid. Also plot mv vs. pH and draw the best-fit straight line (the line may deviate from linearity at high pH). The slope of the plot is the Nernstian slope for the electrode. What is the significance of this slope? What should the value be?

Analysis of the Unknown Compound

Quantitatively transfer 0.5-1 g of the unknown sample into a 250 mL volumetric flask and dissolve with CO_2-free water. Dilute to the mark and mix well.

Check the standardization of the meter. Set up the apparatus as above and pipette a 25 mL aliquot of the unknown solution into the beaker. Determine the pH of the solution. Titrate with standard acid to about pH 1 (it may not be possible to obtain this pH). If increased accuracy is desired, repeat the titration. At any equivalence points, titrate slower.

Titrate another 25 mL aliquot of the unknown solution with standard base. Continue the titration to pH 13 (again, it may not be possible to obtain a pH this high). Correct the pH values for sodium ion error using the attached nomograph, if necessary.

Plot both titration curves on the same graph as pH vs. *moles* of titrant added. The center of the x-axis should be 0.0 moles of titrant added -- increasing moles of base added to the right and increasing moles of acid added to the left. Plotting moles instead of volume of titrant eliminates any differences in concentration of the titrants. Plot the derivative curves also.

Determine from the titration curve the apparent pK_a values of the acid. Compare to those listed in attached table and identify the compound. Calculate the molar mass of the sample for verification. The sample may be a hydrated compound making the molar mass determined higher than the molar mass of the anhydrous compound. The use of appropriate literature to explore this possibility is suggested.

Clean Up

Rinse the electrode well in deionized water. Store the electrode dipped in a beaker of deionized water or electrode storage solution.

Discussion Items

What advantages and disadvantages are inherent in graphically displayed titrations?

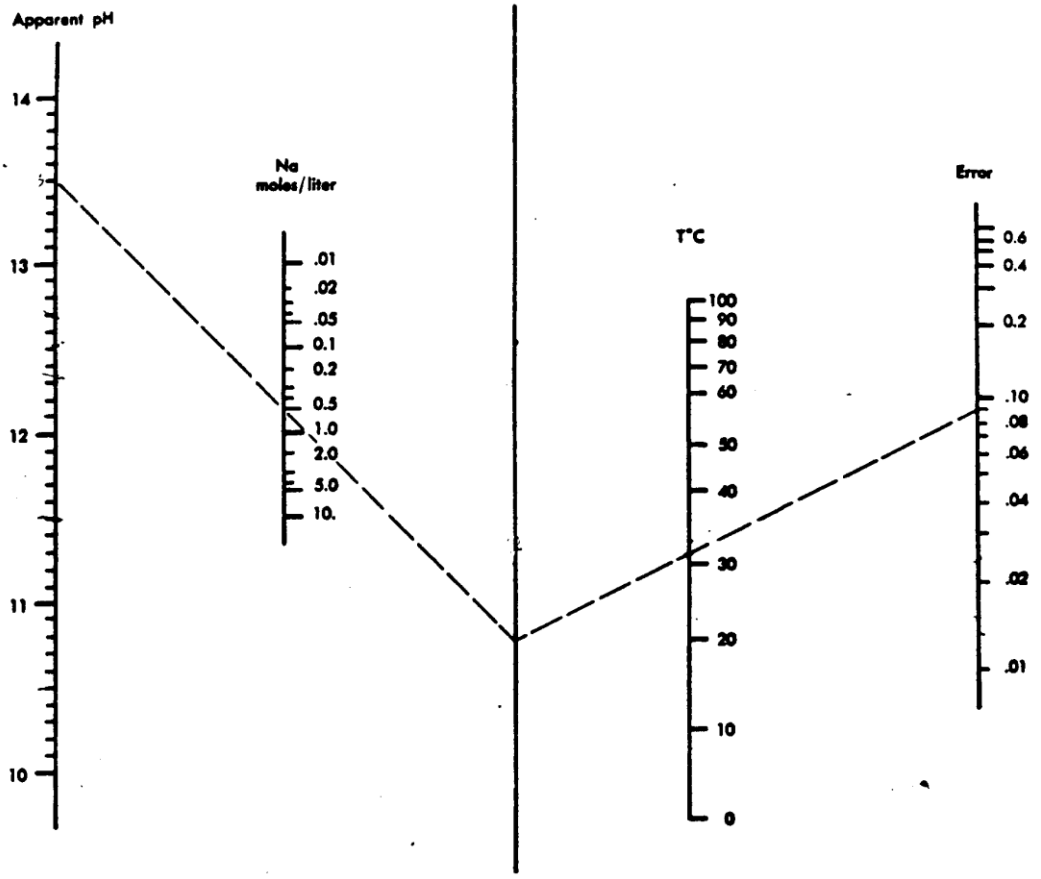
Discuss the usefulness of the derivative plots. How do the different methods of endpoint determination (both graphical methods on pH-vs.-volume curves and algebraic transformations) compare in ease and accuracy? Which would give the least error (if there is any difference)?

If you were to build an automatic computer-controlled titrator, what method of endpoint detection would lend itself to computerized determination?

Nomograph for Sodium Ion Correction of Corning Glass Electrodes

Nomograph for Sodium Ion Correction - Corning Glass Electrode

Taken from Corning Instruction Manual, Model 7 pH Meter



This nomograph may be used to calculate approximate pH corrections for sodium ion error. To determine a correction lay a ruler between the points corresponding to the apparent pH on the left-hand axis and the sodium ion concentration on the Na axis. Mark the point where the ruler intersects the center reference axis. Next lay the ruler between the marked point on the reference axis and the sample temperature. The pH error to be added to the apparent pH is found at the intersection of the ruler and the right-hand axis.

The nomograph is only approximate and is based on the behavior of electrodes intermittently exposed to high pH solutions. Electrodes exposed continuously to high pH and high temperatures may show somewhat higher pH errors. Errors due to other ions are smaller and may usually be neglected.

Potentiometric Titration of a Bromide-Iodide Mixture

Introduction

The purpose of this analysis is to determine the amount of bromide and iodide simultaneously in a mixture of salts using a silver wire as an electrode of the second kind. Additionally, the amount of chloride in tap water or other sources may also be determined.

A metal that is immersed in a solution containing the ions of the metal can be used as an indicator electrode to determine the concentration of the metal. The electrode will behave in a Nernstian fashion such that

$$E = E^{\circ}_{M} - \frac{0.0257}{n} \ln \frac{1}{[M^{n+}]} \qquad (1)$$

at 25°C for the reaction $M^{n+} + ne^- \leftrightarrows M$. The metal is an *electrode of the first kind*; that is, the electrode is being used to measure the concentration (or activity) of the ions of the metal. If the metal is being used in the fashion of an *electrode of the second kind*, the electrode will respond to the concentration (activity) of an anionic species which is only slightly soluble as a salt of the metal.

As an example, a silver wire immersed in a solution containing chloride which is saturated with silver chloride will develop a potential which is determined by the chloride concentration. Of course, the electrode is responding to the concentration of the silver ion but the silver ion concentration is determined by the concentration of the chloride and the magnitude of K_{sp}. The response of the electrode in this case is still Nernstian; however, the Nernst equation becomes[1]

$$E = E^{\circ}_{AgCl} - 0.0257 \ln [Cl^-] + 0.0257 \ln K_{sp} \qquad (2)$$

or, by converting to common log

$$E = E^{*}_{AgCl} + 0.0591\, p\mathrm{Cl} \qquad (3)$$

Such an electrode can be used to monitor the concentration of the Cl^- during a precipitation titration with standard silver ion. A plot of E (or pCl) *vs.* volume (or moles) of titrant will have a sigmoidal shape similar to an acid-base titration.

If other anions in solution are able to precipitate as the silver salt, the electrode will respond to those ions as well. If the solubility products are sufficiently different, the concentrations of different anions in a mixture can be determined from the potentiometric titration curve.

In a potentiometric titration (acid-base, precipitation, etc.) there is a sudden change in the ion concentration (silver ion in this analysis) at the equivalence point(s)

[1]You should derive this form of the Nernst equation yourself.

because the silver ion concentration is always kept small due to the presence of excess anion. The concentration is never zero and is theoretically calculable through K_{sp}. Past equivalence point the [Ag^+] increases dramatically unless another anion is present (with a larger K_{sp} than the first) that will react with the silver ion. If the solubility products of the insoluble silver salts are too similar then the rises observed at the equivalence points will overlap making the analysis difficult or impossible[2].

In the analysis of the bromide-iodide mixture, the AgI will precipitate first since its K_{sp} is about 1/1000 that of AgBr. The addition of the first aliquot of standard Ag^+ will saturate the solution with silver ion to the extent of the solubility product of AgI so the solution does not need to be pre-saturated with the salt. Prior to equivalence point the bromide will be in large excess thereby suppressing the [Ag^+]. Because of this the *E* observed on the potentiometer will change very slowly. At the first equivalence point the potential will change rapidly until the solubility product of the AgBr is reached. Again, the potential will stay relatively constant until the Br^- is completely precipitated, then another sharp increase will be observed signaling the second equivalence point.

Usually potentiometric titrations use a Ag/AgCl or SCE reference electrode. In the high precision analysis of chloride by this technique the use of one of these electrodes would be unsuitable since they leak a small amount of chloride from the liquid junction. The use of a sulfate or nitrate bridge double-junction reference electrode would be appropriate. An alternative reference electrode (though not as convenient) is a glass pH indicating electrode or a combination pH electrode in which the reference fill-solution has been removed. When immersed in a buffered solution the glass electrode generates a very constant potential.

An important advantage of indirect potentiometry is that accurate knowledge of the absolute half-cell potentials of the indicating and reference electrodes is not necessary. A disadvantage is that the ionic strength of the analytical solution must be controlled and be somewhat reproducible from sample to sample. Additionally, a reasonable amount of temperature control must be maintained.

Procedure

Prepare 200 mL of standard 0.1 M $AgNO_3$ from dry primary-standard-grade silver nitrate. Store the solution in an amber reagent bottle. Rinse the bottle with a small quantity of the solution before transferring the bulk[3].

If the unknown is provided as a solid, make 100-200 mL of solution of the unknown such that the concentration of the bromide does not exceed 0.1 M. Assume for the calculation of the mass of unknown necessary that the unknown is 100% NaBr. This assumption will nearly guarantee that the solution does not

[2]Calculate the minimum difference in K_{sp} (to the nearest power of 10) that will still allow a clear differentiation in the equivalence point rises.

[3]Alternatively, a standard silver nitrate solution may be prepared by dissolving an appropriate amount of pure silver wire in a small volume of 6 M nitric acid followed by boiling to remove the dissolved oxides of nitrogen. The solution may then be transferred to a volumetric flask and diluted to the mark with water.

exceed 0.1 M in bromide or iodide. If a percentage composition is known for the sample, prepare the unknown solution based on known composition. If the bromide and iodide are in very low abundance in the sample, it is possible to transfer by mass the solid unknown directly to the titration vessel.

Plug in the pH meter at least 15-20 minutes in advance of using it to take measurements. Support a silver wire electrode and a reference electrode[4] side-by-side. "Short" the meter inputs, set the meter to **mV** and adjust the display to read 0 mV. Attach the silver electrode to the **indicator** jack on the meter and the reference electrode to the **reference** jack. Place a 5-10 mm stack of 11-cm filter paper on a magnetic stirrer to act as thermal insulation (other media may be used as well). Pipette a 20 mL aliquot of the Br-I unknown solution into the beaker. Add a few drops of 6 M HNO_3, ~0.5 g of $Ba(NO_3)_2$, and dilute to 65-75 mL. Stir the solution gently and place the electrodes into the solution taking care that the stir bar does not strike the electrodes. Allow sufficient time for the voltage readings to stabilize. A consistently increasing or decreasing potential usually indicates inadequate thermal insulation from the magnetic stirrer.

Proceed with the titration, using ~0.5 mL additions of the titrant up to near the first equivalence point. Make smaller additions (~0.1 mL) through equivalence point. Continue with the larger additions to the second equivalence point and again make small increments through the equivalence point. Continue the titration several milliliters past the second equivalence point.

Optional

Titrate an aliquot of tap water using the same technique. If the titration is performed as quickly as possible, the effect of the reference electrode fill solution (assuming a single-junction SSC or SCE is being used) will be minimal.

Additionally, the halides in sea water can be determined by this method. It is possible that chloride, bromide, and iodide could all be in sea water. Assume that sea water is approximately 35 parts-per-thousand Cl^- in determining the correct sample size.

Calculations

Plot *E vs.* volume (or moles) of titrant. Determine the end points by any suitable method (including derivative plots). Determine the percentage by mass bromide and iodide in the unknown sample.

Discussion Items

1. What is the purpose of the barium nitrate?

[4]A SSC or SCE may be used in this analysis if the junction leak is very slow (*i.e.* high flowrate and annular junction electrodes should not be used). The use of these single junction reference electrodes may cause a third equivalence point rise due to the chloride in the reference fill-solution if the titration is performed slowly. You are welcome to experiment with building a double-junction attachment for the reference electrode.

2. What percentage of the AgI remains unprecipitated when the first amount of AgBr starts to precipitate?

3. What effect would the presence of 1.0 M ammonia have on the titration? Be specific.

4. How does this method compare with other methods of halogen determinations in terms of precision, accuracy, convenience, etc.?

Fluoride Ion Selective Potentiometry

Introduction

The solid-state fluoride electrode has found extensive use in the determination of fluoride in both static and dynamic systems, in "on-line" analysis of drinking water and foodstuffs, and many other analyses.

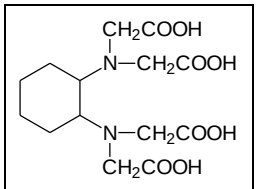
The fluoride ion-selective electrode is based on a crystal of LaF_3 that is doped with Eu^{3+} to improve its conductivity. The selectivity of the F^- electrode is excellent. The main interference is OH^- so F^- determinations are done in neutral to acidic (>pH 5) solutions. The electrode potential is strongly sensitive to ionic strength so all solutions are adjusted to the same ionic strength with *total ionic strength adjustment buffer* (*TISAB*).

The analysis to be performed here is the determination of F^- levels in our local drinking water and in toothpaste.

Procedure

Preparation of TISAB

This solution can be purchased under the trade name *TISAB*. The solution may also be prepared by mixing with stirring 57 mL of glacial acetic acid, 58 g of NaCl, 4 g of 1,2-diaminocyclohexyl-N,N,N',N'-tetraacetic acid (DCTA), and 500 mL of water[1] in a 1 L beaker. After cooling, adjust the pH of the solution to ~5.0-5.5 with 6 M NaOH. Dilute to 1 L and store in a plastic bottle.



DCTA

Preparation of Standards

Prepare no less than four standard solutions of NaF ranging in concentration from 1 x 10^{-3} M to 1 x 10^{-5} M (~50 μg/mL F^--0.5 μg/mL F^-). You should not use the *TISAB* at full strength but rather dilute it during the preparation of solutions. Use the same amount of *TISAB* in each flask (e.g. 10 mL). Complete the dilutions with water. Transfer the standards to plastic for storage between use.

Instrumental Procedure

Set up the instrument as described in pre-lab lecture. Attach the fluoride electrode to the indicating electrode jack on the pH/mV meter and the reference electrode to the reference jack.

Use a magnetic stirrer to stir the solutions while making measurements. Using only plastic beakers, determine the potential of each solution. Allow at least 20 s for the readings to stabilize and wait about the same amount of time for each solution. Make at least 3 replicate readings.

Alternative Instrumental Procedure

[1]Remember to add concentrated acid to water not *vice versa*.

Set up the instrument as described in pre-lab lecture. Attach the fluoride electrode to the indicating electrode jack on the pH/mV meter and the reference electrode to the reference jack.

Use the internal program built into the ion selective electrode meter to calibrate the instrument for direct-reading of fluoride concentration.

Analysis of Unknowns

Tap Water: Transfer a 10-50 mL portion of drinking water to a 100 mL volumetric flask, add *TISAB*, and dilute to the mark. Measure the potential of the water.

Toothpaste: Weigh to the nearest mg about 0.2 g of toothpaste into a 250 mL beaker. Add 50 mL (or the volume you have been using) of *TISAB* and boil for 2-4 min with stirring. Cool and quantitatively transfer the mixture to a 100 mL volumetric flask and dilute to the mark. Determine the potential of the solution.

Carbonated beverage: Remove the carbon dioxide in the solution by heating. Transfer an aliquot to a 100 mL volumetric flask. Add appropriate amount of *TISAB* and dilute to the mark. Determine the potential of the solution.

Analysis of Data

Plot potential (mV) vs. concentration of the standards on suitable graph paper or with an appropriate computer program. Is the plot linear? Is there any nonlinear portion? What is the slope and how is this related to the Nernst equation? What is the correct form of the Nernst equation for this electrode? Report the F^- concentrations of each unknown in ppm.

Electrochemical Methods of Analysis: Polarography

Introduction

Polarographic analysis is an important method for micro- and trace- analysis of many metals. It can also be applied to the electrochemical analysis of organic molecules in nonaqueous media.

In this analysis you will determine the concentration of an electroactive species (Cd^{2+}), the diffusion coefficient of Cd^{2+} in aqueous solution, and apparent *n* for the redox couple

$$Cd^{2+} + n\,e^- \rightleftharpoons Cd$$

The Ilkovic equation defines the diffusion-limited cathodic current, i_d:

$$i_d = knm^{2/3}D^{1/2}t^{1/6}C$$

Where
- i_d = diffusion current, μA
- n = number of electron equivalents/mole
- D = diffusion coefficient of species, cm^2/s
- m = rate of flow of mercury from DME, mg/s
- t = drop time at diffusion current, s
- k = constant – 708 for max current, 607 for average current
- C = concentration, mM

Since i_d is proportional to *C*, a calibration curve of diffusion current vs. concentration should be linear.

Procedure

Set up the polarograph as described in lecture. Never allow the DME to stand in any solution when the mercury is not flowing. Between analyses and at the end of the complete analysis, the cell and electrodes must be washed free of trace materials from prior analyses. Fill the N_2 washing bottles with deionized water. Since the polarographic potentials and currents are sensitive to temperature, best results are obtained by recording calibration data and unknown data on the same day.

Caution: Used mercury should be placed in a beaker containing sufficient water to cover the mercury pool to minimize mercury vapors in the air. Spills must be cleaned up immediately – contact the instructor for procedures.

When ready to proceed with the analysis, open the nitrogen cylinder and adjust the flow rate of the gas to give a gentle stream of bubbles in the gas washing bottles. Do not adjust the mercury column height during analysis.

Determination of an Unknown [Cd^{2+}]

Prepare 2, 4, 6, and 8 mM Cd^{2+} standards from the stock solution or solid reagent. Use 0.1 M HCl prepared with Type-I water as the solvent and to act as the supporting electrolyte.

Place enough sample in the sample cell to cover the electrodes and initiate the N_2 purge. Purge with nitrogen for several minutes to remove oxygen. Discontinue nitrogen purge and turn on the N_2 blanket to maintain an inert atmosphere above the solution. Record the polarogram slowly from -0.1 to -1.2 volts (vs. Ag-AgCl reference)[1] If current-maxima are observed, add 2-4 drops of 0.2% Triton X-100 to the analytical solution and repeat the measurement[2]. Repeat on remaining samples and pure solvent. Between samples wash the inside of the sample cell and the electrodes with Type-I water. Dry the electrodes with a tissue and rinse the cell with a small quantity of sample.

Obtain the unknown containing Cd^{2+} and prepare a sample, dissolved and diluted with 0.1 M HCl, which gives a concentration of approximately 2-8 mM Cd. Transfer a quantity of the solution to the electrochemical cell and scan the polarogram as before.

Measurement of Data Necessary for Calculating *D*

At a fixed potential which gives a diffusion current for one of the standards, determine with a stopwatch, the time for 10 drops to form and release. Calculate the time, *t*, for one drop to form and detach. Next determine the mass of mercury, *m*, dropping from the DME per second. This can best be done by collecting mercury for a known time (about 1 min) in a small beaker containing supporting electrolyte. Wash the collected Hg carefully with Type-I water and acetone then dry (no heat!) and weigh.

Calculations

Calculate the diffusion coefficient for Cd^{2+} in this supporting electrolyte using the Ilkovic equation. Be sure to use the correct constant.

Determine the half-wave potential for Cd^{2+} from the polarogram. Compare the value (adjusted for the reference electrode) to the formal potential for the reduction of cadmium ion.

The equation that defines the polarographic wave of a reversible system is

$$E = E_{1/2} + \frac{RT}{n\mathrm{F}}\ln\frac{i_d - i}{i}$$

[1]The DME has a full range in aqueous solution of +0.3 to -2 V (vs. SCE or SSC). Mercury is oxidized at potentials more positive than +0.3 V and the reduction of water is observed at potentials more negative than -2 V. In 1 M HCl, visible evolution of H_2 is observed from the reduction of H^+ at -1.2 V further limiting the range of the electrode in this supporting electrolyte.

[2]The maxima suppressant should be added to the remaining samples as well so as not to significantly alter the analytical conditions. The addition of excess maxima suppressant may suppress the polarographic wave as well.

where i is the current at a given potential. Thus, a plot of E vs. $\log[(i_d - i)/i)]$ should produce a straight line whose slope is $2.303RT/n$F. From one of your polarograms, select five to ten readings of i (select several from each side of $E_{1/2}$, and plot E vs. $\ln[(i_d - i)/i)]$. From the plot, determine the Nernst slope, n, and $E_{1/2}$. Compare the half-wave potentials determined by graphical analysis of the polarograms and the Nernst plot.

Discussion Items

Research and explain how the addition of a surfactant to the solution suppresses maxima. If you are reading this before you leave the laboratory, you may want to study the effect dissolved oxygen has on the shape of the polarogram. This may be done by recording a polarogram prior to purging with N_2. Discuss other voltammetric electrodes besides the DME.

Optional

Determination of Mixtures

To illustrate the possibility for the analysis of mixtures with the need for separation, record the polarogram from -0.1 to -1.2 V (vs. SSC) for a mixture of Cu^{2+} and Ni^{2+} in 0.5 M NH_3/0.5 M NH_4Cl (supporting electrolyte concentrations approximate). The analytical solution should also contain Triton X-100 to suppress maxima if necessary.

As an interesting study, you may wish to determine the %Cu and %Ni in a sample of Monel metal[3]. Prepare a solution of the metal by dissolving 0.5 g of the sample in about 25 mL of 6 M HNO_3 with gentle heating. After the dissolution of the solid is complete, boil the solution to remove the dissolved oxides of nitrogen. Allow the solution to cool, then add concentrated ammonia to the solution (in the fume hood) dropwise until the solid metal hydroxides just dissolve[4]. Quantitatively transfer the solution to a 100 mL volumetric flask and dilute to the mark with water. Make a 1:10 dilution of the solution into a 100 mL volumetric flask using 0.5 M NH_3/0.5 M NH_4Cl as the diluent[5].

Prepare a standard stock solution of the metals by dissolving approximately 0.7 g of pure Ni metal along with 0.3 g of pure Cu metal in about 25 mL of 6 M HNO_3 followed by the addition of ammonia as in the preparation of the sample. Quantitatively transfer the solution to a 100 mL volumetric flask and dilute to the mark with water. Make four dilutions by transferring 2, 5, 7, and 10 mL of the standard stock solution to four 100 mL volumetric flasks and diluting to the mark with 0.5 M NH_3/0.5 M NH_4Cl.

Using TAST (current-sampled polarography), measure i_d for each wave of the polarogram for the standards and unknown. If necessary, add 0.2% Triton X-100

[3]Monel metal is approximately 70% Ni/30% Cu with trace amounts of several other elements, notably manganese and iron.

[4]The conversion of the copper(II) ion to the tetraminocopper(II) ion shifts the polarographic half-wave potential from +0.04 V to about -0.24 V (vs. SCE).

[5]Verify by calculation that the concentration of the Ni is about 6 mM and the Cu is about 2 mM after the second dilution.

to suppress current maxima. For the second wave, use the extrapolated diffusion current of the preceding wave as a base line. Calculate and report the percentage composition of the two metals in the Monel metal.

Determination of the Formula and Apparent Formation Constant of the Lead-Oxalate Complex Ion by Differential Pulse Polarography

Introduction

Voltammetric methods are important tools in investigating the formation of complex metal ions as well as other analyses such as determining the composition of a sample or studying electrode kinetics. This analysis is based on the fact that the characteristic *half-wave potential* ($E_{1/2}$) of a metal ion is shifted to more negative values (less thermodynamically favored) when the metal ion undergoes complex formation. The extent of the shift varies with the formation constant of the complex. By measuring the shift in the $E_{1/2}$ as a function of ligand concentration, it is possible to obtain information concerning both the formula and the stability of the metal complex.

In this experiment, differential pulse polarography (DPP) will be used to analyze solutions of aqueous Pb^{2+} and the Pb^{2+}-oxalate complex to determine the formula of the complex and the apparent formation constant, K_f, at a fixed ionic strength. The method described here can be extended to the analysis of a wide range of metal complexes and chelates.

Theory I: Measurement of Complex Formation

A short and not entirely rigorous derivation is given here to provide the necessary relationships. The analysis and derivation assume the formation of only one complex species over the entire concentration range of the chelon. For chelates this is often the case but is not always true for metal complexes.

The general equation for the reduction of a metallic ion is:

$$M^{n+} + n\,e^- \rightleftharpoons M \tag{1}$$

where, M^{n+} is the oxidized form and M is the reduced form of the metal. The reduced form of the metal need not be the neutral atom but is assumed as such in this discussion[1]. The corresponding Nernst expression is

$$E = E^\circ - \frac{RT}{n\mathcal{F}} \ln \frac{1}{[M^{n+}]} \tag{2}$$

The complexing agent, X, forms a complex with the metal ion as follows (assuming the ligand is neutral):

$$M^{n+} + pX \rightleftharpoons MX_p^{n+} \tag{3}$$

The formation constant expression is thus written

$$K_f = \frac{[MX_p^{n+}]}{[M^{n+}][X]^p} \tag{4}$$

Solving the K_f expression for $[M^{n+}]$ and substituting into the Nernst equation yields

[1]The concentration of the reduced form of the metal would be in the numerator of the *ln* term in the case of a non-neutral reduced form of the metal.

$$E = E° - \frac{RT}{n\mathcal{F}} \ln \frac{K_f [\mathrm{X}]^p}{[\mathrm{MX}_p]} \qquad (5)$$

By definition at standard state, the half-wave potential, $E_{1/2}$, is equal to the thermodynamic cell potential, $E°$.[2] At finite ionic strength, μ, and nonstandard-state conditions, the tabulated thermodynamic half-cell potential, $E°$, for the reduction of the metal may not be used; however, the $E_{1/2}$ measured under experimental conditions for the uncomplexed metal ion may be substituted for $E°$ (designated here as E'). The value of E' can be called the "apparent" thermodynamic half-cell potential and is the value measured voltammetrically using eq 2.

Using the experimentally determined E' at a fixed and known $[M^{n+}]$ and assuming that K_f is large such that the equilibrium concentration of the complex is equal to the analytical concentration of M^{n+}, eq 5 becomes

$$E_{\frac{1}{2},\mathrm{complex}} = E'_{\mathrm{M}^{n+}/\mathrm{M}} - \frac{RT}{n\mathcal{F}} \ln K_f [\mathrm{X}]^p \qquad (6)$$

Alternatively, if K_f is not large, a great excess of ligand can be used to force the equilibrium to the complex.

Using eq 6 it should become obvious that a plot of the measured values of $E_{1/2,\mathrm{complex}}$ for a set of solutions with constant $[M^{n+}]$ and varying amounts of X *versus* ln[X] should yield a straight line the slope of which equals $-pRT/n\mathcal{F}$. The intercept, where ln[X] = 0, would then evaluate[3] as

$$E_{\frac{1}{2},\mathrm{complex}} = E'_{\mathrm{M}^{n+}/\mathrm{M}} - \frac{RT}{n\mathcal{F}} \ln K_f \qquad (7)$$

Figure 1 shows a hypothetical series of normal pulse polarograms for the complexation of Pb^{2+} with complexing agent, X^-. Curve A is the polarogram of lead ion in the absence of complexing agent and curves B-D represent the waves observed in the presence of increasing $[X^-]$.

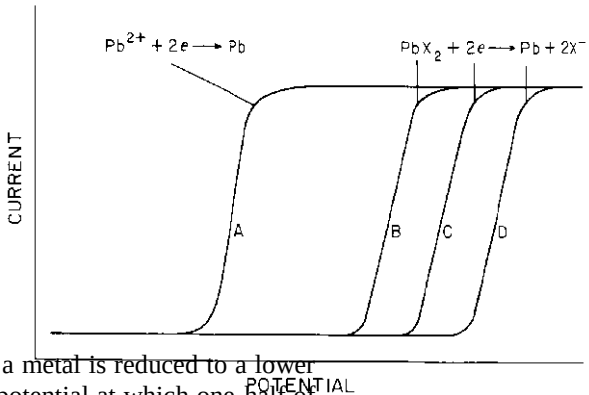


Figure 1. Hypothetical normal pulse polarograms for the reduction of A) Pb^{2+} ion in 0.1 M KNO_3 and B-D) Pb^{2+} ion in the presence of increasing ligand concentration, $[X^-]$.

Theory II: Differential Pulse Polarography

Differential pulse polarography (DPP) is similar to normal polarography in that the potential of the a

[2]This is easily rationalized in a system in which the oxidation state of a metal is reduced to a lower oxidation state rather than to the neutral metal as follows. $E_{1/2}$ is the potential at which one-half of all of the oxidized form of the metal with $n+$ charge reaching the electrode surface is reduced to the $(n-1)+$ state. Thus, at the electrode surface, $[M^{n+}] = [M^{(n-1)+}]$. When this equality is substituted into the Nernst equation written for the reduction

$$M^{n+} + e^- \; M^{(n-1)+}$$

assuming the diffusion coefficients are equal for the two ionic forms, the equality of $E_{1/2}$ and $E°$ becomes obvious. The theoretical reasoning is only slightly more difficult when the reduction of the ion to the neutral metal is considered. Further reading on this subject can found in many books on the theory and applications of electrochemistry. Only one of many references is Sawyer, D.T and Roberts, J.L. *Experimental Electrochemistry for Chemists*, Wiley-Interscience, 1974, Chap. 7.

[3]If log (common log) is used in place of *ln* (natural log), all "pre-log" terms must be multiplied by 2.303 (= ln10).

dropping mercury electrode is scanned; however, in DPP a short potential pulse (excitation pulse) is superimposed on the potential scan prior to mechanical detachment of the mercury drop. The excitation pulse is typically between 5-100 mV and the drop-time typically 0.5-5.0 s. A mechanical "drop-knocker" is often employed to mechanically strike the DME to dislodge the mercury drop. The timing sequence is shown Figure 2.

The current is sampled just prior to the excitation pulse and just prior to the detachment of the mercury drop. The difference in the two sampled currents, Δi (= i_2 - i_1), is recorded for each pulse. This technique results in improved detection limits over normal polarography due to the currents being sample late in the drop life thereby reducing the error from the capacitance current as well as elimination of the "zig-zag" recording due to the current being sampled throughout the lifetime of the mercury drop as in normal polarography. Figure 3 shows the differential pulsed polarogram and normal polarogram for tetracycline in 0.1 M acetate buffer. Note the difference in the concentrations.

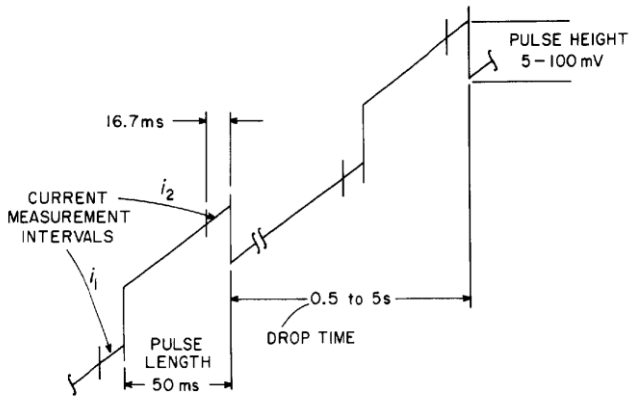


Figure 2. Differential pulse polarographic timing diagram. Based on Flato, J.B. *Anal. Chem.* 1972, *44*, 75A.

DPP can be used for quantitative analysis since Δi is a linear function of concentration of the electroactive species.

The voltage of the peak of the differential pulse polarogram, E_p, closely coincides with $E_{1/2}$ of normal polarography, the relationship of which is

$$E_p = E_{\frac{1}{2}} - \frac{\Delta E}{2} \qquad (8)$$

where ΔE is the excitation pulse voltage. When small excitation pulse voltages are used the resulting difference between $E_{1/2}$ and E_p are small. Notice that when ΔE is large the signal-to-noise ratio is also large but the error in $E_{1/2}$ is also large making the analysis of mixtures difficult.

The width of the wave is related to the electron stoichiometry, *n*. The peak width at one-half height is 90 mV for *n*=1 and 45 mV for *n*=2 when the pulse amplitude is small. Increasing pulse amplitudes also increase the width of the peak.

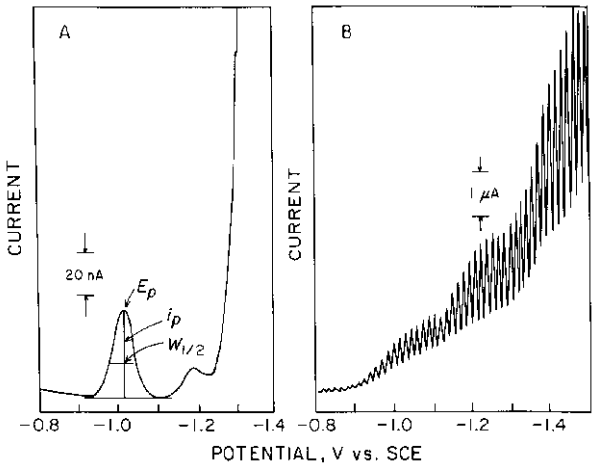


Figure 3. A) Differential pulse polarogram of 0.36 ppm tetracycline hydrochloride in 0.1 M acetate buffer, pH 4 obtained with a 1-s drop time and 50 mV excitation pulse amplitude. B) Normal dc polarogram of 180 ppm tetracycline hydrochloride in 0.1 M acetate buffer, pH 4. From Flato, J.B. *Anal. Chem.* 1972, *44*, 75A.

Procedure

Preparation of Stock Solutions

Prepare 100 mL of 0.020 M $Pb(NO_3)_2$ in a volumetric flask. If the solution is cloudy upon dissolution of the solid, add 1 drop of 6 M HNO_3 prior to diluting to the mark. Prepare 100 mL of 1.0 M potassium nitrate. The concentrations of the $Pb(NO_3)_2$ and KNO_3 should be known accurately.

Prepare 200 mL of 1.0 M potassium oxalate ($K_2C_2O_4$). If solid reagent is not available use the following procedure. Weigh out sufficient solid oxalic acid dihydrate to make 200 mL of 1.0 M oxalic acid into a 200 mL beaker. Add about 50 mL of water and 1 or 2 drops of phenolphthalein indicator and stir the mixture. Verify that it should require approximately 100 mL of 4 M KOH to completely react with the oxalic acid. Add 80 mL of 4 M KOH in 20 mL increments, allowing time between for the KOH to completely react and the any pink color to dissipate. If the solution gets hot to the touch, allow time for the solution to cool. After the addition of the 80 mL of KOH, carefully titrate (using a Pasteur pipette) the solution to a pale-pink phenolphthalein endpoint[4]. Avoid overshooting the endpoint. Allow the solution to cool to room temperature. Quantitatively transfer the solution to a 200 mL volumetric flask and dilute to the mark with water.

Preparation of Analytical Solutions

Obtain and number five 100 mL volumetric flasks. Into each flask, pipette 2.00 mL of the 0.020 M $Pb(NO_3)_2$ solution. Into each of the flasks also pipette the following reagents:

Flask	Reagent

[4]The quantity of phenolphthalein is so small that it will not affect the rest of the analysis.

1	10 mL of 1.0 M KNO_3
2	10 mL of 1.0 M $K_2C_2O_4$
3	20 mL of 1.0 M $K_2C_2O_4$
4	40 mL of 1.0 M $K_2C_2O_4$
5	80 mL of 1.0 M $K_2C_2O_4$

Dilute each to the mark with water and mix well. Calculate the concentration of the Pb^{2+} in the first flask and the concentration of the oxalate in the remaining flasks.

Analytical Procedure

Set up the voltammetric analyzer for differential pulse polarography (DPP) as described in lecture. Use a 20 mV excitation pulse and 1-s drop time. Scan no faster than 0.2 V/min, and the slower the better. Record the temperature with the thermometer near the instrument.

Place enough of the solution in the first flask into the electrolysis cell to cover the electrodes and degas with nitrogen for 10 minutes to remove dissolved oxygen. Record the differential pulse polarogram from -0.2 to -1.0 V (vs. SSC or SCE). Be careful to record the exact voltage at which the polarogram starts since accurate $E_{1/2}$ values are essential for this analysis. Repeat the measurement to verify that position of the peak remains constant. If the variability of the measurement is small, it is not necessary to make repeated measurements. If two DPP[5] waves are observed for the Pb^{2+}, one of the waves will be due to a current maximum. Add 0.2% Triton X-100 five drops at a time with mixing until the current maximum is removed or 25 drops have been added. Do not add a large excess and the same amount of the surfactant should be added to each solution for consistency.

Acquire the polarograms on the remaining solutions, rinsing and drying the electrodes between analytical solutions. The polarographic wave for the solutions containing the lead-complex will be more negative than the lead ion solution.

Calculations

The peak voltage, E_p, observed in the differential pulse polarogram corresponds to the $E_{1/2}$ of the normal dc polarogram[6]. Measure the E_p for every polarogram accurately, to the thousandth of a volt if possible. From the slope of the plot of $E_{1/2}$ vs. $\ln[C_2O_4^{2-}]$, determine the stoichiometry of the oxalate in the lead-oxalate complex. From the intercept and E' determined from the lead ion polarogram, determine the apparent formation constant, K_f, for the lead-oxalate complex at the experimental temperature. Rigorous error analysis is not necessary for this experiment.

Discussion Items

Explore the effects of changing the excitation pulse voltage, scan speed, and drop time (one at a time, of course). Describe the effects observed qualitatively and quantitatively. Explain why it is not necessary to know the potential of the

[5]It is easier to observe the presence of current maxima using TAST or normal pulse polarography.
[6]Recall that the peak potential is actually $E_{1/2} - \Delta E/2$ where ΔE is the excitation pulse height.

reference electrode accurately or even at all. What effect might be observed if the analysis was performed at pH 4?

Conductimetry: Analysis of Aspirin

Introduction

Solutions that contain many mobile ions conduct electric current well, and solutions that contain few or relatively immobile ions conduct electric current poorly. In this manner the conductance of solutions provides the analyst with another method by which changes in the composition of solutions can be detected and thus with another method of end point detection in a titration.

The conductance of a solution varies with the concentration, size, and charge of the ions and also with some characteristics of the solvent, such as viscosity. Thus, ions of different species would be expected to contribute differently to the total conductivity of a given solution, so that if one ionic species were replaced by another ionic species of different size or charge through a chemical reaction, a noticeable change in the conductivity of the solution would result.

More specifically, the conductance L of a solution can be represented by the expression

$$L = B\sum C_i \lambda_i |Z_i|$$

where *B* is a constant characteristic of the geometry and size of the conductance cell, *C* is the molar concentration of the individual ions in the solution, λ is the equivalent ionic conductance of the individual ions, and *Z* is the ionic charge for the individual ions.

Table 1 lists some equivalent ionic conductances, which illustrate the relative values for the various ions.

Table 1. Equivalent Ionic Conductance at 25°C

Cations	λ_+°	Anions	λ_-°
H_3O^+	349.8	OH^-	198
Li^+	38.7	Cl^-	76.3
Na^+	50.1	Br^-	78.4
K^+	73.5	I^-	76.8
NH_4^+	73.4	NO_3^-	71.4
Ag^+	61.9	ClO_4^-	68.0
$\frac{1}{2}Mg^{2+}$	53.1	$C_2H_3O_2^-$	40.9
$\frac{1}{2}Ca^{2+}$	59.5	$\frac{1}{2}SO_4^{2-}$	79.8
$\frac{1}{2}Ba^{2+}$	63.6	$\frac{1}{2}CO_3^{2-}$	70
$\frac{1}{2}Pb^{2+}$	73	$\frac{1}{2}C_2O_4^{2-}$	24
$\frac{1}{3}Fe^{3+}$	68	$\frac{1}{4}Fe(CN)_6^{4-}$	110.5
$\frac{1}{3}La^{3+}$	69.6		

The analyst makes use of the conductance of ionic solutions by devising a system so that the ionic species to be determined is replaced by another species of significantly different conductance. In this way, by following the changes in the conductance of the solution as the titration proceeds leads to a curve which has a break, bend, or discontinuity when the replacement is complete. This point is taken as the end point of the titration. Thus, in the titration of HCl by NaOH, the addition of the NaOH decreases the hydrogen ion concentration by the formation of water:

$$H^+ + Cl^- + Na^+ + OH^- \rightarrow H_2O_{(l)} + Na^+ + Cl^-$$

Figure 1a shows a theoretical titration curve, and Fig. 1b indicates how each of the ions contributes to the conductance. The shape of the titration curve can be predicted by summing the ionic conductances of the various species at any point during the course of the titration; the resulting summation gives the titration curve.

Although the hydrogen ions are replaced by sodium ions, the conductance of the solution is decreased because the sodium ion has a lower mobility than the hydrogen ion and thus conducts less current. After the equivalence point is passed, the addition of NaOH results in an increase in conductance because of the addition of the highly mobile hydroxyl ions. The end point of the titration is taken as the intersection of the two straight lines in Fig. 1a.

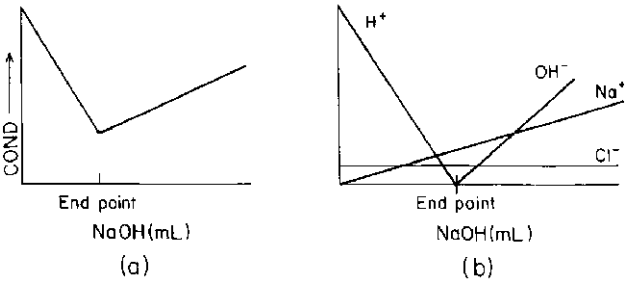


Figure 1. Theoretical conductimetric titration of HCl with NaOH. a) Observed titration curve. b) Relative contributions of each ion to conductance during the titration.

A conductance titration of any two substances is possible if a reaction occurs in which one ion is substituted for a second ion of different mobility either before or after the equivalence point. Thus not only may acids be titrated with bases but salts of weak acids may be titrated with strong acids. Precipitation reactions may also be followed conductimetrically.

The conductance of a solution is equal to the reciprocal of the resistance of the solution. For a column of solution *A* square centimeters in cross-sectional area (that is, the area of the electrode surface) between two electrodes *d* centimeters apart, the resistance *R* is given by

$$R = \rho \frac{d}{A} \tag{1}$$

where ρ is the specific resistance in ohm-centimeters. Therefore the conductance is given by

$$L = \frac{1}{R} = \kappa \frac{A}{d} \tag{2}$$

where κ is the specific conductance in Ω^{-1}-cm^{-1}. The quantity d/A is called the cell constant and is specific for a given conductance cell. It can be measured by determining the conductance for a cell containing an exact known concentration of a solution, usually potassium chloride. The equivalent conductance is equal to the conductance of a solution containing 1 mol of solute between electrodes separated by a distance of 1 cm. If *C* is the concentration of the solution in mol per liter, the volume of solution in cubic centimeters per mol is equal to 1000/C and the equivalent conductance Λ is described by the following equation:

$$\Lambda = \frac{1000\kappa}{C} \tag{3}$$

Substituting for κ gives

$$\Lambda = \frac{1000Ld}{CA} \tag{4}$$

so

$$L = \frac{A\Lambda C}{1000d} \tag{5}$$

If κ is known for a solution of concentration *C*, Λ can be determined from Eq. (3). Λ increases with decreasing concentration due to decreasing interionic attraction and repulsion forces. At low concentrations, for strong electrolytes, the increase in Λ with dilution is linear and

$$\Lambda = \Lambda^{\circ} - B\sqrt{C} \tag{6}$$

where Λ° is the equivalent conductance at infinite dilution, and *B* is a constant. The value of Λ° can be obtained by extrapolation to zero concentration of the line resulting a plot of measured equivalent conductances vs. $\sqrt{C}$ fit to Eq. (6). For weak electrolytes, an increase in the degree of dissociation occurs with increasing dilution, and Λ° may be obtained by the relationship

$$\Lambda^{\circ} = \lambda_{+}^{\circ} + \lambda_{-}^{\circ} \tag{7}$$

where λ_{+}° and λ_{-}° are the equivalent ionic conductances for the cation and anion, at infinite dilution, and

$$\Lambda = \lambda_{+} + \lambda_{-} \tag{8}$$

where λ_{+} and λ_{-} are the equivalent ionic conductances at a given concentration.

In actual practice of a conductimetric titration, the dilution of the sample solution by the titrant causes the observed conductance to be smaller than would be expected were the volume to remain unchanged during the titration. In order to correct for dilution, the observed conductance at each point should be multiplied by the ratio $(V + v)/V$, where *V* is the volume of the original solution and *v* is the volume of titrant added. Notice that this ratio effectively increases the measured value of *L* at all titrant volumes, *v*. The correction can be ignored by using a titrant at least 20 times more concentrated than the sample.

Procedure

Preparation of 0.01 M KHP

Prepare 200 mL of 0.01 M potassium hydrogen phthalate (KHP) by dissolving an appropriate amount of primary standard grade reagent in a volumetric flask and diluting to the mark with water.

Preparation of 0.01 M NaOH

Being careful to avoid pipetting any solid, transfer about 0.5 mL (graduated pipette or cylinder) of 50% NaOH solution to about 1 L of boiled deionized water in a plastic container. Mix thoroughly and keep the bottle closed except when transferring liquid.

Standardization of NaOH

Set up the conductivity meter as described in pre-lab lecture. Take care to not damage the conductivity cell and rinse the cell thoroughly between analyses.

Pipette 15.00 mL of the standard KHP solution into a plastic beaker followed by a measured amount of water necessary to completely cover the conductivity electrodes. Record the volume of water added. Since only relative conductivities are being measured, high precision in the water volume measurement is not necessary so a graduated cylinder may be used.

Stir the solution with a magnetic stirrer and titrate with NaOH in 0.5-1.0 mL increments. Record the conductivity after each addition, allowing sufficient time between additions for the system to equilibrate. No particular extra care need be taken around the end point; this is one of the primary advantages of conductimetric titrations. Repeat the standardization at least two additional times.

Correct each measured conductivity for dilution and plot the corrected-conductance values vs. volume of titrant added. Draw straight lines through the linear portions of the data[1] giving little weight to the data in the immediate neighborhood of end point. End point is determined by the intersection of the lines extrapolated until they meet. Calculate the molar concentration of the base.

Analysis of Aspirin

Weigh one aspirin tablet and transfer it to a 250 mL volumetric flask. Add about 15 mL of water and swirl until the tablet breaks up and is partially dispersed. Add about 30 mL of 95% ethanol and swirl again until the solid is finely dispersed. Dilute to the mark with water.

Pipette a 15 mL aliquot of the aspirin solution into the plastic beaker followed by a measured amount of water necessary to completely cover the conductivity electrodes. Titrate with the standard NaOH in 0.5 to 1.0 mL increments until end point has been past[2] by at least 5 mL. Repeat the titration at least two more times.

Plot corrected-conductance vs. volume of NaOH and determine the end point as before.

Calculations

Calculate the mass of acetylsalicylic acid in the original tablet. Calculate and compare the number of grains of aspirin in the original tablet. Report the values with confidence calculated from the repeated measurements.

Discussion Items

[1]Alternatively, perform a least-squares fit of the linear portions of the data. The volume obtained by setting the two fit equations equal to each other is the end point volume.

[2]Knowing that 1 g = 15.4 grains, verify that approximately 10-15 mL of titrant will be necessary to give a 5 mL excess.

Explain the shapes of the titration curves. Estimate the titration curve for the titration of 0.01 M KHP with 0.01 M NaOH based on the equivalent conductances for each ion.

Do the results of this analysis necessarily represent the aspirin content of the tablets in the bottle? Why or why not?

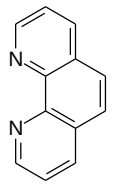
What experimental modification could be made to negate the necessity of performing a dilution correction? Determine the relative percentage error in the concentration of the NaOH if the dilution correction were neglected in this analysis?

Spectrophotometric Methods of Analysis

Determination of Iron with 1,10-Phenanthroline

Introduction

The reaction between Fe^{2+} and 1,10-phenanthroline (phen) to form a red complex of $Fe(phen)_3^{2+}$ serves as a good sensitive method for determining iron. The molar absorptivity of $Fe(phen)_3^{2+}$ is 11,100 at 508 nm. The intensity of the color is independent of pH in the range of 2-9. The complex is extremely stable and the iron remains in the +2 oxidation state for very long periods of time.



1,10-phenanthroline

The iron must be in the +2 oxidation state, hence a reducing agent is employed. Tin(II) ion is an acceptable reducing agent, however, hydroxylamine hydrochloride can also be used. The reaction of Fe(III) with hydroxylamine is

$$2\ Fe^{3+} + 2\ NH_2OH + OH^- \rightarrow 2\ Fe^{2+} + N_2 + H_2O$$

The pH is adjusted and moderately buffered to a value between 6 and 9 by the addition of sodium acetate. The $Fe(phen)_3^{2+}$ complex obeys Beer's law.

Procedure

Preparation of Solutions

Dissolve (with heating, if necessary) 0.1 g of 1,10-phenanthroline monohydrate in 100 mL of deionized water.

Dissolve 10 g of hydroxylamine hydrochloride in 100 mL of deionized water.

Dissolve 10 g of sodium acetate in 100 mL of water.

Weigh to the best precision possible 0.07-0.11 g of pure iron(II) ammonium sulfate. Dissolve the solid in water and transfer to a 1 L volumetric flask. Carefully add ~2.5 mL of concentrated sulfuric acid and dilute to the mark. This is a standard stock solution.

Preparation of Standard Dilutions

Make *at least* 5 dilutions of the standard stock solution. At the very least, make the following dilutions. Into five 100 mL volumetric flasks, pipette 1, 5, 10, 25, and 50 mL aliquots of the standard stock solution. To each flask, add 1 mL of the hydroxylamine solution, 10 mL of the 1,10- phenanthroline solution, and 8 mL of the sodium acetate solution. Dilute to the mark in each flask with water. Allow the solutions to stand for 10 min to develop the color.

Prepare a blank by placing 50 mL of water in a 100 mL volumetric flask, adding reagents as above, and diluting to the mark.

Preparation of the Unknown Solutions

Obtain a numbered vial containing a weighed (but unknown) amount of iron(II) ammonium sulfate and prepare as the standard solution above. Make a single dilution of between 1 and 50 mL into a 100 mL volumetric flask. Prepare the dilution identical to the standard dilutions.

Dissolve 0.1 g of iron ore in 6 M HCl, heating gently. Cover with a watch glass and boil to near dryness to remove most of the HCl. Quantitatively transfer to a 1 L volumetric flask, add H_2SO_4, and dilute to the mark.

Prepare a standard dilution of the solution into a 100 mL volumetric flask. Prepare the complex as above.

Preparation of the Standard Curve

Turn on the Spectronic 20 spectrophotometer and allow it to warm up for 15 min. Obtain 2 matched cuvettes. Dedicate one cuvette as the reference (blank solution) and the other to sample. Wipe the outside of the cuvette to remove fingerprints, etc.

Using the blank as the reference and any one of the iron solutions prepared above, measure the absorbance at wavelengths from 400 to 600 nm at an interval of not more than 25 nm. In the region of maximum absorbance (minimum transmittance), decrease the wavelength interval.

Plot the absorbance vs. wavelength and draw a smooth curve through the points. Select the proper wavelength to use for the analysis based on the visible light spectrum.

Using the selected wavelength, measure the absorbance of each standard solution and the unknown(s). You should repeat the measurements at least once (twice is better) and average. Check the 0.0 au reference occasionally.

Calculations

Data tables should have concentrations of all species after dilution. Prepare a Beer's law plot from the absorbance vs. concentration data. Determine the equation of the line from linear regression. From the absorbance and dilution data, calculate the original amount of iron(II) ammonium sulfate in the vial. From absorbance and dilution data, calculate the %Fe in the iron ore.

Discussion Items

What is the purpose of the acid added in the beginning?
How was the buffer formed? What is the approximate pH of the buffer?

Determination of the Formula of a Complex by the Method of Continuous Variations

Introduction

The *method of continuous variations* is a simple and widely used method for the determination of the formula of a complex. It may also be used to determine the formation (stability) constant, K_f, for the complex. It is reliable, though, only when a single complex is formed -- it will not yield useful information in the study of stepwise complex formation.

In the method of continuous variations, the total concentration, *C*, of metal, M, and complexing agent (ligand, L) is held constant,

$$C_M + C_L = Constant \qquad (1)$$

and only their ratio is varied. A wavelength is selected where only the complex absorbs and the ligand and metal do not or, at least, only weakly. Thus, over the mole fraction range, *X*, of 0 to 1, metal or ligand is in excess except at the mole fraction corresponding to the complex formula. At this mole fraction, absorbance is maximized (prove this); at any other *X* absorbance will be lower (see Figure 1).

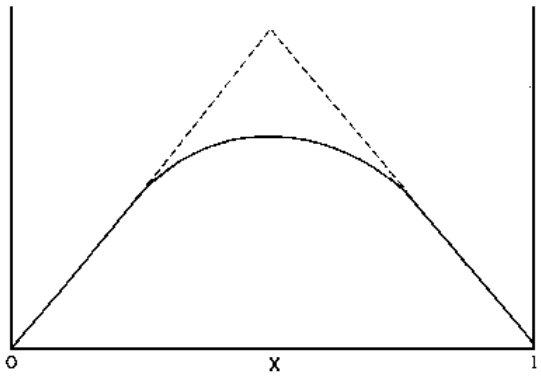


Figure 1. Continuous-Variations Plot

If K_f is not large, then at the optimal mole fraction the absorbance will be somewhat lower than the absorbance expected for the pure complex. The intersection of the lines extrapolated from the straight-line portions of the curve give the absorbance of the complex assuming no dissociation. It is this feature of continuous variations that makes it possible to calculate K_f for the complex.

Theory

For a 1:1 complex represented by the formation reaction

$$M + L \rightleftharpoons ML$$

the ratio of true absorbance, *A*, to the extrapolated absorbance, A_{ext}, is the mole fraction of the complex formed:

$$X_{ML} = \frac{A}{A_{ext}} = \frac{[ML]}{C} \quad (2)$$

Where C is the concentration of complex assuming no dissociation and [ML] is the equilibrium concentration of the complex. Thus,

$$[ML] = \frac{A}{A_{ext}} C \quad (3)$$

and

$$[M] = C_M - [ML] = C_M - \frac{A}{A_{ext}} C \quad (4)$$

$$[L] = C_L - [ML] = C_L - \frac{A}{A_{ext}} C \quad (5)$$

Neglecting any pH effects on K_f, the equilibrium expression is written

$$K_f = \frac{[ML]}{[M][L]} \quad (6)$$

Substituting eqs 2-4 into eq 5 yields[1]

$$K_f = \frac{(\frac{A}{A_{ext}})C}{[C_M - \frac{A}{A_{ext}} C][C_L - \frac{A}{A_{ext}} C]} \quad (7)$$

It is important to realize that stability (formation) constants are usually quite dependent on ionic strength, μ. Stability constants are, therefore, often determined at high and constant ionic strength (often in the form of a pH buffer) and reported at that ionic strength.

In this analysis, the formula and formation constant of the iron(III)-sulfosalicylate[2] complex will be determined.

Procedure

Prepare the following solutions:

1 L of 0.1 M $HClO_4$

100 mL of 0.01 M iron(III) chloride in 0.1 M $HClO_4$ (Fe)

100 mL of 0.01 M sulfosalicylic acid in 0.1 M $HClO_4$ (SSA)

Prepare the following mixtures in 50 mL volumetric flasks. Dilute each to the mark with 0.1 M $HClO_4$.

[1]In the case of, for example, a 1:3 complex, C_M = C and C_L = C where C_M and C_L are *analytical concentration* of metal and ligand, respectively. Of course, *equilibrium concentration* of each will be much smaller than this.

[2]Other iron(III) complexes may be studied instead of the sulfosalicylate complex.

Flask	Volume (mL) Fe	Volume (mL) SSA
1	1	9
2	2	8
3	3	7
4	4	6
5	5	5
6	6	4
7	7	3
8	8	2
9	9	1
10	10	0

Allow the solutions to stand for ~30 min to establish equilibrium. Measure the absorbance spectrum for each over a suitable wavelength range using 0.1 M $HClO_4$ as the blank. Select an appropriate wavelength for quantitative analysis[3].

Prepare 5 solutions similar to the above containing only iron. Construct a Beer's law plot of absorbance at the analytical wavelength(s) used above vs. Fe(III) concentration.

Tabulate the absorbances, corrected absorbances, and mole fractions iron, X_{Fe}. For each sample where iron is in excess, subtract the calculated absorption due to excess iron, if necessary. Make a plot of corrected absorbance vs. X_{Fe} and determine the formula of the complex.

Construct tangent lines along the straight-line portions of the corrected curve and determine the absorbance at the intercept (A_{ext}). From A_{ext} and actual absorbance, A, at the same X determine the concentration of the complex. Calculate the value of K_f for the complex. If necessary, derive the correct formula for the determination of K_f. Compare the value obtained to the literature value[4], at the same ionic strength if possible.

Discussion Items

What is (are) the purpose(s) of the 0.1 M $HClO_4$? How could the analysis be modified to determine the formation constant, K_f, at infinite dilution?

What is the structure of sulfosalicylic acid and the expected structure of the chelate?

Justify that the maximum absorbance will occur at the mole fraction corresponding to the formula of the complex. What conditions are necessary for the application of the method of continuous variations to be valid?

[3]If you are using a nonscanning instrument, you may wish to perform the analysis at several wavelengths, especially if one can be found which has no interference due to Fe(III).

[4]*Lange's Handbook of Chemistry* is one source for formation constants.

What other method might be used to determine the formula of a complex? What method(s) would be best suited to the determinations of stepwise formation constants? Why?

What happens to the shape of the curve as K_f becomes smaller? ... larger? Why?

Determination of the Manganese in Steel

Introduction

Manganese can be determined spectrophotometrically after oxidation to permanganate with potassium periodate:

$$2\ Mn^{2+} + 5\ IO_4^- + 3H_2O \rightarrow 2\ MnO_4^- + 5\ IO_3^- + 6\ H^+$$

The purple permanganate ion absorbs strongly at 525 nm and the absorption of permanganate obeys Beer's law. The addition of a small excess of periodate maintains the manganese in the +7 oxidation state thus avoiding the formation of MnO_2.

The analysis of alloys containing manganese (e.g. steel) present problems which must be dealt with either chemically or instrumentally. Iron(III) absorbs weakly at the same wavelength so phosphoric acid is added to form the nearly colorless iron-phosphate complex. Nickel, cobalt, and chromium all exhibit some absorbance at 525 nm. The interferences from these last two ions may be minimized in one of several ways:

1. The addition of the same amount of these ions to the standards as are in the sample. This, of course, requires prior knowledge of the composition of the sample for these ions.

2. Prepare the reference blank identically to the sample without the oxidation of manganese with periodate.

3. Use the method of *standard additions*.[1]

Matrix-matching of the standards to the sample as described by method 2 is used in this analysis. The standard solutions will be prepared from a sample of steel of known composition. After appropriate treatment of the sample, the visible light spectrum from 400 to 600 nm will be determined and the calibration curve prepared at the wavelength of maximum absorption.

Procedure

Preparation of Standard Solutions

From the manganese content of the standard steel sample, calculate the amount of steel necessary to give an absorbance of about 0.5 to 1 when the sample is dissolved, the manganese is converted to MnO_4^-,and the resulting solution diluted to 250 mL. The molar absorptivity of permanganate is 2360 $M^{-1}cm^{-1}$ at 525 nm.

Weigh to the best precision possible the amount of standard steel calculated above. Dissolve the sample in ~50 mL of 6 *M* HNO_3 with gentle heating and finally boiling to remove the oxides of nitrogen. Remove from the heat and add ~1 g of

[1] The method of standard additions is a convenient and powerful way to perform an analysis when matrix matching is not possible.

ammonium persulfate[2]. Boil for a further 10-15 min to decompose the excess persulfate. If a precipitate forms or the solution is imparted with even the faintest color of permanganate, add a small amount of sodium sulfite (10-20 mg should be sufficient, add more as necessary[3]). Continue boiling to remove sulfur dioxide.

Dilute the solution with ~50-100 mL of water, add 10 mL of 85% H_3PO_4 (concentrated). Allow the solution to cool, quantitatively transfer to a 250 mL volumetric flask, and dilute to the mark. Calculate the concentration of the manganese in the standard solution.

Calculate the volumes of the standard solution necessary to make 4 dilutions (not exceeding 100 mL each) with the lowest concentration one-tenth or less the standard concentration.

Into 4 labeled beakers, pipette the calculated amounts of standard solution and increase the volume with addition of deionized water. Take care not to add so much water that a quantitative transfer to the volumetric flask will not be possible. To each beaker, add about 0.15 g of potassium periodate[4] and heat the solutions to nearly boiling to allow the reaction to go to completion (3 min is sufficient). Quantitatively transfer the solutions to the appropriate size volumetric flask and dilute to the mark.

Beer's Law Calibration Curve

Use untreated standard steel solution as the reference blank. With the spectrophotometer set to 525 nm, determine the absorbance of each of the standard solutions. Plot the data as absorbance vs. concentration manganese and determine the equation of the best-fit straight line.

Analysis of Sample

Weigh a sample of steel whose manganese content is to be determined. Prepare the sample as for the standard steel sample. Make a dilution which will nearly insure that the absorbance of the sample will be bracketed by the standards.

Measure the absorption of the solution and determine the concentration of the manganese from the equation of the least-squares fit calibration line. Calculate the %Mn in the steel. If the absorbance exceeds the calibration data, perform a standard dilution.

Report and Discussion

Report the %Mn in the steel sample. As part of the discussion, explore the use and effects of transition metals in steel alloys.

[2]Ammonium persulfate is added to oxidize any carbon present.

[3]Sulfite converts the manganese ion to the +2 state.

[4] Potassium periodate is often labeled potassium meta-periodate.

Spectrophotometric Analysis of a Complex Mixture

Introduction

The analysis of a mixture of compounds is often difficult by optical methods if the UV-visible spectra overlap appreciably. For example, both potassium dichromate and potassium permanganate absorb strongly in the visible and ultraviolet region. Their spectra overlap sufficiently so that the presence of one interferes with the quantitative analysis of the other. Accurate analysis of the mixture is, however, possible by employing multiwavelength techniques.

If a solution is a binary mixture of two components in which the optical spectra only partially overlap, only two wavelengths are necessary for a complete analysis. The determination of the molar absorptivities of both pure components at both wavelengths followed by the measurement of the absorbance of the mixture leads to 2 equations with 2 unknowns – an algebraically simple system of equations to solve. In the analysis of a ternary system, 3 wavelengths are necessary. As the number of components in the mixture increases, it becomes increasingly difficult to analyze for the concentration of the components.

Multicomponent Analysis (MCA) allows an analyst to analyze for many components in a mixture as long as the components of the mixture are known and spectra of each pure component can be obtained. MCA can be performed "by hand" but lends itself well to automation by computer. The spectrum of each component with known concentration is obtained and stored in computer memory. Being digitally stored, the molar absorptivity at each wavelength interval over a wide range of wavelengths is determined by the program for each component. Finally, the spectrum of the solution of the complex mixture is sampled and stored. The program then, using least squares fitting or other error minimization technique, reconstructs the spectrum of the sample from the spectra of the pure components. The calculated concentrations of each component necessary to reconstruct the sample spectrum are reported by the computer in the units selected by the operator.

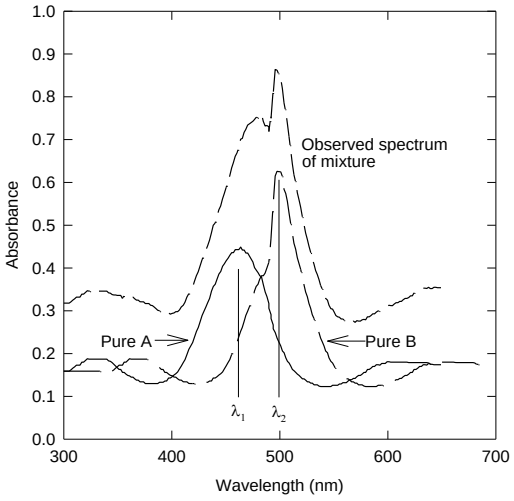


Figure 1. Absorption spectra of pure compounds A and B and the observed absorption spectrum of a mixture of A and B.

In the analysis to be performed, the amount of $KMnO_4$ and $K_2Cr_2O_7$ in a binary mixture will be determined by two-wavelength analysis. This type of analysis could be used in, for example, the simultaneous analysis of Mn and Cr in steel.

Theory

In the absence of molecular interactions of the absorbing species in a mixture, the observed absorption spectrum at a given wavelength for the mixture is the sum of the absorbance contribution of each species. Thus, the observed spectrum of a mixture of the two species in Figure 1 would be a wavelength-by-wavelength sum of the absorbances due to each species. This being the case, it is possible to analyze for both species in solution almost simultaneously by measuring the absorbance of the mixture at two wavelengths (λ_1 and λ_2 in Figure 1). If the molar absorptivity of each component is known, then the concentration of each component can be determined algebraically. The following is a derivation of the Beer's law analysis of a two-component mixture. A 1.0 cm pathlength sample cell will be assumed for all equations.

The Beer-Lambert law for a single component is

$$A = \varepsilon l C \tag{1}$$

Where A is the observed absorbance for concentration C, ε is the molar absorptivity, and l is the pathlength of the sample cell. The quantity εl is a constant at a given wavelength and can be denoted as k.

For a solution containing two absorbing species, the absorbance at a given wavelength is

$$A_\lambda = A_A + A_B \tag{2}$$

Which can be rewritten

$$A_\lambda = k_A^\lambda C_A + k_B^\lambda C_B \tag{3}$$

Since this is an equation with 2 unknowns (C_A and C_B) it is necessary to take measurements at 2 wavelengths.

$$A_1 = k_A^1 C_A + k_B^1 C_B \tag{4}$$

$$A_2 = k_A^2 C_A + k_B^2 C_B \tag{5}$$

Then by the solution of two simultaneous equations, the concentrations of species A and B can be calculated. The values of k are determined from the slopes of the calibration plots prepared at the wavelengths of interest.

It should be clear that the spectra must have sufficient "non-overlap" so that only one species is the primary contributor to the observed absorbance at a given wavelength. It should also be evident that better accuracy would be achieved if multiple wavelengths were chosen and concentrations determined by multiple rather than two equations[1].

[1]Simultaneous determinations by this method can be done on mixtures containing up to 4 components. It is only necessary to have as many standards as there are components and to use at least as many wavelengths as components present. When 3 or more components are present, it is normally better to solve the simultaneous equations by matrix methods.

Procedure

Preparation of Standard Solutions

Prepare 100 mL of 3-7 x 10^{-3} M $K_2Cr_2O_7$ by a suitable dilution of a stock solution. The concentration of this dilution should be known to the best possible precision. Use 0.5 M H_2SO_4 as the diluent.

Prepare 100 mL of 3-7 x 10^{-3} M $KMnO_4$ by a suitable dilution of a stock solution as above. Use 0.5 M H_2SO_4 as diluent also. The standard $KMnO_4$ should contain ~0.2% KIO_4 added as a stabilizer; no additional precautions need be taken to prevent decomposition.

Prepare four dilutions of the standard solutions. Into four 100 mL volumetric flasks, pipette 2, 5, 7, and 10 mL of the $K_2Cr_2O_7$. Dilute with 0.5 M H_2SO_4 to the mark. Repeat with the $KMnO_4$ standard solution. Calculate the concentrations to the appropriate precision.

Analysis of Standards

Rinse and fill one cuvette of a matched pair with 0.5 M H_2SO_4 (**Reference**). Rinse and fill the other with the most concentrated dilution of $K_2Cr_2O_7$ (**Sample**). Measure and plot the absorption spectrum from 200-700 nm against the reference. Rinse and fill the sample cuvette with the most concentrated dilution of $KMnO_4$. Measure and plot the absorption spectrum on the same page with the same scale over the same wavelength range (Disable autoscaling if necessary).

Select two wavelengths for analysis. Scan the remaining standards and obtain absorbance readings for each dilution at both wavelengths. Plot the absorbance vs. concentration (Beer's law plot) at both wavelengths for both solutions. Calculate by the method of least-squares, the molar absorptivities, ε, of both anions at both wavelengths.

Analysis of the Unknown Mixture

Prepare 100 mL of the unknown based on the reported %Cr and %Mn using 0.5 M H_2SO_4 as the solvent. If necessary, make a more concentrated sample solution and perform a standard dilution. Measure and plot the absorbance spectrum from 200-700 nm as above. If the maximum absorbance in the region of interest is above 3.5 au, perform a standard dilution of the unknown solution and rerun the spectrum. Determine the absorbance at the two analytical wavelengths.

Calculations

Calculate the quantities of the $KMnO_4$ and $K_2Cr_2O_7$ in the original dilution. Report the number of percentage of each in the original sample to the appropriate significance. Estimate and report the precision of your answer. Report the measured ε values.

Discussion Items

What would be the effect on the analysis if the two absorbing species interacted chemically with each other (chemical noise)? How would pH changes effect this analysis?

Optional

Determine the equilibrium constant for the reaction

$$2\ CrO_4^{2-} + 2\ H^+ \quad Cr_2O_7^{2-} + H_2O$$

Prepare $Cr_2O_7^{2-}$ standard solutions ranging in concentration from 0.5 to 5 mM using primary standard grade $K_2Cr_2O_7$ and 0.5 M H_2SO_4 as the solvent. Prepare CrO_4^{2-} standard solutions in the same concentration range using 0.1 M NaOH as the solvent. Obtain the spectra of each anion at each concentration.

Prepare a solution of about 5 mM $K_2Cr_2O_7$ in water but, prior to dilution, adjust the pH of the solution with either acid or base until a color change is just observed. If too much acid or base is added, shift the equilibrium back with base or acid. Quantitatively transfer to a volumetric flask and dilute to the mark with water. Measure and record the pH of the equilibrium solution just prior to analysis.

From the spectrum of the mixture, determine the equilibrium concentration of each chromium species. Using the measured pH, calculate the apparent equilibrium constant. If the ionic strength can be approximated, determine the activity coefficients of each species and calculate the thermodynamic equilibrium constant. Finally, compare the value(s) obtained to one found in the literature or, alternatively, calculate the thermodynamic equilibrium constant from other thermodynamic quantities such at $E°$ values. Consult an appropriate text for the relationship of $E°$ and K.

Another Optional

Perform the simultaneous analysis on another sample such as an Fe(II)/Fe(III) mixture, metals in steel, Monel metal, Cu/Zn in brass[2], etc.

[2]This is an interesting problem since Zn^{2+} has no absorption band in the UV-visible above 200 nm. It is possible however to make a colored zinc solution by use of the appropriate organic ligand. Other methods are better for Zn analysis than optical spectroscopy.

Spectrophotometric Determination of the pK_a of an acid-Base Indicator

Introduction

The pK_a of an acid-base indicator may be determined spectrophotometrically. The absorbance vs. pH curve can be analyzed in a similar fashion to titration curve. Alternatively, since pH indicators usually have 2 or more distinct colors determined by the pH of the solution, it is possible to use methods similar to those used in the analysis of complex mixtures to determine the equilibrium concentrations of the indicator species in solution. It is necessary, however, to analyze only one wavelength because of the stoichiometric relationship of the two forms of the indicator in solution. It is critical that the pH of the indicator solution be known accurately so buffered solutions are used.

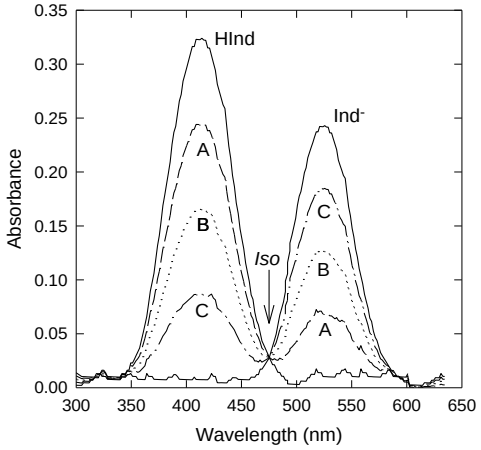


Figure 1. Spectra of an acid-base indicator in solutions of varying pH. *HInd* is the spectrum in 0.1 M HCl and *Ind*$^-$ is the spectrum in 0.1 M NaOH. A) -0 .5 pH units from the p*K*; B) pH = p*K*; C) +0.5 pH units from the p*K*. All spectra are of 0.1 mM indicator.

Theory

The absorption spectrum of the indicator can be determined by UV-visible spectrophotometry. Then from absorbance data, the p*K* of the indicator determined. Assume the equilibrium to be

$$\text{HInd} \quad \text{Ind}^- + \text{H}^+$$

Since the concentration of HInd and Ind$^-$ are coupled by stoichiometry, it is possible to analyze the sample at only one wavelength. The absorbances at low and high pH are due entirely to the HInd and Ind$^-$, respectively. The absorbance at the intermediate pH values will be due to a mixture of HInd and Ind$^-$, so

$$A = \varepsilon_{\text{HInd}} \times l \times [\text{HInd}] + \varepsilon_{\text{Ind}} \times l \times [\text{Ind}] \qquad (1)$$

and

$$C_{\text{HInd}} = [\text{HInd}] + [\text{Ind}] \qquad (2)$$

C_{HInd} is the analytical concentration and l is the pathlength. Rearranging (2), substituting into (1), and assuming a 1 cm cuvette,

$$[\text{HInd}] = \frac{A - \varepsilon_{\text{Ind}} C_{\text{HInd}}}{\varepsilon_{\text{HInd}} - \varepsilon_{\text{Ind}}} \qquad (3)$$

Figure 1 shows spectra obtained for an acid-base indicator HInd at various pH values. The dashed lines represent the spectra of HInd in 0.1 M HCl (left) and Ind$^-$ in 0.1 M NaOH (right), both at the same concentration (0.1 mM). Spectrum A is the observed spectrum 0.5 pH units more acidic than the p*K* of the indicator, spectrum B is at a pH exactly equal to p*K*, and C is the spectrum obtained when the

pH of the solution is buffered at 0.5 pH units more basic than p*K*. All spectra are taken on samples of identical concentration as the spectra of the pure compounds. The point at which all of the curves intersect (labeled *iso*) is called the *isosbestic point*. The isosbestic point contains no information concerning the p*K* of the indicator but does obey Beer's law and can be used to determine the total concentration of the indicator in solution regardless of pH.

Procedure

Weigh to the best precision possible 0.050-0.1 g of an acid-base indicator into a 100 mL volumetric flask. Add a few mL of ~0.1 M NaOH to dissolve. If the indicator does not immediately go into solution, add ethanol until the solid is dissolved. Dilute to the mark with water. Calculate the concentration and label this as the "stock" solution. Make a dilution of the stock solution by pipetting 1.00 mL of the stock into a 50 mL volumetric flask.

Record the absorption spectrum of the dilution from 300-700 nm using water as the reference solvent. If any absorption in the spectrum exceeds 2 au, determine the dilution of the stock necessary to keep the absorbance below 2 au. All subsequent dilutions will be made to this concentration.

Transfer a ~25 mL quantity of the stock indicator solution to a small beaker. Add acid dropwise until a color change is just detected. Determine this pH; three pH buffers will be prepared around this value.

Prepare two standard dilutions, one in acid and one in base, as follows. Pipette 1 mL of the stock solution into a 50 mL volumetric flask and dilute to the mark with water[1]. Make a similar dilution, diluting to the mark with 0.1 M hydrochloric acid. Calculate[2] the amounts of 0.1 M H_3PO_4 and 0.1 M K_2HPO_4 or 0.1 M K_2HPO_4 and 0.1 M KH_2PO_4 solutions needed to make 3 buffer solutions ranging in pH of about ±1 units around the color-change pH.

For example:

1 mL of indicator + 5.0 mL $H_2PO_4^-$ + 1.0 mL HPO_4^{2-} diluted to 50 mL

$$pH = 7.2 - \log \frac{5.0\ \text{mL} \times 0.1M}{1.0\ \text{mL} \times 0.1M} = 6.5$$

Add the appropriate amounts of each reagent to the 25 mL volumetric flask and dilute to the mark with water. Measure the pH of the solution just prior to spectroscopic analysis.

Record the absorption spectrum from 300-700 nm for the pure HInd and the pure Ind^-. Select two wavelengths -- one at or near λ_{max} for the low pH solution (HInd) and one at or near λ_{max} for the high pH solution (Ind^-). Determine the molar absorptivities, ε, at the selected wavelengths from concentration and absorbance[3].

[1]If the 1:50 dilution is too concentrated, use the appropriate dilution ratio.

[2]The Henderson-Hasselbach equation is convenient for this calculation. The pH of the buffer will not be exactly the calculated pH.

[3]Using one concentration and one absorbance reading to calculate ε is essentially calculating a point-slope from a single point on the Beer's law calibration graph. It should be immediately apparent the pitfalls of this type of calibration. A high level of accuracy in the standard solution is necessary to achieve accurate analytical results. Except for situations in which exacting results are

Record the absorbance spectra for each of the buffered solutions. Determine the absorbance at the two selected wavelengths.

Analysis of Data

Determine p*K* from each spectrum in which the pH was buffered using one or both of the wavelengths. Report an average value with precision and temperature (since K_a is temperature dependent). Show your spectra and derivation of your working equation (eq 3). If you derive equation 3 differently, the equation may look different but will be numerically equivalent.

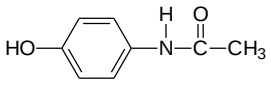
not required or where it is being used as a "calibration check", the one-point calibration should be avoided.

Multicomponent Drug Analysis by UV-Visible Spectroscopy

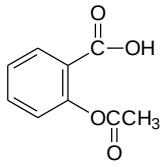
Introduction

It is necessary to evaluate content uniformity and stability of pharmaceutical products to protect the general public. Many prescription and nonprescription drugs are mixtures of active and inactive compounds, often making the analysis of the pharmaceutical product difficult. High Performance Liquid Chromatography (HPLC) is one excellent method for analyzing multicomponent mixtures; however, UV-visible spectroscopic techniques can be successfully applied to multicomponent mixtures if spectral overlap of the components is minimal. In fact, even this last restriction has been partially overcome by using multiple linear regression, principle component regression (PCR), and full spectrum quantitation (FSQ) with spectral preprocessing in the Fourier domain. PCR can be used to quantitate multicomponent samples with impurities, components with strongly overlapping spectra, and even components which interact chemically.

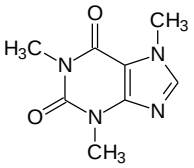
The analysis to be performed here is the quantitation of the components of a mixture containing acetaminophen, aspirin, and caffeine found in an over-the-counter analgesic. In addition, any salicylic acid present due to the degradation of the aspirin will be determined. For a formulation to be acceptable, each component must fall within 85-110% of label claim[1]. Likewise, the salicylic acid content of an aspirin-containing analgesic must not exceed the aspirin content by more than 3%[1].



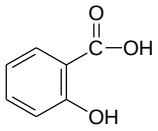
4-acetamidophenol



Acetylsalicylic acid



Caffeine



Salicylic acid

Theory

A detailed description of multicomponent analysis can be found in the literature[2]. In essence, the number of standards or mixture of standards must be equal to or greater than the number of components in the mixture. Over-specification of the number of standards increases the accuracy of the analysis. Rather than selecting an equal number of analytical wavelengths as components, a range of wavelengths is used. The wavelength range must include analytically useful wavelengths for each standard and about 10-20% baseline. The component concentration range must, of course, bracket the range expected in the unknowns to assure accurate quantitation. Close bracketing increases the accuracy in the final result.

Calibration can be validated by analysis of a prepared set of mixtures with component concentrations different than those used for calibration. Once the calibration has been validated, samples can be analyzed quickly.

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[1]*The United States Pharmacopeia XXII - The National Formulary XVI* (United States Pharmacopeial Convention, Rockville, MD, 1991).

[2]Donahue, S.M.; Brown, C.W.; Obremski, R.J. *Appl. Spectrosc. 42*, **1988**, 353-359.

Procedure

All standard samples, validation mixtures, and samples are to be prepared in water, methanol, glacial acetic acid (69:28:3 by volume).

Standards

Prepare at least 4 standard solutions each of acetaminophen[3] and aspirin[4] ranging in concentration up to 20 μg/mL. Prepare standard solutions of caffeine and salicylic acid ranging up to 5 μg/mL. Make no more than 100 mL of each standard to preserve reagents and work at the maximum precision possible.

Validation Mixtures

Prepare a 3-component validation mixture which contains all components except salicylic acid and a 4-component validation mixture which contains all components. All concentrations should be in the bracket range of the standards.

Samples

Weigh three to five tablets of an analgesic[5] together. Determine the average mass of tablet. Lightly grind[6] the tablets together into a powder then weigh the equivalent of one tablet into a small beaker. Add a small quantity of solvent (water:MeOH:HOAc 69:28:3) to dissolve the components. Undissolved material is the "binder" which can be removed by gravity filtration through a "medium" or "fast" filter paper. Rinse the filter paper quantitatively with solvent, keeping all washings. Quantitatively transfer the clarified filtrate into a 100 mL volumetric flask and dilute to the mark with solvent. Based on the "label" masses of drug content, make a 1:100 dilution or the dilution necessary of the sample to get the concentrations of each component bracketed by the standards. If desired, make replicate samples from the powdered tablets.

Spectroscopic Analysis

Turn on the HP8452A diode-array spectrophotometer optical bench and the computer. If not already active, start Windows™ and after the optical bench completes its internal diagnostics (the "Busy" light will go out), start the spectrophotometer software. The login window will open shortly. Your name as it will appear on analytical print-outs is necessary; however, no password is required and none should be given. Allow the instrument to warm-up for 20-30 minutes prior to quantitative analysis.[7]

Note: It is important that the spectrometer software not be started until the "Busy" light is extinguished on the spectrometer. The spectrometer software communicates through the optical bench's GPIB interface which is not active until

[3]4-Acetamidophenol

[4]Acetylsalicylic acid

[5]such as Excedrin Extra Strength Pain Reliever (Bristol-Myers)

[6]It is not necessary to grind the tablets to a fine powder and, in fact, doing so may promote the decomposition of the aspirin to salicylic acid by the introduction of moisture.

[7]The spectrometer will operate during this time, however, and is useful time to explore the software, setup the method, etc.

the optical bench internal diagnostics are completed. If the software is started prior to completion of the diagnostic routines, the computer and optical bench will not be able to communicate with each other. ***In this event, turn off the spectrometer, terminate the spectrometer software, and start over.***

A feature of the HP8452A spectrometer is that it is possible to acquire all of the standard spectra in one spectrum file (a "standards" file) then acquire samples, analyzing each individually or as a block of spectra.

The remaining instructions given here on operating the instrument are supplements and reminders of the instructions given in laboratory lecture[8].

Dedicate one of two matched cuvettes as a reference containing the solvent. The other cuvette will be used for all samples. Between samples, rinse the sample cuvette twice with small quantities of the next solution. When changing solutions, rinse an additional time to avoid cross-contamination. Acquire a "blank" spectrum after every group of standards, every 30 minutes, or whenever results obtained are erratic or outside of expected confidence.

Acquire the standard spectrum (**Measure Standard**) of the first standard solution. Fill in the data windows with the appropriate identity, concentration, etc. Some of the same data must be typed into two different windows. Acquire and annotate the spectra of the remaining samples. It is possible, though not necessary, to acquire the spectra of all standards of all four compounds into the same file. It is recommended that the standard spectra of each compound be saved to a disk file then cleared before starting the next compound. Once all of the standards have been acquired, the standard spectra of each compound may then be loaded (**File Load Standards**) together for the analysis[9].

When all spectra are acquired and loaded as the standards (whether loaded from disk or acquired *en masse*), set up the method for multicomponent analysis (MCA) from the **Method Data-Analysis Evaluation** pull-down menu. From the wavelength "setup", set the "range" of wavelengths around 240-400 nm. Verify from your data the actual wavelengths to be used, remembering to include sufficient baseline. It is not necessary nor desirable to include too much baseline.

From the **Method** window, select **Calibrate**. The calibration will take place using the standards loaded in the Standard Spectra window and the parameters set in the **Data-Analysis** menu. The calibration may take awhile especially if the wavelength range selected is large. The results of the calibration should be printed.

Once the calibration is complete, the method is ready for validation. Place the validation solution in the sample cuvette and **Measure** the spectrum to **Sample**. The spectrum of the sample will be displayed in a sample window. Select **Method Analyze**. When the MCA analysis of the sample spectrum is complete, the results

[8]If all else fails, try reading the manual entitled *Getting Started With Your Quantitation Software* and *Using Your Quantitation Software*.

[9]If you intend to store the spectra for later retrieval (*highly* recommended), use a floppy disk. All spectra will then be recorded on the A: floppy drive.

will be displayed in 3 windows. The first window is the reconstructed spectrum of the sample. Along with the reconstruction will be a plot of the residuals of the errors[10]. The results window will display the calculated concentrations of each component in the mixture. Compare the calculated results with analytical concentrations. Print the results.

Clear the sample spectrum (**Edit Clear**) and repeat the validation with the second standard mixture[11]. If the results of the two validations are not satisfactory, determine the appropriate action necessary to achieve acceptable analytical results.

Scan and analyze the unknown sample. If replicates are being determined, simply record the spectra of all samples then analyze once. Report the percentage difference of the average content of each component from the product label. Account for any deviation greater than ±10%.

Optional

I. Explore the effect of the number of standards on the final results. Start with one standard spectrum of each component. If the standards have been stored on disk, simply load them into the standard spectra window then delete all but one of each standard. Calibrate the method and reanalyze the two validation spectra and the sample(s). Clear the standards, then use 2 spectra for each component, and so on. Discuss the accuracy and precision obtained based on the data obtained.

II. Contact the Hewlett-Packard Corporation, Technical Service, for information on the method used by their system for multicomponent analysis. To get you started: their headquarters office is in Van Nuys, California.

Discussion Items (not necessarily a complete list)

What problems would be encountered by using *too little* or *too much* baseline in the FSQ analysis?

How would the data be affected if, between collection of standards and samples, instrument conditions changed such that the background spectrum was no longer valid but was not rescanned?

Why is this method satisfactory up to about 6 components. Assume the average absorption band is ~100 nm FWHM.

[10]The residuals spectrum will be displayed only if that selection is turned on in "method display" parameters. The default setting is "on" unless changed by a previous operator.

[11]Actually, it is not necessary to clear the spectrum before proceeding. The first sample spectrum will simply be reanalyzed with the second validation solution.

Determination of the Spectrochemical Series

Introduction

Many transition metal complexes are highly colored due to the presence of unfilled d-orbitals. In the free atom or ion, it is usually assumed that the d-orbitals are degenerate. In complexes of the transition elements, the negative charge or negative end of the ligand is electrostatically attracted to the metal ion. This is the force holding the ligand to the metal. However, there is electrostatic repulsion between the bonding electron pairs of the ligand and the d-electrons. It is the magnitude of this repulsion which determines the amount by which the d-orbitals lose their degeneracy – called the *crystal-field splitting*. Often the difference in energy between the non-degenerate orbitals is such that electronic transitions within the d-orbital manifold is in the visible light.

In this experiment, the crystal-field splitting created by each of six different ligands on a transition metal ion will be measured. From the crystal-field splitting data, the spectrochemical series for the six ligands will be developed.

You will also develop a spectrochemical series for several metal ions and correlate the experimentally derived series to the ion's d- orbital configuration and nuclear charge.

Procedure

Initially prepare all solutions at a concentration of ~2 x 10^{-4} M and dilute if necessary. In some cases, it may be necessary to make more concentrated solutions then perform a standard dilution.

Preparation of the aquo complexes

Dissolve an appropriate amount of the chloride, nitrate, or sulfate salt of the transition metal with water in a volumetric flask. Acidify the solution with a small quantity of concentrated nitric acid to prevent hydrolysis then dilute to the mark.

Preparation of the chloro complexes

Dissolve an appropriate amount of the chloride, nitrate, or sulfate salt in 12 M HCl (CAUTION). If necessary, make a more concentrated stock solution and dilute a aliquot. Determine the formula of the chloride complex from an appropriate reference book.

Preparation of the amino complexes

Dissolve the salt in a small amount of water. When solution is complete, add concentrated NH_3 until the color of the solution remains constant in color with additional NH_3. Add a small (few mL's) excess of the reagent. If the metal hydroxide forms, continue adding NH_3 until it dissolves. Transfer to the volumetric flask and dilute to the mark with water. If while diluting, the equilibrium shifts back towards the aquo- complex, add additional NH_3 and continue diluting to the mark. Determine the formula for the complex.

Preparation of the thiocyanato complexes

Dissolve the metal salt in water. Determine, from any available reference book, the coordination number for thiocyanate for your metal ion. Calculate the amount of KSCN necessary for complete reaction. Add an excess of KSCN if K_f for the complex is not large.

Preparation of the cyanido complexes

CAUTION: Do not add KCN to <u>any</u> solution containing <u>any</u> acid. HCN gas will be formed. HCN is a poisonous gas. If in doubt, check the acidity with litmus paper.

Dissolve the metal salt in water. Avoid any addition of acid. Look up the coordination number for CN^- for your M-CN complex. Calculate the amount of KCN necessary to form the complex. Add the KCN and a small excess if necessary. Dilute to the mark with water.

Preparation of the ethylenediamine (en) complexes

Dissolve the metal complex in water. From knowledge of the coordination number for the metal, calculate the amount of en necessary to completely react with the metal. From mass of en necessary, determine and pipette the equivalent volume[1].

Spectral Analysis

Using the UV-visible spectrophotometer of your choice, record the visible light absorption spectrum of each of your solutions[2]. You may which to overlay the six spectra or record them separately. From each spectrum, determine λ_{max}. From absorption at λ_{max} calculate ε.

Using the spectra, qualitatively relate the color of the solution with the absorption spectrum.

From λ_{max}, calculate (in kJ [per atom]) the crystal-field splitting of the d-orbitals. Order the ligands form low crystal-field ligand to high crystal field ligand. Compare your spectrochemical series with that found in an inorganic or spectrochemical analysis text.

Using the data from other analysts in the laboratory, develop the spectrochemical series for the metal ions[3].

Discussion Items

[1]Alternatively, add en dropwise to the solution until no further color change is observed. Add a small excess.

[2]What are the visible wavelengths? You may wish to go into the UV region a little.

[3]From data collected in the laboratory, there are six series for each metal.

What factors make a ligand a low or high crystal-field ligand? What factors influence the spectrochemical series of the metal ions (keeping the ligand constant)? What determines the value of for a complex? Using what you now know, place EDTA in the spectrochemical series. You may perform the experiment to verify if you would like.

Determination of Acetylsalicylic and Salicylic Acid in Aspirin by Fluorescence Spectroscopy

Introduction

Fluorescence is the emission of light by a molecule which has absorbed a electromagnetic radiation. The emitted light is usually at a longer wavelength (lower energy) than the excitation energy.

An electron can be excited from singlet ground state, S_o, to a singlet excited state, S_1, by the incident radiation if the molecule has an electronic transition of the same energy as the wavelength selected. The electron may be excited to higher vibrational states in S_1 as dictated by the vibrational wavefunction overlap (refer to the Jablonski diagram from lecture). Because of collision with the solvent, etc. the electron will vibrationally relax to the lowest vibrational state in S_1. The electron can now return to S_o by (1) emission of light at a longer wavelength (fluorescence), (2) emission of heat or other non-radiative energy loss, or (3) cross over to T_1 via intersystem crossing[1] with the subsequent slow emission of light (phosphorescence). The lifetime of the electrons in S_1 before fluorescence is about 10^{-8} s while the lifetime of the electrons in T_1 is on the order of 10^{-4} s. *Fluorescence quenching* (S_1 deactivation) can be realized by the presence of certain ions or molecules.

In fluorometry, the intensity of fluorescent light detected, *F*, is a function of the radiant power of the source, P_o, and the concentration of the fluorescing species, *c*:

$$F = P_o(1 - 10^{-\varepsilon lc})Q_f k \qquad (1)$$

In dilute solution ($c < 0.01$ M), F can be approximated

$$F = P_o(2.303\ \varepsilon lc)Q_f k \qquad (2)$$

Where ε = molar absorptivity
l = path length
Q_f = quantum efficiency = photons emitted/photons absorbed
k = photons measured/photons emitted

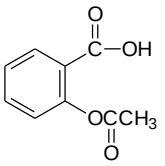
It is clear from eq 2 that a plot of *F* vs. *c* should be a straight line. Extremely dilute samples can be analyzed because of the high sensitivity of the method. In fact, fluorescence analysis is among the most favored instrumental techniques for the trace analysis of many organic compounds. However, in extremely dilute solutions, care must be taken to avoid conditions which would give extraneous background emission (e.g. suspended particles or other light scatterers) or suppression of the fluorescence (e.g. quenchers, etc.).

Fluorometers are available in two types: (1) the filter fluorometer, where the source light is made nearly monochromatic by interference filters and the detected

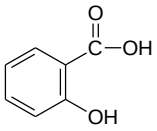
[1]Intersystem crossing is rare and phosphorescence has historically had little analytical use.

fluorescence wavelength is selected by similar filters, and (2) the spectrofluorometer, where the source beam and sample emission wavelengths are selected by independent monochromators. The spectrofluorometer has a decided advantage over the filter fluorometer in that scanning is possible. Scanning makes the selection of excitation and emission wavelengths fast and simple.

In this analysis, acetylsalicylic acid (ASA) and salicylic acid (SA) will be determined by fluorometry. A scanning spectrofluorometer will be used to select the appropriate wavelengths for sample excitation and emission. A calibration plot of emission intensity versus concentration will be constructed from standard solutions and a pain reliever sample analyzed.



Acetylsalicylic acid



Salicylic acid

Procedure

Standard Solutions

Prepare about 1 L of 1% acetic acid in chloroform (1% HOAc/$CHCl_3$). Use this solution for all dilutions. The acetic acid improves the quantum efficiency of the ASA and SA fluorescence. Using the 1% HOAc/$CHCl_3$ as solvent, prepare 100 mL stock solutions of the following:

2.22×10^{-3} M ASA (0.40 g/L)
5.43×10^{-3} M SA (0.75 g/L)

It may be necessary to make a more concentrated stock solution and perform a standard dilution. Prepare 1:100 dilution standards of each of the stock solutions to give

2.22×10^{-5} M ASA (4.0 μg/mL)
5.43×10^{-5} M SA (7.5 μg/mL)

Pipette 5, 10, 15, and 20 mL of the 4.0 μg/mL ASA standard into 25 mL volumetric flask, successively. Dilute to the mark and identify the flasks.

Likewise, pipette 5, 10, 25, and 20 mL of the 7.5 μg/mL SA standard into 25 mL volumetric flasks and dilute to the mark.

Weigh 3-5 aspirin tablets and grind to an extremely fine powder. Using the average weight of a single tablet, weigh a mass of about 1 tablet into a 100 mL volumetric flask (weighing paper can be used). Dissolve and dilute to the mark. The binder will not dissolve so gravity filter rapidly through a "fast" paper, such as Whatman #1. This solution must not be allowed to stand for more than 1 h from the time of dissolution or the analysis of ASA will be low[2].

[2]The acid hydrolysis of ASA is fairly facile. While there should be little water in the solvent, there is probably enough to undergo reaction with the ASA.

The aspirin solution can be used "as-is" for SA determination. The ASA concentration must be reduced by a factor of 1000 for analysis. Do three successive dilutions of 10 mL diluted to 100 mL.

Analysis

Salicylic Acid

Set up the instrument as described. Set the emission monochromator to around 450 nm. Set the range of the excitation monochromator to scan from 250 to 350 nm. Zero the instrument with solvent as the blank. Place the most concentrated SA standard in the cuvette holder. Scan the excitation monochromator slowly to obtain the optimum excitation wavelength. Holding the excitation wavelength constant, scan the emission monochromator slowly from 400 to 500 nm.

Analyze the SA standards, recording emission intensity for each concentration. Repeat the series of standards 3 times. Analyze the aspirin solution. Take 3 replicate readings.

Acetylsalicylic acid

Determine the optimum monochromator settings as before. The excitation wavelength is between 250 and 300 nm and the emission wavelength is between 300 and 350 nm.

Analyze the ASA standards as before. Determine the fluorescence emission of the 1:1000 dilution.

Report the amount of ASA and SA in mg per aspirin tablet. Compare with the value reported on the bottle.

Discussion Items

1. Discuss why the emission spectra of these molecules in condensed phase are broad while the emission spectra of elements (such as copper or nickel) in atomic absorption or emission are narrow.

2. Show the structure of ASA and the reaction that converts ASA to SA. Research the efficacy of SA as a pain reliever.

Determination of the Critical Micelle Concentration of Sodium Dodecyl Sulfate Using a Fluorescent Probe

Introduction

Fluorescence is the emission of light by a molecule which has absorbed a electromagnetic radiation. The emitted light is usually at a longer wavelength (lower energy) than the excitation energy.

An electron can be excited from singlet ground state, S_0, to a singlet excited state, S_1, by the incident radiation if the molecule has an electronic transition of the same energy as the wavelength selected. The electron may be excited to higher vibrational states in S_1 as dictated by the vibrational wavefunction overlap (refer to the Jablonski diagram) and appropriate selection rules. Because of collision with the solvent, etc., the electron will vibrationally relax to the lowest vibrational state in S_1. The electron returns to S_0 by (1) emission of light at a longer wavelength (fluorescence), (2) emission of heat or other non-radiative energy loss, or (3) cross over to T_1 via intersystem crossing[1] with the subsequent slow emission of light (phosphorescence). The lifetime of the electrons in S_1 before fluorescence is about 10^{-8} s while the lifetime of the electrons in T_1 is on the order of 10^{-4} s and longer. *Fluorescence quenching* (S_1 deactivation) can be realized by the presence of certain ions or molecules.

In fluorometry, the intensity of fluorescent light detected, P, is a function of the radiant power of the source, P_o, and the concentration of the fluorescing species, c:

$$P = P_o(1 - 10^{-\varepsilon lc})\Phi_f k$$

In dilute solution ($c < 0.01$ M), P can be approximated

$$P = P_o(2.303\varepsilon lc)\Phi_f k$$

Where ε = molar absorptivity
l = path length
Φ_f = quantum efficiency = photons emitted/photons absorbed
k = photons measured/photons emitted

It is clear from eq 2 that a plot of P vs. c should be a straight line. Extremely dilute samples can be analyzed because of the high sensitivity of the method. In fact, fluorescence analysis is among the most favored instrumental techniques for the trace analysis of many organic compounds. However, in extremely dilute solutions, care must be taken to avoid conditions which would give extraneous background emission (e.g. suspended particles or other light scatterers) or suppression of the fluorescence (e.g. quenching agents, etc.).

Fluorometers are available in two types: (1) the filter fluorometer, where the source light is made nearly monochromatic by interference filters and the detected

[1]Intersystem crossing is rare and phosphorescence has historically had little analytical use.

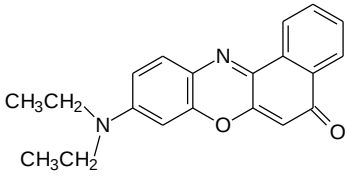
fluorescence wavelength is selected by similar filters, and (2) the spectrofluorometer, where the source beam and sample emission wavelengths are selected by independent monochromators. The spectrofluorometer has a decided advantage over the filter fluorometer in that scanning is possible. Scanning makes the selection of excitation and emission wavelengths fast and simple.

Background

Soaps and detergents are in a class of compounds known as surfactants (*surf*ace *act*ivators). Surfactants normally are long-chain hydrophobic molecules with an ionic hydrophilic moiety at one end and work by forming *micelles*. In aqueous solution, a micelle consists of an aggregation of the molecules into a sphere with the hydrophobic "tails" pointing into the sphere and the hydrophilic "heads" facing out to the solvent. Because of the nonpolar region of the micelle, aqueous solutions of soaps and detergents are able to dissolve normally water-insoluble compounds such as oils.

In dilute solution (that is, below the critical micelle concentration), the surfactant is non-aggregated and most resembles a solution of unassociated molecules. At the critical micelle concentration (**CMC**) micelle formation begins and a micellar colloid forms. Micellar colloids have been used as model systems of biological systems such as the lipid bilayer present in the cell membrane, for understanding the properties of detergents, and have been used in extraction and purification.

The **CMC** is an important property of any surfactant and has been measured in a variety of ways including conductimetrically and by surface tension techniques. Unmatched in sensitivity, however, is the use of a fluorescent "probe" which has increased solubility in nonaqueous media characterized by the core of the micelle. The method exploits the enhanced fluorescence of the probe normally exhibited in nonaqueous solutions. The fluorescence intensity of the probe thus serves as an indicator of molecular environment in which the fluorescent molecule is found. Probe concentrations must be kept low so as to not alter the properties of the system being studied and, often, the fluorescent molecules are only slightly soluble in water. Fluorescence spectroscopy, with its intrinsic sensitivity, is highly suited to studying these systems.



Nile Red

In this experiment[2,3], the phenoxazone Nile Red will be used as the fluorescent probe to study the micelle formation of sodium dodecyl sulfate (**SDS**). **SDS** is a very common detergent used in household formulations as well as in techniques such as gel electrophoresis. The concentration of the probe will be held constant in solution at about 30 μM and the concentration of the **SDS** varied from about 0 to 20 mM. A plot of relative fluorescence intensity vs. **SDS** concentration yields a sigmoidal curve qualitatively similar to a titration curve. The inflection point of the curve is taken as the **CMC**.

[2]For similar references see Rujimethabhas, M.; Wilairat, P. *J. Chem. Educ.* **1978**, *55*, 342 and
[3]Goodling, K.; Johnson, K.; Lefkowitz, L.; Williams, B.W. *J. Chem. Educ.* **1994**, *71*, A8.

Procedure

Carefully prepare 1-L of 30 μM solution of Nile Red (**NR**). Start with a more concentrated stock solution (make much less of the stock) and perform a standard dilution. If necessary, in preparing the more concentrated stock solution use a small amount of methanol to dissolve the solid, then dilute to the mark with water. This solution will be denoted as "labeled deionized water."

Prepare 500 mL of 0.05 M (50 mM) solution of sodium dodecyl sulfate. Use labeled deionized water to dissolve the SDS. Being a detergent solution, it will foam is shaken vigorously, therefore, agitate gently when dissolving the solid.

Obtain eleven 25 mL volumetric flasks. Into the flasks, successively pipette[4] 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 mL of the **SDS** solution. Dilute to the mark with labeled deionized water and gently mix. Calculate the millimolar concentration of the **SDS** in each flask. Place the flasks in the dark for about 1 hour to allow for adequate time to establish labeled micelles. These solutions remain stable for up to about 3 days if stored in the dark.

Analysis

Set up the spectrofluorometer as described in pre-lab lecture. Use deionized water as the blank and use the most concentrated **SDS** solution to determine the optimum excitation and emission wavelengths[5]. Be sure to use a manually fixed display gain rather than an automatic gain setting so that all measurements are consistent. Determine the relative fluorescence of each solution including the labeled deionized water.

Plot relative fluorescence vs. concentration **SDS**. Using techniques similar to those used to graphically determine equivalence point in a potentiometric titration, determine the **CMC**. The literature value, determined conductimetrically, is 8.1 mM at 25°C[6].

Alternatively, use the equation for a sigmoidal curve and determine the best nonlinear fit of the equation to the data and from the equation determine the **CMC**. The equation for a sigmoid is

$$f(x) = \frac{a}{1+e^{-b(x-c)}} + d \qquad (3)$$

where

f(*x*) is relative fluorescence,
a is the fluorescence range,
b is the slope at inflection point,
c is the CMC, and,
d is the minimum fluorescence.

[4]Alternatively, use a class-A burette.

[5]Nile Red has a excitation wavelength around 550 nm and emission wavelength maximum around 630 nm.

[6]Mukerjee, P.; Mysels, K.J.; *Critical Micelle Concentrations of Aqueous Surfactant Systems*; NSRDS-NBS *36*, U.S. Dept. of Commerce: Washington, 1971.

Use the computer to optimize the constants a, b, c, and d. Since four variables are being optimized simultaneously, it may be necessary to constrain certain variables to a specific range of values to avoid "local minima" during the optimization.

Optional

Determine the **CMC** of another pure detergent-type molecule such as stearic acid. Compare hydrophobic chain-length to the **CMC**.

Discussion Items (not a complete list)

Discuss why the emission spectra of molecules in condensed phase are broad while the emission spectra of elements (such as copper or nickel) in atomic emission are narrow.

Atomic Absorption Spectroscopy

Introduction

Atomic spectroscopy is used for the quantitative analysis of trace levels of around 65 metallic elements in all types of materials and solutions. In atomic absorption spectroscopy (AAS), measurement is made of the radiation absorbed by ground state atoms in the vapor state. Flame emission spectroscopy (FES) is used to measure the emission of radiation from atoms excited above ground state by the flame. If the flame temperature (~2300°C for air-acetylene) is higher energy than an atomic transition, then flame emission techniques are preferred over atomic absorption. However, few elements have strong electronic transitions at temperatures obtainable in air-acetylene flames, making atomic absorption more often the preferred method.

At the much higher energies of the spark and plasma sources (e.g. inductively-coupled plasma, 8000-10000 K), emission methods are used almost exclusively. This convenience comes at the expense of more complex emission line spectra and more complex and expensive instrumentation to adequately resolve and distinguish the many lines. There are other flameless methods available; for example, the graphite furnace and flameless Hg-vapor analysis.

The source in atomic absorption must produce a narrow band of light corresponding to an absorption energy of the gas phase atom. The ordinary monochromator is incapable of delivering a line as narrow as an atomic absorption band. To obtain a monochromatic source beam of narrow bandwidth, the hollow-cathode lamp (HCL) is utilized. The cathode of the HCL is constructed of the same metal as is to be analyzed, placed in a glass envelope with a quartz window, and filled with argon or neon at low pressure. The inert gas is ionized when a voltage is applied across the anode and cathode. The inert gas ions strike the cathode, causing it to sputter. The sputtered metal atoms are excited by collision with the argon ions and emit light when returning to ground state. Some cathodes are made of 2 or more metals, making them multielement sources. Since there is less individual metal present in the cathode, the lifetimes of the multielement HCL's are often shorter than single-metal sources.

A critical part of the AA instrument is the sample introduction system to deliver solution to the flame and its vaporization and atomization to ground state atoms. In the typical flame atomizer, all or part of a solution is sprayed as a mist into the flame. Often the fuel-oxidant gas mixture is used to aspirate the solution into the flame. There are two basic types of burner systems: (1) total consumption burner, in which fuel and oxidizing gas come through separate passages at the base of the flame with the sample aspirated by one of the gases, and (2) the premix, or laminar flow, burner in which sample mist, fuel and oxidant are mixed prior to arrival at the burner head.

The source beam is monochromated after the flame by a conventional grating monochromator. Single-beam and double-beam configurations of optics are available. Accessories are available to eliminate signal due to background emission such as deuterium lamp emission background correction and Zeeman

background correction. The absorbed intensity obeys Beer's law as in molecular spectroscopy.

Interferences in AA are few since most compounds are completely atomized in the flame. However, metal oxides and some other ionic compounds, such as refractory phosphates and hydroxides, are not completely decomposed to atoms which leads to deviations from Beer's law due to a fewer number of atoms in the flame. Other interferences include:

- Light scattering by solids and unvaporized solvent
- Ionization of the atoms due to flame temperature
- Background absorption

Factors affecting the amount of sample which reaches the flame include:

- rate of aspiration of the solution
- viscosity of the analytical solution: *high viscosities affect flow rate, causing the solution to move more slowly through the aspirator.*
- solvent choice: *some organic solvents improve aspirator efficiency and may also increase the absorbance.*
- surface tension of the solvent: *Lower surface tensions allow smaller droplet size and more sample to reach the flame.*

Ideally, the calibration standards are prepared in a matrix similar to that of the sample solution. Concentrations which can be analyzed by AAS are typically limited to less than about 50-100 μg/mL (50-100 ppm). Above 50 ppm most elements deviate from Beer's law significantly but can be analyzed if calibration *curves* are employed.

When standards cannot be prepared identical to the unknown sample matrix, the ***method of standard additions*** should be used. For example, in the analysis of nickel in a coinage metal alloy, a known amount of nickel is added to the sample[1] so that it contains, say, 10 μg/mL additional nickel. To another portion of sample, Ni is added to increase the concentration by 20 μg/mL, and so on. The absorbance of each solution, including the untreated solution, is measured and a plot of absorbance versus concentration of added nickel is constructed. The straight line extrapolated to the x-axis intercept indicates the amount of Ni present in the original sample. An added benefit of standard additions is that, if desired, only one standard addition sample need be prepared. It is possible to derive a simple relationship between the absorbances of the two solutions (that is, the original and "spiked" solution) and the concentration of the original solution. A disadvantage of standard additions is that the spiked and unspiked samples must obey Beer's law.

[1]Sometimes called "spiking" the sample.

Procedure

Preparation of Stock Solutions

Solutions of metal ions with concentration known with high accuracy are often made from the pure metal.

Transfer about 100 mg of clean copper shot[2] to a small beaker. Add enough 0.001-0.1 M HNO_3 to cover the shot. With occasional stirring, allow the copper to remain in the acid for a few minutes. This treatment will remove surface oxidation from the metal.

Filter the shot over a course sintered-glass crucible. Wash the metal with deionized water. Wash the solid with acetone and continue suction to dry the metal. If the copper metal is to be stored for an extended amount of time, it should be placed in a sample vial to protect it from air. It is not advisable to dry the freshly cleaned metal in the oven since this will speed the reoxidation of the metal.

Clean the nickel metal by the same treatment.

Weigh to the best precision possible 50.0 mg of the clean and dry copper metal into a 250 mL beaker. Add about 50 mL of 6 M nitric acid, cover with a watch glass, and heat the mixture to dissolve the metal. When the metal has completely dissolved, boil the solution to remove the oxides of nitrogen. Rinse the watch glass with deionized water. Quantitatively transfer the solution to a 500 mL volumetric flask and dilute to the mark with water. Calculate the concentration in mg Cu/1000g solution (ppm) assuming the density of the solution is 1.00 g/mL.

Prepare a solution of 100 ppm Ni similarly.

Preparation of the Solution of an Unknown Metal Alloy[3]

Accurately weigh a sample of Monel metal of 0.1 g. Place the sample into a beaker, add 30 mL of 6 M HCl and warm on a hot plate (do not boil). After the metal has been allowed to react for several minutes, add 20-30 mL of 6 M HNO_3, cover with a watch glass, and heat to a gentle boil to complete the dissolution of the alloy and drive off the nitrogen oxides. Cool, wash down the watch glass, and quantitatively transfer the sample to a 500 mL volumetric flask and dilute to the mark with water. Transfer a 10 mL aliquot to an acid cleaned 250 mL volumetric flask and dilute to the mark with 1% HNO_3[4].

Analysis

Select either analysis by calibration or by standard additions. Optionally, compare the results of the analysis performed by both methods.

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[2]Alternatively, pure copper foil or wire may be used.

[3]If the sample is not Monel metal, use the approximate percentages given or assume the sample is approximately 50% in each metal.

[4]The sample is ~30-70% in each metal so the concentration needs to be lowered to less than 50 mg/mL. The second dilution achieves this desired concentration. 1% HNO_3 is used to keep the pH low to prevent hydrolysis of the metal.

Calibration Method

Preparation of Standards

Prepare 4 dilutions of 1, 2.5, 5, and 10 μg/mL Cu. This can be best accomplished by pipetting 10 mL of the 100 ppm stock solution into a 100 mL volumetric flask and diluting to the mark with 1% HNO_3. The resulting solution is nominally 10 μg/mL. You may wish to make a larger quantity of the 10 μg/mL solution. From the 10 μg/mL solution, transfer 10, 25, and 50 mL aliquots to separate 100 mL volumetric flasks and dilute. Prepare similar dilutions of the nickel solutions. If the standards are to be stored for longer than a few hours, transfer them to acid-cleaned plastic bottles.

Analysis by the Calibration Method

Into 6 disposable beakers place one sample each of 1% HNO_3 (blank), each Cu standard, and the unknown solution. Set up the instrument for copper analysis as described in lecture. Aspirate the blank and set the 0.0 A on the instrument (ZERO). Aspirate the least concentrated dilution and record the absorbance. Repeat on the remaining standards. Aspirate a small amount of blank solution between each sample, wiping off the siphon tube between samples. Aspirate the unknown last. Perform three repeats of standards and unknown.

Set up the instrument for Ni and repeat with the nickel standards and the unknown solution. When finished, aspirate deionized water to clean the premix burner and flow-spoilers.

Construct Beer's law plots for the Cu and Ni. From the Beer's law plots and dilution data, determine the %Cu and %Ni in the alloy. The values may not add to 100% due to impurities present in the alloy.

Standard Additions

Analysis by Standard Additions

Prepare a standard 1 μg/mL solution of Cu and a similar solution of Ni. To a 5 mL aliquot of the solution of Monel metal in a disposable beaker, add 2 mL of the 1 μg/mL Cu standard and 3 mL of 1% HNO_3. Repeat, on 5 mL aliquots of the unknown, with 3, 4, and 5 mL of the 1 μg/mL stock solution. Dilute each of the samples with enough 1% HNO_3 to give 10 mL total. Prepare one solution which is not "spiked" but is diluted with 1% HNO_3. Calculate the increase in Cu concentration in each beaker. Set up the instrument for Cu analysis as described in lecture, zero the instrument on 1% HNO_3, and measure the absorbance for each solution. Aspirate a small amount of blank solution between each sample, wiping off the siphon tube between samples. Repeat with the nickel standard. When finished, aspirate deionized water to clean the premix burner and flow-spoilers.

Plot absorbance versus concentration of added metal. Determine the unknown metal concentration from the measured or computed x-axis intercept. From dilution data and original sample mass, calculate %Cu and %Ni in the original sample. The values may not add to 100% due to impurities present in the alloy.

Discussion Items

1. The premix burner has been known to explode due to the aspiration of air from the solvent drain. What has been done to prevent this? Why not simply use the total consumption burner to avoid this?

2. Metal oxides often cause low responses in AAS. What other anions cause poor response? Why?

3. Explain why atomic absorption spectra in the flame are so sharp whereas molecular spectra in solution phase are very broad.

4. Discuss the differences in AAS, FES, and inductively-coupled plasma spectroscopy.

Fourier Transform Infrared Spectroscopy - FT-IR

Reading: *Theory of FT-IR*, Nicolet, July, 1986.

Procedure

In this lab, we will cover basic instrument operation, different forms of sampling, data manipulation, and sample identification by spectral searching.

Several of the important data station commands will be found in the "Abridged Instructions". Realize that you can do no damage to the instrument from the keyboard. However, care should be exercised anytime the sample or instrument cover is open.

CAUTION: The instrument uses a low power HeNe laser for frequency calibration, mirror alignment, etc. The laser beam is coincident with the sample beam. With the sample compartment open, reflections of the laser may be observed. While damage to the eyes is not immediate, prolonged staring into even a low power laser or its reflections should be avoided.

Sampling

Samples for IR spectrometry may be prepared several different ways: a) neat liquid film between salt plates, b) liquid dissolved in minimally IR absorbing solvent, c) solid ground to a fine powder in suitable non- absorbing salt (e.g. KBr) and pressed into a pellet, d) solid ground into a fine powder then mulled with a heavy oil such as mineral oil, Nujol, or perfluorokerosene (PFK), and e) neat powdered solid or solid powdered with a suitable salt and measured by diffuse reflectance (DRIFTS). Other sampling techniques exist such as attenuated total reflectance (ATR), gas cell, GC-IR, etc. but these will not be performed in this exercise.

Neat Liquid

Place one salt plate in the cell holder. Place a drop of sample on the salt plate and carefully place the other salt plate on the sample. Put the retaining plate over the mounting screws and screw the knurled nuts down gently and evenly.

Acquire the spectrum from 4000-400 cm^{-1}. If the absorbances are too strong, the pathlength may be reduced by tightening the knurled nuts slightly tighter. You may wish to store the spectrum to disk for later viewing and analysis.

Identify the liquid from the spectrum.

KBr Pellet

Make a mixture of approximately 0.1% of the solid sample in dry KBr. You may wish to make 0.5-1 g to make weighing easier. Weigh either on tared weighing paper or directly into the milling vial. Place a ball bearing into the milling cup and grind the sample on the "Wig-l-Bug" for 30-45 seconds.

Assemble the KBr die holder and place one plate, shiny side up, into the holder. Transfer 100 mg of the ground mixture into the die assembly and tap gently on a hard surface (not the bench top) to flatten the pile. Place the other die, shiny side down, in the holder followed by the plunger. Place the plunger in the holder so that the beveled side is up.

Center the die holder on the Carver press. Close the pressure knob on the jack "thumb tight" and apply 8 metric tons of pressure. Apply the pressure slowly -- increase the pressure from 0 to 8 metric tons over a period of 15-30 seconds.

Wait at least one minute then release the pressure over a period of at least one minute. Remove the base and invert the base of the die. Replace the die in the jaws of the press and squeeze the dies from the barrel of the die holder.

Mount the pellet in the pellet holder and acquire the spectrum from 4000-400 cm^{-1}. You may wish to store the spectrum to disk for later viewing and analysis.

Identify the solid from the spectrum.

Mineral Oil Mull

Grind ~50-100 mg of solid sample in an agate mortar to as fine a powder as possible. Add one drop of mineral oil (or other mulling oil) and continue grinding to the consistency of toothpaste (the paste variety, not gel).

Acquire a reference spectrum of the pure mineral oil. Store the background ratioed spectrum in the *REFERENCE* file.

Clean the salt plates. Transfer a drop of the mull to a clean salt plate. Sandwich the mull with the other salt plate. Mount the sample in the plate holder and acquire the spectrum from 4000-400 cm^{-1} into the *SAMPLE* file.

Subtract the *REFERENCE* from the background ratioed *SAMPLE* spectrum to remove the solvent absorptions. Compare the solid sample pelleted in KBr with the mulled sample.

Diffuse Reflectance

Remove the transmission cell holder from the instrument and carefully set aside. Carefully place the DRIFTS accessory in the sample compartment. Close the sample door and purge for several minutes.

Place the alignment mirror on the sample stage. Close the aspherical focusing mirrors and the sample doors. Using the automatic alignment command (ALN), optimize the position of the return mirror of the interferometer.

Grind a sample of solid similar to the pelleting technique. If sufficient solid remains from the grinding of the pellet, you may use that material. Grind a sample of pure KBr for the background spectrum.

Place the ground pure KBr in one sample cup and place the cup on the sample stage. Using the align mode of the spectrometer, peak the interferogram energy by raising or lowering the sample stage. When through, close the sample compartment, end the align routine, and acquire the background spectrum.

Place the ground sample in the other sample cup and the cup on the sample stage. Peak the interferogram energy as before. Acquire the diffuse reflectance spectrum of the solid. Convert the spectrum into Kubelka-Monk units for display.

Spectral Searching

Convert one of your transmission spectra into absorbance. Use the Search And Display (SAD) macro to get closest library matches of your compound. Use library number 10. Your sample must be in the *DESTINATION* file.

Discussion Items

1. Comment on the differences between the different sampling methods and give examples of situations where one would be superior than others.

2. Often in the spectroscopy of organometallic compounds containing weakly bound ionic ligands, it is highly desirable to NOT use KBr pelleting for sample preparation. Discuss the reason(s) for this.

3. Diffuse reflectance spectra do not obey Beer's law. How does light intensity vary with concentration in the diffuse reflectance experiment. Why?

4. What is...

   a) Felgett's advantage?
   b) Jaquinot advantage?
   c) Connes' advantage?

   What role do they play in non-dispersive instrumentation?

Analysis Based on Separation Science

Gas Chromatography: Mechanistic Study of the Dehydration of Butanol

Introduction

Gas chromatography is a particularly useful tool for the analysis of small samples containing mixtures of several components which are volatile liquids or gases. Because of the high efficiency of the method, compounds with very similar properties (*e.g.* boiling point, dipole moment, etc.) can be separated with relative ease. It is the ability of the gas chromatograph to separate components with very similar boiling points that make the analysis of the isomers formed from the dehydration 1- and 2-butanol possible.

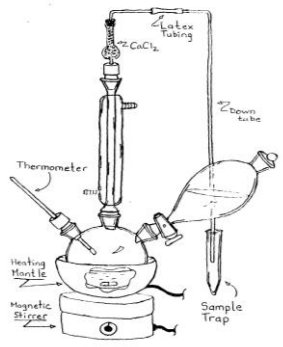
Recall from your studies of organic chemistry that a straight-chain alkyl alcohol may be dehydrated with concentrated sulfuric acid to give mixtures of alkenes.

$$CH_3CH_2CH_2CH_2OH + H_2SO_4 \rightarrow CH_3CH_2CH{=}CH_2 + CH_3CH{=}CHCH_3$$
(*cis*- and *trans*-)

$$CH_3CH_2\underset{\displaystyle OH}{\underset{|}{C}}HCH_3 + H_2SO_4 \rightarrow CH_3CH_2CH{=}CH_2 + CH_3CH{=}CHCH_3$$
(*cis*- and *trans*-)

In the reaction illustrated here, 1- and 2-butanol undergo elimination to give four isomers of butene: 1) 1-butene, 2) *cis*-2-butene, 3) *trans*-2-butene, and 4) 1,1-dimethylethylene (isobutylene). The 1,1-dimethylethylene is a minor product.

From a study of the products formed and their relative amounts, conclusions may be made concerning the mechanisms for the elimination reactions as well as an understanding of the steric and other effects that control the product outcome.



Procedure

Set up the chromatograph as described pre-lab lecture. Start with a oven temperature of about 50°C and a helium flowrate appropriate for the column. It may be necessary to further optimize the analysis.

Assemble the apparatus shown using ground-glass glassware. Use a 250 or 500 mL three neck round-bottom flask for the reaction vessel. Use only a minimum amount of grease on the joints. Assemble the sample trap as shown in the figure. Fill the drying tube with anhydrous calcium chloride. Use latex rubber tubing at the joint of the drying tube and "down-tube". A conical graduated centrifuge tube works well as the sample trap.

The trap is cooled with a dry ice-acetone slush[1]. The slush is prepared by placing acetone in a small Dewar flask and adding small amounts of dry ice slowly until vigorous bubbling ceases. Dry ice may then be added as needed to keep the slush cold. The collection vessel can then be placed in the slush and supported with a clamp.

Place 100 mL of 70% sulfuric acid[2] in the round-bottom flask. Check that the sample trap is in place in the dry ice bath. Place 25 mL of 1-butanol in a 100 mL separatory funnel, making sure the stopcock is closed. Attach the separatory funnel to the round-bottom flask. Turn on the water to the condenser and adjust the thermometer so that it is not dipped into the liquid. Stir the acid gently and, using the Variac, adjust the temperature until the sulfuric acid refluxes at ~105°C.

When the temperature has stabilized, open the stopcock so that the alcohol is added *no faster than* 1 drop per second. As the reaction proceeds, products will condense in the trap. After 2 mL of butenes have been collected, insert the syringe needle through the rubber tubing above the drying tube and collect a 2 mL gas sample.

Immediately inject this sample into the chromatograph and acquire the chromatogram. There should be at least 3 peaks[3] in addition to the air peak (if present). If the chromatogram is unacceptable, make necessary changes in the chromatograph to improve efficiency. Make at least three injections of the head-space gasses onto the optimized chromatograph. If time permits and the 1-butene and 1,1-dimethylethylene are not resolved, attempt to separate the two isomers.

After the chromatograms have been collected and are determined to be acceptable, turn off the heat to the reaction system and allow it to cool. Clean the reaction and sample condensation systems. **Take extreme care in disposing of the sulfuric acid.** Repeat the analysis using 2-butanol.

Analysis

The order of appearance of the peaks in the gas chromatogram should in the same as the order of increasing boiling points of the components. Devise a simple experiment to verify this.

The boiling points for the four butene isomers[4] are

1,1-dimethylethylene	-6°C
1-butene	-5°C
trans-2-butene	1°C
cis-2-butene	2.5°C

Determine the relative areas for each of the peaks. From the tabulated data, determine which is the predominate isomer for the dehydration of each alcohol and the ratios of the isomers. Postulate and defend a mechanism which is consistent

[1]The dry ice-acetone slush is -80°C. Severe frostbite can occur if the dry ice or slush comes in contact with skin. The dry ice should be handled with gloved hands or tongs.

[2]**CAUTION:** 70% H_2SO_4 is still relatively concentrated sulfuric acid. Take extreme care to avoid drips, splashes, or spills. If it gets on skin, wash it off immediately. Sulfuric acid is essentially nonvolatile. Dilute solutions of the acid will concentrate as the water evaporates.

[3]The 1-butene and 1,1-dimethylethylene have very similar boiling points so they may coelute.

[4]The *cis*- and *trans*- isomers are sometimes reversed in the literature.

with the data for the dehydration of the butanol isomers. References and texts may be consulted. The discussion should account for all reaction products observed.

Identification of an Unknown Mixture by Gas Chromatography

Introduction

Gas Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of the phases constituting a stationary bed of large surface area, contained in the chromatographic column, the other being an inert carrier gas, *e.g.* helium, that percolates through and along the stationary phase.

The sample mixture is introduced on the column by use of a syringe. After introduction, the sample is vaporized and swept by the mobile carrier gas on to the column. The sample is kept in the vapor state during the entire analysis by placing the column in an oven whose temperature is kept above the dewpoint of the compounds in the sample.

As the sample mixture begins to dissolve in the stationary phase, the solvent selectively retards movement of the sample components down the column. Since the sample components are soluble in both the stationary and mobile phases, partitioning takes place causing the formation of distinct and, hopefully, separated component bands. The components are eluted from the column in the carrier gas stream and detected by a suitable detector.

This analysis demonstrates the utility of quantitating a mixture of pure compounds by their mole fractions in the mixture. This analysis also takes advantage of the normally linear relationship of peak area and concentration in the chromatogram for quantitation.

Procedure

Set up the gas chromatograph using conditions and methods outlined in prelaboratory lecture.

Obtain a small amount of each of the pure reagents and a mixture of the components into small labeled beakers. Inject 1 or 5 μL[1] of the mixture to check the separation. Inject the same amount of each individual reagent and acquire the chromatogram. Allow at least 1 min of baseline after the peak elutes to flush the column. Neatly label each chromatogram with all pertinent data. It is important to include the volume injected and instrument settings. Calculate the detector response factors for each compound using the one-point calibration method.

From the chromatogram of the mixture, determine the retention time (t_R), capacity ratio (k'), plate height (H), and plate number (N_{eff}) for each peak. Correct the retention times to the air peak, t_M. For any pair of peaks that are not baseline resolved, calculate the resolution for the pair. It may be necessary to replot the

[1]If a packed column is used in the instrument, inject 5-10μL.

chromatogram at different scales to make the appropriate measurements. Explain any deviations using theoretical principles.

Inject a sample of the same size of your unknown mixture on the appropriate column and determine the identity of your mixture from the retention times of the peaks. Since each sample is a mixture of the components with no support matrix (*i.e.* solvent), calculate the volume ratio of the components and percentage by weight of each component.

Optional

Select one compound from those available and acquire the data to construct the Van Deempter plot. Acquire no fewer than 15 flow rates.

Determination of Caffeine in Beverages by HPLC

Introduction

High performance liquid chromatography (HPLC) is a technique used to separate the chemical components in a solution matrix by taking advantage of differential physico-chemical properties of the components. The properties by which separation takes place may include hydrophobicity/hydrophilicity, polarity, ionic charge density, or the molecular size. The amount of resolution[1] between the components is important and depends strongly upon the extent of interaction between the solutes and the stationary and mobile phases. The mobile phase in HPLC is a liquid and the interaction of the solute with column stationary phase and liquid mobile phase can be manipulated by careful choice of solvent and column packing. As a result, HPLC offers the analyst versatility not found in many other techniques. HPLC lends itself well to the separation and analysis of virtually every class of compounds. HPLC also offers versatility in analytical scale: 1) trace and micro (ng to μg) scale, semi-preparative (milligram quantities), and even preparative scale (g quantities).

The essential components of the typical HPLC system include the solvent reservoir(s), pump, injector system, column, detector, and recorder. Optionally, some systems include a solvent mixing system for performing solvent gradient elution and a thermostatted column oven. The figure shows a schematic diagram of a complete HPLC system.

The heart of any chromatographic system in the column. HPLC columns are usually packed with stationary phase particles which are 3-10 μm in diameter. Many different packings may be employed in the column which is determined by the separation intended.

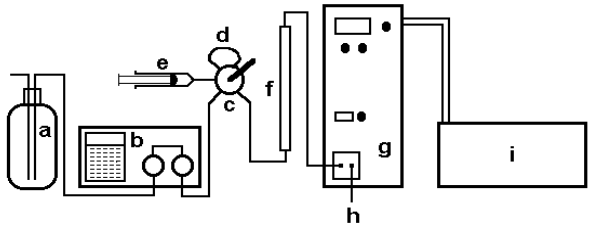


Figure. Diagram of a typical HPLC system. a. Solvent reservoir with tubing for solvent delivery and He-sparging. b. High pressure pump. c. Injector system. d. Sample loop. e. Sample syringe to fill sample loop. f. Column. g. Detector. h. Liquid outlet from detector to waste bottle. i. Output device such as a chart recorder, integrator, computer, etc.

Since the "back-pressure" developed by the column is often very high, sometimes reaching several thousand pounds-per-square-inch[2], a high pressure pump is required to move the solvent through the column. Additionally, the stainless steel

[1]Resolution may be defined as the degree of separation of two solute bands from each other and is given the symbol *R*.

[2]14.7 psi = 1.01 bar = 1 atm

tubing most often used[3] has an internal diameter of 0.001 inches (0.03 mm) which provides relatively high resistance to fluid flow.

An analysis begins by introducing the sample onto the column. An injection valve is most commonly used to deliver the sample into the solvent stream without interrupting the solvent flow. When the valve is placed in the "load" position, a syringe may be used to fill a stainless steel loop, the "sample loop", with the solution to be analyzed. The injector valve is then set to the "inject" position at which time the sample loop is placed in-line with the mobile phase stream.

After the mixture is separated on the column, detection of the individual components is important and selection of the detector is often made by the types of analytes to be determined. A variety of detectors are currently available making use of, for example, UV-visible spectrophotometers, refractive index, fluorescence, electrochemical properties, conductivity, radioactivity, and recently, even nuclear magnetic resonance and mass spectrometry. Ideally, the detector is sensitive and the response linear with concentration of analyte. The output of the detector is normally displayed on a chart recorder, integrator (which combines a chart recorder and electronic integration system), or a computer.

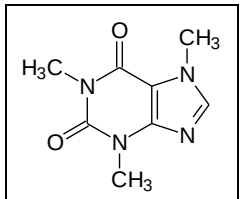
Each component in the mixture can be qualitatively identified by its retention time, t_R, or retention volume, V_R. Identification by t_R necessarily requires knowledge of the retention time of the pure component as determined by an injection of a sample of the pure compound. The strategy of comparing t_R values of the unknown components with t_R values of standards works only if every component has a unique retention time.

Quantitative analysis can be performed by injecting standards of known concentration, measuring the chromatogram, and determining the area under the chromatographic peaks. The area under the peak is proportional to the component's concentration; thus, a calibration curve may be established and solutes of unknown concentration in a mixture determined.

The theory and background of chromatography is well-established and discussed more fully in the textbook and many other excellent sources and will not be reviewed here.

[3]Sometimes titanium tubing and pump heads are used for biological samples since titanium is less reactive than steel.

The analysis to be performed is the determination of caffeine in beverages by reversed-phase[4] HPLC. The chromatographic separation described will allow the analyst to resolve caffeine from the matrix of water, colorings, and sugars found in soft drinks, tea, and coffee. Standard solutions of caffeine will be prepared and injected into the HPLC. From each of the peak areas, a calibration curve of peak area versus concentration will be made. Solutions of each of the beverages to be analyzed will be prepared and injected. From the retention time, the caffeine in each beverage will be identified and the calibration curve employed to quantitate the caffeine.



Caffeine

The mobile phase system employed will be 20% methanol:80% water and the column will be ODS bonded to 5 μm silica. Because of the small size of the stationary phase particle, care must be taken to avoid pumping solvents or injecting samples which contain particulate matter larger than 0.5 μm so solvents and samples will be filtered though at least 0.45 μm filters. UV detection at 254 nm will be used for detecting eluted analytes since caffeine has a reasonably large molar absorptivity at this wavelength while the solvents are very nearly completely transparent and many of the matrix analytes are only weakly absorptive in the UV region.

Procedure

If an isocratic HPLC system is to be used for analysis, prepare by volume, not mass, 2 L of 20% methanol:80% H_2O; 1 L for sample preparation and 1 L as the HPLC solvent. If a gradient system is to be used, prepare only 1 L of the solvent for sample preparation. The 20:80 methanol:H_2O mixture will be referred to as "chromatographic solvent" or just "solvent". Use only HPLC quality solvents or, alternatively, the highest quality solvents available filtered through a 0.2 μm membrane.

Preparation of Standard Solutions

Accurately prepare 4 or 5 solutions of caffeine ranging in concentration from 0.025 to 0.125 mg/mL in chromatographic solvent. Only 50-100 mL of each need be prepared but because of the low concentrations, it may be necessary to prepare them by standard dilutions from a stock solution.

Preparation of Analytical Solutions

For samples of coffee, make a 1:10 dilution[5] of the coffee in a volumetric flask using chromatographic solvent as the diluent. Prepare 1:5 dilutions of tea for analysis.

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[4]The "phase" of the chromatographic separation is normally defined by the solvent and stationary phase polarities. Normal phase chromatography typically uses a polar stationary phase (*e.g.* silica or alumina) and relatively nonpolar mobile phase (*e.g.* hexane). Reverse phase chromatography takes advantage of a nonpolar stationary phase (*e.g.* octadecylsilane, ODS, a waxy long-chain alkane) and a polar mobile phase (*e.g.* water, alcohols, acetonitrile, etc.).

[5]For example, 5 mL of coffee diluted to 50 mL.

Soft drinks must be "decarbonated" before analysis. That is, the dissolved carbon dioxide must be removed to the greatest extent possible. The CO_2 can be removed simply by pouring the soft drink back and forth between two beakers or by sonication until the beverage no longer produces bubbles.

Make 1:4 to 1:3 dilutions of the soft drinks to be analyzed, using chromatographic solvent as the diluent.

Analysis

The HPLC will be set up for use with a UV detector and silica-based C_{18} (ODS) reversed-phase column.

Turn on the UV detector and set the wavelength to 254 nm and 0.1 AUFS (absorbance units full scale). Make sure the chart recorder or computing integrator, if either is used, is set to 0.5 or 1.0 cm/min chart speed. If an isocratic pump is used place prepared mobile phase in the solvent reservoir. If a gradient pump is used, set the gradient controller to deliver 20% methanol and 80% water from the appropriate solvent reservoirs. Continually degas the solvents by helium sparging the mobile phases in the reservoir bottles. Turn the pump on and set the flowrate to 1.00 mL/min. Allow the column to equilibrate with mobile phase for at least 15 minutes.

Obtain a small syringe and a 0.2 or 0.45 μm syringe-tip filter. Draw up at least 25-50 μL (or more) of the most concentrated caffeine standard. Attach the syringe filter and blunt-tip needle to the syringe and inject more than 10 μL of sample into the sample loop while the injector is in the LOAD position. The sample loop needs only 10 μL of sample; however, somewhat more than 10 μL is necessary to assure that the loop is completely filled with solution.

Turn the injection valve to the INJECT position. If the chart recorder or computing integrator do not automatically indicate the start of an analysis, simultaneously press the MARK button on the detector, while injecting, to leave a mark on the chromatogram signaling the start of the analysis.

Allow the entire chromatogram to be recorded. If the peak of the first standard is too small or off scale, adjust the detector AUFS scale and/or the computing integrator's attenuation setting to give the best full-scale deflection of the chromatogram and rerun the standard again. It should not be necessary to readjust the sensitivity of the detector or attenuation of the integrator again. Inject the remaining standards, allowing each chromatogram to complete before starting the next. If time permits, acquire 3 chromatograms for each standard and average the peak areas.

If a computing integrator is used, use the integrated peak areas for each standard and prepare a calibration graph of peak area vs. concentration. If a chart recorder is utilized, use any convenient method available for determining peak area[6] or simply use peak height.

[6]Relative peak area can, for example, be determined by the cut-and-weigh method, planimeter, height x 1/2 base, or other electronic methods.

Inject each of the samples in a similar manner and acquire the chromatograms. Use the retention time obtained for the standard caffeine injections to identify the caffeine peak in the beverage samples. Integrate the peak areas due to caffeine in each sample and use the calibration curve to determine the concentration (in mg/mL) of the caffeine in each. Do not forget to account for any dilutions. Time permitting, acquire 3 chromatograms for each sample and average the peak areas.

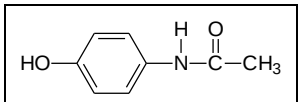
Discussion

Explain the rationale for using reversed-phase chromatography for this analysis. What differences would you predict if a C_8 rather than a C_{18} (ODS) column were used.

Determination of Doxylamine Succinate and 4-Acetamidophenol in an OTC Pharmaceutical by HPLC

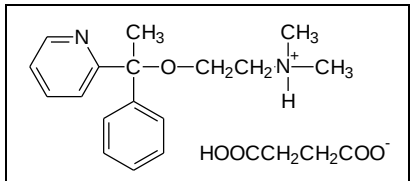
Introduction[1]

Many common over-the-counter (OTC) formulations for those suffering from the symptoms of allergies, colds, and other ailments contain more that a single pharmaceutical agent. This allows a person to treat with a single dose multiple ailments such as headache, sinus congestion, and coughing. Many physicians recommend purchasing multiple OTC medications each one specifically for the symptom being treated so as not to introduce unnecessary chemical substances into the body. Nonetheless, complex OTC formulations are routinely and successfully marketed regardless of their "overkill" in treating a person's ailments, in part because of their speed in bringing relief and few, if any, unwanted side effects.



4-acetamidophenol

One such example of a successful OTC product is Vicks® Nyquil™ which has been advertised as the "nighttime, sniffling, sneezing, coughing, aching, stuffy head, fever, so you can rest medicine™."[2] Two of the active ingredients in Nyquil™ are 4-acetamidophenol (also known as acetaminophen) for pain and doxylamine succinate[3], an antihistamine and mild sedative. Additionally, in many liquid OTC cold medications, pharmaceutical companies will add up to – and often more than – 10% ethanol. The ethanol (a sedative) coupled with antihistamines (most of which have sedative properties) helps the cold or allergy sufferer to rest through the night.



Doxylamine succinate

Quality assurance analysis of OTC medications is not only valuable to the consumer to ensure value but also regulated by the Food and Drug Administration to protect consumers from under- or overdose[4].

Because of the complexity of the sample (from the analytical point-of-view), spectroscopic analysis of the sample would yield only poor results. Utilization of the power of high performance liquid chromatography, however, makes the analysis of the 4-acetamidophenol and doxylamine succinate straight forward. Basic compounds are often best separated by ion-exchange chromatography but

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[1]For a more complete introduction to the method of high performance liquid chromatography (HPLC), refer to the ***Introduction*** in the analysis entitled *Determination of Caffeine in Beverages by HPLC* earlier in this section.

[2]Vicks, Nyquil, and the advertisement phrase are all registered trademarks of the Proctor and Gamble Company.

[3]Doxylamine succinate is the succinate acid salt of N,N-dimethyl-2-[1-phenyl-1-(2-pyridinyl)ethoxy]ethanamine.

[4]*The United States Pharmacopeia XXII - The National Formulary XVI* (United States Pharmacopeial Convention, Rockville, MD, 1991).

good separations can often be achieved by reversed-phase chromatography with a buffered acidic mobile phase.

Procedure

This procedure requires the use of a chromatographic pump capable of gradient solvent delivery. Since an inorganic salt buffer will be used, care must be taken to not leave mobile phase in the pump and column for extended periods of time when the pump is idle to avoid precipitation of salts which can corrode metal parts (even stainless steel) or damage pump seals. Also, take care to avoid a large percentage of organic solvent as a mobile phase modifier to prevent salt precipitation. When the analysis is complete, pump 50 mL of water through the system followed by 50 mL of methanol or acetonitrile.

Preparation of Mobile Phase Buffer

Prepare 2 L of 50 mM KH_2PO_4 in deionized water. Adjust the pH of the solution to 3.5 with concentrated phosphoric acid then filter the mobile phase through a 0.45 μm nylon membrane filter. Transfer one liter of the buffer to a clean dry bottle and install the bottle on the liquid chromatograph pump. Degas the solution with helium and fill the solvent line with buffer solution.[5]

Ensure that there is acetonitrile in another solvent reservoir bottle and that the solvent line is filled.

Preparation of Standard Solutions

Accurately prepare at least 4 standard solutions of doxylamine succinate in chromatographic solvent ranging in concentration from 0.04 to 1 mg/mL. Less than 25 mL of each need be prepared but because of the low concentrations, it may be necessary to prepare the standards by dilutions from a stock solution. Doxylamine succinate is an expensive reagent so take care to avoid waste.

Likewise, prepare at least 4 standard solutions of 4-acetamidophenol in chromatographic solvent with concentrations in the range of 0.2 to 0.7 mg/mL. As before, it may be necessary to make suitable dilutions from a stock solution.

Preparation of Analytical Solutions

Determine from the label on the bottle of Vicks® Nyquil™ (or equivalent OTC formulation) the expected concentrations of the doxylamine succinate and 4-acetamidophenol. Normally, the 4-acetamidophenol will be in such a larger concentration than the doxylamine succinate[6] that the two components cannot easily be simultaneously determined. As such, two different analyses need to be performed on the sample: first on a dilution of the OTC product that gives a concentration of the doxylamine succinate in the range of the standards and a

[5]If you are unsure as to how to fill the solvent lines and prime the pump, ask the instructor.

[6]In fact, except for the alcohol, the 4-acetamidophenol is likely to be the predominant active ingredient.

second chromatographic analysis on a dilution of the product that gives a concentration of 4-acetamidophenol in the range of the standards.

Based on the expected concentration of the doxylamine succinate, prepare a dilution of the OTC product such that the doxylamine succinate concentration will be in the range of the standards. Prepare a second dilution, either a standard dilution from the OTC product or a serial dilution from the prior solution, which will bring the expected concentration of the 4-acetamidophenol into the range of the standards.

Analysis

The HPLC instrument will be set up for use with a UV detector, silica-based 150 x 4.6 mm 5 μm C_{18} (ODS) reversed-phase column, and an injector equipped with a 10 μL sample loop.

Turn on the UV detector and set the wavelength to 242 nm and 0.1 AUFS (absorbance units full scale). Make sure the chart recorder or computing integrator, if either is used, is set to 0.5 or 1.0 cm/min chart speed. Program the pump to provide a solvent gradient of 100% KH_2PO_4 to 70% KH_2PO_4/30% CH_3CN in 15 min. Continually degas the solvents by helium sparging the mobile phases in the reservoir bottles. Turn the pump on and set the flowrate to 1.00 mL/min. Allow the column to equilibrate with mobile phase for at least 15 minutes.

Obtain a small syringe and a 0.2 or 0.45 μm syringe-tip filter. Draw up at least 100 μL (or more) of the most concentrated doxylamine succinate standard. Attach the syringe-tip filter to the syringe and a blunt-tip needle to the filter and inject more than 10 μL of sample into the sample loop while the injector is in the LOAD position. The sample loop needs only 10 μL of sample; however, somewhat more than 10 μL is necessary to assure that the loop is completely washed and filled with solution.

Turn the injection valve to the INJECT position while simultaneously starting the gradient program. Allow the entire chromatogram to be recorded. If the peak of the first standard is too small or off scale, adjust the detector AUFS scale and/or the computing integrator's attenuation setting to give the best full-scale deflection of the chromatogram and rerun the standard again. It should not be necessary to readjust the sensitivity of the detector or attenuation of the integrator again. Inject the remaining standards, allowing each chromatogram to complete before starting the next. If time permits, acquire 3 chromatograms for each standard and average the peak areas.

If a computing integrator is used, use the integrated peak areas for each standard and prepare a calibration graph of peak area vs. concentration. If a chart recorder is utilized, use any convenient method available for determining peak area[7] or simply use peak height.

Inject the sample in a similar manner and acquire the chromatogram. There should, of course be more peaks in the sample due to the presence of other components.

[7]Relative peak area can, for example, be determined by the cut-and-weigh method, planimeter, height x 1/2 base, or other electronic methods.

Use the retention time obtained for the standard doxylamine succinate injections to identify the doxylamine succinate peak in the diluted OTC sample. Integrate the peak area due to doxylamine succinate in the sample and use the calibration curve to determine its concentration in units of mg/mL.

Repeat on the 4-acetamidophenol standards and the more dilute sample preparation.

Calculations

Based on the concentrations of the doxylamine succinate and 4-acetamidophenol in the dilutions, calculate the mass of each per recommended dose volume and determine the percentage error from the expected quantity.

Discussion

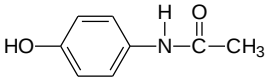
Explore and discuss the use of the two OTC drugs analyzed.

HPLC of an Over-The-Counter Analgesic

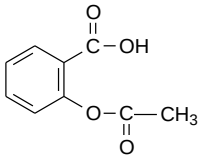
Introduction[1]

Many common over-the-counter analgesic[2] formulations for those suffering from sickness, joint or muscle pain, and other ailments contain more that a single pharmaceutical agent. Over-the-counter (OTC) pain relievers containing multiple analgesic compounds can target different sources of pain in a single tablet or capsule.

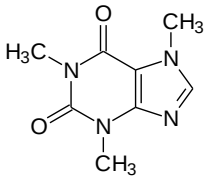
One such example of a successful OTC product is one manufactured by Excedrin®. The two active ingredients in Excedrin® are 4-acetamidophenol (also known as acetaminophen) and acetylsalicylic acid (aspirin). Additionally, many OTC analgesics which contain aspirin also contain a small amount of caffeine to counteract the mild sedative effects of the aspirin.



4-acetamidophenol



Acetylsalicylic Acid



Caffeine

Depending upon the age of the medication, any formulation which contains aspirin may also contain small to significantly larger quantities of salicylic acid. The salicylic acid is a by product of the decomposition of acetylsalicylic acid when exposed to moisture.

Quality assurance analysis of OTC medications is not only valuable to the consumer to ensure value but also regulated by the Food and Drug Administration to protect consumers from under- or overdose[3].

Because of the complexity of the sample (which may contain 3 or more water soluble components), high performance liquid chromatography is a method of choice for the analysis of the analgesic These compounds are best separated by reversed-phase chromatography on a C_{18} (ODS) stationary phase with a slightly acidic mobile phase.

Procedure

Prepare 3 standard solutions each of acetylsalicylic acid and 4-acetamidophenol (acetaminophen) ranging from 0.001 to 0.01 mg/mL. Prepare 3 standard solutions each of caffeine and salicylic acid 0.00025 to 0.0025 mg/mL. If necessary, prepare

[1]For a more complete introduction to the method of high performance liquid chromatography (HPLC), refer to the ***Introduction*** in the analysis entitled *Determination of Caffeine in Beverages by HPLC* earlier in this section.

[2] An analgesic is a mild pain-reliever.

[3]*The United States Pharmacopeia XXII - The National Formulary XVI* (United States Pharmacopeial Convention, Rockville, MD, 1991).

a stock solution and make standard dilutions. Do not exceed 100 mL in volume for any solution.[4]

Alternative Preparation of Standards

Prepare all four standard compounds in the same solution in the desired concentration range.

Obtain an average mass of tablet from a bottle of OTC analgesic. Determine the mass of tablet necessary to prepare a solution of the OTC analgesic such that the concentration of the most abundant component falls within the range of the corresponding standard. If necessary, prepare a more concentrated solution and perform a standard dilution.

Set up the HPLC instrument with a UV detector and silica-based C_{18} (ODS) reversed-phase column. The mobile phase is 40:60 MeOH:1%HOAc with a flowrate of 1.00 mL/min.

Turn on the UV detector and set the wavelength to 280 nm and 0.1 AUFS (absorbance units full scale). Make sure the chart recorder or computing integrator, if either is used, is set to 0.5 or 1.0 cm/min chart speed. Continually degas the solvents by helium sparging the mobile phases in the reservoir bottles. Turn the pump on and set the flowrate to 1.00 mL/min. Allow the column to equilibrate with mobile phase for at least 15 minutes.

Obtain a small syringe and a 0.2 or 0.45 μm syringe-tip filter. Draw up at least 25-50 μL (or more) of the most concentrated caffeine standard. Attach the syringe filter and blunt-tip needle to the syringe and inject more than 10 μL of sample into the sample loop while the injector is in the LOAD position. The sample loop needs only small amount of sample; however, somewhat more than than the loop volume is necessary to assure that the loop is completely filled with solution.

Turn the injection valve to the INJECT position. Allow the entire chromatogram to be recorded. If the peak of the first standard is too small or off scale, adjust the detector AUFS scale and/or the computing integrator's attenuation setting to give the best full-scale deflection of the chromatogram and rerun the standard again. It should not be necessary to readjust the sensitivity of the detector or attenuation of the integrator again. Inject the remaining standards, allowing each chromatogram to complete before starting the next. If time permits, acquire 3 chromatograms for each standard and average the peak areas.

If a computing integrator is used, use the integrated peak areas for each standard and prepare a calibration graph of peak area vs. concentration. If a chart recorder is utilized, use any convenient method available for determining peak area[5] or simply use peak height.

Inject the sample in a similar manner and acquire the chromatogram. Use the retention times obtained for the standard injections to identify the peaks in the OTC

[4]Alternatively, prepare the standard solutions with all four standard compounds in the same solution in the desired concentration range.

[5]Relative peak area can, for example, be determined by the cut-and-weigh method, planimeter, height x 1/2 base, or other electronic methods.

analgesic samples. Integrate the peak areas due to caffeine in each sample and use the calibration curve to determine the concentration (in mg/mL) of the caffeine in each. If present in the sample, quantitate the salicylic acid decomposition product. Do not forget to account for any dilutions. Time permitting, acquire 3 chromatograms for each sample and average the peak areas.

Calculations

Based on the concentrations of the analgesics and caffeine in the dilutions, calculate the mass of each per recommended dose and determine the percentage error from the expected quantity.

Discussion

Explore and discuss the use of the two OTC drugs analyzed.

Computers in Analytical Chemistry

Mathcad Problem Session: Equilibrium

In this problem session, you will explore the use of Mathcad for solving difficult problems easily. This is only an introduction, but you will see some of the powerful features of Mathcad.

Basics

Read and perform the exercises in Mathcad Basics. This will give you an introduction to inputting equations and solving simple problems.

Note that there are 3 different kinds of "equal" signs.

- = This is the immediately computed "equals". Typing an equation and pressing <=> immediately evaluates the equation.

```
5 - 2 x 2 = 1
```

- := This is the assignment "equals". This *defines* or *assigns* one side of an equality to be equal to the other. The keystroke for assignment is to press the colon key  .

```
x := 5
y := 2
x - y x y = 1
```

- **=** The bold "equals" is the *constrained to be* assignment. This constrains an equality for use in simultaneous equations. The keystroke sequence for *constrained equals* is  .

Statistics

Explore the properties of the Gaussian curve. The equation for the Gaussian curve is

$$f(x) = \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{(x-\mu)^2}{2\sigma^2}}$$

If the curve is integrated from -∞ to +∞, the result should be unity.

$$\int_{-\infty}^{+\infty} \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{(x-\mu)^2}{2\sigma^2}} = 1$$

If you are not already running Mathcad, run the program. If you are, start a new worksheet. Type in the equation for the integration of the Gaussian curve. The key sequences follows. You can use the mouse and the symbol buttons if you wish. Pressing a letter then ctrl-g makes the font Greek.

```
&             (integration)
(             (open a parenthesis)
type "1" in the top of the fraction
/             (make a fraction)
```

```
move the cursor focus to the denominator of the fraction
s<ctrl>g (create variable σ)
*              (multiply)
\              (square root symbol)
type "2" inside the square root symbol
*              (multiply)
p<ctrl>g       (internally defined symbol π [=3.1415…])
)              (close the parenthesis)
*              (multiply)
e^-            (create the exponential function)
/              (create a fraction)
move the cursor focus to the numerator of the fraction
(x-m<ctrl>g)   (start of numerator of the exponetial term)
^2             (completes the numerator term)
move the cursor focus to the denominator
2*s<ctrl>g^2   (completes the fraction in exponential term)
move the cursor focus to the bottom limit of the integral
type "-10"
move the cursor focus to the top limit of the integral
type "10"
move the cursor focus to the placeholder after the
differential
type "x"
type "="
```

Notice that when you press the equals key, some of the symbols are highlighted indicating that these variables have not yet been defined. Move the crosshairs above the equation and input the following equalities:

$$\mu := 0 \qquad \sigma := 1$$

Change the numerical format to display 10 decimal places. Is the result of the integration exactly 1? Because a computer cannot evaluate infinity, integrating from -10 to +10 closely approximates infinity. The integration implies that the probability of obtaining a measurement within $\pm 10\sigma$ of the true value is about 100%.

Now change the limits to $\pm 1\sigma$, $\pm 2\sigma$, and $\pm 3\sigma$. Are the probability values (integrations under the curve over these limits) similar to anything that you have seen in lecture?

Annotate and print the calculations. Briefly describe (1-5 sentences) your observations and conclusions.

Equilibrium

Monoprotic Acid

Using a *solve block*, solve rigorously for the pH of 0.1, 0.01, 0.001, 0.0001 M trichloroacetic acid. You can use the your textbook, the *CRC Handbook of Chemistry and Physics*, or any other appropriate handbook for the acid dissociation constant.

Instructions for setting up the solve block and finding the solutions to the equations will be given in pre-lab lecture and can be found in the MathCad instruction manual.

Compare the results you obtain with Mathcad to those obtained when you make simplifying assumptions and perform the calculation "by hand." Experiment with the solver to observe the effects of changing variables such as initial guess, TOLerance, etc. Select another acid with a different K_a and repeat the calculations with Mathcad.

Annotate and print the results of each calculation.

Diprotic Acid

Determine the pH of 0.01 and 0.0001 M oxalic and 0.01 and 0.0001 M succinic acid making no simplifying assumptions using Mathcad. Compare the result you obtain with Mathcad to that which you obtain using simplifying assumptions. Annotate and print your results.

Sparingly Soluble Salt of a Weak Acid

Determine the theoretical solubility of $BaCO_3$ in aqueous solution with no simplifying assumptions. Repeat with $CaCO_3$. Annotate and print the results.

Spreadsheets in Chemistry: An Introduction

Introduction

The presence of the personal computer and modern software has revolutionized the field of chemistry over the last ten or more years. Of particular importance is spreadsheet software. Originally introduced into the business community in the early 1980's, spreadsheet software has become extremely useful in the scientific community, especially where tables of data are acquired and analyzed. Modern spreadsheet software such as Microsoft Excel, Corel QuattroPro, and Lotus 1-2-3 to name a few, all contain most of the mathematical and statistical functions necessary for the practicing scientist. Often, when a necessary function does not exist designed into the program itself, the operator can "write" a macro or design into the spreadsheet itself the necessary computation.

Further, modern spreadsheet programs have very good graphical capabilities which allow the user to produce graphical plots of varying kinds quickly. The plots produced with a good spreadsheet program will often be of a quality suitable for publication.

Spreadsheet programs allow the user to perform simple calculations on tables of numbers and "what if" calculations by changing variables, determine the best-fit equation of linear *and* nonlinear x-y data, perform theoretical modeling of complex systems, as well as many other applications. As you increase the level of your knowledge of the utility of the spreadsheet, you will learn to design your own approaches and solutions to complicated chemical systems with increasing sophistication.

The exercises illustrated here will be presented using a generic spreadsheet "engine" but virtually any high quality spreadsheet software for the Microsoft Windows™ or Macintosh® platform will work nearly the same. Consult the manual for the specific software if a command is unclear. It is possible that the inexpensive "integrated" software often bundled with new computer systems[1] may not be able to handle some of the more advanced graphical and numerical processing available in more sophisticated spreadsheet programs.

It will be assumed that you are able to start the program and input data into spreadsheet cells and have at least a basic knowledge of "cutting", "copying", and "pasting" information from one place to another. If you are unsure of any of the basics of the operation of the program, consult your instructor or colleague in the class or consult the operations manual for the computer and software package you are using. You will quickly learn the basics and more advanced techniques by simply trying operations in the program.

[1]For example, the spreadsheets packaged Microsoft® Works™ and Claris® Works™ may not be able to perform all of the functions described here.

Remember: It is nearly impossible to damage the computer from anything you do at the keyboard; on the other hand, it *is* possible to render a program unusable by indiscriminately executing programs, erasing files, etc. Please be careful when executing any command that writes to the hard disk or floppy disk drives or which erases files from any disk drive.

The exercises presented are all real examples taken from laboratory data analyses and other sources to which spreadsheets can be easily and usefully applied. Feel free to explore beyond the examples.

Basics

It is reasonable to expect that every worksheet that you work on have a title and date. This author nearly always reserves the first few lines of cells in upper left corner of the worksheet for descriptive data. Fortunately, blank lines of cells can be added after you start a sheet.

Get into the habit of *never* removing a floppy disk from the disk drive unless the all files which are stored on the floppy disk are **saved** and **closed**. This will avoid corruption of the file and possibly the entire disk. This is true of all computer platforms and software programs. Computers are notorious for damaging files for apparently no reason when, in fact, it may be as simple as the user removing a floppy disk before the drive is finished writing to the disk or before the user has saved and closed all open files.

Furthermore, get into the habit of saving files to a floppy disk or, if saving to the hard disk drive, transferring the files to a floppy disk. In many cases, personal files are not allowed to be stored on public computer hard disk drives and will be erased without notice by the system operator or maintenance personnel.

A Word On Computer Viruses. Fortunately, computer viruses are relatively rare. Unfortunately, they exist. A computer virus is a small program written either by a person researching computing viruses or by a vandal bent on creating a nuisance. In either event, the virus can be something as innocuous as a little statement which flashes onto the screen at a particular time or as devastating as a complete destruction of the files on the hard disk drive. In all cases, computer viruses ***must be avoided*** at any cost.

If you get a virus on your own computer, please do not use any floppy disk from that computer on any other computer until the disk is repaired. If you notice a virus on *any* computer or disk, notify the instructor immediately. Much damage can be avoided if the virus is caught and eradicated early. Intentionally introducing a virus into any publicly used computer is considered very bad form and, in some environments, may be cause for legal action.

1. Data Treatment: Weighted-Average Atomic Mass

The determination of the atomic mass for an element is among the most fundamental measurements in chemistry. Every atomic mass measured and

reported on the periodic table is a weighted-average mass of the isotopes of the specific element, each with its own isotopic mass. Table 1 shows the isotopic number, abundance, and mass for the seven predominant isotopes of mercury[2].

Table 1. Pertinent data for each isotope of mercury.

Isotope	Abundance	Mass (u)
196	0.140%	195.965
198	10.039%	197.967
199	16.830%	198.967
200	23.120%	199.968
201	13.230%	200.97
202	29.790%	201.97
204	6.850%	203.973

The average mass of an element can be calculated from the isotopes but not by simply taking the average of each of the masses. Since the abundance of each isotope is not equal to the other, a *weighted-average* must be calculated. This is done by multiplying the individual isotopic masses by their fractional-equivalent abundances[3] then summing each contribution to the average mass to get the weighted-average atomic mass[4].

Table 2. Example view of the isotopic data in a spreadsheet.

	A	B	C	D
1	Your name			
2	Date			
3	Weighted-Average Atomic Mass of Mercury			
4				
5		Abundance	Mass	
6	Isotope	(%)	(u)	
7	196	0.140	195.965	
8	198	10.039	197.967	
9	199	16.830	198.967	
10	200	23.120	199.968	
11	201	13.230	200.97	
12	202	29.790	201.97	
13	204	6.850	203.973	

Start the spreadsheet program and set up a spreadsheet that looks similar to the example spreadsheet in Table 2. When text is larger than the normal cell width, it may appear to "spill" over into adjacent cells. The line that starts in cell A3 is entirely in cell A3. In some spreadsheet programs, it is necessary to type either an

[2]From *Handbook of Chemistry and Physics 56th Ed.* CRC Press, 1976.

[3]that is, the percentage abundance divided by 100

[4]In reality, all averages are weighted-averages but in the case of the simple average or mean, the "abundance" of each contributor to the average is equal thus the average is simply the sum of all contributors divided by the number of contributors.

apostrophe ('), carat (^), or double-quote (") before the first parenthesis in the column headings to indicate the that the heading is text and not an equation. Center the column titles by highlighting cells A5 to C6 by clicking in cell A5 with the left mouse button and dragging to cell C6, releasing the mouse button, and finally pressing the "center cell contents button" on the tool bar. Highlight cells A6 to C6 and place a thin solid line on the bottom of the cells as a divider between the headings and values.

What you should notice immediately is that all trailing zeroes past the decimal point are dropped. This is remedied by highlighting those cells in which there should be a fixed number of decimal digits and setting the **Numeric Format** to a fixed 3 decimal digits. It should be clearly understood that this procedure "fixes" the number of decimal digits; it does *not* set the number of significant digits. Also, "fixing" the decimal digits to a specific number of places does not round the number for calculations; all digits are used, just not necessarily displayed. Do not fix the decimal places for the mass column since ^{201}Hg and ^{202}Hg are reported to only 2 decimal digits.

If you wish, you can change the font and font size as well as the column width and alignment inside the column to improve the esthetics of the table. It is not necessary for this example but improves the look of manuscripts and laboratory reports, giving them a more polished appearance.

Table 3. The example spreadsheet with an added formula.

	A	B	C	D
4				Isotopic Mass
5		Abundance	Mass	Contribution
6	Isotope	(%)	(u)	(u)
7	196	0.140	195.965	=B7/100*C7
8	198	10.039	197.967	
9	199	16.830	198.967	
10	200	23.120	199.968	
11	201	13.230	200.97	
12	202	29.790	201.97	
13	204	6.850	203.973	

Modify the sheet by adding, centering, and underlining the titles in cells D4 to D6 as shown. Write the equation in cell D7 exactly as shown in Table 3 and press the **Enter** key.

What you should see is that the equation is replaced by a number calculated from the abundance (in decimal rather than percentage form) and isotopic mass. An alternative way to write the equation is to press the "plus" (+) key then use the arrow keys or mouse to highlight cell B7 then type "/100*" followed by using the arrow keys or mouse again to highlight cell C7 and finally pressing the **Enter** key. You will learn many shortcuts as you use this and other software more.

At this point, your sheet should look similar to the following (Table 4):

Table 4. See text for details.

	A	B	C	D
1	Your name			
2	Date			
3	Weighted-Average Atomic Mass of Mercury			
4				Isotopic Mass
5		Abundance	Mass	Contribution
6	Isotope	(%)	(u)	(u)
7	196	0.140	195.965	0.274351
8	198	10.039	197.967	

Rather than retype the formula for calculating the mass contribution for each isotope, it is better to copy the formula to each cell. One feature of the spreadsheet program is that when the formula is copied then pasted, the cell references will be updated to reflect the new position(s) of the formula. If cell D7 is not the active cell (*i.e.* the highlighted cell), click in cell D7 with the mouse or move to it with the arrow keys. Use the mouse or shortcut key and click on **Edit** and then **Copy**. Highlight cells D8 to D13 and click on **Edit** and then **Paste**.

Notice that the numeric contents of cell D8 are 19.87391 and the formula (as seen in the formula edit line below the tool bar indicates the formula to be +B8/100*C8. The cell row numbers automatically updated to those of the next line down. If you copy and paste across a row, the cell column numbers will automatically update. You can override this feature when necessary by placing a dollar sign ($) before the letter and number in the cell reference. Thus, if $C$8 where in a formula and the formula was to be copied to other cells, the reference to cell C8 would remain unchanged.

If everything has worked so far, your spreadsheet should appear as in Table 5.

Table 5. Example spreadsheet after copying the equation in cell D7 to cells D8 through D13

	A	B	C	D
1	Your name			
2	Date			
3	Weighted-Average Atomic Mass of Mercury			
4				Isotopic Mass
5		Abundance	Mass	Contribution
6	Isotope	(%)	(u)	(u)
7	196	0.140	195.965	0.274351
8	198	10.039	197.967	19.873907
9	199	16.830	198.967	33.486146
10	200	23.120	199.968	46.232602
11	201	13.230	200.97	26.588331
12	202	29.790	201.97	60.166863
13	204	6.850	203.973	13.972151
14				
15				

Realistically, each Isotopic Mass Contribution should have no more than 3 decimal digits. Fix the number of decimal digits in the column as before to reflect this.

Finally, we calculate the weighted-average atomic mass which is simply the sum of the mass contributions. Place the formula[5] =@SUM(D7..D13) in cell D15. So as to leave no doubt as to the identity of the number, type the phrase "**The atomic weight is**" starting in cell B15.

Is the calculated atomic weight of mercury consistent with that reported on the periodic table? If not, check to see that every number is input correctly. When the sheet is satisfactory, save the spreadsheet to a file on a floppy disk and print the spreadsheet. As your skills with the spreadsheet program improve, treating data tables such as this will become very easy, perhaps even trivial.

Most modern spreadsheet programs stand out in their ability to make excellent graphs and plots quickly[6]. One useful example is a bar graph of percentage abundance *vs.* isotope number.

Start the graph construction by clicking on the **Graph Wizard** button then selecting **New**. Using the sequence of commands appropriate for the spreadsheet program, select the bar or column type graph, the percentage abundance column for the values (or series) to plot, and the isotope number as the x-axis category labels (or x-axis values).

Axes ranges, fonts, attributes, etc. can be modified by clicking on the axis with the right mouse button and simply altering the desired property. Graphs can be annotated with text, drawings, arrows, etc. Feel free to experiment.

2. Data Analysis: Least-Squares Fit

Among the most common statistics performed in analytical chemistry is the linear least-squares fit of presumably straight-line data. As an example, the data acquired for a Beer's Law calibration plot of concentration and absorbance are presumed to obey Beer's law, $A = \varepsilon l C$, where A is absorbance, ε is molar absorptivity, l is the pathlength, and C is the molar concentration of the absorbing species.

The following dataset was collected by a chemistry student for the percentage transmittance of 4 standard solutions of potassium permanganate with water as a blank using a Bausch & Lomb Spectronic 20 spectrophotometer and a 1.00 cm cuvette.

[5]There are many built-in functions, known as @-functions, that can be utilized for many operations such as adding lists of numbers, averages, standard deviations, trigonometric functions, and many others. Check the software manual or help screens in the program.

[6]It is in graphical operations where spreadsheet programs often differ the most.

Table 6. Spectrophotometric results for 4 $KMnO_4$ standards and solvent blank.

Concentration (M)	Percentage Transmittance (%*T*)
0.00	100.0
3.40×10^{-5}	80.3
9.02×10^{-5}	51.9
2.67×10^{-4}	19.4
3.70×10^{-4}	8.5

The student measured percentage transmittance, %*T*, since the %*T* scale on the instrument is more accurate and easier to read than the absorbance (*A*) scale. It is easy to convert %*T* to *A* through the relationship of

$$A = -\log\left(\frac{\%T}{100}\right)$$

Start a new spreadsheet (**File, New**) and place the appropriate titles in the upper-left corner cells. Make columns for concentration, percentage transmittance, and absorbance all side-by-side so that the first numerical datum will be in cell A6. Input the entire dataset (Table 6) into the appropriate cells[7]. Into the first cell of calculated absorbance, type in the formula for converting %*T* to *A*. The common log @-function is **@LOG**[8]. Copy the formula to all of the necessary cells and fix the calculated results to 2 decimal digits.

To determine the least-squares fit equation of the line which represents the data, use the **Regression** selection. Regression analysis is found in Excel, for example, as a menu item under **Tools|Data Analysis** while in QuattroPro it is found under **Tools|Numeric Tools**. The independent[9] (or x-axis) variable range is concentration, the dependent (or y-axis) variable range is absorbance, and the output data array should be placed in cell in a convenient area of the worksheet. The y-intercept selection must be set to be computed so that the y-intercept is not forced through zero. When complete, press **OK** and the results of the linear least-squares fit will be displayed below the data table.

There is much information in the results table but for now the only important values are the **intercept** or **constant** which corresponds to the y-intercept of the best-fit line and the **x-coefficient** which corresponds to the slope of the equation. In this example, the slope of the line should be 2814 $M^{-1}cm^{-1}$ and the intercept 0.00426 absorbance units. A non-zero intercept is not unusual for laboratory data as long as the intercept is nearly zero.

[7]A number written in scientific notation, 3.40×10^{-5} for example, is entered as 3.40E-5.

[8]The natural log @-function is @LN.

[9]The *independent* variable is normally the variable controlled by the analyst. The *dependent* variable is the value determined by the independent variable. In the example presented, the analyst prepared a solution to 3.40×10^{-5} M $KMnO_4$ which was measured to have a %*T* of 80.3. The %*T* depends on the concentration.

An alternative to using the advance math tools is to use the @-functions for slope (@slope) and intercept (@intercept). The instructions for using these functions are in the software manuals on on-line help screens.

Can you trust the results of the analysis? Humans are very visually oriented and can spot errors very quickly in graphs or pictures - much faster than in a column of numbers. Let's plot the *A* versus *C* data and superimpose the best-fit line over the data.

Start by titling a new column to the right of the Absorbance column with Absorbance (fit). Your sheet may look something like the following.

	A	B	C	D
4	Concentration			Absorbance
5	(M)	%T	Absorbance	(fit)
6	0.00	100	0.00	
7	3.4E-5	80.3	0.10	

Type the following formula in cell D6.

=$C$20*A6 + $D$14

Which corresponds to εlC + intercept. Notice the use of the dollar signs ($) to make the slope and intercept constants. Make sure to use the correct references if your sheet does not look exactly like this example. Copy the formula into all of the appropriate cells and fix the results to 2 decimal places.

To visually observe if the data match and best-fit line are satisfactory, it is important to graph the data and superimpose the best fit line. In many programs dedicated to plotting data and functions, this is very easy - usually just a mouse click. In spreadsheet programs it is necessary to have both the data and results of the fit equation plotted simultaneously.

Start a new graph. Use the range A6..A10 for the X-axis series, C6..C10 for the 1st y-axis series, and D6..D10 for the 2nd y-axis series. Press **OK** to build the graph. If necessary, change the graph type to a X-Y scatter graph[10]. It is customary to remove the lines connecting the symbols representing the data. Set the properties of the 1st series to have a line style of "no line". It is also customary to remove the symbols from the best fit line. Set the properties of the 2nd series to have a marker size of zero. Graph and axis titles should be added to identify the plot and axes. The font sizes can be modified to better fit the printed page if desired.

You should print the spreadsheet and graph. The graph will be saved with the spreadsheet when it is saved to the floppy disk.

[10]There are two types of graphs that *appear* similar but are very different: 1) X-Y scatter graph and 2) Line graph. The Line graph type is very rarely ever used in chemistry. Be careful to select the X-Y scatter graph type for all y-versus-x plots.

2. Numerical Analysis: Nonlinear Fit

Many times it is necessary to fit an equation to nonlinear data. Examples include first-order decay such as found in kinetics, sigmoidal relationships as often observed in chemical probe binding to macromolecular substrates, titration curves, and many others. For this exercise, you will apply a numerical method to determine the first-order rate constant for a decay process.

First-order decay processes obey the integrated rate law

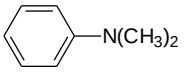
$$C_t = C_0 e^{-kt} \qquad (1)$$

where

C_0 is initial concentration,
C_t is the concentration at any time, t, and
k is the first-order rate constant

The method to be employed is the minimization of the sum of the squared residuals. A residual is the difference between an experimental and calculated datum[11]. The individual squared residuals for all data points are added together to get the sum of the squared residuals, R^2. By systematically changing the user-defined variable in the fit equation, the R^2 value may improve (or get worse). The spreadsheet program continues to refine the variables until there are insignificant changes in R^2.

The example to be studied is the photochemical destruction of dimethylaniline (DMA) in aqueous solution. Decomposition progress was monitored by gas chromatography-mass spectrometry and the results are summarized in Table 7.



Dimethylaniline

Table 7. [DMA] over time during photochemical destruction.

Time (min)	[DMA] (mM)
0	0.0606
5	0.0535
10	0.0447
15	0.0306
30	0.0257
45	0.0093
60	0.0049
90	0.0016
120	0.0015

[11]See also Anderson, Robert L., *Practical Statistics for Analytical Chemists*, Van Nostrand Reinhold, Co., New York, 1987, your textbook, or other source of statistical analysis.

After creating a plot of [DMA] *vs.* Time, it becomes very obvious that the data do not obey a linear equation (see Figure 1). Knowing that these data obey first-order decay kinetics, it is possible to perform a nonlinear fit to the integrated first-order rate law (eq 1). On a new spreadsheet, build a data table containing the data of table 7. Leave 3 extra rows empty below the sheet's identifying information for the results of the analysis. Include a column for the "fit" equation and another for "residual squared."

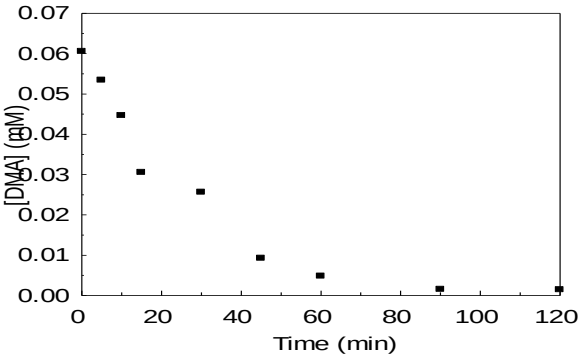


Figure 1. Plot of [DMA] *vs.* Time. See text for details.

Table 8 shows a possible view of the spreadsheet with the results area labeled. The formulas in cells C10 (integrated rate law) and D10 (squared residual) were copied into the cells beneath. The values in cells D4 (initial concentration) and D5 (rate constant) are initial "guesses" to give the numerical processor a starting point. Cell D6 is the sum of the squared residuals. The labels in cells C4 to C6 are for identification only but should be included for clarity. The formulas shown written out will display as values. Notice the use of constants in the fit equation. For consistency, fix the number of decimal places in the "fit" cells to four places.

Table 8. Example of spreadsheet prior to numerical optimization to determine the rate constant for a first-order decomposition reaction. See text for details.

	A	B	C	D
3			***RESULTS***	
4			Co =	0.06
5			k =	0.05
6			R^2 =	=@SUM(D10..D18)
7				
8	Time	[DMA]	[DMA]	Residual
9	(min)	(mM)	(fit)	squared
10	0	0.0606	=$D$4*@exp(-$D$5*A10)	+(C10-B10)^2
11	5	0.0535	=$D$4*@exp(-$D$5*A11)	+(C11-B11)^2
12	10	0.0447	=$D$4*@exp(-$D$5*A12)	+(C12-B12)^2
13	15	0.0306	=$D$4*@exp(-$D$5*A13)	+(C13-B13)^2
14	30	0.0257	=$D$4*@exp(-$D$5*A14)	+(C14-B14)^2
15	45	0.0093	=$D$4*@exp(-$D$5*A15)	+(C15-B15)^2
16	60	0.0049	=$D$4*@exp(-$D$5*A16)	+(C16-B16)^2
17	90	0.0016	=$D$4*@exp(-$D$5*A17)	+(C17-B17)^2
18	120	0.0015	=$D$4*@exp(-$D$5*A18)	+(C18-B18)^2

Before proceeding, create a X-Y scatter graph with Time as the x-axis, [DMA] as the 1st series, and [DMA] (fit) as the 2nd series. Set the properties of the 1st series to symbols only (turn off the lines) and the properties of the 2nd series to lines only (turn off the markers). This is consistent with the prior example where the fit line has no markers and the data themselves are not connected with lines.

Notice in the plot that the line is not very close to the data points, indicating a poor "goodness-of-fit." Since Co and k are the constants in the fit equation, it would be possible to refine the model by adjusting Co and k to improve (reduce) the sum of the squared residuals, R^2. Try it; change k to 0.04 and observe how R^2 changes. View the graph; notice how the fit line gets closer to the data points. It is possible to systematically change k and Co by hand until R^2 is as small as you can make it but it is repetitive tasks such as these where spreadsheet software shines.

In Excel, go to the **Solver** selection under the **Tools** menu item (**Tools|Numeric Tool|Optimizer** in QuattroPro). Set the Target Cell (or solution cell) to D6 (*i.e.* R^2) so as to minimize it with a **target value** of zero. Set the variable cells to D4..D5 then click **OK**. In a moment, the computer will complete the numerical optimization of the variables to minimize the sum of the squared residuals. When the computer is done, press the **Close** button. Occasionally, a solution cannot be found immediately. In such a case, convergence can be obtained by subsequent attempts at achieving a solution.

Observe how small the value for R^2 is and the values of Co and k. Look at the graph. While the line does not go through every point, it is as close as it can get to every point. The sum of the squared residuals has been minimized thus the fit must be as good as possible.

You may be asking why optimize the initial concentration when it was known experimentally. By optimizing both C_0 and the rate constant, k, we are able to get a better goodness-of-fit. After saving and printing the spreadsheet and graph, reset the value of C_0 to the experimentally determined initial concentration and optimize again, but only by optimizing the rate constant. How are the values for the rate constant and R^2 different this time?

Conclusion

After completing these exercises, you have the necessary tools to experiment and explore with a powerful computer tool. Mastery of the spreadsheet program will allow you to explore many sophisticated chemical and physical systems rapidly and by changing parameters see sometimes subtle and sometimes profound changes in the system. Also, as you gain experience, you will discover the limitations of the program and will be able to make decisions about software possibly more appropriate to the system under study.

For credit, turn in all spreadsheets and plots with appropriate annotations.

A Problem of the Least-Squares Fit When You Don't Look at Your Data

Introduction

With the convenience and speed of computer programs the user is often apt to believe whatever the result the computer gives. This is no less true in scientific applications. It seems strange, perhaps, but otherwise meticulous and detail-oriented scientists will often blindly trust that they entered data correctly into the computer, that the mathematical model is use is correct, and that the results of a calculation are necessarily correct. There is a danger in analyzing data without actually looking carefully at the data. Many mistakes can be avoided by simply taking the time the prepare a plot and check the results obtained from the computer.

The Anscombe dataset[1] is a useful tool for learning some of the difficulties that can occur in least-squares fitting of x-y data. The datasets are 4 pairs of x-y data (Table 1.) For the moment, disregard your scientific nature to analyze numbers and trends and enter the four datasets into spreadsheet columns.

Table 1. The Anscombe Dataset

X_1	Y_1	X_2	Y_2	X_3	Y_3	X_4	Y_4
10	8.04	10	9.14	10	7.46	8	6.58
8	6.95	8	8.14	8	6.77	8	5.76
13	7.58	13	8.74	13	12.75	8	7.70
9	8.81	9	8.77	9	7.11	8	8.84
11	8.33	11	9.26	11	7.81	8	8.47
14	9.96	14	8.10	14	8.84	8	7.04
6	7.24	6	6.13	6	6.08	8	5.25
4	4.26	4	3.10	4	5.39	19	12.50
12	10.84	12	9.13	12	8.15	8	5.56
7	4.82	7	7.26	7	6.42	8	7.91
5	5.68	5	4.74	5	5.73	8	6.89

Without graphing, use the spreadsheet program to determine the equation of the best-fit straight line for each individual x-y dataset by least-squares fit. What are the results of the least-squares fit? If all of the values were transferred correctly, a somewhat surprising result should be observed.

Now graph the data. Plot all with the same axes:

x-range = 0 to 20
y-range = 0 to 15

You will find that the graphs look very different.

Not only do these four data-sets all fit to the same equation

[1]F. J. Anscombe, "Graphs in Statistical Analysis", *American Statistician*, 1973, v. 27, pp. 17-21.

$$Y = 0.50X + 3.0$$

but the have all the same *goodness-of-fit* parameters, namely the same correlation coefficient, the same uncertainties of slope and intercept and the same standard deviation of residuals. Thus, the four least-squares regressions agree exactly.

- The first set does fit a straight-line model, though there is a lot of random scatter typical of an analysis near the limit of detection.
- The second set does not agree with a straight-line model; the pattern is strongly curved, and a quadratic model

  $$Y = aX^2 + bX + c$$

  would clearly be more appropriate[2].
- The third set does fit a straight-line model, but the data-set includes one outlier (or deviant) point, which pulls the fitted line systematically away from the trend of all the other points.
- The fourth set has most of the data-points clustered together at the low end (x = 8) of the data-range, so that the fitted line is entirely determined by a single high-leverage point at x = 19.

Conclusion

The Anscombe data-set is valuable in demonstrating that computers must never treated as infallible oracles. Computer statistics can be misleading. The best instrument for data analysis is still the ACME Mark-I human eyeball.

Turn in for credit the computer worksheet and annotated plots with best-fit straight lines.

[2]You may wish to determine the values of a, b, and c numerically by fitting the data to a quadratic equation. Verify that the results obtained are a = -0.1268, b = 2.7815, and c = -5.999.

Appendices

Appendix 1

Formula Weights of Selected Compounds

$AgBr$	187.78	$HClO_4$	100.46	$Mg_2P_2O_7$	222.57
$AgCl$	143.32	$H_2C_2O_4 \cdot 2H_2O$	126.07	$MgSO_4$	120.37
Ag_2CrO_4	331.73	H_5IO_6	227.94	MnO_2	86.94
AgI	234.77	HNO_3	63.01	Mn_2O_3	157.88
$AgNO_3$	169.87	H_2O	18.015	Mn_3O_4	228.81
$AgSCN$	165.95	H_2O_2	34.01	$Na_2B_4O_7 \cdot 10H_2O$	381.37
Al_2O_3	101.96	H_3PO_4	98.00	$NaBr$	102.90
$Al_2(SO_4)_3$	342.14	H_2S	34.08	$NaC_2H_3O_2$	82.03
As_2O_3	197.85	H_2SO_3	82.08	$Na_2C_2O_4$	134.00
B_2O_3	69.62	H_2SO_4	98.08	$NaCl$	58.44
$BaCO_3$	197.35	HgO	216.59	$NaCN$	49.01
$BaCl_2 \cdot 2H_2O$	244.28	Hg_2Cl_2	472.09	Na_2CO_3	105.99
$BaCrO_4$	253.33	$HgCl_2$	271.50	$NaHCO_3$	84.01
$Ba(IO_3)_2$	487.14	KBr	119.01	$Na_2H_2EDTA \cdot 2H_2O$	372.2
$Ba(OH)_2$	171.36	$KBrO_3$	167.01	Na_2O_2	77.98
$BaSO_4$	233.40	KCl	74.56	$NaOH$	40.00
Bi_2O_3	466.0	$KClO_3$	122.55	$NaSCN$	81.07
CO_2	44.01	KCN	65.12	Na_2SO_4	142.04
$CaCO_3$	100.09	K_2CrO_4	194.20	$Na_2S_2O_3 \cdot 5H_2O$	248.18
CaC_2O_4	128.10	$K_2Cr_2O_7$	294.19	NH_4Cl	53.49
CaF_2	78.08	$K_3Fe(CN)_6$	329.26	$(NH_4)_2C_2O_4 \cdot H_2O$	142.11
CaO	56.08	$K_4Fe(CN)_6$	368.38	NH_4NO_3	80.04
$CaSO_4$	136.14	$KHC_4H_4O_4$ (phthalate)	204.23	$(NH_4)_2SO_4$	132.14
$Ce(HSO_4)_4$	528.4	$KH(IO_3)_2$	389.92	$(NH_4)_2S_2O_8$	228.18
CeO_2	172.12	K_2HPO_4	174.18	NH_4VO_3	116.98
$Ce(SO_4)_2$	332.25	KH_2PO_4	136.09	$Ni(C_4H_7O_2N_2)_2$	288.93
$(NH_4)_2Ce(NO_3)_6$	548.23	$KHSO_4$	136.17	$PbCrO_4$	323.18
$(NH_4)_4Ce(SO_4)_4 \cdot 2H_2O$	632.6	KI	166.01	PbO	223.19
Cr_2O_3	151.99	KIO_3	214.00	PbO_2	239.19
CuO	79.54	KIO_4	230.00	$PbSO_4$	303.25
Cu_2O	143.08	$KMnO_4$	158.04	P_2O_5	141.94
$CuSO_4$	159.60	KNO_3	101.11	Sb_2S_3	339.69
$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$	392.14	KOH	56.11	SiO_2	60.08
FeO	71.85	$KSCN$	97.18	$SnCl_2$	189.60
Fe_3O_3	159.69	K_2SO_4	174.27	SnO_2	150.69
Fe_3O_4	231.54	$La(IO_3)_3$	663.62	SO_2	64.06
HBr	80.92	$Mg(C_9H_6ON)_2$	312.39	SO_2	80.06
$HC_2H_3O_2$ (acetic acid)	60.05	$MgCO_3$	84.32	$Zn_2P_2O_7$	304.68
$HC_7H_5O_2$ (benzoic acid)	122.12	$MgNH_4PO_4$	137.35		
HCl	36.46	MgO	40.31		

Appendix 2

Concentrations of Some Common Acids and Bases

	Molar Mass	mol/L	g/L	$\%^{w}/_{w}$	density g/mL
acetic acid, glacial	60.05	17.4	1045	99.5	1.05
acetic acid		6.27	376	36	1.045
butyric acid	88.1	10.3	912	95	0.96
formic acid	46.02	23.4	1080	90	1.20
		5.75	264	25	1.06
hydroiodic acid	127.9	7.57	969	57	1.70
		5.51	705	47	1.50
		0.86	110	10	1.1
hydrobromic acid	80.92	8.89	720	48	1.50
		6.82	552	40	1.38
hydrochloric acid	36.5	11.6	424	36	1.18
		2.9	105	10	1.05
hydrocyanic acid	27.03	25	676	97	0.697
		0.74	19.9	2	0.996
hydrofluoric acid	20.01	32.1	642	55	1.167
		28.8	578	50	1.155
lactic acid	90.1	11.3	1020	85	1.2
nitric acid	63.02	15.99	1008	71	1.42
		14.9	938	67	1.40
		13.3	837	61	1.37
perchloric acid	100.5	11.65	1172	70	1.67
		9.2	923	60	1.54
phosphoric acid	98	14.7	1445	85	1.70
sulfuric acid	98.1	18.0	1766	96	1.84
sulfurous acid	82.1	0.74	61.2	6	1.02
aqueous ammonia	17.0	14.8	252	28	0.898
potassium hydroxide	56.1	13.5	757	50	1.52
		1.94	109	10	1.09
sodium carbonate	106.0	1.04	110	10	1.10
sodium hydroxide	40.0	19.1	763	50	1.53
		2.75	111	10	1.11

Appendix 3

Some Important Statistical Tables[1]

Table 1: Confidence Levels for Various Values of *z*

Confidence Levels %	*z*
50	0.67
68	1.00
80	1.29
90	1.64
95	1.96
96	2.00
99	2.58
99.7	3.00
99.9	3.29

Table 2: Critical Values for Rejection Quotient, *Q*

Number of Observations	Q_{crit} 90% Confidence	95% Confidence	99% Confidence
3	0.941	0.970	0.994
4	0.765	0.829	0.926
5	0.642	0.710	0.821
6	0.560	0.625	0.740
7	0.507	0.568	0.680
8	0.468	0.526	0.634
9	0.437	0.493	0.598
10	0.412	0.466	0.568

(Results of test are: OK to reject if $Q_{exp} > Q_{crit}$)

Table 3: Values of Student's-*t* at Various Levels of Probability

Degrees of Freedom	*t* at Confidence Interval 80%	90%	95%	99%	99.9%
1	3.08	6.31	12.7	63.7	637
2	1.89	2.92	4.30	9.92	31.6
3	1.64	2.35	3.18	5.84	12.9
4	1.53	2.13	2.78	4.60	8.60
5	1.48	2.02	2.57	4.03	6.86
6	1.44	1.94	2.45	3.71	5.96
7	1.42	1.90	2.36	3.50	5.40
8	1.40	1.86	2.31	3.36	5.04
9	1.38	1.83	2.26	3.25	4.78
10	1.37	1.81	2.23	3.17	4.59
11	1.36	1.80	2.20	3.11	4.44
12	1.36	1.78	2.18	3.06	4.32
13	1.35	1.77	2.16	3.01	4.22
14	1.34	1.76	2.14	2.98	4.14
∞	1.29	1.64	1.96	2.58	3.29

[1]Adapted from Anderson, Robert L., *Practical Statistics for Analytical Chemists*, Van Nostrand Reinhold, Co., New York, 1987.

Table 4: Critical Values for the Rejection Quotient, *Tn*

Number of Observations	T_n 95% Confidence	97.5% Confidence	99% Confidence
3	1.15	1.15	1.15
4	1.46	1.48	1.49
5	1.67	1.71	1.75
6	1.82	1.89	1.94
7	1.94	2.02	2.10
8	2.03	2.13	2.22
9	2.11	2.21	2.52
10	2.18	2.29	2.41

Table 5: Critical Values for *F* at the 5% Level

Degrees of Freedom (Denominator)	Degrees of Freedom (Numerator) 2	3	4	5	6	12	20	∞
2	19.00	19.16	19.25	19.30	19.33	19.41	19.45	19.50
3	9.55	9.28	9.12	9.01	8.94	8.74	8.66	8.53
4	6.94	6.59	6.39	6.26	6.16	5.91	5.80	5.63
5	5.79	5.41	5.19	5.05	4.95	4.68	4.56	4.36
6	5.14	4.76	4.53	4.39	4.28	4.00	3.87	3.67
12	3.89	3.49	3.26	3.11	3.00	2.69	2.54	2.30
20	3.49	3.10	2.87	2.71	2.60	2.28	2.12	1.84
∞	3.00	2.60	2.37	2.21	2.10	1.75	1.57	1.00

Appendix 4

Acid Dissociation Constants of Selected Weak Acids

Acid	Formula	K_a
acetic	$HC_2H_3O_2$	1.8×10^{-5}
acrylic	$HC_3H_3O_2$	5.5×10^{-5}
p-aminobenzoic	$HC_7H_6NO_2$	1.2×10^{-5}
arsenic	H_3AsO_4	6.0×10^{-3}
	$H_2AsO_4^-$	1.0×10^{-7}
	$HAsO_4^{2-}$	3.2×10^{-12}
arsenous	H_3AsO_3	6.6×10^{-10}
ascorbic	$H_2C_6H_6O_6$	7.9×10^{-5}
	$HC_6H_6O_6^-$	1.6×10^{-12}
benzoic	$HC_7H_5O_2$	6.3×10^{-5}
bromoacetic	$HC_2H_2BrO_2$	1.3×10^{-3}
butyric	$HC_4H_7O_2$	1.5×10^{-5}
carbonic	H_2CO_3	4.4×10^{-7}
	HCO_3^-	4.7×10^{-11}
chloroacetic	$HC_2H_2ClO_2$	1.4×10^{-3}
chlorous	$HClO_2$	1.1×10^{-2}
citric	$H_3C_6H_5O_7$	7.4×10^{-4}
	$H_2C_6H_5O_7^-$	1.7×10^{-5}
	$HC_6H_5O_7^{2-}$	4.0×10^{-7}
cyanic	HOCN	3.5×10^{-4}
dichloroacetic	$HC_2HCl_2O_2$	5.5×10^{-2}
fluoroacetic	$HC_2H_2FO_2$	2.6×10^{-3}
formic	HCO_2H	1.8×10^{-4}
fumaric	$H_2C_4H_2O_4$	9.3×10^{-4}
	$HC_4H_2O_4^-$	3.6×10^{-5}
hydrazoic	HN_3	1.9×10^{-5}
hydrocyanic	HCN	6.2×10^{-10}
hydrofluoric	HF	6.6×10^{-4}
hydrogen peroxide	H_2O_2	2.2×10^{-12}
hydroselenic	H_2Se	1.3×10^{-4}
	HSe^-	1×10^{-11}
hydrosulfuric	H_2S	1.0×10^{-7}
	HS^-	1×10^{-19}
hydrotelluric	H_2Te	2.3×10^{-3}
	HTe^-	1.6×10^{-11}
p-hydroxybenzoic	$H_2C_7H_4O_3$	3.3×10^{-5}
	$HC_7H_4O_3^-$	4.8×10^{-10}
hypobromous	HOBr	2.5×10^{-9}
hypochlorous	HOCl	2.9×10^{-8}
hypoiodous	HOI	2.3×10^{-11}
hyponitrous	HON=NOH	8.9×10^{-8}
	$HON=NO^-$	4×10^{-12}
iodic	HIO_3	1.6×10^{-1}
lactic	$HC_3H_5O_3$	8.4×10^{-4}
maleic	$H_2C_4H_2O_4$	1.4×10^{-2}
	$HC_4H_2O_4^-$	8.6×10^{-7}
malic	$H_2C_4H_4O_5$	3.9×10^{-4}
	$HC_4H_4O_5^-$	7.8×10^{-6}
malonic	$H_2C_3H_2O_4$	1.5×10^{-3}
	$HC_3H_2O_4^-$	2.0×10^{-6}
nitrous	HNO_2	7.2×10^{-4}
oxalic	$H_2C_2O_4$	5.4×10^{-2}
	$HC_2O_4^-$	5.3×10^{-5}
phenol	HOC_6H_5	1.0×10^{-10}
phenylacetic	$HC_8H_7O_2$	4.9×10^{-5}
phosphoric	H_3PO_4	7.1×10^{-3}
	$H_2PO_4^-$	$6.3 \times l0^{-8}$
	HPO_4^{2-}	4.2×10^{-13}
phosphorous	H_3PO_3	3.7×10^{-2}
	$H_2PO_3^-$	2.1×10^{-7}
o-phthalic	$H_2C_8H_4O_4$	1.3×10^{-3}
	$HC_8H_4O_4^-$	$3.9 \times l0^{-6}$
picric	$HC_6H_2N_3O_7$	4.2×10^{-1}
propionic	$HC_3H_5O_2$	1.3×10^{-5}
pyrophosphoric	$H_4P_2O_7$	3.0×10^{-2}
	$H_3P_2O_7^-$	4.4×10^{-3}
	$H_2P_2O_7^{2-}$	2.5×10^{-7}
	$HP_2O_7^{3-}$	5.6×10^{-10}
selenic	H_2SeO_4	strong
	$HSeO_4^-$	2.2×10^{-2}
selenous	H_2SeO_3	2.3×10^{-3}
	$HSeO_3^-$	5.4×10^{-9}
succinic	$H_2C_4H_4O_4$	6.2×10^{-5}
	$HC_4H_4O_4^-$	2.3×10^{-6}
sulfuric	H_2SO_4	strong
	HSO_4^-	$1.1 \times l0^{-2}$
sulfurous	$H_2SO_3^-$	$1.3 \times l0^{-2}$
	HSO_3^-	6.2×10^{-8}
thiophenol	HSC_6H_5	3.2×10^{-7}
trichloroacetic	$HC_2Cl_3O_2$	3.0×10^{-1}

Base Ionization Constants of Selected Weak Bases

Base	Formula	K_b
ammonia	NH_3	1.8×10^{-5}
aniline	$C_6H_5NH_2$	7.4×10^{-10}
codeine	$C_{18}H_{21}O_3N$	$8.9 \times l0^{-7}$
diethylamine	$(C_2H_5)_2NH$	3.1×10^{-4}
dimethylamine	$(CH_3)_2NH$	5.9×10^{-4}
ethylamine	$C_2H_5NH_2$	4.3×10^{-4}
hydrazine	NH_2NH_2	8.5×10^{-7}
	$NH_2NH_3^+$	8.9×10^{-16}
hydroxylamine	NH_2OH	6.6×10^{-9}
isoquinoline	C_9H_7N	2.5×10^{-9}
methylamine	CH_3NH_2	4.2×10^{-4}
morphine	$C_{17}H_{19}O_3N$	7.4×10^{-7}
piperidine	$C_5H_{11}N$	1.3×10^{-3}
pyridine	C_5H_5N	1.5×10^{-9}
quinoline	C_9H_7N	6.3×10^{-10}
triethanolamine	$C_6H_{15}O_3N$	5.8×10^{-7}
triethylamine	$(C_2H_5)_3N$	5.2×10^{-4}
trimethylamine	$(CH_3)_3N$	$6.3 \times l0^{-5}$

Appendix 5

Solubility Products of Selected Compounds

Solubility Product Constants Measured at or near 25°C

Compound	*Ksp*
Aluminum hydroxide	3.7×10^{-15}
Barium carbonate	8.1×10^{-9}
Barium chromate	2.4×10^{-10} (28°)
Barium fluoride	1.7×10^{-6} (25.8°)
Barium iodate dihydrate	6.5×10^{-10}
Barium sulfate	1.1×10^{-10}
Calcium carbonate	8.7×10^{-9}
Calcium fluoride	4.0×10^{-11} (26°)
Calcium oxalate hydrate	2.6×10^{-9}
Calcium sulfate	2.5×10^{-5}
Copper(II) iodate	1.4×10^{-7}
Copper(II) oxalate	2.9×10^{-8}
Iron(II) oxalate	2.1×10^{-7}
Lead fluoride	3.7×10^{-8} (26.6°)
Lead iodate	2.6×10^{-13} (25.8°)
Lead iodide	1.4×10^{-8}

Compound	*Ksp*
Lithium carbonate	1.7×10^{-3}
Magnesium ammonium phosphate	2.5×10^{-13}
Magnesium fluoride	6.4×10^{-9} (27°)
Mercury(I) bromide	1.3×10^{-21}
Mercury(I) chloride	2×10^{-18}
Mercury(I) iodide	1.2×10^{-28}
Silver bromate	5.8×10^{-5}
Silver bromide	7.7×10^{-13}
Silver carbonate	6.2×10^{-12}
Silver chloride	1.6×10^{-10}
Silver chromate	9×10^{-12}
Silver dichromate	2×10^{-7}
Silver iodide	1.5×10^{-16}
Silver thiocyanate	1.2×10^{-12}
Strontium carbonate	1.6×10^{-9}

Solubility Product Constants Measured at Other Than Standard Temperature

Compound	*Ksp*
Aluminum hydroxide	4×10^{-13} (15°)
Aluminum hydroxide	1.1×10^{-15} (18°)
Barium carbonate	7×10^{-9} (16°)
Barium chromate	1.6×10^{-10} (18°)
Barium fluoride	1.6×10^{-6} (9.5°)
Barium fluoride	1.7×10^{-6} (18°)
Barium iodate dihydrate	8.4×10^{-11} (10°)
Barium oxalate, $BaC_2O_4 \cdot 3½H_2O$	1.6×10^{-7} (18°)
Barium oxalate dihydrate	1.2×10^{-7} (18°)
Barium oxalate hemihydrate	2.2×10^{-7} (18°)
Barium sulfate	8.7×10^{-11} (18°)
Barium sulfate	2.0×10^{-10} (50°)
Cadmium oxalate trihydrate	1.5×10^{-8} (18°)
Cadmium sulfide	3.6×10^{-29} (18°)
Calcium carbonate (calcite)	9.9×10^{-9} (15°)
Calcium fluoride	3.4×10^{-11} (18°)
Calcium iodate hexahydrate	2.2×10^{-7} (10°)
Calcium iodate hexahydrate	6.4×10^{-7} (18°)
Calcium oxalate hydrate	1.8×10^{-9} (18°)
Calcium tartrate dihydrate	7.7×10^{-7} (18°)
Cobalt sulfide	3×10^{-26} (18°)
Copper(II) sulfide	8.5×10^{-45} (18°)
Copper(I) bromide	4.2×10^{-8} (18-20°)
Copper(I) chloride	1.0×10^{-6} (18-20°)
Copper(I) iodide	5.1×10^{-12} (18°)
Copper(I) sulfide	2×10^{-47} (18°)
Copper(I) thiocyanate	1.6×10^{-11} (18°)
Iron(III) hydroxide	1.1×10^{-36} (18°)

Compound	*Ksp*
Iron(II) hydroxide	1.6×10^{-14} (18°)
Iron(II) sulfide	3.7×10^{-19} (18°)
Lead carbonate	3.3×10^{-14} (18°)
Lead chromate	1.8×10^{-14} (18°)
Lead fluoride	2.7×10^{-8} (9°)
Lead fluoride	3.2×10^{-8} (18°)
Lead iodate	5.3×10^{-14} (9.2°)
Lead iodate	1.2×10^{-13} (18°)
Lead iodide	7.5×10^{-9} (15°)
Lead oxalate	2.7×10^{-11} (18°)
Lead sulfate	1.1×10^{-8} (18°)
Lead sulfide	3.4×10^{-28} (18°)
Magnesium carbonate	2.6×10^{-5} (12°)
Magnesium fluoride	7.1×10^{-9} (18°)
Magnesium hydroxide	1.2×10^{-11} (18°)
Magnesium oxalate	8.6×10^{-5} (18°)
Manganese(II) hydroxide	4×10^{-14} (18°)
Manganese(II) sulfide	1.4×10^{-15} (18°)
Mercury(II) sulfide	4×10^{-53} (18°)
Nickel(II) sulfide	1.4×10^{-24} (18°)
Potassium hydrogen tartrate	3.8×10^{-4} (18°)
Silver bromate	4.0×10^{-5} (20°)
Silver bromide	4.1×10^{-13} (18°)
Silver chloride	2.1×10^{-11} (4.7°)
Silver chloride	3.7×10^{-11} (9.7°)
Silver chloride	1.3×10^{-9} (50°)
Silver chloride	2.2×10^{-8} (100°)
Silver chromate	1.2×10^{-12} (14.8°)

Compound	*Ksp*
Silver cyanide	2.2×10^{-12} (20°)
Silver hydroxide	1.5×10^{-8} (20°)
Silver iodate	9.2×10^{-9} (9.4°)
Silver iodide	3.2×10^{-17} (13°)
Silver sulfide	1.6×10^{-49} (18°)
Silver thiocyanate	4.9×10^{-13} (18°)
Strontium fluoride	2.8×10^{-9} (18°)

Compound	*Ksp*
Strontium oxalate	5.6×10^{-8} (18°)
Strontium sulfate	2.8×10^{-7} (2.9°)
Strontium sulfate	3.8×10^{-7} (17.4°)
Zinc hydroxide	1.8×10^{-14} (20°)
Zinc oxalate dihydrate	1.4×10^{-9} (18°)
Zinc sulfide	1.2×10^{-23} (18°)

When K_{sp} is known at two or more temperatures, the value of K_{sp} at other temperatures can be estimated using the Arrhenius equation:

$$\ln \frac{K_{sp,1}}{K_{sp,2}} = \frac{\Delta H}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right)$$

Appendix 6

Stability Constants for Selected Complex-Ions

Formula	K_f	Formula	K_f	Formula	K_f
$[Ag(CN)_2]^-$	5.6×10^{18}	$[Co(ox)_3]^{3-}$	10^{20}	$[HgI_4]^{2-}$	6.8×10^{29}
$[Ag(EDTA)]^{3-}$	2.1×10^{7}	$[Cr(EDTA)]^-$	10^{23}	$[Hg(ox)_2]^{2-}$	9.5×10^{6}
$[Ag(en)_2]^-$	5.0×10^{7}	$[Cr(OH)_4]^-$	8×10^{29}	$[Ni(CN)_4]^{2-}$	2×10^{31}
$[Ag(NH_3)_2]^+$	1.6×10^{7}	$[CuCl_4]^{2-}$	5×10^{5}	$[Ni(EDTA)]^{2-}$	3.6×10^{18}
$[Ag(SCN)_4]^{3-}$	1.2×10^{10}	$[Cu(CN)_4]^{3-}$	2.0×10^{30}	$[Ni(en)_3]^{2+}$	2.1×10^{18}
$[Ag(S_2O_3)_2]^{3-}$	1.7×10^{13}	$[Cu(EDTA)]^{2-}$	5×10^{18}	$[Ni(NH_3)_6]^{2+}$	5.5×10^{8}
$[Al(EDTA)]^-$	1.3×10^{16}	$[Cu(en)_2]^{2+}$	1×10^{20}	$[Ni(ox)_3]^{4-}$	3×10^{8}
$[Al(OH)_4]^-$	1.1×10^{33}	$[Cu(NH_3)_4]^{2+}$	1.1×10^{13}	$[PbCl_3]^-$	2.4×10^{1}
$[Al(ox)_3]^{3-}$	2×10^{16}	$[Cu(ox)_2]^{2-}$	3×10^{8}	$[Pb(EDTA)]^{2-}$	2×10^{18}
$[CdCl_4]^{2-}$	6.3×10^{2}	$[Fe(CN)_6]^{4-}$	10^{37}	$[PbI_4]^{2-}$	3.0×10^{4}
$[Cd(CN)_4]^{2-}$	6.0×10^{18}	$[Fe(EDTA)]^{2-}$	2.1×10^{14}	$[Pb(OH)_3]$	3.8×10^{14}
$[Cd(en)_3]^{2+}$	1.2×10^{12}	$[Fe(en)_3]^{2+}$	5.0×10^{9}	$[Pb(ox)_2]^{2-}$	3.5×10^{6}
$[Cd(NH_3)_4]^{2+}$	1.3×10^{7}	$[Fe(ox)_3]^{4-}$	1.7×10^{5}	$[Pb(S_2O_3)_3]^{4-}$	2.2×10^{6}
$[Co(EDTA)]^{2-}$	2.0×10^{16}	$[Fe(CN)_6]^{3-}$	10^{42}	$[PtCl_4]^{2-}$	1×10^{16}
$[Co(en)_3]^{2+}$	8.7×10^{13}	$[Fe(EDTA)]^-$	1.7×10^{24}	$[Pt(NH_3)_6]^{2+}$	2×10^{35}
$[Co(NH_3)_6]^{2+}$	1.3×10^{5}	$[Fe(ox)_3]^{3-}$	2×10^{20}	$[Zn(CN)_4]^{2-}$	1×10^{18}
$[Co(ox)_3]^{4-}$	5×10^{9}	$[Fe(SCN)]^{2+}$	8.9×10^{2}	$[Zn(EDTA)]^{2-}$	3×10^{16}
$[Co(SCN)_4]^{2-}$	1.0×10^{3}	$[HgCl_4]^{2-}$	1.2×10^{15}	$[Zn(en)_3]^{2+}$	1.3×10^{14}
$[Co(EDTA)]^-$	10^{36}	$[Hg(CN)_4]^{2-}$	3×10^{41}	$[Zn(NH_3)_4]^{2+}$	4.1×10^{8}
$[Co(en)_3]^{3+}$	4.9×10^{48}	$[Hg(EDTA)]^{2-}$	6.3×10^{21}	$[Zn(OH)_4]^{2-}$	4.6×10^{17}
$[Co(NH_3)_6]^{3+}$	4.5×10^{33}	$[Hg(en)_2]^{2+}$	2×10^{23}	$[Zn(ox)_3]^{4-}$	1.4×10^{8}

Appendix 7

Standard Reduction Potentials at 25°C

(In Order of Decreasing Potential)

Half-reaction	*E*° vs. *NHE* (volts)
$F_2 + 2H^+ + 2e \leftrightarrows 2HF$	+3.06
$S_2O_8^{2-} + 2e \leftrightarrows 2SO_4^{2-}$	+2.01
$Ag^{2+} + e \leftrightarrows Ag^+$	+1.98
$Co^{3+} + e \leftrightarrows Co^{2+}$	+1.842
$H_2O_2 + 2H^+ + 2e \leftrightarrows 2H_2O$	+1.77
$IO_4^- + 2H^+ + 2e \leftrightarrows IO_3^- + H_2O$	+1.70
$MnO_4^- + 4H^+ + 3e \leftrightarrows MnO_2 + 2H_2O$	+1.695
$PbO_2 + 4H^+ + SO_4^{2-} + 2e \leftrightarrows PbSO_4 + 2H_2O$	+1.685
$NiO_2 + 4H^+ + 2e \leftrightarrows Ni^{2+} + 2H_2O$	+1.68
$Ce^{4+} + e \leftrightarrows Ce^{3+}$	+1.61
$NaBiO_3 + 6H^+ + 3e \leftrightarrows Bi^{3+} + Na^+ + 3H_2O$	+1.59
$2BrO_3^- + 12H^+ + 10e \leftrightarrows Br_2 + 6H_2O$	+1.52
$MnO_4^- + 8H^+ + 5e \leftrightarrows Mn^{2+} + 4H_2O$	+1.51
$PbO_2 + 4H^+ + 2e \leftrightarrows Pb^{2+} + 2H_2O$	+1.455
$Cl_2 + 2e \leftrightarrows 2Cl^-$	+1.360
$Cr_2O_7^{2-} + 14H^+ + 6e \leftrightarrows 2Cr^{3+} + 7H_2O$	+1.33
$Tl^{3+} + 2e \leftrightarrows Tl^+$	+1.25
$MnO_2 + 4H^+ + 2e \leftrightarrows Mn^{2+} + 2H_2O$	+1.23
$O_2 + 4H^+ + 4e \leftrightarrows 2H_2O$	+1.229
$2IO_3^- + 12H^+ + 10e \leftrightarrows I_2 + 6H_2O$	+1.195
$ClO_4^- + 2H^+ + 2e \leftrightarrows ClO_3^- + H_2O$	+1.19
$Br_2(l) + 2e \leftrightarrows 2Br^-$	+1.065
$VO_2^+ + 2H^+ + e \leftrightarrows VO^{2+} + H_2O$	+1.00
$NO_3^- + 3H^+ + 2e \leftrightarrows HNO_2 + H_2O$	+0.94
$2Hg^{2+} + 2e \leftrightarrows Hg_2^{2+}$	+0.920
$2Cu^{2+} + 2I^- + 2e \leftrightarrows Cu_2I_2$	+0.86
$Hg^{2+} + 2e \leftrightarrows Hg$	+0.854
$Ag^+ + e \leftrightarrows Ag$	+0.799
$Hg_2^{2+} + 2e \leftrightarrows 2Hg$	+0.789
$Fe^{3+} + e \leftrightarrows Fe^{2+}$	+0.771
$Quinone + 2H^+ + 2e \leftrightarrows H_2Quinone$	+0.699
$O_2 + 2H^+ + 2e \leftrightarrows H_2O_2$	+0.682
$Ag_2SO_4 + 2e \leftrightarrows 2Ag + SO_4^{2-}$	+0.653
$AgOAc + e \leftrightarrows Ag + OAc^-$	+0.643
$UO_2^+ + 4H^+ + e \leftrightarrows U^{4+} + 2H_2O$	+0.62
$MnO_4^- + e \leftrightarrows MnO_4^{2-}$	+0.564
$H_3AsO_4 + 2H^+ + 2e \leftrightarrows HAsO_2 + 2H_2O$	+0.559
$2Cu^{2+} + 2Cl^- + 2e \leftrightarrows Cu_2Cl_2$	+0.538

Half-reaction	*E*° vs. *NHE* (volts)
$I_2 + 2e \leftrightarrows 2I^-$	+0.536
$I_3^- + 2e \leftrightarrows 3I^-$	+0.536
$Cu^+ + e \leftrightarrows Cu$	+0.521
$Ag_2CrO_4 + 2e \leftrightarrows 2Ag + CrO_4^{2-}$	+0.446
$VO^{2+} + 2H^+ + e \leftrightarrows V^{3+} + H_2O$	+0.361
$Fe(CN)_6^{3-} + e \leftrightarrows Fe(CN)_6^{4-}$	+0.36
$Ag_2O + H_2O + 2e \leftrightarrows 2Ag + 2OH^-$	+0.344
$Cu^{2+} + 2e \leftrightarrows Cu$	+0.337
$UO_2^{2+} + 4H^+ + 2e \leftrightarrows U^{4+} + 2H_2O$	+0.334
$BiO^+ + 2H^+ + 3e \leftrightarrows Bi + H_2O$	+0.31
$Hg_2Cl_2 + 2e \leftrightarrows 2Hg + 2Cl^-$	+0.268
$H_3AsO_3 + 3H^+ + 3e \leftrightarrows As + 3H_2O$	+0.24
$AgCl + e \leftrightarrows Ag + Cl^-$	+0.222
$HgBr_4^{2-} + 2e \leftrightarrows Hg + 4Br^-$	+0.21
$SO_4^{2-} + 4H^+ + 2e \leftrightarrows H_2SO_3 + H_2O$	+0.17
$Cu^{2+} + e \leftrightarrows Cu^+$	+0.153
$Sb_2O_3 + 6H^+ + 6e \leftrightarrows Sb + 3H_2O$	+0.152
$Sn^{4+} + 2e \leftrightarrows Sn^{2+}$	+0.15
$Hg_2Br_2 + 2e \leftrightarrows 2Hg + 2Br^-$	+0.140
$CuCl + e \leftrightarrows Cu + Cl^-$	+0.137
$TiO^{2+} + 2H^+ + e \leftrightarrows Ti^{3+} + H_2O$	+0.1
$H_2O + HgO + 2e \leftrightarrows Hg + 2OH^-$	+0.098
$AgBr + e \leftrightarrows Ag + Br^-$	+0.095
$S_4O_6^{2-} + 2e \leftrightarrows 2S_2O_3^{2-}$	+0.08
$UO_2^{2+} + e \leftrightarrows UO_2^+$	+0.05
$CuBr + e \leftrightarrows Cu + Br^-$	+0.033
$2H^+ + 2e \leftrightarrows H_2$	0.000
$HgI_4^{2-} + 2e\ Hg + 4I^-$	-0.04
$Hg_2I_2 + 2e \leftrightarrows 2Hg + 2I^-$	-0.04
$Pb^{2+} + 2e \leftrightarrows Pb$	-0.126
$Sn^{2+} + 2e \leftrightarrows Sn$	-0.136
$AgI + e \leftrightarrows Ag + I^-$	-0.151
$Cu_2I_2 + 2e \leftrightarrows 2Cu + 2I^-$	-0.185
$Mo^{3+} + 3e \leftrightarrows Mo$	-0.2
$N_2 + 5H^+ + 4e \leftrightarrows N_2H_5^+$	-0.23
$Ni^{2+} + 2e \leftrightarrows Ni$	-0.250
$V^{3+} + e \leftrightarrows V^{2+}$	-0.255
$PbCl_2 + 2e \leftrightarrows Pb + 2Cl^-$	-0.268

Half-reaction	*E*° vs. *NHE* (volts)
$Co^{2+} + 2e \leftrightarrows Co$	-0.277
$PbBr_2 + 2e \leftrightarrows Pb + 2Br^-$	-0.280
$Ag(CN)_2^- + e \leftrightarrows Ag + 2CN^-$	-0.31
$Tl^+ + e \leftrightarrows Tl$	-0.336
$Cu_2O + H_2O + 2e \leftrightarrows 2Cu + 2OH^-$	-0.34
$PbSO_4 + 2e \leftrightarrows Pb + SO_4^{2-}$	-0.356
$PbI_2 + 2e \leftrightarrows Pb + 2I^-$	-0.365
$Ti^{3+} + e \leftrightarrows Ti^{2+}$	-0.37
$Cd^{2+} + 2e \leftrightarrows Cd$	-0.403
$Cr^{3+} + e \leftrightarrows Cr^{2+}$	-0.41
$Fe^{2+} + 2e \leftrightarrows Fe$	-0.440
$2CO_2 + 2H^+ + 2e \leftrightarrows H_2C_2O_4$	-0.49
$Ga^{3+} + 3e$ Ga	-0.53
$TlCl + e \leftrightarrows Tl + Cl^-$	-0.557
$U^{4+} + e \leftrightarrows U^{2+}$	-0.61
$TlBr + e \leftrightarrows Tl + Br^-$	-0.658
$Cr^{3+} + 3e \leftrightarrows Cr$	-0.74
$TlI + e \leftrightarrows Tl + I^-$	-0.753
$Zn^{2+} + 2e \leftrightarrows Zn$	-0.763
$Cd(OH)_2 + 2e \leftrightarrows Cd + 2OH^-$	-0.809
$2H_2O + 2e \leftrightarrows H_2 + 2OH^-$	-0.828

Half-reaction	*E*° vs. *NHE* (volts)
$TiO^{2+} + 2H^+ + 4e \leftrightarrows Ti + H_2O$	-0.89
$Cr^{2+} + 2e \leftrightarrows Cr$	-0.91
$V^{2+} + 2e \leftrightarrows V$	-1.18
$Mn^{2+} + 2e \leftrightarrows Mn$	-1.18
$Ti^{2+} + 2e \leftrightarrows Ti$	-1.63
$Al^{3+} + 3e \leftrightarrows Al$	-1.66
$U^{3+} + 3e \leftrightarrows U$	-1.80
$Be^{2+} + 2e \leftrightarrows Be$	-1.85
$Np^{3+} + 3e \leftrightarrows Np$	-1.86
$Th^{4+} + 4e \leftrightarrows Th$	-1.90
$AlF_6^{3-} + 3e \leftrightarrows Al + 6F^-$	-2.07
$Pu^{3+} + 3e \leftrightarrows Pu$	-2.07
$Mg^{2+} + 2e \leftrightarrows Mg$	-2.37
$Ce^{3+} + 3e \leftrightarrows Ce$	-2.48
$La^{3+} + 3e \leftrightarrows La$	-2.52
$Na^+ + e \leftrightarrows Na$	-2.714
$Ca^{2+} + 2e \leftrightarrows Ca$	-2.87
$Sr^{2+} + 2e \leftrightarrows Sr$	-2.89
$Ba^{2+} + 2e \leftrightarrows Ba$	-2.90
$K^+ + e \leftrightarrows K$	-2.925
$Li^+ + e \leftrightarrows Li$	-3.045

Standard Reduction Potentials at 25°C

(Sorted in Alphabetical Order)

Half-reaction	*E*° vs. *NHE* (volts)
$Ag^+ + e \leftrightarrows Ag$	+0.799
$Ag^{2+} + e \leftrightarrows Ag^+$	+1.98
$AgBr + e \leftrightarrows Ag + Br^-$	+0.095
$Ag(CN)_2^- + e \leftrightarrows Ag + 2CN^-$	-0.31
$Ag_2CrO_4 + 2e \leftrightarrows 2Ag + CrO_4^{2-}$	+0.446
$AgCl + e \leftrightarrows Ag + Cl^-$	+0.222
$AgI + e \leftrightarrows Ag + I^-$	-0.151
$Ag_2O + H_2O + 2e \leftrightarrows 2Ag + 2OH^-$	+0.344
$AgOAc + e \leftrightarrows Ag + OAc^-$	+0.643
$Ag_2SO_4 + 2e \leftrightarrows 2Ag + SO_4^{2-}$	+0.653
$Al^{3+} + 3e \leftrightarrows Al$	-1.66
$AlF_6^{3-} + 3e \ Al + 6F^-$	-2.07
$H_3AsO_3 + 3H^+ + 3e \leftrightarrows As + 3H_2O$	+0.24
$H_3AsO_4 + 2H^+ + 2e \leftrightarrows HAsO_2 + 2H_2O$	+0.559
$Ba^{2+} + 2e \leftrightarrows Ba$	-2.90
$Be^{2+} + 2e \leftrightarrows Be$	-1.85
$BiO^+ + 2H^+ + 3e \leftrightarrows Bi + H_2O$	+0.31
$NaBiO_3 + 6H^+ + 3e \leftrightarrows Bi^{3+} + Na^+ + 3H_2O$	+1.59
$Br_2(l) + 2e \leftrightarrows 2Br^-$	+1.065
$2BrO_3^- + 12H^+ + 10e \leftrightarrows Br_2 + 6H_2O$	+1.52
$Quinone + 2H^+ + 2e \leftrightarrows H_2Quinone$	+0.699
$2CO_2 + 2H^+ + 2e \leftrightarrows H_2C_2O_4$	-0.49
$Ca^{2+} + 2e \leftrightarrows Ca$	-2.87
$Cd^{2+} + 2e \leftrightarrows Cd$	-0.403
$Cd(OH)_2 + 2e \leftrightarrows Cd + 2OH^-$	-0.809
$Ce^{3+} + 3e \leftrightarrows Ce$	-2.48
$Ce^{4+} + e \leftrightarrows Ce^{3+}$	+1.61
$Cl_2 + 2e \leftrightarrows 2Cl^-$	+1.360
$ClO_4^- + 2H^+ + 2e \leftrightarrows ClO_3^- + H_2O$	+1.19
$Co^{2+} + 2e \leftrightarrows Co$	-0.277
$Co^{3+} + e \leftrightarrows Co^{2+}$	+1.842
$Cr^{2+} + 2e \leftrightarrows Cr$	-0.91
$Cr^{3+} + e \leftrightarrows Cr^{2+}$	-0.41
$Cr^{3+} + 3e \leftrightarrows Cr$	-0.74
$Cr_2O_7^{2-} + 14H^+ + 6e \leftrightarrows 2Cr^{3+} + 7H_2O$	+1.33
$Cu^+ + e \leftrightarrows Cu$	+0.521
$Cu^{2+} + e \leftrightarrows Cu^+$	+0.153
$Cu^{2+} + 2e \leftrightarrows Cu$	+0.337

Half-reaction	*E*° vs. *NHE* (volts)
$CuBr + e \leftrightarrows Cu + Br^-$	+0.033
$CuCl + e \leftrightarrows Cu + Cl^-$	+0.137
$2Cu^{2+} + 2Cl^- + 2e \leftrightarrows Cu_2Cl_2$	+0.538
$2Cu^{2+} + 2I^- + 2e \leftrightarrows Cu_2I_2$	+0.86
$Cu_2I_2 + 2e \leftrightarrows 2Cu + 2I^-$	-0.185
$Cu_2O + H_2O + 2e \leftrightarrows 2Cu + 2OH^-$	-0.34
$F_2 + 2H^+ + 2e \leftrightarrows 2HF$	+3.06
$Fe^{2+} + 2e \leftrightarrows Fe$	-0.440
$Fe^{3+} + e \leftrightarrows Fe^{2+}$	+0.771
$Fe(CN)_6^{3-} + e \leftrightarrows Fe(CN)_6^{4-}$	+0.36
$Ga^{3+} + 3e \leftrightarrows Ga$	-0.53
$2H^+ + 2e \leftrightarrows H_2$	0.000
$2H_2O + 2e \leftrightarrows H_2 + 2OH^-$	-0.828
$H_2O_2 + 2H^+ + 2e \leftrightarrows 2H_2O$	+1.77
$2Hg^{2+} + 2e \leftrightarrows Hg_2^{2+}$	+0.920
$Hg^{2+} + 2e \leftrightarrows Hg$	+0.854
$Hg_2^{2+} + 2e \leftrightarrows 2Hg$	+0.789
$Hg_2Br_2 + 2e \leftrightarrows 2Hg + 2Br^-$	+0.140
$HgBr_4^{2-} + 2e \leftrightarrows Hg + 4Br^-$	+0.21
$Hg_2Cl_2 + 2e \leftrightarrows 2Hg + 2Cl^-$	+0.268
$Hg_2I_2 + 2e \leftrightarrows 2Hg + 2I^-$	-0.04
$HgI_4^{2-} + 2e \leftrightarrows Hg + 4I^-$	-0.04
$HgO + H_2O + 2e \leftrightarrows Hg + 2OH^-$	+0.098
$I_2 + 2e \leftrightarrows 2I^-$	+0.536
$I_3^- + 2e \leftrightarrows 3I^-$	+0.536
$2IO_3^- + 12H^+ + 10e \leftrightarrows I_2 + 6H_2O$	+1.195
$IO_4^- + 2H^+ + 2e \leftrightarrows IO_3^- + H_2O$	+1.70
$K^+ + e \leftrightarrows K$	-2.925
$La^{3+} + 3e \leftrightarrows La$	-2.52
$Li^+ + e \leftrightarrows Li$	-3.045
$Mg^{2+} + 2e \leftrightarrows Mg$	-2.37
$Mn^{2+} + 2e \leftrightarrows Mn$	-1.18
$MnO_2 + 4H^+ + 2e \leftrightarrows Mn^{2+} + 2H_2O$	+1.23
$MnO_4^- + e \leftrightarrows MnO_4^{2-}$	+0.564
$MnO_4^- + 4H^+ + 3e \leftrightarrows MnO_2 + 2H_2O$	+1.695
$MnO_4^- + 8H^+ + 5e \leftrightarrows Mn^{2+} + 4H_2O$	+1.51
$Mo^{3+} + 3e \leftrightarrows Mo$	-0.2
$N_2 + 5H^+ + 4e \leftrightarrows N_2H_5^+$	-0.23

Half-reaction	*E*° vs. *NHE* (volts)
$NO_3^- + 3H^+ + 2e \leftrightarrows HNO_2 + H_2O$	+0.94
$Na^+ + e \leftrightarrows Na$	-2.714
$Ni^{2+} + 2e \leftrightarrows Ni$	-0.250
$NiO_2 + 4H^+ + 2e \leftrightarrows Ni^{2+} + 2H_2O$	+1.68
$Np^{3+} + 3e \leftrightarrows Np$	-1.86
$O_2 + 2H^+ + 2e \leftrightarrows H_2O_2$	+0.682
$O_2 + 4H^+ + 4e \leftrightarrows 2H_2O$	+1.229
$Pb^{2+} + 2e \leftrightarrows Pb$	-0.126
$PbBr_2 + 2e \leftrightarrows Pb + 2Br^-$	-0.280
$PbCl_2 + 2e \leftrightarrows Pb + 2CI^-$	-0.268
$PbI_2 + 2e \leftrightarrows Pb + 2I^-$	-0.365
$PbO_2 + 4H^+ + 2e \leftrightarrows Pb^{2+} + 2H_2O$	+1.455
$PbO_2 + 4H^+ + SO_4^{2-} + 2e \leftrightarrows PbSO_4 + 2H_2O$	+1.685
$PbSO_4 + 2e \leftrightarrows Pb + SO_4^{2-}$	-0.356
$Pu^{3+} + 3e \leftrightarrows Pu$	-2.07
$SO_4^{2-} + 4H^+ + 2e \leftrightarrows H_2SO_3 + H_2O$	+0.17
$S_2O_8^{2-} + 2e \leftrightarrows 2SO_4^{2-}$	+2.01
$S_4O_6^{2-} + 2e \leftrightarrows 2S_2O_3^{2-}$	+0.08
$Sb_2O_3 + 6H^+ + 6e \leftrightarrows 2Sb + 3H_2O$	+0.152
$Sn^{2+} + 2e \leftrightarrows Sn$	-0.136
$Sn^{4+} + 2e \leftrightarrows Sn^{2+}$	+0.15

Half-reaction	*E*° vs. *NHE* (volts)
$Sr^{2+} + 2e \leftrightarrows Sr$	-2.89
$Th^{4+} + 4e \leftrightarrows Th$	-1.90
$Ti^{2+} + 2e \leftrightarrows Ti$	-1.63
$Ti^{3+} + e \leftrightarrows Ti^{2+}$	-0.37
$TiO^{2+} + 2H^+ + 4e \leftrightarrows Ti + H_2O$	-0.89
$TiO^{2+} + 2H^+ + e \leftrightarrows Ti^{3+} + H_2O$	+0.1
$Tl^+ + e \leftrightarrows Tl$	-0.336
$Tl^{3+} + 2e \leftrightarrows Tl^+$	+1.25
$TlBr + e \leftrightarrows Tl + Br^-$	-0.658
$TlCl + e \leftrightarrows Tl + Cl^-$	-0.557
$TlI + e \leftrightarrows Tl + I^-$	-0.753
$U^{3+} + 3e \leftrightarrows U$	-1.80
$U^{4+} + e \leftrightarrows U^{2+}$	-0.61
$UO_2^+ + 4H^+ + e \leftrightarrows U^{4+} + 2H_2O$	+0.62
$UO_2^{2+} + 4H^+ + 2e \leftrightarrows U^{4+} + 2H_2O$	+0.334
$UO_2^{2+} + e \leftrightarrows UO_2^+$	+0.05
$V^{2+} + 2e \leftrightarrows V$	-1.18
$V^{3+} + e \leftrightarrows V^{2+}$	-0.255
$VO^{2+} + 2H^+ + e \leftrightarrows V^{3+} + H_2O$	+0.361
$VO_2^+ + 2H^+ + e \leftrightarrows VO^{2+} + H_2O$	+1.00
$Zn^{2+} + 2e \leftrightarrows Zn$	-0.763