CH456 Lab Manual

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CH456 Lab Manual
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Grading Policy

During the course of the semester, you will submit 8 lab reports and 3 assignments for grading. *Unless explicitly stated otherwise, all lab reports are due within one week of completion*. Your lab reports will be graded by your lab TA using a scale from 0 to 25. Up to 2 of the 25 points for each lab report is based on the presentation of your report (e.g., writing efficiency, legibility, formatting, etc.), and up to 4 of the 25 points is based on your prelab performance. Make sure your pre-lab score is written on your ***Lab Report Title Page*** *(available on blackboard)* before you leave the lab. You must print off the Lab Report Title pages (available on Canvas) and bring them with you to lab.

The breakdown of the remaining points is divided between each section of the lab report and varies with experiment, with higher marks being allocated to the more challenging parts of each report. The accuracy of your results will also impact your scores, so take care in the lab! A report receiving full points will be clear, concise, have all required attachments (e.g., sample calculations, tables, graphs), include all the required sections, reported experimental results and statistical analysis, show thought and understanding of the science presented, and be well presented with a clear writing style. You will lose points for things such as: incorrect significant figures, missing or incorrect units, sections from textbooks that are not referenced, cluelessness, etc. You will lose 2.5 points off the lab report, if you fail to shut down and clean up your lab area properly at the end of each lab session.

A number of experiments evaluate your lab technique, as demonstrated by the accuracy of your results. If your results are poor due to "bad" data, you are expected to consider possible sources of experimental error and discuss how you could eliminate these errors in future experiments.

Graded reports will be available one week after submission. It is your responsibility to notify the lab director if your lab reports are not returned within one week. **You have one week from when your report grade is made available to request a re-grade**. **No re-grade requests will be considered after the last class day of the semester**. *Re-grade requests* must clearly specify, in writing, your grade dispute and the reasons behind it. *Re-grade requests* must clearly specify, in writing, your grade dispute and the reasons behind it (a simple "Re-grade it!" won't be considered).Specify which section(s) you want re-graded and why you disagree with the grading of those sections. Re-grade requests must be within the constructs of the grading rubrics available on Canvas under "Lab Documents". Attach your re-grade request to the front of your graded report, and resubmit it to your TA to consider.

The 3 assignments are worth a total of 50 points: a literature search exercise worth 10 points, a rewrite of a lab report (JACs style communication) worth 15 points, and a proposal worth 25 points. Your final lab score is the sum of your lab report and assignment scores. In addition, your TA will provide a "lab performance score" to the course instructor. This score is a subjective grade, given by your TA, based on participation in round-robin labs, preparedness, understanding of the concepts presented, attitude, efficiency, organization, etc. Your TA will determine this grade by observing you throughout the semester and asking you questions

during lab sessions. This score will be used by the instructor as she sees fit, and is particularly important in the consideration of borderline cases when assigning final grades.

The total lab score is worth 250 possible points.

The instructor of record will determine how your lab score counts toward your course grade.

Grading Policy

Library Exercise	***1 x 10 = 10 pts***
JACs rewrite	***1 x 15 = 15 pts***
Proposal	***1 x 25 = 25 pts***
Lab Reports	***8 x 25 = 200 pts***
Total	***250 pts***

TA grading is monitored to ensure consistency between sections; however, in cases of obvious incongruity, final lab scores may be adjusted.

Your course instructor determines the portion of your total course grade that comes from your lab score, but keep in mind that y**ou will FAIL this course if you do not attend every lab and turn in a COMPLETE report for each experiment**; i.e., a report that demonstrates a serious attempt at all the sections described in the *Lab Report Format* section of this manual. In addition, y**ou must make at least 50% (125 points) in the lab component to pass this course.**

Attendance and Make-up Labs

You are expected to attend every lab session on time. If you arrive more than 15 minutes late, you will not be permitted in the lab. In this case, follow up with the lab director; you will also have to make up the experiment. If you are unable to attend your lab session, you must give your TA *written notice* (e.g., by email) before your lab time, or as soon as possible after an emergency prevents you from attending.

You may miss ONE lab with a valid, official, documented excuse (Ex. Doctor's note signed and dated), and may make up that lab at the first available make-up session for full credit for the missed lab. If you failed to provide a valid, official excuse to your TA, you will be assessed a 10% penalty on your lab report.

During the long semesters (Fall and Spring), there are three make-up dates listed in the lab schedule. During the summer, there are two. All make-up sessions during the Fall and Spring semester are on Saturdays from 9 to 12, and must be completed on those dates. The makeup sessions during the Summer semester are on Fridays from 1 to 5pm. If you do not

perform all the labs in this course you will fail, so do not use the make-up option frivolously. **The final makeup lab of the semester is reserved for Round Robin labs ONLY**. If you miss an early lab, you will need to make it up at the earliest possible time.

To reserve a space at a make-up session, you must sign up at the stockroom by 5:00 pm day prior to the make-up session. If you are unprepared, you will not allowed into lab and must attend another make-up session (in the case of the final make-up lab, your failure to be prepared will result in failing the course).

Due dates and late penalties

Late lab reports are strongly discouraged. Each report is due within the first 5 minutes of your lab period the week it is due —reports submitted after this time will be considered late. *Late reports are penalized in the following way:* **Late reports will lose 2.5 points per day that it is late. Weekend days *count* as late days; however, official UT holidays (e.g., Thanksgiving, Spring Break, etc.) do not count as late days.**

The stockroom report box is checked between 9 am and 10 pm weekday Monday-Thursday, but is not checked between Friday and 9 am Monday. If submitting at this time, your Blackboard time stamp will serve as time of submission, provided the electronic copy matches the hard copy. Please note that after ten 24-hour periods, you will get zero for that report; **however, you must still submit a complete report before the end of the semester in order to pass the course**.

No reports will be accepted after 12:00 pm (NOON) on the last class day of the semester.

Academic Dishonesty

Cheating will not be tolerated. Labs will be submitted electronically through Blackboard and will be compared to old lab reports, internet content, and other available materials to ensure that you have not plagiarized. If you are caught plagiarizing on a lab report, you will receive a zero for that lab report, you will be given a written warning, and your name will be on file with the dean's office. **If you commit academic dishonesty on more than one lab report, you will automatically fail the course even if you have not received a prior warning.**

Anyone handing in copies of the same spreadsheets and/or graphs will receive zero for the lab report; repeat offenders will auto-fail the course. We expect you to create all your own computer files from scratch. Using Word or Excel documents created by somebody else, even if you have edited them extensively, is still scholastic dishonesty. Reusing old lab reports is not allowed.

You may debate the discussion questions with each other, and come to the same conclusion about the most appropriate answer, but you must express your answer **in your own words** in your report. We recommend that you do not compare your reports to each other directly and do not read each other's work.

Doing your own research in the library, or on the web, is encouraged. Information used in your report that is not considered "common knowledge in the field," and that is not your own original idea, *must* be cited and references provided, you have to reference your textbook if you use it for get any kind of information. We recommend that you also reference any source that you read, other than your textbook or the lab manual that results in an improvement of your understanding of the science. It is rare that a direct quote of a source would be used in most scientific writing unless the statement is so profound or unique that it must be used verbatim to have the full impact. Plagiarism and paraphrasing are forms of scholastic dishonesty that will be dealt with accordingly.

Ignorance is not an excuse for academic dishonesty. Failure to cite other work or paraphrasing too closely from published sources and the web are both examples of scholastic dishonesty. *There is almost never a reason to cut and paste or to use someone's words verbatim—even with quotes and appropriate referencing—unless the quoted statement is unique (e.g., "Give me liberty or give me death.") or a verbatim quote is needed to insure accurate representation of an individual's statement (e.g., Jones notes that "Gravity is inoperative for light masses".) The latter is often used when such a statement is controversial, paradigm shifting, central point of a discussion, etc. and is almost certain not to occur in a CH 456 lab report.* If you are unsure about how to cite other scientists' work or want more on the distinction between paraphrasing and quoting, ask your TA or Lab Director.

See http://deanofstudents.utexas.edu/sjs/scholdis.php for additional information.

References

Your references should be numbered in consecutive series in order of mention in your report. If a reference is repeated, do not give it a new number; use the original reference number. Reference numbers in the text should be superscripted outside the punctuation of the sentence or phrase to which the citations applies, or after the name of the author if referred to in the text. For example:

Quantitation of chlordane in freshwater fish has been reported previously.[1]
When compared to previous reports of chlordane in fish, [1] the results reported here ……
Work of Doe *et al.*[1] has shown that …

The complete list of literature citations should be placed at the end of your report, after the conclusions, but before the appendices. List references in numerical order and use the following formats.

Journal references must include the author names, abbreviated journal title, year of publication, volume number (if applicable), and the range of pages of the cited article. For example:

- Smith, A. D.; Moini, M. *Anal. Chem.* 2001, **73**, 240−246.

Book references must include the author or editor's name, book title, publisher, city of publication, and year of publication. For example:

- Skoog, D. A.; Holler, F. J.; Nieman, T. A. *Principles of Instrumental Analysis*, 5th ed.; Saunders College: USA, 1998; Chapter 7.

Web site references must include the author (if known), title of site from browser window title bar, URL, and date accessed. For example:

- U.S. Environmental Protection Agency Home Page, http://www.epa.gov/ (accessed July 1996).

Failure to cite references in the above manner will result in a zero for the lab report. No exceptions!!!! If you have any questions about citing references, ask your TA or the Lab Director.

For more information on citation formats for journals and other types of publications use Chapter 14 of the *ACS Style Guide* (3rd edition), which is available on reserve in the Chemistry Library. Some other examples based on ACS style of referencing can be found at: http://chemistry.library.wisc.edu/writing/acs-style-guidelines.html

Online Submission of Lab Reports

You are required to submit an electronic copy of your lab reports to Canvas on the same day you turn in the hard-copy to your TA. All rules concerning due-dates and late penalties apply to online submission as well as hard-copy submission.

Online Lab Report Submission Instructions:

1. Save the report as one of the following file types:
    a. .doc
    b. .docx
    c. .rtf
    d. .pdf
2. Name the file as "firstinitialLastname_report" (Ex: JDee_NaOH lab, JDee_GC)
3. Go to Blackboard under your unique section number.
4. Select the Online Submission tab on the menu. (Left side)
5. Click on View/Complete link under the lab to be submitted.
6. On the submission screen:
    a. Verify you are attaching the correct report.
    b. Attach the report file.
    c. Click Submit at the bottom of the page.
7. Make sure that you submitted the report.

You are responsible for making sure that the lab report is submitted properly.

Pre-lab preparation

The Pre-Lab helps you understand and answer the scientific concept (s), the objectives, the overall purpose for this lab and your hypothesis for the lab experiment. You are expected to have a good understanding of the purpose, experimental details, chemicals and hazards, and the underlying science of the experiment when you come to lab. Each pre-lab will be worth 4 points of your lab report grade for that experiment.

- Before each lab session, you should prepare by reading the lab manual *and the required reading from your textbook*.
- You must submit a *written pre-lab. The written pre-lab consists of the writing experimental procedure and answering the questions under the pre-lab in the lab manual. These items must be in your lab notebook*.
- *You must also pass an oral Pre-lab Quiz* at the start of each lab session to demonstrate your understanding of the material. Quiz questions are in this manual.

Pre-lab quizzes will be administered by a lab TA. TAs may choose to give quizzes individually or in small groups. **If it is clear to the TA that you have not read the manual and textbook, or have not attempted to understand the experiment, you will not be permitted to perform the experiment. In this case, you will have to perform the experiment during a makeup session.**

Checking out Equipment from the Analytical Stockroom

The manual indicates what is needed from the stockroom for each experiment. Stockroom procedures are as follows:

1. *Pick up and sign for* the equipment/chemicals you need (make sure to take appropriate clean, dry glassware to collect chemicals, if so specified in the procedure).
2. *In some of the titration lab and the round robin labs, your equipment will already be in the lab, but need to be returned to the stockroom at end of the lab.*
3. *Clean and return* all borrowed items to the stockroom at the end of your lab session (unless noted otherwise in the procedure). If you fail to do this, you will be charged for them.

***Do not* wear gloves when you go the stockroom (or any time you leave the lab).**

Lab Notebook

Your notebook should be a journal in which you record all aspect of the experiments you perform—what was done, when and how it was done, *what was observed*, and what data was taken. **All raw data, *including* observations, should be recorded immediately in your lab notebook**. Your notebook should be the *original record of the experiment*; copying results into your notebook from scraps of paper is *not acceptable*. When working with others, **each member of the group must have his or her own record of all data, in his or her own notebook, before leaving the lab**. A photocopy of someone else's lab notebook page is not appropriate scientific reporting.

Each page of your lab notebook should contain your name, any partners' names, and the date, page number, and title of the experiment. Create a table of contents on the first page of your notebook and keep it updated! Use the pages in your notebook sequentially—don't leave space for calculations to go back and fill in later. Use ink—never pencil or whiteout! Mistakes should be crossed out with one line and initialed, and an explanation for the deletion should be noted. No pages may be torn out; one diagonal mark through a page will indicate that the page should not be used.

The lab notebook is not just a convenient place to scribble down notes and numbers for you. It is a legal document that should contain an accurate record of each experiment performed and be understandable to a complete stranger—if your TA can't follow it, you have a problem.

You are required to have a new lab notebook at the beginning of the semester!!!

Shutdown

At the end of each lab session, your TA will check that you have washed your glassware, returned items to the stockroom, disposed of waste appropriately, and cleaned up your area. The TA will check and initial your data in your notebook and sign your Summary Sheet. **It is your responsibility** to make sure your lab notebook is signed and a ***Lab Report Title Page*** is completed by your TA before you leave at the end of the lab session. If you fail to properly shut down equipment (***instrument specialist will do so***) and clean up your area (this includes returning *clean* items to the stockroom), you will lose 2.5 points off the lab report score for each incident.

Teaching Assistants

Your TA is one of your most valuable resources for any questions concerning the lecture and lab portion of the class. If you have questions, please feel free to ask them. You are given your TA's email during the first day of lab. If you email them, give them 24 hours to answer your question. They are not required to answer email on the weekend. Do not ask your TA for their office number and location.

Each week your TA will hold an office hour outside the labs in the second floor hallway. Please feel free to ask them questions regarding the lab. Be advised of office hour etiquette (See below).

Office Hour Etiquette

1. Use office hours for specific questions about the data analysis. Students should not show up with nothing done and expect the TA to explain each step of the data analysis section.

2. If you feel you have bad results, you should not expect the TA to look over your entire report in search of possible errors in the data analysis. You should do this and then ask the TAs questions about specific parts that you feel may be incorrect. Also, if you conclude all calculations were done correctly but the data is still bad, you should try to come up with sources of experimental error on your own without relying on your TA for these ideas.

3. Do not expect the TAs to give answers to discussion questions. TAs will attempt to lead students in the right direction, but never flat out give the answer.

4. TA do not "pre-grade" discussion questions or any other section of the lab report.

5. Please bring the lecture textbook to office hours if you are asking questions about theory and the discussion questions.

Safety & Waste Disposal

Unsafe, imprudent and careless activities will result in you having points deducted from the lab report score or be removed from the lab. Continued noncompliance could result in course failure.

Personal Safety

- **Safety goggles are provided and must be worn at all times in the labs**. Contact lenses should not be worn in the lab because chemicals and particulates can get caught behind them, causing severe eye damage. Failure to wear safety goggles will result in point deductions for the lab.
- **You must wear lab appropriate attire in the lab**. The following is not permitted in the labs: open-toed shoe, sandals, or other uncovered footwear, shorts or skirts, shirts that do not cover the torso and upper arms. The stockroom has a pair of rubber boots if appropriate footwear is not worn. We will sell you a "bunny suit" to wear if you do not wear appropriate clothing. You may store protective clothing in your lab drawer for use in class.
- Long hair must be tied back while in the lab. Beware of dangling jewelry, loose clothing, and anything else that may get caught in equipment, or dipped in chemicals.
- Eating, chewing gum, and drinking in the lab are not permitted.

- Wash your hands and arms with soap and water as soon as possible after leaving the lab.
- **Never enter the lab without your TA or the Lab Director present.**
- No horseplay, pranks, or other acts of mischief will be tolerated in the lab.
- If you are uncertain about any safety aspect of an experiment, please ask your TA or the Lab Director

LAB SAFETY

- Make sure you know the exact locations of the safety features of the lab: eyewash fountains, safety showers, chemical spill kits, fire extinguishers, fire alarms, fire blankets. You will receive site-specific training on the safety procedures of this teaching lab in your introductory class.
- **Do not deal with incidents on your own**. Your TA and the stockroom staff are all trained to respond to the sort of incidents that may occur in this lab, for example, chemical spills, cuts, burns, fires, medical emergencies, and so on. Emergency contact numbers are listed near the phone.
- Keep your work area clean and organized to reduce the possibility of accidents. Know what you are doing and don't be careless. Label all containers.
- **Avoid unnecessary exposure to chemicals. Never pipette by mouth. Never taste or inhale a chemical. Wear gloves when directly working with hazardous chemicals. Use hoods when appropriate.**
- Take appropriate precautions. Keep flammables away from hot plates and open flames. Wear gloves when using toxic, carcinogenic, or other hazardous chemicals. Take care with corrosive acids and bases. Always pour concentrated acid slowly into water (never water into acid). Read the **Safety Issues** section at the beginning of each experiment.
- Be informed. Material Safety Data Sheets (MSDSs) summarize known hazards associated with every chemical and are available from the UT web site at http://www.utexas.edu/safety/ehs/msds/. Review these before your lab.
- Chemicals and equipment may not be removed from the lab without permission.

DISPOSAL OF CHEMICAL WASTE

It is very important to properly dispose of the chemical waste you generate.

- Generate as little waste as possible. It is expensive to have hazardous waste removed and disposed of. Don't prepare more of a chemical than you expect to use.
- **Never return unused portions of chemicals to the reagent bottle.** At the end of your experiment, unused reagent must be disposed of as waste, so don't pour out more than you need.
- **Do not discard chemicals down the sink or in the wastebasket**, unless you are explicitly told that it's okay to do so. Most of your chemicals will pose a threat to the environment if disposed of improperly.
- Place chemical waste in the appropriate container. Often, more than one waste container is provided to separate certain chemicals for safety or easier disposal. Pay attention to the **Waste Disposal** information for each experiment in this lab manual, and use the waste

containers indicated. If you cannot find a waste bottle labeled with your particular chemical, ask your TA where to dispose of it.

- Do not overfill a waste container. Tell the stockroom the bottle is getting full and they will replace it.
- Use the clearly marked GLASS containers to dispose of broken glass and Pasteur pipettes. Do not place broken glass in the sink or wastebasket, to avoid serious injury to an unsuspecting person.
- Use the clearly marked WASTE SOLIDS wide-mouth bottles/buckets to dispose of waste solids. Waste solids include excess solid chemicals, filter paper, and weighing paper.
- If you realize that you disposed of a chemical in the wrong container, use the waste inventory list provided by the waste container to let us know.
- If you have waste whose identity you can't recall, you can often test your waste (e.g., with litmus paper) to deduce its identity. If you still can't tell, ask your TA or the stockroom staff to help you identify the waste. Do not add unidentified waste to the waste bottles.

Computers

- Don't use lab computers for checking your email, playing games, or browsing the internet. Repeated violation of this policy will result in point deductions or removal from lab.
- Communications between students, TAs, and Instructors will make use of your official UT email address, with the expectation that you will check your email daily.
- For some of the round robin experiments, you are expected to use UT Webspace to save data.

Computers for data analysis

The data analysis for most experiments can be done more quickly using a computer. UT has a helpful Excel tutorial available at http://www.utexas.edu/its/training/handouts. Also, your text includes brief introductory sections on Excel (Sections 2-10 and 2-11). It is recommended that you do all calculations and graphing in Excel.

A number of experiments produce data in digital form. We recommend that you upload this data directly into your UT web space, http://www.utexas.edu/its/webspace/, where your files may be stored so that you can access them from anywhere. You are expected to use your UT web space to store all computer files generated by this course for the duration of the semester.

Lab Performance Points

5 of the 25 points available for each lab report are assessed in lab by your TA. Your lab performance is determined by your TA based on several factors, including, but not limited to:

Adherrence to safety rules (wearing goggles at all times in the lab, not wearing gloves on keyboards, proper waste disposal, etc.)

Knowledge of analytical glassware protocol (not pipetting from stock solutions, making "volumetric" solutions in a volumetric flask instead of a beaker, properly labeling all vials/beakers/flask with the contents, etc.)

Ability to function as a cohesive group. Most of the labs will be performed in groups of two or more. Is there a proper delegation of tasks, or is one person performing the experiment, while the others stand around and watch?

Finishing the experiment on time (barring instrumental irregularities: eg. a leaking pump or empty gas cylinder) and leaving the area prepared for the next lab session.

Following the TA's instruction

Your TA will record your lab performance points on the ***Lab Report Title Page*** at the end of lab, so it is very important that you retain the title page and submit it with your written lab report. Any disagreements about assignment of points will NOT be addressed during lab, and a time outside of lab must be scheduled with your TA.

Lab Reports

It is very important to be able to communicate your work to others. Writing that is unclear or confusing will affect your grade, as will lack of regard for grammar, punctuation, and spelling. You should aim to be brief, precise, and unambiguous. The reader should clearly understand what you are trying to say. Keep your verb tense consistent and appropriate. You may use either passive or active voice, but be consistent. Avoid using jargon or slang and always use full sentences. It is rare that you would need to use first person; that is, try not to refer to "I", "we", "our", "us"; nor should you speak about yourself, for example, "the student."

A portion of each lab report grade is based on your presentation, formatting, and layout. We recommend that you use a word processor and spreadsheet software to produce your reports. All graphs must be created using graphing software, and may not be hand-drawn. Any section not produced on a computer must be hand-written in your lab notebook. Do not use pencil. See the ***Your Lab Notebook*** section of this manual for more detail. Lab Reports will not be graded if portions are handwritten even if your writing is neat, legible, well organized and clearly laid out. On the other hand, using a computer does not automatically guarantee you full presentation points. Use a legible (at least 12 pt) fonts, lay things out well, label table columns, and so on.

When handing in your report, submit the carbon copies from your lab notebook, and attach printouts of all work done on a computer. Make sure you keep an electronic copy of all work submitted. You will have a copy of all handwritten work in your notebook; thus, any report you have submitted should be easily recreated. Any line-outs in your lab report must be initialed by your TA.

You have two options to deal with figures, graphs, and tables in your lab reports: (1) You may place them all in an appendix at the end of your report, or (2) you may include your figures, graphs, and tables in sequence in your report (this is our preference). Cut your tables and graphs out of the programs you used to create them and paste them into your word processor document.

No matter which option you choose for positioning your figures, graphs, and tables, all *figures* (meaning all diagrams and graphs) should be numbered consecutively, be referred to by number (e.g., Figure 1, Table 5, etc.) in the appropriate part of your report, and have a caption that clearly explains the figure. Similarly, all tables should have an appropriate title, and should be numbered consecutively in the order of reference in the text. Note that table numbering should be independent of figure numbering.

If a plot is included in the experimental procedure (e.g., in the data analysis section), then you must include the plot in your report and discuss it. Do not include figures or tables that are not explicitly required in your report. Any graphs, tables, or diagrams that you have copied from another source must be referenced appropriately in the caption or title. Hand-drawn (use a scanner to create a pdf image and insert) or computer-generated diagrams are equally acceptable as long as they are neat and clearly legible. All graphs must be computer generated. Tables should also be prepared in a consistent format, labeling each column clearly and showing units. A table should have a specific theme; don't put 10 columns of unrelated data in a single table.

You will write a lab report for each experiment performed during this course following the ***Lab Report Format*** guidelines, as described next.

Appeals

Teaching assistants will make decisions concerning grades, attendance, and other policy matters for their sections. If your TA does not return your report in a timely manner, contact the Lab Director immediately. Should you disagree with a TA, you are welcome to take the matter to the Lab Director. All appeals must be in writing and refer to the grading rubrics posted on Canvas. <u>All appeals must be initiated within one week of receiving the graded lab report.</u>

All lab grades will be submitted (by your TA) directly to Canvas. No lab reports will be accepted after the last class day, and all lab grades will be submitted to Canvas by the Wednesday following the last class day of the semester. Any issues/appeals with lab grades must be addressed with the lab director by the Friday following the last class day of the semester.

<u>CH 456: Lab Report Format</u>

Title Page (ALL 8 reports)

- Available on CH 456 Canvas Master Site (You must bring these to lab as well)

Abstract (Reports 4-8)

- Briefly summarize experiment in under 200 words
- Follow same order as the entire report
- *Include*:
    - Purpose of research
    - Analyte and sample investigated
    - Experimental approach used (instrumentation and/or method)
    - Summary of principal findings
    - Reliability of findings
    - Conclusions and significance

Introduction (All 8 reports)

- Present problem, its significance and provide background information (this section should be ½ - 1 page of text – <u>diagrams do not count as text</u>)
- *Include*:
    - Experimental approach and theory
        - Define variables and scientific terms
        - Show important equations (number them so you can reference them; e.g., E1, E2, etc.)
        - Show relevant chemical reactions (number them so you can reference them later; e.g., R1, R2, etc.)
        - Describe principles of instrument operation

        - Include labeled block diagram of the instrument
    - Scope and limits of approach
        - What assumptions have been made?
        - Under what conditions is the technique applicable?
        - To what extent is the technique limited?
    - Relationship to other experiments
        - How is your method related to other work?
        - Why is this method preferable to others?

Experimental (Reports 4-8)

- Provide enough information so experiment can be repeated by another scientist
- *Include*:
    - Concise description of procedure (in paragraph form!!)
    - Specific instrument information
        - Make and model
        - Parameters used
    - Chemicals used
        - Name, formula, purity
        - Safety considerations and special precautions

Results (ALL 8 reports)

- Report the results of your analysis
- *Include*:
    - Tabular report of results with appropriate significant figures and statistics
    - Calibration curves (if used)

Discussion (All 8 reports)

- Analyze and interpret your results
- Demonstrate understanding of purpose, technique, instrumentation and results
- *Include*:
    - Summary of experimental results (SD, RSD, 90% Confidence Interval)
        - Reference to any relevant plots or tables which summarize your results
        - Relevant observations and deviations from procedure
    - Summary of calculation results
        - Reference to significant calculations used
        - Important assumptions and data rejection
    - Discussion of findings
        - Sources of error (systematic?, random?, gross?)
        - Most significant source of error
        - Reasonable methods to improve experiment
        - Consider experiment in terms of: accuracy, precision, reproducibility, sensitivity and analysis time
        - Implications of results

Discussion questions (Reports 4-8)

Discussion questions should be answered in this section in paragraph form, incorporated into the discussion of results. Answers should be identified by placing the question number in brackets at the beginning of the answer, i.e., [#]. If your TA cannot find the answer, *it's your problem*, so make sure they're clearly identified.

Conclusion (All 8 reports)

- Concise summary of Results and Discussion (~½ page of text)
- *Include*:
    - Summary of results
        - Reasonable?
        - Accurate?
        - Precise?
        - Reproducible?
        - Selective?
    - Comparison to "known" or expected values
    - Significant deviations from planned procedure
    - Most significant sources of error and their effect on results

References (ALL 8 reports)

- Cite all references used (use proper citation format!). Examples:

    Harris, Daniel C., *Quantitative Chemical Analysis,* 6th Ed. W.H. Freeman and Company: New York, 2003, pp. 81-91.

    UT Dept. of Chemistry (2006). *CH456 Analytical Lab Manual, Experiment #1: NaOH Standardization*. Retrieved August 24, 2006, from University of Texas, Department of Chemistry Web site: http://courses.cm.utexas.edu

Appendix (ALL 8 reports)

- Raw data, calculations, notes – attached in the following order (remember, if your TA can't find it, *that's your problem*)
    - Complete, numbered list of all attachments
    - Graded Pre-lab materials (chemicals list and wet-lab strategy)
    - Lab notebook pages (**carbon copies**)
        - Raw data and observations
    - Typed calculations in equation editor or similar program
    - Chromatograms, spectra, etc.

*Note: If you fail to attach your original data and calculations, you will get **no credit** for your results.*

NOTES ON LAB REPORTS

- DO NOT plagiarize right out of the lab manual. Put it in your own words!
- Always calculate and report the %RSD (% Relative Standard Deviation) and the 90% confidence interval using Student's t.

JACS Communication-style reports

You will complete your Round-Robin reports in the style of a JACs communication. The format template will be available on Canvas. The first JACs lab report will be a rewrite of the Fluoride ISE lab. This rewrite is designed to try to get you in the habit of condensing a lot of information while conveying the most pertinent material. The Round-Robin lab reports must be in the format of a JACS communication style paper. You will need to include all of the pertinent information (clearly identified and labeled) listed in the previous section of this lab manual.

Proposal Writing Exercise

The Proposal is broken down into 2 different assignments and worth a total of 25 points.

Pre-proposal	***5 points***
Full Proposal	***20 points***

All parts of the assignment are due at the beginning of lab the week that it is due. A hard copy turned into your TA and electronic copy submitted to Canvas for each part of the assignment are required. Proposals turned in after noon on the last class day will not be graded. Late pre-proposals and progress reports will be a zero.

The project proposal: You will write a proposal mimicked after a journal article in the literature. You will find a peer-reviewed article in the literature and complete the following exercise. The article should not come from the Journal of Chemical Education (although this is a good journal to get starting ideas). The articles must be current (last 10 years). The project proposal should describe, in detail, how you plan to *reproduce* the experiment outlined in the article. You must flesh out the details and design your own methodology for the project utilizing *only the equipment available in the analytical laboratory*. (You will not carry out the experiment). This assignment is meant to aid in proposal writing for CH376K. Find a topic that interests you. There are examples of topics found on Blackboard. If you have problem finding a topic, talk to your TA or the lab director.

Pre-proposal Assignment

Find a current peer-reviewed article in the literature using one of the instruments listed below. Make sure that the article is detailed enough to write a full proposal. An example of a detailed article can be found on Blackboard.

Instrumentation in the Analytical Labs
GC-FID, HPLC, CE, Electrochemistry (CV and ASV), UV-Vis Spectroscopy, FAAS, IR, Fluorescence, GC-MS (First four techniques are from CH456; last techniques are covered in CH376K. (chose your technique wisely))

Please answer the following questions for your pre-proposal on the pre-proposal form

Define the problem:

- Describe the sample.
- State the analyte being tested.
- Specify which instrument utilized. (Does the article give instrument parameters?)
- State the hypothesis of the research.
- List why the research is important (give some background).
- Provide a photocopy of the journal article to your TA.
- You will get feedback by the following lab session, so you can begin your full proposal.

Proposal Progress

- You are required to meet with your TA in lab to discuss your progress on completing the proposal assignment during your off weeks in the Round Robin series.

Full proposal

A full project proposal should contain the following elements:

- **Title Page**
- **Project Summary**
- **Statement of Problem (Background)**
- **Goals and Objectives**
- **Literature Review**
- **Methodology**
  - **Experimental Procedure**
  - **Evaluation of Results**
- **Modifications**
- **References**
- **List of Needs (Appendix)**

Further information about what is expected in each section of the proposal is detailed below. *You are REQUIRED to meet with Dr.Herath the week of lab checkout to discuss your proposal before points can be assigned*. More details will be provided in Lab.

Proposal Guidelines:

Project Summary: The summary should clearly state the project objective and the hypothesis to be tested, and provide an overview of the experimental procedure. The procedural details should identify the analyte to be measured, the instrument to be used, the sample(s) to be collected and the methods (for prep and analysis) to be employed. The summary should conclude by stating the purpose of the project and the expected results (Don't just guess, find values from the literature, the web, environmental studies, labels, etc.). **[~1 paragraph]**

Statement of Problem (Background): This section should provide the "whys" of the project. This section of the proposal should contain a discussion of the problem the project is addressing and the hypothesis to be tested. Scientific literature and other resources should be used to gather the background information on the project. This section should conclude by stating how the expected results may address the problem. **[Typically 2-3 paragraphs, but yours should be ~1 paragraph for the purpose of this exercise]**

Goals and Objectives: The overall objective of the project should be stated here. Specific goals (steps) that must be met as the project progresses toward that objective should also be clearly defined. A timeline for the project should also be included here. **[~1 paragraph + timeline] (A hint for 376k: No matter how much time you think it will take to do the project, double it. Your timeline for 376k is ~6 weeks)**

Literature Review: A review of the literature concerning the analytical methods and procedures employed in the project – this section describes how each reference supports the project methodology. It is usually the case that several references are used; however, for this exercise, find *ONE* scientific, refereed journal article *(aside from the one chosen)* that supports the analytical

procedure to be used in the project. **[*minimum,* 1 paragraph per reference]** *The supporting article must be submitted with the proposal.*

Methodology: This section should justify the analytical approach, address potential difficulties and demonstrate that the methods to be used are appropriate to the analysis. Discuss details about *how* the technique/instrument employed in your proposal works, types of samples it can run, analytes it's used for, etc. You may use your textbook (Harris) to get much of this information. Please do not copy directly from Harris.

Experimental Procedure

Instrumentation – Provide the analytical parameters to be employed for the instrument (e.g., temperature ramp for GC, mobile phase for HPLC, column type, length, etc.). State the instrument/technique LOD for the analyte (compare this to what is expected to be found in the samples).

Sample collection – State where and how sample(s) will be collected. How will they be stored/preserved prior to analysis? Cite appropriate sample collection techniques.

Sample preparation – How will the samples be treated to get them in the proper form for analysis (e.g., digestion, dissolution, extraction)? Are blanks, spikes or other controls necessary?

Analysis – State whether multiple samples will be run, how many replicates of each, what standards or reference materials will be used, etc. Discuss how the results will be validated by describing the appropriate use of blanks and controls in each analysis.

Evaluation of Results

Discuss how the results will be evaluated as to whether they have properly tested the hypothesis. Explain how statistical analysis will be used to evaluate the data and results.

Modifications: This section will address a single modification that you can make to the experiment that will make the project uniquely yours. For example, you might have found an experiment that determines the amount of lycopene in different types of tomatoes. Your modification to the experiment could include determining the amount of lycopene in a tomato after it has been baked, broiled, microwaved, stored on a counter for 3 weeks, etc.. The point is: what can you add to the scientific community? **[~1 paragraph]**

References: All citations should be in ACS format according to the *ACS Style Guide*.

List of Needs (Appendix): Detailed list of materials, etc. needed to complete the project.

Chemicals / Reagents (and quantities)

Make a table of the chemicals, reagents, and equipment needed to conduct the project (only raw materials, pure reagents and solvents should be listed). Indicate whether a specific chemical grade is required (e.g., HPLC grade, spectroscopic grade, ultrapure water, etc.) The table should provide the following information for *each required chemical and reagent*: CAS number, vendor name, cost of item, amount & unit of measure (e.g., 10 grams, 50 milliters, etc.), quantity ***needed*** (this is not the minimum quantity to purchase). The table should include specific consumable items that are required for instrumental analysis, such as a CE capillary, electrode, etc. This also includes items needed for sample prep, such as a rotary evaporator, centrifuge (+ tubes), furnace (w/ temp., vol. and time req'd), oven, sonicator, lyophilizer, soxhlets, etc.

Pre-lab Quiz Questions

Preparation of NaOH Standard Solution and Determination of Aspirin Content

What is the purpose of this lab?
Define: buret, titration, primary standard, equivalence point, endpoint, indicator, standardization
What strong acid(s) will you be using today?
What strong base(s) will you be using today?
What are the chemical reactions taking place in this experiment?
What are the products when you react a strong acid and a strong base?
What indicator is being used in this experiment?
How will you know when you've reached the endpoint?
What is equal to half the equivalence point?
What is the difference between the equivalence point and the end point?
What is the error between the equivalence and end point called?
What do you expect physically to happen when you reach the endpoint of the titration?
Why does the indicator change color at the end point?
What is the primary standard in this experiment?
What are some desirable characteristics of a primary standard?
What does KHP stand for? Why do we keep it in a desiccator?
What do you do if the solution level in the buret approaches the 50mL mark?
What would be the effect on your calculated concentration of NaOH if unbeknownst to you, someone left the KHP out and it had absorbed some water?
What is the effect on the calculated concentration of NaOH if you used some NaOH that had been sitting out on the bench (ie: not sealed in a container) and had absorbed a lot of water?
If you titrated until the solutions were magenta instead of pale pink but still used the data, what would be the effect on your calculated value of concentration of NaOH?
What is weighing by difference? Why do we use this technique?
What are the physical and health hazards of NaOH and HCl?
Why is it not necessary to fill the burette exactly to the "0.00" level
Define back titration.
Explain the difference between a direct titration and a back titration.
Why do we use a back titration for the analysis of aspirin?
Define percent composition.
How can you determine the percent composition of aspirin in a tablet?
Define Le Chatelier's principle.
How many moles of aspirin react with each mole of NaOH?
What is occurring chemically when you reach the endpoint of your titration?
Why is it so important to heat the solution in a hot water bath after initial titration with NaOH?
What indicator are we using this week?
Define transition range of an indicator.

Identify the color changes expected during each step of the aspirin titration.
Will all of the aspirin tablet dissolve? Does this matter for your experiment?
Explain why ethanol is used in this experiment.
Say you did not clean the beaker from last week's lab and it has some HCl left in it. How does that affect your aspirin titration?
Where should you dispose of your aspirin solid waste at the conclusion of this lab?
Are there any hazards associated with aspirin?
Where do you put your waste at the end of lab?

Potentiometric Titration

Define: pH, strong acid, weak acid
What is a polyprotic acid?
Explain conjugate acids and bases, giving an example.
What are the three ionization steps of phosphoric acid?
What are the three acid dissociation constants and/or pKa values for phosphoric acid?
How many endpoints would you expect to observe in the titration of a triprotic acid with a strong base?
How many endpoints will you observe in the experiment today?
Describe what is happening chemically at each of the endpoints.
How will you know when you reached the first and second endpoints?
Why do we use potentiometric titration for this experiment instead of just using an indicator?
What color will your solution be at the first endpoint? The second endpoint? (ha ha ha)
Why don't we calibrate the pH meters?
Describe how you will determine the concentrations of the mixture of two acids by a single titration.
Explain how determining the first and second derivative will aid you in finding the experimental endpoints.
How does a pH meter work?
What does a pH meter measure?

Water Hardness

Definitions: chelating ligand, complexometric titration, formation constant, masking agent, water hardness, and solubility product.
Explain the purpose of the experiment.
What metal ions generally contribute to water hardness?
What metal ions are you going to test for in your experiment?
What indicator will you be using for the purposes of this experiment?
How will you know you have reached the titration endpoint?
Is EDTA a strong or a weak acid?
How many pKa values does EDTA have?
Why is EDTA chosen for this titration?

How many deprotonations need to occur in order for EDTA to effectively bind to a metal? (This varies for different metals; for calcium EDTA needs to be almost entirely deprotonated-which is why we work at pH=10-but it can bind most metals at lower pH values.)
Why do we use buffers for the titrations?
Why is all glassware acid washed?
Does EDTA bind equally to Ca^{2+} and Mg^{2+}? Which has the higher complex ion formation constant?
Which metal ion must be present in the water for a color change to be produced and constant?
Which metal ion must be present in the water for a color change to be produced and observed?
When would you use a masking agent?
Why do multidentate ligands form more stable metal complexes than similar monodentate ligands?
Why do we use EBT as an indicator? What does the end point signify?
Why do we use murexide as the indicator for the second set of titrations?
How can you determine the water hardness due to calcium and magnesium individually?
What causes water hardness in nature? Why don't we measure the water hardness of distilled water?
What is the most hazardous chemical used in this experiment?
What should potassium cyanide never come in contact with?
Where does the cyanide waste go at the end of lab?

Potentiometric Determination of Fluoride

Define buffer,ISE.
What is the purpose of this experiment?
What does an ISE measure?
What would the presence of species which complexes to fluoride ion have on your results?
What is the pKa value for HF?
What is the purpose of adding the buffer to your water sample?
Why is it important to maintain constant temperatures between measurements?
What methods will you use to analyze your results?
What are some advantages to potentiometric methods?
When should the standard additions method be used over the calibration curve method?
Why should the electrode be washed with DI water and blotted dry between measurements?
Describe how you will determine the concentrations of fluoride ions using the calibration curve method.
If you record a measurement that falls outside of the linear range of a calibration curve, what should you do?
If you record a measurement that falls outside of the linear range of the instrument itself, what should you do?

Cyclic Voltammetry

What is the purpose of this experiment?
What is standard potential?
What are the electrodes in a three-electrode cell? What is the function of each?
What are the characteristics of a good reference electrode?
What reference electrode is being used? What is its reduction potential?
What is the purpose of the supporting electrolyte and what are you using in this experiment?
How do we vary the applied potential in a chronoamperometry experiment?
What process is responsible for the shape of the chronoamperometry curve after the potential step?
How do we vary the applied potential in a cyclic voltammetry experiment?
What does a cyclic voltammogram for a reversible reaction look like?
How would the cyclic voltammogram shapes differ for reversible and irreversible reactions?
When a substance is being reduced, what type of current is produced?
When the reaction begins, how does the current change?
How do you convert from the standard potential to the potential vs. the Ag/AgCl electrode?
By which three methods can an ion in solution reach an electrode surface?
How are we eliminating electromigration and convection in this experiment?
Draw a block diagram of the potentiostat/electrode setup.
Can you roughly draw a CV for ferricyanide? Explain each part of the cyclic voltammogram.
What considerations are there with regard to the waste generated in this lab? (stress the importance of not mixing cyanide and acid)

Gas Chromatography-Mass Spectrometry

Define gas chromatography.
Describe the components of a typical GC-MS
What is the purpose of the control sample?
What is the difference between HPLC and GC? When should a HPLC be used over a GC?
Compare/contrast GC/MS with GC-FID.
What is the purpose of a solvent delay?

Gas Chromatography-FID

Define gas chromatography.
What are some advantages for using a GC?
Describe the components of the GC instrument.
How are components separated while passing through the column?
What is your internal standard?
Explain the internal standard method.
How does your internal standard make your analysis more accurate?
Describe the detector used in this experiment.
Which gas is used as the carrier gas?
What are some of the parameters that can be adjusted to achieve better separation?
What property of the compounds in this experiment determines their elution order?
Explain what a response factor is and how it is used in to calculate the concentrations of the compounds in this experiment.
Why are we increasing the temperature during a sample run?

High Performance Liquid Chromatography

What is reverse phase HPLC? What is normal phase HPLC?
What can you tell me about the polarities of each phase in reverse phase HPLC? Relate this to stationary phase and mobile phase composition.
Define spike recovery and explain why we use it in this lab.
Describe how you will construct your calibration curve.
Why can't the hot sauce be injected directly into the instrument?
What must you do to your sample before injecting it into the HPLC?
Describe how to use solid phase extraction (SPE).
What are the major components of an HPLC system and what is the function of each?
Describe how the detector used in this experiment works.
What is the primary reason to use HPLC instead of GC?
Based on the structures of capsaicin and dihydrocapsaicin, which will elute first and why?
How might the mobile phase be modified to provide greater separation of the two peaks?
What are the health hazards of capsaicins?
Where should you dispose of used filters and SPE cartridges?

Preparation of a NaOH Standard Solution and Determination of Aspirin Content

This experiment is divided into two parts. The first part introduces the basic principles of titrimetric analysis and the concept of standardizing a NaOH solution by *direct titration* against a *primary standard*. Acid and base solutions are generally prepared to an approximate concentration (typically within ~10%), and the exact concentration is then determined by comparison to a known standard. Since the NaOH solution will be used in future experiments, it is critical to standardize it accurately. Using this method, the standardized NaOH concentration should fall within ± 0.5% of the actual concentration.

In the second part experiment, you will determine the aspirin content per tablet using a common *indirect*, or *back titration,* technique. Initially, you will use the NaOH you previously standardized to directly titrate an aspirin solution past its endpoint. Because this acid hydrolysis is a kinetically slow process, you will then heat the solution to force the reaction to completion. Hydrochloric acid can then be used to *back titrate* any excess, unreacted NaOH to accurately determine the concentration of aspirin in an ordinary analgesic tablet. You will be graded on your accuracy in this experiment.

Required Reading

Quantitative Chemical Analysis by Daniel C. Harris

7th Edition
Review: Ch. 1 Measurement, Sect. 1-3 (Preparing Solutions)
Technique: Ch. 2 Tools of the Trade, Sect. 2-3 (esp. Weighing by Difference), 2-4 (Operating a Buret), 2-5.
Theory/Data analysis: Ch. 7 Let the Titrations Begin, Sect. 7-1, 7-2, and Ch. 11 Acid−Base Titrations, Sect. 11-1, 11-6, 11-7.
8th Edition

Review: Ch. 1 Measurement, Sect. 1-3 (Preparing Solutions)
Technique: Ch. 2 Tools of the Trade, Sect. 2-3 (esp. Weighing by Difference), 2-4 (Operating a Buret), 2-5.
Theory/Data Analysis: Ch 1 Chemical Measurements, Sect. 1-5 and 1-6; Ch. 10 Acid-Base Titrations, Sect. 10-1, 10-6, and 10-7.

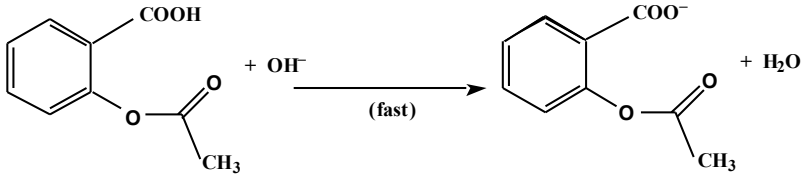
Pre-Lab

Written: (1) Write out each *balanced* titration reaction, (2) Write out the step-by-step directions for *weighing by difference*, (3) List the properties that make KHP a good *primary standard*. (4) Explain why it is necessary to use *back titration* for the aspirin analysis (i.e., – why direct titration alone is not a practical method to determine the aspirin content per tablet). (5) Step-by-Step Procedure

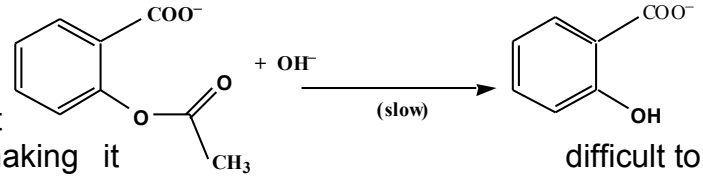
Background

Direct titration is a useful analytical technique for reactions with fast kinetics and a clear end point. However, when studying reactions that are either slow or have unstable equilibria, back titration is a more sensible and accurate technique. Acetylsalicylic acid, the active ingredient in aspirin, is a weak acid capable of reacting *stoichiometrically* with a hydroxide ion in *two* separate steps.

Step #1: Acid-Base Neutralization



Step #2: Hydrolysis



The slow kinetics of the hydrolysis step can cause uncertainty about the completeness of the reaction, making it difficult to determine the endpoint using direct titration. To ensure that the aspirin and base fully react, *excess* NaOH is added to the sample solution, which is then heated to force the reaction to completion. The exact amount of unreacted (excess) base can then be determined by back-titrating the sample solution with HCl. Subtracting the amount of unreacted base from the total amount of base added to the solution will result in the amount of base that actually reacted *stoichiometrically* with the aspirin sample.

Chemicals & Their Location

Balance Room	Lab Bench
Potassium hydrogen phthalate, KHP, (dessicator) Aspirin tablets	Phenolphthalein, indicator soln. 0.1 M NaOH (aq) (In box at end of bench). 0.1 M HCl (aq) (In box at end of bench) Ethanol

Equipment & its Location

Stockroom	Your Lab Drawer
Buret (2) Weigh Bottle (2)	All other glassware

Lab Bench
Boiling Chips Iron Rings (Return to Stockroom after lab) Mortar and pestle (return to Stockroom after lab)

Safety Issues & Chemical Hazards

Chemical	Health Hazards	Physical Hazards
Ethanol	irritant	flammable
Hydrochloric acid (HCl)	toxic, irritant	water-reactive, corrosive
Phenolphthalein	irritant	none
Potassium hydrogen phthalate	irritant	none
Sodium hydroxide (NaOH)	toxic, irritant	water-reactive, corrosive

Procedure

If all the balances are in use, begin with preparing your burets for the lab by filling one with 0.1 M HCl and the other with 0.1 M NaOH. You may start on either the Standardization of NaOH or the Determination of Aspirin lab to minimize crowding at the balance stations and hot water baths. Your TA will tell you which step to begin on.

Please remember to bring your ***Lab Report Title page*** (available on Canvas) with you to lab.

Standardization of NaOH

Preparation of a Primary Standard (KHP)

1. Because KHP can take a while to dissolve, these solutions should be made first. The KHP is pre-dried and stored in desiccators in the balance room. To avoid contamination, do not use your own spatula, a spatula has been provided for you to use in the desiccator. Into a labeled, clean 250 mL Erlenmeyer flask, accurately weigh **by difference** ~0.7 g of KHP from a clean, dry weigh bottle. Repeat twice, to have a total of **three** separate flasks with ~0.7 g KHP. Add 75 mL of distilled water (measure with a graduated cylinder) and three drops of the phenolphthalein indicator solution. Cover each flask with a small beaker on the top, leave to dissolve, and swirl occasionally.

Obtaining the NaOH Solution

The 1 L bottle of NaOH solution that you obtain in this step **must be saved** after this experiment and stored in your drawer for future experiments. Do not throw the NaOH solution away.

2. The stockroom has already prepared a set of NaOH solutions (all ~0.1 M). Select one of these solutions (record the identifying letter or number in your notebook. The identifying mark will be on the top of the box**.**), and fill a clean 1 L bottle from your drawer. Note - if you choose a glass bottle, use a rubber stopper – alkaline solutions can "freeze" ground glass joints. Clean the buret you checked out from the stockroom first with water and then rinse using a small amount of your NaOH solution. Fill your buret with the NaOH solution and record the volume of the liquid (within two decimal places – all buret readings should have two decimal places).

Standardization of NaOH with KHP

3. Begin to titrate the KHP solution in your first flask by adding NaOH until the solution has changed from colorless to the first hint of pink. Placing a piece of white paper under the titration flask will help in detecting the slight color change. Proceed slowly by adding the NaOH drop-wise, stirring frequently, until the entire solution remains a *light* pink for at least 60 seconds. At this point, the **endpoint** of the titration has been reached. Record the final volume of NaOH solution in the buret to two decimal places. If your solution is bright pink, you have gone too far. The correct endpoint is a very faint pink. Titrate the other two flasks of KHP solution in a similar manner. **Be careful** not to let the fluid level in the buret drop below the 50 mL line. If more solution is required to complete a titration, stop the titration prior to reaching the line, record the exact volume, refill the buret, record the exact volume, and continue the titration. The overall volume can then be obtained by adding the two volumes to calculate the total titrant volumes.

Determination of Aspirin Content

Aspirin Sample Preparation

1. Accurately record the *combined* weight of three unbroken, uncrushed tablets. This mass will be used to calculate the average mass of a tablet.

2. Use a mortar and pestle to crush enough tablets to produce ~ 1 g of aspirin tablet powder. Transfer the powder to a clean, dry weighing bottle.

3. Accurately weigh *by difference*, ~ 0.3 g of tablet powder into a labeled 250 mL Erlenmeyer flask. Repeat two more times to have a total of *three* separate aspirin samples.

4. To each of the three flasks, add ~ 20 mL of ethanol and three drops of phenolphthalein indicator. Swirl gently to dissolve the aspirin powder as much as possible in the ethanol solution. Ethanol is used because aspirin is not very soluble in water. Aspirin tablets also have other insoluble ingredients, which can result in a somewhat cloudy solution.

Aspirin Titration with NaOH

5. Directly titrate the first aspirin sample with NaOH until you observe the first *permanent* cloudy pink color change (remaining pink for at least 60 seconds).

At this color change, it can be assumed that the first step of the reaction (the acid-base neutralization) is complete, and that the second reaction (hydrolysis) may be underway. An excess of base is needed to help force this kinetically slow step to completion (think about Le Chatelier's Principle; excess reactant drives the equilibrium towards the products).

6. To make sure you add excess base to get past the final end point, add the same amount of base (from Step #5) + 10 mL. Add the base using the buret. For example, if you added 15.30 mL of NaOH to reach the first permanent, cloudy pink color (Step #5), you need to add 25.30 additional mL of NaOH (15.30mL + 10mL) to complete the hydrolysis step.

7. Titrate the remaining two flasks by adding the **same total volume** you did to flask #1 to each, recording the amount to two decimal places. Be careful not to let the level of NaOH in the buret fall below the 50 mL marking. To add enough NaOH to the two flasks, you may need to record an intermediate volume (BEFORE you hit the 50 mL marking), refill the buret, and add the rest of the difference.

Heating the Reaction to Completion

8. Slide a weighted iron ring over each flask's neck (to prevent it from floating and spilling), and place each sample in a water bath to speed up the hydrolysis step of the reaction. Do not boil the sample, as that may cause some components to decompose. While heating, swirl the flasks periodically. Allow the samples to heat for 15 minutes, remove them from the water bath, and allow them to cool for ~ 5 minutes prior to back titrating them with HCl.

9. If the solution turns from pink to colorless at any time during or after the heating (before titrating), add 2-3 additional drops of phenolphthalein. If it is still colorless after adding phenolphthalein, add 10 mL more NaOH (with your buret), and reheat the flask. If you add more NaOH, remember to record it and add the extra volume to the total volume for that flask.

Back Titration with Acid

10. Using the first buret that you filled with the ~0.1 M HCl, titrate the excess NaOH with acid in each flask until the pink color *just disappears*. The endpoint for this point will be a "cloudy white" color. (Remember, color changes are often easier to detect if you have a white piece of paper underneath the flask). Record the volume of acid used to *back titrate* each sample to two decimal places.

Shutdown Procedures

IMPORTANT NOTE: <u>Save your remaining NaOH solution</u> in your drawer after this experiment; you will need it again for a future experiment.

11. Dispose of all waste in the appropriate waste containers. Clean and dry all glassware. Clear up your work area. Clean and return any items borrowed from the Stockroom.

12. Double check that you have recorded *all* information about your sample, including the brand name, manufacturer, and the reported amount of aspirin per tablet from the label. The label should be posted on the hallway bulletin board. Make sure that you have the concentration for the HCl before leaving lab.

Waste Disposal

Waste Chemical	Waste Container	Location
Neutral solutions after titration	Down the Drain	Sink
HCl; unused KHP solution	Acidic waste	Waste Cabinet
KHP Solid	Solid KHP	Balance Room
NaOH	Basic waste	Waste Cabinet

Data Analysis

Standardization of NaOH

1. Calculate the number of moles of KHP in each flask. To do so, you will need: the weight of KHP you added to each flask and the molecular weight of KHP (Note – the MW is NOT 71 g/mol).

2. Using the total volume of NaOH used to titrate each KHP flask to its endpoint, calculate the molarity of your NaOH solution. Make sure you use the proper units (ie – convert the volume from milliliters to liters). Report the concentration of your standardized NaOH as the average of your *three* replicates with statistical analysis. (Include the standard deviation, %RSD, and the 90% Confidence Interval. Show your work for all calculations. If you suspect that you have an outlier, perform and show the work for the Q-Test).

Determination of Aspirin

3. Calculate the total number of moles of NaOH added to each aspirin sample. Next, calculate the number of moles of excess NaOH left in each flask from the volume of HCl needed to back titrate each flask and the HCl concentration. Find the difference between the total number of moles and excess moles of NaOH to determine the number of moles of NaOH that reacted with the aspirin in each sample. Using the reaction stoichiometry, calculate the number of moles of aspirin in each flask.

4. Calculate the mass of aspirin in each flask. Use the mass of crushed tablet powder in each flask to calculate the weight percent of aspirin in each of your three samples.

5. Calculate the *average* mass of an individual aspirin tablet from the combined mass you recorded in Step #1 of the procedure. Use the average tablet mass and the weight percentages (Step #3) for each sample to calculate the average mass of aspirin in a tablet. Report the final aspirin content per tablet, in *mg*, as the average of your three replicates with the appropriate number of significant figures and statistical analysis.

References

(1) Day, R.A. Jr.; Underwood, A.L. *Quantitative Analysis*, 4th ed.; Prentice-Hall, Inc.: Englewood Cliffs, NJ, 1980.
(2) Harris, D.C. *Quantitative Chemical Analysis*, W.H. Freeman, NY, 2003.
(3) Skoog, D.A.; Holler, F.J.; Crouch, S.R. *Principles of Instrumental Analysis*, 6th ed.; Brooks Cole: USA, 2006.

Determining the Concentration of a Mixture of HCl and H_3PO_4 by Potentiometric Titration

Potentiometric titration is a powerful electroanalytical technique based on the same fundamental principles as a direct titration. Potentiometric detection of a reaction's endpoint offers a number of advantages in comparison to standard visual-based detection approaches. This week, you will use *potentiometry* to determine the amount of hydrochloric (strong) and phosphoric (weak) acids in a mixture. You will use your standardized NaOH to titrate the acid mixture and a pH meter to detect the endpoints of the titration. It is essential to have a good understanding of the experimental logic, as you will simultaneously analyzing two components of a mixture. You will make first and second derivative graphs of your data to accurately determine the endpoints of the titration. The experiment will be done in pairs. You will be graded on your accuracy in this experiment.

Required Reading

Quantitative Chemical Analysis by Daniel C. Harris

7th Edition

Technique: Ch. 2 Tools of the Trade, Sect. 2-4 (Operating a Buret), 2-5.
Theory/Data analysis: Ch. 7 Let the Titrations Begin, Sect. 7-5 (Titration of a Mixture).Ch. 10 Polyprotic Acid−Base Equilibria, Sect. 10-1 through 10-5.
Ch. 11 Acid−Base Titrations, Sect. 11-4, 11-5 (Using Derivatives to Find the Endpoint). Ch. 15 Electrodes and Potentiometry, Sect. 15-5 (pH Measurement with a Glass Electrode).

8th Edition

Technique: Ch. 2 Tools of the Trade, Sect. 2-4 (Operating a Buret), 2-5.
Theory/Data Analysis: Ch. 26 Gravimetric Analysis, Precipitation Titrations, and Combustion Analysis, Sect. 26-6 (Titration of a Mixture). Ch. 9 Polyprotic Acid-Base Equilibria, Sect. 9-1 through 9-5. Ch. 10 Acid-Base Titrations, Sect. 10-4 and 10-5 (Using Derivatives to Find the End Point). Ch. 14 Electrodes and Potentiometry, Sect. 14-5 (pH Measurements with a Glass Electrode).

Pre-Lab

Written: (1) Write out each *balanced* titration reaction. (2) Look up the equilibrium constants for the *three* ionization steps of phosphoric acid and make a table of the values in your notebook. (3) Step-by-Step Procedure

Background

Phosphoric acid is triprotic and will undergo three dissociation steps in the titration with NaOH. At any given pH, there will be no more than *two* phosphate species (a *conjugate acid-base pair*) present because its equilibrium constants are separated by more than four orders of magnitude (10^4). Protons are removed from the phosphoric acid in a stepwise fashion as NaOH is added to the sample solution during the titration (causing the pH to change). The complete removal of each phosphoric acid proton will cause a significant change in pH and result in an inflection point on your titration curve. Although phosphoric acid has three removable protons, you will only observe the removal of the first *two*.

The unknown sample solution you will be given is a mixture of hydrochloric acid (HCl) and phosphoric acid (H_3PO_4). HCl is a stronger acid than H_3PO_4, but the dissociation constant of the first H_3PO_4 proton is large enough that its removal is impossible to differentiate from the HCl proton. Though HCl actually reacts with the NaOH first, no distinct endpoint is observed; and the first endpoint in your titration curve represents the sum of the amounts of HCl and H_3PO_4 in the mixture. Consequently, the volume of NaOH required to reach the first endpoint corresponds to the total amount of acid in the unknown solution (HCl plus H_3PO_4); and the volume of NaOH needed to reach the second endpoint from the first represents the amount of NaOH that reacts with H_3PO_4 alone. **The information in this paragraph is important, if you do not follow the logic for how to determine the concentration of both acids in the mixture, read the paragraph again.** ***If you still don't understand, ask your TA for further clarification before you begin the experiment.***

Additional Notes:

You will use a pH meter to detect *changes* in the pH of the solution as NaOH is added. Use your textbook as a reference for information on the principles of pH meters. Ask your TA if you have any questions on how to operate the pH meter.

You will collect a large number of data points for each titration. Furthermore, the analysis for this experiment requires a good deal of data manipulation. A spreadsheet program (i.e., – Excel, or similar software) is required for the data analysis. If you do not know how to use a spreadsheet, ask a TA for help in office hours BEFORE your report is due. The UT ITS site (http://www.utexas.edu/its/training/handouts/UTOPIA_ExcelGS/) and your textbook have helpful handouts and tutorials.

Chemicals & Their Location

Stockroom	
Unknown Hydrochloric-Phospohoric Acid Mixture	
Balance Room	Your Drawer
EDTA dihydrate, disodium salt	Sodium hydroxide, standardized solution

Equipment & its Location

Stockroom	Above / On Lab Bench
For EDTA lab (each <u>student</u> needs) Acid-washed 250-mL Vol. flask Weighing bottle **For Potentiometric lab (each <u>group</u> needs)** Buret (50 mL) 25.0 mL Volumetric Pipet	Electrode Holder Magnetic Stir Plate Stir bar pH meter and probe (return to stockroom) <u>Please return the pH meter to the stockroom like you found them!!</u>

Safety Issues & Chemical Hazards

Chemical	Health Hazards	Physical Hazards
*Hydrochloric Acid**	toxic, irritant, sensitizer	water-reactive, corrosive
*Phosphoric Acid**	none	corrosive
Sodium hydroxide	toxic, irritant	water-reactive, corrosive

** Both hydrochloric and phosphoric acids are highly corrosive. Be careful, and wear gloves while working with these acids at all times. Change your gloves often!*

Procedure

<u>Preparation for the Next Experiment – each group must prepare one EDTA solution for next week's lab.</u>

<u>Water Hardness Prep (required)</u>: EDTA salts dissolve slowly. To prepare a *standard* EDTA solution to use for the water hardness lab, make this solution at the *beginning* of today's lab. If the balances are busy, proceed with the potentiometric titration, but <u>do not forget to make the EDTA solution during the f</u>**<u>irst half</u>** <u>of the lab period</u>.

Disodium EDTA dihydrate ($Na_2H_2Y{\cdot}H_2O$) is a primary standard. EDTA is pre-dried and stored in desiccators in the balance room. To prevent contamination, <u>do not use your own spatula</u>, one has been provided for you the desiccator. <u>Return the EDTA salt to the desiccator whenever it is not being used!</u>

1. Use a clean, dry weigh bottle to *<u>accurately weigh</u>, by difference*, ~ 0.25 g of disodium EDTA into the *acid-washed 250 mL volumetric flask* that you checked out from the

stockroom. Make sure that the EDTA is *quantitatively transferred* to the flask (you may need to use a clean, dry funnel, and rinse it with a small amount of distilled water into the flask). Add ~ 200 mL of distilled water to the flask, and swirl occasionally until the solid EDTA has dissolved.

2. *After the EDTA has completely dissolved*, dilute to the mark on the flask's neck using a plastic pipet, and mix thoroughly by shaking and inverting the flask (with the stopper in place). If you add water above the mark on the flask, you will have to remake your solution.

3. Use a series of *three* small portions of the EDTA solution (**< 20 mL each**) to rinse a clean polyethylene 500 mL bottle. Swirl the bottle with each aliquot to ensure that the entire inner surface of the polyethylene bottle has been rinsed. These rinses remove any residual metal ions from the bottle's inner surface. Transfer all of the remaining EDTA solution from the volumetric flask to the polyethylene bottle for storage in your drawer until next week. The polyethylene bottle is preferable to glass because EDTA solutions can leach metal ions from glass surfaces.

Potentiometry Setup

1. This experiment will be done in pairs. Determine whose NaOH solution to use (based on the volume left). **Record the concentration of the NaOH solution**. Your TA will give you the correct concentration of the NaOH if the experimental value is inaccurate. Clean the buret that you checked out from the stockroom first with water and then rinse using a small amount of the NaOH solution. Fill your buret with the NaOH solution and record the volume of the liquid to two decimal places.

2. Pick out a pH meter from the front of the lab. *Note – the pH electrode is kept in a storage buffer solution that protects it by keeping it clean and hydrated. It is important that the electrode be immersed in solution as much as possible. Do not leave the electrode exposed to air for long periods of time; try to keep it in either the storage buffer or your experimental solution at all times. Whenever you move the electrode from one solution to another, you should rinse it well with your squirt bottle (filled with distilled water) to prevent cross-contamination between your solutions and the storage buffer. Please return the pH meter and probes to the stockroom at the end of the lab.*

3. For the titrations in today's experiment, you will use beakers (instead of an Erlenmeyer flask, see Figure 1), so that you can fit the buret tip and the pH electrode into the titration solution. The beaker (250 mL) will sit on the center of the stir plate with a stir bar inside it. Clamp the buret over the beaker. Use the electrode holder to carefully position the electrode in the beaker so that it does not interfere with the titration process and the stir bar does not hit it.

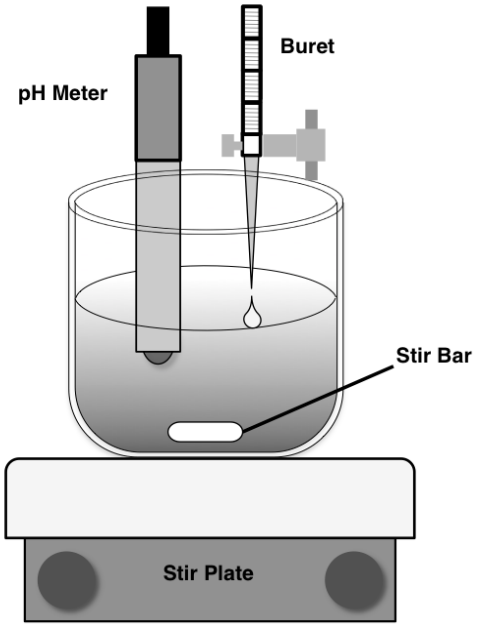


Figure 1: Potentiometry Setup.

Sample Preparation

4. Bring a clean, dry, labeled 100 mL beaker to the stockroom to get the unknown acid mixture sample. Record the unknown mixture's identifying number or letter in your notebook.

5. Transfer 25.0 mL of the unknown acid mixture into a clean, dry, labeled 250 mL beaker for the titration using the volumetric pipet. Take care not to aspirate acid into the pipet bulb. Dilute with ~ 40 mL of distilled water. Repeat twice more to have a total of *three* samples to titrate.

6. Position the first beaker approximately in the middle of the stir plate. Add the stir bar to the solution, and set the stirrer so it *gently* mixes the solution. Position the buret over the beaker and the pH electrode in the solution.

Potentiometric Titration

In this titration, you will add titrant past the endpoint. You will then determine the endpoint from your data. The pH will change quickly at each endpoint, and remain relatively constant between the two endpoints.

7. The first titration is a fast and dirty titration to give you an idea of where the endpoints are located on the titration curve. Begin to titrate the first sample. Record both the buret volume and the pH after each NaOH addition. You will not have as much data for this sample, so you will not use this titration in your calculations.

8. Now that you have the approximate positions of the endpoints, the remaining two samples should be titrated with more accuracy and precision. Begin the titration using somewhat large volumes (1 mL) of NaOH for each addition. Record the volume in the buret and the pH for each addition. Once the pH starts to change, slow down and add only enough NaOH to change the pH by ~ 0.1 units per addition. Stop and wait for the stir bar to mix the solution well prior to recording the data for that addition. As you get closer to the endpoint, titrate drop-wise and record the data. Titrate in this manner near each of the endpoints. You should have a large number of data points in these two regions. Once you are past the first endpoint, gradually increase the volume of the additions until you near the second endpoint. Titrate until you have passed the second endpoint and there is no significant pH change observed for each addition mL of NaOH added.

Shutdown Procedures

IMPORTANT NOTE: You may dispose of your NaOH solution after this lab **IF** you have completed the first lab. Do not dispose of the NaOH if you missed the first lab.

9. Dispose of all waste in the appropriate waste containers. Clean and dry all glassware. Clear up your work area. Clean and return any items borrowed from the Stockroom. Make sure you return the pH meter and probe as you found them to the stockroom. Make sure the probe is in the storage buffer solution.

10. Double check that you have recorded the identifying number or letter for your unknown acid mixture, your lab partner's name, and all of the volume and pH measurements from the *three* titrations. Do not leave the lab without having all of the data recorded in your lab notebook.

Waste Disposal

Waste Chemical	Waste Container	Location
All solutions after titration	Potentiometric	Waste Cabinet
Excess unknown solution	Acid	Waste Cabinet
Unused HCl, dilute or concentrated	Acid	Waste Cabinet
Unused NaOH, dilute or concentrated	Base	Waste Cabinet

Data Analysis

Endpoint Determination

1. Since the first titration was approximate, you will only be using the data from the second two titrations to calculate the acid concentrations. You will use a spreadsheet (i.e., – Excel) to analyze the data.

2. Begin by entering the data from the second and third titrations in a spreadsheet as shown below. The first two columns are the buret reading and pH for each addition, respectively. In the third column, use Excel to calculate the total volume of base added up to that point, by subtracting the initial buret reading from the reading at each data point (sample spreadsheet shown below). *Note: you will need to correct for cases in which you had to refill the buret.*

Sample Excel Spreadsheet

	A	B	C	D	E	F	G
1	Buret Reading	pH	Total Vol. NaOH Added	ΔpH/Δvol	Average Volume	Δ²pH/Δvol²	Average Volume
2	0.90	2.86	0				
3	1.72	2.87	=A3-A2	=(B3-B2)/(C3-C2)	=(C3+C2)/2		
4	2.44	2.88	=A4-A2	=(B4-B3)/(C4-C3)	=(C4+C3)/2	=(D4-D3)/(E4-E3)	=(E4+E3)/2

Each time you take a derivative, one data point at each end of the data set will be lost (here, cells D2 and F3). Once a formula is entered in a column, you can highlight the cell and position the mouse at the bottom right corner (the cursor will change from a hand to a cross), left click (the cursor will change from the cross to a box with arrows at two corners), and drag the box down through the cells you want the formula to apply to.

3. For the second and third titrations, construct a *titration curve* by plotting pH *vs.* total volume added (Figure 2a). The inflection points on the graph (the midpoints of the steepest regions) correspond with the two endpoints of the titration (where the largest pH change is observed for the smallest amount of NaOH added). Approximate the two volumes that correlate to the two inflection points on the titration curve. Label the two endpoints on your titration curve.

4. Create two new plots for each titration using the same data you used for Step #2. For the new plots, use your titration curve from Step #2 to determine a smaller volume range that expands the areas of interest near the endpoints (i.e.; – 9-11 mL and 13-15 mL volume added, example shown in Figure 2*b*). Use the same x-axis scale for each of the four total graphs so that they can be directly compared to each other.

5. The two endpoints fall where the localized slope of the titration curve is the highest. Mathematically, this is the point where the *derivative* (ΔpH/ΔVol) reaches a local maximum. In your spreadsheet, create a new column (Sample Spreadsheet Column

"D"), and calculate the first derivative of the data for each titration by dividing the *change* in added volume by the *change* in pH between two consecutive data points.

6. Create another column to calculate the average volume corresponding to each derivative point by finding the average of each pair of consecutive readings (Sample Spreadsheet Column "E").

7. Make a first derivative plot for each titration by graphing the ΔpH/ΔV (Column "D") *vs*. V_{avg} (Column "E"). The first derivative of a curve is a measure of its *slope*. The slope of the titration curves you plotted in Step #2 should peak at each of the endpoints (Figure 2*c*). Find and label the two endpoints from each titration on the first derivative plots. (You should have 4 of these plots.)

8. An alternative mathematical method to accurately determine the endpoint(s) from a titration curve is through a second derivative (Δ^2pH/ΔVol^2) analysis. The second derivative measures the *rate of change* in the slope of the titration curve. Calculate Δ^2pH/ΔVol^2 by dividing the change in ΔpH/ΔVol between two consecutive data points by the change in the average volume added between two readings (Sample Spreadsheet Column "F").

9. Calculate the "average average" volume for each second derivate point from Step #7 by averaging each pair of consecutive average volumes from Step #5 in a new column in your spreadsheet (Sample Spreadsheet Column "G").

10. Create a second derivative plot for each titration by graphing Δ^2pH/ΔVol^2 (Column "F") *vs*. V_{avg2} (Column "G"). Since the second derivative curve represents the rate of change in the slope of a curve, it should be equal to zero whenever the first derivative reaches a maximum or minimum. Therefore, the second derivative graph should cross the x-axis at each endpoint (Figure 2*d*). (You should have 4 of these plots.)

11. For a good titration, the second derivative plot will only cross the x-axis once for each endpoint. If your plot has multiple crossings, use your best judgment to determine the two endpoint volumes for each titration. *Label each endpoint on your graphs.* The second derivative graphs should provide the most accurate and precise means of determination for the endpoints. *Report the volume, one for each endpoint and explain how each was determined*.

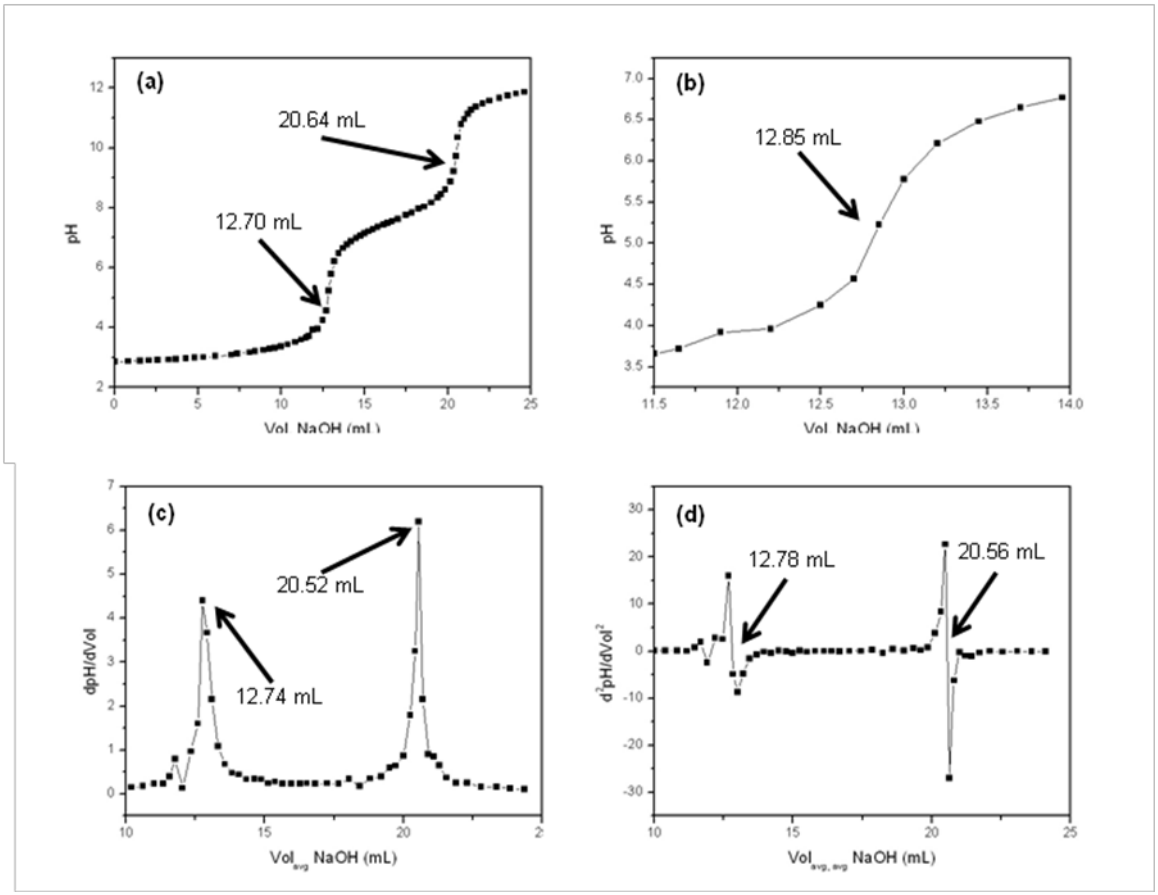


Figure 2: Sample potentiometric titration data analysis plots. (a) raw data, (b) raw data zoomed in on first endpoint, (c) first derivative curve, (d) second derivative curve.

Concentration Determination

12. Use the volume of NaOH required to reach the first endpoint to determine the number of moles of NaOH that reacted with *both* acids in the mixture. This will represent the number of moles of both acids present in 25 mL.

13. Next, using the volume required to reach the second endpoint <u>from the first endpoint</u>, calculate the moles of NaOH that reacted with *only* the $H_2PO_4^-$ in the 25 mL mixture.

14. Calculate the number of moles of HCl in the 25 mL sample by finding the difference between the total moles of reacted NaOH (Step #12) and the number of moles of NaOH that reacted only with the $H_2PO_4^-$ (Step #13).

15. Use the number of moles of each acid in the 25 mL aliquot to calculate the molarity of each acid in the unknown sample.

References

(1) Day, R.A. Jr.; Underwood, A.L. *Quantitative Analysis*, 4th ed.; Prentice-Hall, Inc.: Englewood Cliffs, NJ, 1980.
(2) Harris, D.C. *Quantitative Chemical Analysis*, W.H. Freeman, NY, 2003.
(3) Skoog, D.A.; Holler, F.J.; Crouch, S.R. *Principles of Instrumental Analysis*, 6th ed.; Brooks Cole: USA, 2006.

Determination of Water Hardness of Filtered and Unfiltered Water using Complexometric Titration: What Effect will a Brita® Filter have on Water Hardness?

In the first portion of this experiment, you will use an *EDTA complexometric titration* to determine the hardness of a water sample. You will measure the *total hardness* as well as the *individual* hardness of calcium and magnesium, the two metal ions responsible for making water "hard". Similar to the acid-base and indirect titration techniques employed in your first experiment, the endpoint of these titrations is determined by a color change of an indicator. Here, a *metallochromic indicator* undergoes a visible color change when EDTA replaces the indicator molecule as the ligand in the divalent metal ion complex. Eriochrome Black T is the indicator used to measure the *total hardness*; and *solubility product* concepts along with a Murexide/NaCl indicator mixture are used to determine the *individual calcium hardness*. In the second part of this lab, you will determine the effect running the sample through a Brita® filter has on water hardness.

Required Reading

Quantitative Chemical Analysis by Daniel C. Harris

7th Edition

Review: Ch. 1 Measurements, Sect. 1-2 (Parts per Million and Parts per Billion), 1-3.
Theory/Data Analysis: Ch. 6 Chemical Equilibrium, Sect. 6-3 (Solubility Product), Ch. 12 EDTA Titrations (all).

8th Edition

Review: Ch. 1 Measurements, Sect. 1-2 (Parts per Million and Parts per Billion), 1-3.
Theory/Data Analysis: Ch. 6 Chemical Equilibrium, Sect. 6-3 (Solubility Product), Ch. 11 EDTA Titrations (all).

Pre-Lab

Written: (1) Write out the *balanced* reaction for each titration. (2) Draw the structure of EDTA and the structure of EDTA complexed to a (typical) metal ion. (3) Find (and write out) the reactions and equilibrium constants of the complex formation between EDTA and (i) calcium, and (ii) magnesium. (4) Find (and write out) the pK_a values for EDTA, Eriochrome Black T, and Murexide (they are all in the *Harris* text). (5) Step-by-Step procedure

Background

In nature, water is hardened by the passage of rainwater containing dissolved carbon dioxide through layers of stone, such as chalk, gypsum, and limestone. "Hard" water contains multiply charged and heavy metal ions. These ions replace sodium and potassium ions in soaps and detergents, and form precipitates that can interfere with cleaning, leave rings and "scum" in bathtubs and sinks, and clog pipes. *Total water hardness* is defined as the sum of the calcium and magnesium concentrations simply because the concentrations of Ca^{2+} and Mg^{2+} ions are much higher than any other ions contributing to water hardness. Calcium carbonate ($CaCO_3$) is the most common precipitate of hard water, and total water hardness is normally expressed as the milligrams of $CaCO_3$ equivalent to the total mass of calcium and magnesium measured in one liter of water (mg/L = ppm). Depending on the water source, total water hardness can range anywhere from zero to hundreds of ppm. The US Geological Survey (USGS) classification of degree of water hardness is as follows:

Degree of Hardness	**Measured Total Water Hardness (ppm $CaCO_3$)**
Soft	0-60
Moderately Hard	61-120
Hard	121-180
Very Hard	> 181

A *Complexometric titration* is based on the formation of a complex ion. Metal ions are Lewis acids, and are capable of accepting electrons from donating ligands, forming *complex metal ions*. When hard water is titrated with a ligand capable of donating electrons, the metal ions (calcium and magnesium) bind the ligand and form complex ions. Ethylenediaminetetraacetic acid (EDTA, or H_6Y^{2+}, where $\mathbf{Y = C_{10}H_{12}N_2O_8}$) is a widely used synthetic complexing agent specifically designed to bind metal ions quantitatively. EDTA forms stable, water-soluble complexes with a 1:1 stoichiometry for most metal ions (i.e., one EDTA molecule binds to one metal ion). EDTA forms stable complexes with both calcium and magnesium, but binds more tightly to calcium; therefore:

$$Ca^{2+} + Y^{4-} \rightarrow CaY^{2-}$$
$$Mg^{2+} + Y^{4-} \rightarrow MgY^{2-}$$
$$Ca^{2+} + MgY^{2-} \rightarrow CaY^{2-} + Mg^{2+}$$

A *metallochromic indicator* is often used to detect the endpoint of a complexometric titration of metal ions. Metallochromic indicators change color depending on whether they are bound or unbound to a metal. When EDTA is used to titrate a sample containing calcium and magnesium ions, the calcium is preferentially complexed by the EDTA, while the magnesium is complexed by the metalllochromic indicator. Once EDTA binds all of the free calcium and free magnesium, additional EDTA extracts the magnesium ions from the metallochromic indicator, restoring it to its *uncomplexed* color; and the endpoint of the titration is observed. In

this experiment, *Eriochrome Black T* is used as the indicator to determine the endpoint for the *total water hardness* titration:

$$MgIn^{-1} \text{ (purple-red)} + H^{+} + Y^{4+} \text{ (clear)} \rightarrow MgY^{2-} \text{ (clear)} + HIn^{2-} \text{ (blue)}$$

Eriochrome Black T does not give a sharp color change for water containing calcium, but no magnesium. To make sure that there is some magnesium present in your water sample, you will add a *small* amount of Mg-EDTA (magnesium already complexed to EDTA). Since you are adding the same amount of EDTA as magnesium, the addition of the Mg-EDTA to the water sample has no net effect on the titration. EDTA and both of the metallochromic indicators you will use are *weak* acids, and their reactivity is pH dependent. Therefore, it is critical that we use a *buffer* to hold the sample solutions at an appropriate pH for the EDTA and indicators to work properly (pH ~10 is suitable for EDTA and Eriochrome Black T).

Some metal ions, such as nickel, occasionally present in water from different types of sources, can interfere with the titration of calcium and magnesium with EDTA and Eriochrome Black T. These interfering ions can cause indistinct endpoints or complex with either EDTA or the indicator more strongly than the analytes. A *masking agent* is chemical that can be added to the sample solution to prevent the interfering ion(s) from binding with the EDTA, allowing it to complex the analytes. You will use a series of test tube reactions prior to the titrations to observe the color changes of the two indicators and demonstrate the effect of a masking agent.

The second portion of this lab uses a Brita® water filtration system to see the effect that it has on water hardness. Brita® filtration systems are certified to reduce zinc, mercury, copper, and lead as well as improve the overall taste and odor of the water. The filter is constructed of activated carbon and ion-exchange resin. The activated carbon removes compounds that affect the taste and odor of the water. The ion-exchange resin will exchange protons or other cations for cations in the water.

Chemicals & Their Location

Lab Bench	Balance Room
Eriochrome Black T, 0.5% wt/vol. in ethanol Hydrochloric acid, 0.1 M Magnesium chloride, 0.01 M Magnesium-EDTA, 0.01 M Nickel sulfate, 0.1 M Potassium cyanide, 5% Sodium hydroxide, 0.1 M Sodium hydroxide, 1 N Sodium hydroxide, 50%	Murexide
	In the Hood (keep in the hood)
	Ammonia/ammonium chloride buffer, pH 10
	You Supply It
	1 L unknown water sample

We encourage you to bring a water sample from a location of your choosing, especially if you have access to a well or bore water. If you prefer, you may take a water sample from the sink in the lab or the building. ***DO NOT*** *attempt to measure the hardness of water collected from the distilled water tap OR a bottled water sample (unless the label explicitly says that it was calcium and/or magnesium in it, or that it is spring water). If you bring your own water sample, you must bring at least one liter*

Safety Issues & Chemical Hazards

Chemical	Health Hazards	Physical Hazards
Ammonia	toxic	corrosive
Ammonium chloride	irritant	none
EDTA dihydrate, disodium salt	irritant	none
Eriochrome Black T	irritant	none
Hydrochloric acid	toxic	water-reactive, corrosive
Magnesium chloride	irritant	none
Murexide	irritant	none
Nickel sulfate	toxic, sensitizer, carcinogen, mutagen	none
Nitric acid	toxic	strong oxidizer, water-reactive, corrosive
Potassium cyanide	**extremely toxic**, corrosive	corrosive
Sodium chloride	irritant	none
Sodium hydroxide	toxic, irritant	water-reactive, corrosive

Potassium cyanide is a swift acting, highly toxic poison. You will be using very small amounts of a weak solution, but you still need to use extreme care. Poisoning may occur from ingestion, skin absorption, or inhalation. ***UNDER NO CIRCUMSTANCES ALLOW CYANIDE AND ACID TO COME INTO CONTACT****. The mixture of cyanide with acid will create hydrogen cyanide, a very deadly gas. Some of these chemicals are corrosive. Wear gloves*

and take care not to breathe the vapor when working with these chemicals. If you used the KCN, please change your gloves after use.

Equipment & its Location

Stockroom	Lab Bench
50mL Buret (2) 50 mL volumetric pipet (1), acid washed	Test tubes (7), acid washed Test tube rack Brita® Pitcher and filter

Procedure

Important Note: Metal ions can adsorb onto the walls of glassware. Any metal ions present in your glassware will react with EDTA, and lead to poor results. Ideally, all glassware in this experiment would be washed with 50% nitric acid, followed by distilled water to remove any metal ions. The stockroom has cleaned the test tubes and your 50mL volumetric pipet and 250mL flask in this manner. However, due to the large number of Erlenmeyer flasks required for the titrations, acid washing is not feasible. You should simply rinse the Erlenmeyer flasks from your drawer thoroughly with distilled water before each titration.

At Least One Week Before Lab *(you should have done this last week)*

IF YOU DID NOT DO THIS LAST WEEK, IT NEEDS TO BE **THE FIRST** THING YOU DO WHEN YOU BEGIN THE EXPERIMENT.

Making the Standard EDTA Solution:

Disodium EDTA dihydrate ($Na_2H_2Y{\cdot}2H_2O$) is a primary standard. The dihydrate form is used because the free acid is nearly insoluble. The EDTA has been pre-dried and is stored in desiccators in the Balance Room. To prevent contamination, do not use your own spatula, one has been provided for you in the desiccator. Return the EDTA salt to the desiccator when it is not being used!

4. Use a clean, dry weigh bottle to accurately weigh, **by difference**, ~ 0.25 g of disodium EDTA into the acid-washed 250 mL volumetric flask that you checked out from the stockroom. Make sure that the EDTA is *quantitatively transferred* to the flask (you may need to use a clean, dry funnel, and rinse it with a small amount of distilled water into the flask). Add ~ 200 mL of distilled water to the flask, and swirl occasionally until the solid EDTA has dissolved.

5. *After the EDTA has completely dissolved*, dilute to the mark on the flask's neck, and mix thoroughly by shaking and inverting the flask (with the stopper in place). Use a series of *three* small portions of the EDTA solution (**< 20 mL each**) to rinse a clean polyethylene 500 mL bottle. Swirl the bottle with each aliquot to ensure that the entire inner surface of the polyethylene bottle has been rinsed. These rinses remove any

residual metal ions from the bottle's inner surface. Transfer all of the remaining EDTA solution from the volumetric flask to the polyethylene bottle.

Day of the Lab

Test Tube Reactions

You will perform a number of test tube reactions to examine the various color changes involved in this experiment. While the color changes are described in the procedure, you need to record your own observations after each chemical is added. Make sure you fully mix the solutions by swirling each test tube after each reagent is added. Do not use your thumb as a stopper to mix the test tubes. The amount of water used in each reaction is not critical. Use distilled water to wash down the inner walls of the test tubes if necessary. Keep all of the test tubes for color comparison until you have completed the experiment. You may perform this part of the experiment only in groups of 4, The titration portion of the experiment will be done in pairs.

1. pH Effects on Eriochrome Black T Indicator: Place *4 drops* of Eriochrome Black T into each of three test tubes. Fill each test tube ~half full with distilled water. At this point, the contents of the three test tubes should be the same color. *If any of the tubes are a different color, it isn't clean enough*. If necessary, exchange the tube for a newly acid-washed tube from the Stockroom.

   Tube #1: Add *1 drop* of 0.1 M HCl (pink-red).

   Tube #2: Add *1 drop* of 0.1 M HCl. Add 0.1 M NaOH drop-wise until the color changes (blue).

   Tube #3: Add *1 drop* of 50% NaOH (orange-red).

   Do not discard these solutions.

2. Eriochrome Black T Indicator Complexation with Metals: Add *1 drop* of 0.01 M $MgCl_2$ solution to Tubes 1-3.

   Tube #1: (pink-red, no significant color change).

   Tube #2: Add *5 drops* of pH 10 buffer (blue changes to purple-red). *Note – the ammonia/ammonium chloride pH 10 buffer has a pungent odor, and should be kept in the hood at all times. Take the tubes to the hood to add the drops of buffer.*

   Tube #3: (orange-red, no significant color change).

3. Eriochrome Black T Indicator Action: Place *5 drops* of pH 10 buffer into each of three more test tubes (#s 4-6). Fill each tube ~half full with distilled water.

   Tube #4: Add *4 drops* of Eriochrome Black T and *5 drops* of EDTA solution (blue).

Tube #5: Add *5 drops* of EDTA solution and *1 drop* of 0.01 M $MgCl_2$ solution (colorless).

Tube #6: Add *4 drops* of Eriochrome Black T (blue), *1 drop* of 0.01 M $MgCl_2$ (purple-red), and *5 drops* of EDTA (blue).

4. Masking: Place *5 drops* of pH 10 buffer into a test tube (#7), and fill ~half full with distilled water.

   Tube #7: Add *4 drops* of Eriochrome Black T (blue), *1 drop* of 0.1 M nickel sulfate (blue changes to purple-red), and *~15 drops* of EDTA (purple-red, no significant color change). *The indicator is now "blocked".* Add *several drops* of 5% KCN (purple-red changes to blue). ***What happened?***

Filtering water with Brita Filtration System

(all remaining work is done in pairs)

5. Filter 600 mL of tap water using one of the supplied Brita® filters at the end of the bench. Record the letter (or number) of the pitcher.

Total Hardness Determination for Unfiltered Water

6. Check out an acid-washed 50.0 mL volumetric pipette and 2 burets from the Stockroom.

7. Clean the burets you checked out from the Stockroom as follows: Shake the bottle of EDTA solution to make sure solution is thoroughly mixed. Rinse the burets with a small aliquot of EDTA (to remove any adsorbed metals from the glass) and drain the rinsate to a waste beaker. Fill the buret with your EDTA solution, and record the initial volume.

8. Use the acid-washed 50.0 mL volumetric pipet to transfer 50.0 mL of your water sample into one of your cleaned 250 mL Erlenmeyer flasks. Add ~2 mL of pH 10 buffer, 0.5 mL of Mg-EDTA solution, and 5 drops of Eriochrome Black T indicator, and mix well (purple-red).

9. Titrate the water sample with your EDTA solution until the color changes to *clear blue* (uncomplexed indicator, recall your test tube reaction observations). This reaction is slow at the endpoint. To avoid over-shooting the endpoint, add the titrant slowly (wait 3-5 seconds between drops towards the end) and swirl the flask periodically. First, the color will change from purple-red to purple. One more drop of EDTA will change the color to a clear blue.

Note: If your titration required less than 10 mL to reach the endpoint, double the volume of your water sample for the remaining titrations (to increase the precision of your measurement).

***** *If you do not observe a color change after adding ~50 mL of your EDTA solution, your water sample may have metals blocking the indicator. Tell your TA; you may need to use a masking agent. If this is the case, remember the hazards of potassium cyanide, and dispose of it properly in the cyanide waste container.*

10. Repeat Steps 6-8 on *two* more aliquots of your water sample to *accurately* determine the *total hardness*.

11. Rinse the three 250 mL Erlenmeyer flasks very well before the next set of titrations.

Total Hardness Determination for Filtered Water

12. Pipet and discard some distilled water to rinse your 50mL pipet. Use the rinsed 50.0 mL volumetric pipet to transfer 50.0 mL of your filtered water sample into one of the 250 mL Erlenmeyer flasks. Add ~2 mL of pH 10 buffer, 0.5 mL of Mg-EDTA solution, and 5 drops of Eriochrome Black T indicator, and mix well (purple-red).

13. Titrate the water sample with your EDTA solution until the color changes to *clear blue* (uncomplexed indicator, recall your test tube reaction observations). This reaction is slow at the endpoint. To avoid over-shooting the endpoint, add the titrant slowly (wait 3-5 seconds between drops towards the end) and swirl the flask periodically. First, the color will change from purple-red to purple. One more drop of EDTA will change the color to a clear blue.

14. Repeat Steps 12-13 on *two* more aliquots of your filtered water sample to *accurately* determine the *total hardness*.

15. Rinse the three 250 mL Erlenmeyer flasks very well before the next set of titrations.

Individual Calcium Hardness for the Unfiltered Water

(using Murexide indicator)

16. Pipet and discard some distilled water to rinse your 50mL pipet. Use the pipet to transfer a 100.0 mL aliquot of your water sample into each of the three freshly rinsed 250 mL Erlenmeyer flasks. Add 2 mL of 1 N NaOH to each flask and mix well. Use pH paper to confirm that the pH = 12-13. *If the pH is not high enough, add more NaOH until you reach pH = 12-13.* Note how much NaOH you added and use this amount for your filtered and unfiltered replicates. There is no need to confirm the pH for each replicate.

17. Measure ~2.4 g of the Murexide/NaCl indicator mixture into a weigh boat.

18. Add approximately one-sixth of the indicator mixture from the weigh boat to one of the flasks.

19. Titrate the flask to the endpoint with your EDTA solution. You have reached the endpoint when you observe a color change in the flask from pink to purple. Record the volume of EDTA required to reach the endpoint. Confirm that you have actually reached the endpoint by adding another 1-2 drops of titrant; if there is no further color change, then use the original volume you recorded.

Note: If your titration required less than 10 mL to reach the endpoint, double the volume of your water sample for the remaining titrations (to increase the precision of your measurement).

20. Repeat Steps 18-19 with the other *two* aliquots of your water sample to *accurately* determine the *individual calcium hardness*. Note – the indicator mixture should be added to the water sample *just before* the start of each titration.

Individual Calcium Hardness for the Filtered Water

(using Murexide indicator)

21. Pipet and discard some distilled water to rinse your 50mL pipet. Use the pipet to transfer a 100.0 mL aliquot of your filtered water sample into each of the three freshly rinsed 250 mL Erlenmeyer flasks. Add the amount of 1 N NaOH to each flask, as you did in the previous section, and mix well.

22. Add approximately one-sixth of the Murexide indicator mixture from the weigh boat to one of the flasks. Be careful not to add too much Murexide to the flask, or the solution will appear to be dark pink and determining the color change will be difficult.

23. Titrate the flask to the endpoint with your EDTA solution. You have reached the endpoint when you observe a color change in the flask from pink to purple. Record the volume of EDTA required to reach the endpoint. Confirm that you have actually reached the endpoint by adding another 1-2 drops of titrant; if there is no further color change, then use the original volume you recorded.

24. Repeat Steps 13-14 with the other *two* aliquots of your water sample to *accurately* determine the *individual calcium hardness*. Note – the indicator mixture should be added to the water sample *just before* the start of each titration.

Shutdown Procedures

25. Put all waste in the appropriate waste containers. Clean and dry all glassware. Clear up your work area, and return any items you checked out from the Stockroom.

26. Make sure that you have recorded all data from the experiment and information about the sample you studied (the source of your water sample, and anything about the source that could affect the expected hardness of your sample).

Waste Disposal

Waste Chemical	Waste Container	Location
All waste	EDTA	Under workbenches

Data Analysis

1. Calculate the molarity of your EDTA standard solution. Recall, an EDTA salt, $Na_2H_2Y \cdot 2H_2O$, was used to prepare the solution. (Hint: Y is <u>not</u> yttrium.)

Unfiltered Water

2. *From your first set of data using the unfiltered water for total hardness titration*, calculate the number of moles of metal ions (Ca^{2+} and Mg^{2+}) in each flask containing an aliquot of your water sample. *If you had to increase your sample volume, the number of moles will increase in those flasks*.

3. *Total hardness* is expressed in equivalents of mg/L (ppm) $CaCO_3$ (this represents how many metal ions of either calcium *or* magnesium are bound since they both have the same effect on water hardness). Calculate the *total water hardness* of your water sample using the results from the previous step, the volume of water in each flask, and the molecular weight of $CaCO_3$. Report the *total hardness* of your water sample in mg/L (ppm) of $CaCO_3$ equivalents as the average of your three replicates with the correct number significant figures and appropriate statistical analysis. *If you had to increase your sample volume, remember to factor this into your calculation.*

4. *From your individual calcium hardness titrations using the unfiltered water*, calculate the number of moles of calcium ions in each flask containing an aliquot of your water sample. *If you have to increase your sample volume, the number of moles will increase in those flasks. If you had to dilute your EDTA solution for these titrations, remember to <u>use the diluted concentration in your calculation</u>.*

5. Calculate the *calcium hardness* of your water sample using the results from the last step, the volume of water in each flask, and the molecular weight of $CaCO_3$. Report the *calcium hardness* of your water sample in ppm of $CaCO_3$ equivalents as the average of your three replicates with the correct number significant figures and appropriate statistical analysis. *If you had to dilute your EDTA <u>or</u> increase your sample volume, remember to factor this into your calculation*.

6. Convert the *calcium hardness* (ppm of $CaCO_3$) to *calcium content* (ppm Ca). (Hint: you will need to use the atomic mass of Ca). Report the *calcium content* as the average of your three replicates with the correct number significant figures and appropriate statistical analysis.

7. From the *average total hardness* and *average calcium hardness*, calculate the *magnesium hardness* of your water sample (in ppm $CaCO_3$ equivalents).

8. Convert the *magnesium hardness* (ppm of $CaCO_3$ equivalents) to *magnesium content* (ppm Mg). (Hint: you will need to use the atomic mass of Mg).

Brita® Filtered Water

9. Repeat steps 2-8 for the filtered water.

References

(1) Brita® Home Page, http://www.brita.com (accessed December 2010).
(2) Day, R.A. Jr.; Underwood, A.L. *Quantitative Analysis*, 4th ed.; Prentice-Hall, Inc.:Englewood Cliffs, NJ, 1980.
(3) Harris, D.C. *Quantitative Chemical Analysis*, W.H. Freeman, NY, 2003.
(4) Skoog, D.A.; Holler, F.J.; Crouch, S.R. *Principles of Instrumental Analysis*, 6th ed.; Brooks Cole: USA, 2006.
(5) U.S. Geological Survey Water Hardness and Alkalinity Page, http://www.usgs.gov/owq/hardness-alkalinity.html#hardness (accessed December 2010).

Potentiometric Determination of Fluoride in Solutions

This experiment is designed to introduce the basic operational principles and illustrate an application of *ion selective electrodes* (ISEs) for the determination of fluoride ion (F^-) in water. The water supplies of most cities contain fluoride, which is added to prevent tooth decay for the population as a whole. You will use a fluoride ISE connected to a simple millivolt/pH meter to record the potentials of a series of aqueous standards having known fluoride activities to generate a calibration curve as well as a standard additions curve. You will then measure the fluoride ion activity in a sample of tap water, bottled water and mouthwash to determine the amount of fluoride ion present using both the calibration and standard additions curve.

Required Reading

Quantitative Chemical Analysis by Daniel C. Harris[1]

7th Edition

Introduction: Ch. 14 Fundamentals of Electrochemistry, Sect. 14-4 through 14-6
Theory: Ch. 15 Electrodes and Potentiometry, Sect. 15-4 through 15-7
Analysis: Ch. 4 Statistics, Sect. 4-7 through 4-9, and Ch. 5 Quality Assurance and Calibration Methods, Sect. 5-2, 5-3.

8th Edition

Introduction: Ch. 13 Fundamentals of Electrochemistry, Sect. 13-4 through 14-6
Theory: Ch. 14 Electrodes and Potentiometry, Sect. 14-4 through 14-7
Analysis: Ch. 4 Statistics, Sect. 4-7 through 4-9, and Ch. 5 Quality Assurance and Calibration Methods, Sect. 5-2, 5-3.

Pre-Lab

Written: (1) Complete the calculations required for Steps 5, 8, and 14 of the sample preparation procedure. (2) Step-by Step procedure

Background

Potentiometry allows for the determination of concentration in a variety of samples over a fairly large range. Some advantages of using an ISE (ion selective elect rode) as means of analysis incude efficiency, selectivity, and lack of interference with the sample itself. In today's lab, you will determine the amount of fluoride ion present in Austin tap water as well as bottled water and mouthwash. You will quantify the fluoride present by creating calibration curves from a supplied standard stock solution and using the standard addition method.

The concentration of fluoride ions is approximately 1 ppm in most city water supplies. Fluoridation of drinking water occurs to help lower rates of tooth decay in the general

population. Concentrations higher than 1 ppm can lead to pitting of teeth, and in some instances bone cancer.

The electrode used in these experiments contains a lanthanum fluoride crystal as a membrane. Fluoride ions are able to freely flow through this membrane. A potential will develop across the membrane when measured against a constant reference potential. It takes a while (approximately 1 minute) for equilibrium to be established across the membrane and result in a stable electrode measurement.

Potential is measurement of ion activity (mV) rather than concentration. However, the ion activity is proportional to concentration when the ionic strength is held constant. This is usually accomplished in ISE experiments by adding TISAB (total ionic strength adjustment buffer). The TISAB that you will be using in this lab consists of a mixture of glacial acetic acid, concentrated NaOH, NaCl, and 1,2-diaminocyclohexanetetraacetic acid (CDTA). CDTA is a complexiometric salt which binds selectively to metal ions and keeps them from interfering with the ionic activity registered on the multimeter.

Ionic strength is defined as:

$$I = \tfrac{1}{2} \sum c_i z_i^2 \quad (1)$$

where c represents the ion concentration and z its charge.

Ionic activity is also a function of concentration:

$$a_i = \gamma_i c_i \quad (2)$$

where γ is the activity coefficient. Making substitutions into the Nernst equation:

$$E = E_0 - E_J + (RT/ZF) \ln a_i \quad (3)$$

Where,

E = measured electrode potential

E_0 = reference potential (a constant)

E_J = junction potential

R = 8.314 J/K*Mole

T = Temperature in Kelvin

Z = charge (Fluoride ion = -1)

F = Faraday's constant (96, 485 C/ mole electron)

a = ion activity

the equation becomes:

$$E = E_0 - E_J + (RT/ZF) \ln \gamma_F + (2.303\ RT/ZF) \log C_F \quad (4)$$

or more generally,

$$\text{Signal} = \text{constant} + m \log C_F \quad (5)$$

Converting the concentration of fluoride ions (C_F) into pF, and collecting terms, the following equation can be obtained:

$$E = (59.16)\ pF + \textit{constant} \quad (6)$$

This equation shows that measured potential should change by 59 mV when the concentration is changed by a factor of 10. This is a check to ensure that the electrode is functioning properly and good "Nernstian" behavior is observed. The results of potentiometric measurements are usually expressed in terms of a parameter, the *p-function*, which is directly proportional to the measured potential.[2]

For standard additions, the math becomes much more complicated. If the observed signal of the solution (I_x) is proportional to the concentration of the unknown $[X]_i$ and the signal

of the unknown + standard (I_{x+s}) is proportional to the final concentration of the unknown + standard ($[X]_f + [S]_f$).

The following equation was derived based on the preceding assumptions:

$$\underbrace{V_{tot} * 10^{\frac{E}{m}}}_{y} = \underbrace{10^{\frac{k}{m}}[X]_i V_X}_{b} + \underbrace{10^{\frac{k}{m}}[S]_i}_{+m'} \underbrace{V_S}_{x} \quad (7)$$

Where V_{tot} is the total volume (unknown + standard), E is the voltage recorded on the potentiostat, m is the slope of the calibration curve generated by plotting voltage vs. + log $[F^-]$ (approximately -59 if graphed in mV or -0.059 if graphed in Volts), k is the y-intercept of the calibration curve, $[X]_i$ and $[S]_i$ are the initial concentrations of the unknown and standard respectively, and V_x and V_s are the Volumes of your unknown and standard respectively. By plotting the above equation (y vs. x), one will be able to extract the ionic concentration in the original solution ($[X]_i$). More details of extraction are given in the Data Analysis section.

Standard additions in potentiometry are useful for quantitative measurements when the matrices are complex. However, the standard addition plot requires the use of calibration parameters and their associated error.

Additional Notes:

You will use a multimeter to detect *changes* in the ion activity of the solution, measured in mV. Use your textbook as a reference for information on the principles of multimeters. Ask your TA if you have <u>any</u> questions on how to operate the multimeter. **Note: make sure the multimeter is set to record mV and NOT pH**. Also, some of the values you will observe are negative numbers. Just remember that for very 10x change in concentration, the mV recording should change by ~60 mV.

Chemicals & Their Location

Lab Bench	Quantities needed (per group)
TISAB (total ionic strength adjustment buffer)	~100 mL
2000 ppm NaF	~1 mL

Equipment & its Location

Stockroom	Above / On Lab Bench
For Fluoride ISE lab (each group needs) 25-mL Vol flask 50-mL Vol flask 10-mL beaker (6) 10-mL Vol flask (6) 0.5 mL Vol pipet 1 mL Vol pipet 2 mL Vol pipet 5 mL Vol pipet (2) Magnetic stir bars (3)	Electrode Holder Magnetic Stir Plate Stir bar ISE meter and probe (return to stockroom) Please return the meter and probe to the stockroom like you found them!!

Safety Issues & Chemical Hazards

Chemical	Health Hazards	Physical Hazards
Sodium Fluoride *TISAB*	Irritant, toxic Irritant, mutagenic (investigated)	None None

Wear gloves *and take extreme care while working with these chemicals. Sodium fluoride is* ***toxic*** *in high concentrations. Do not touch your face or eyes with gloves on. Change your gloves often.*

Procedure

All sample preparation needs to be done in the wet lab. You will work in groups of 2 for this lab. If there is an odd number of people in the lab, there will be one group of 3.

There are multiple components to this procedure. To make the most effective use of time, the work should be divided evenly among group members (i.e., – the solutions can be prepared while the electrode is recording. Though the work will be split up, each group member is responsible for understanding every step of the procedure. In order to run all of the samples and finish on time, it is important that one person is rinsing the volumetric flasks while the other is recording the potentials.

Potentiometry Setup

1. This experiment will be done in pairs.

2. Pick out a multimeter from the front of the lab. *Note – the fluoride electrode is kept in a storage buffer solution that protects it by keeping it clean and hydrated. It is important that the electrode be immersed in solution as much as possible. Do not leave the electrode exposed to air for long periods of time; try to keep it in either the storage buffer or your experimental solution at all times. Whenever you move the electrode from one solution to another, you should rinse it well with your squirt bottle (filled with distilled water) to prevent cross-contamination between your solutions and the storage buffer. Please return the multimeter and probes to the stockroom at the end of the lab.*

3. For the titrations in today's experiment, you will use beakers (instead of an Erlenmeyer flask, see Figure 1), so that you can fit the fluoride electrode into the titration solution. The beaker (10 mL) will sit on the center of the stir plate with a stir bar inside it. Use the electrode holder to carefully position the electrode in the beaker so that it does not hit the stir bar. The linear range of the fluoride ISE is 0.2 to 19,000 ppm.

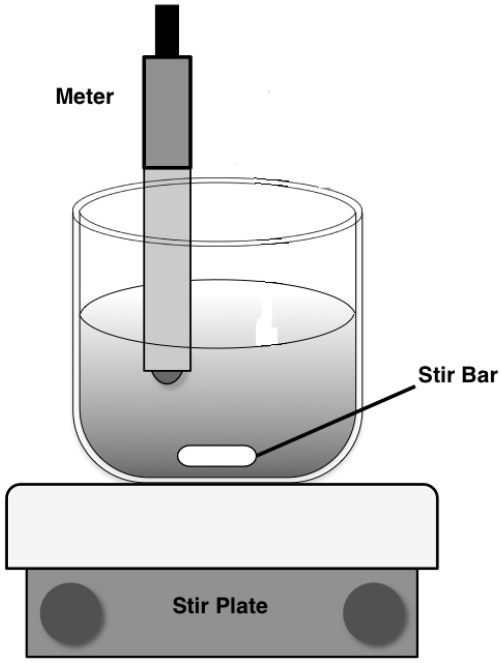


Figure 3: Potentiometry Setup.

Calibration Curve Method

1. Volumetrically prepare 10.0 mL of a solution that is ~200 ppm in fluoride ions from the sodium fluoride solution provided (transfer a small amount of the stock solution into a beaker before using a pipet). The stock solution will be approximately 2000 ppm in fluoride ions. Record the actual concentration for you calculations . Before class, calculate the amount of stock solution you will need to make the dilution. Use DI water as your diluent.

2. Use a volumetric pipet to transfer 4.0 mL of your 200 ppm fluoride solution to a 10 mL beaker.

3. Use a separate volumetric pipet to transfer 4.0 mL of your buffered solution (TISAB) to your 10 mL beaker. Use the stir bar/plate to gently swirl the solution before taking readings of your sample. No vortexes. Use the fluoride ISE and meter to record the potential of the solution (mV). Rinse the electrode with DI water in between measurements of each of the solutions you will test.

4. Use the 200 ppm fluoride solution made in Step #1 to make 10 mL of 20 ppm dilution with DI water. Repeat steps 2 and 3 and measure the potential of the newly made solution.

5. Continue to make serial dilutions until you have a total of 4 solutions (including the previous two). The solutions should range from 200 to 0.2 ppm in fluoride ions. You MUST show TA roadmap of how you will prepare your samples PRIOR to beginning lab. Each solution should be made in a 10 mL volumetric flask. You will transfer 5 mL of each solution (Do NOT discard the remaining solutions until after the standard additions solution has been made) and 5 mL of the buffered solution to a 10 mL beaker before you record the potential (from Steps 2 and 3).

Unknown Fluoride Sample Preparation by Calibration Curve Method

6. The three samples to be analyzed for fluoride are mouthwash, tap water, and bottled water. For each, take 4 mL of each unknown and add 4 mL of your buffered solution within a 10 mL beaker. Make each unknown in triplicate for a total of 9 samples.

7. Measure the potential of each of these nine samples, while stirring the samples in a similar fashion to Step 3.

Standard Addition Method

8. You will need to make a standard (25 mL) that is approximately 20 ppm F^-. You may use any of the remaining solutions (unbuffered) from the calibration curve method section to aid in your preparation. You MUST show TA roadmap of how you will prepare this solution PRIOR to beginning lab.

9. Prepare four samples for analysis using four 10.0-mL volumetric flasks. In each flask, add 5 mL of buffered solution and 1.0 mL of tap water.

10. One sample will have 0.5 mL of standard (~ 20 ppm) added to it and then diluted to 10.0-mL with DI water.

11. Before diluting the other three samples add 1 mL of standard to one, 2 mL to another, and 3 mL to the last one.

12. Repeat Steps 9-11 for the other unknown (mouthwash) in place of the tap water.

13. Transfer each solution to a clean 10 mL beaker and use the Fluoride ISE and meter to record the potential (mV) of each solution while magnetically stirring. You should have a total of four recordings for each unknown (eight in total).

14. *Standard Additions Solutions*: All calculations and solutions should be made to have a *final* volume of 10.0 mL, and all dilutions need to be made with DI water. Each *standard additions* solution should result in the unknown sample being diluted by a factor of *ten*. Include a table such as the following one in your notebook as part of your pre-lab (filling in the blanks). Your TA must verify these calculations before you prepare these solutions.

Flask #	**Volume of Unknown (V_x)**	**Volume of ~20 ppm F^- (V_s)**	**Total Solution Volume (V_{tot})**	**mV from Fluoride ISE**
A			10.0	
B			10.0	
C			10.0	
D			10.0	

Ex. Sample Standard Additions Table

Shutdown Procedures

15. Put all waste in the appropriate waste container.

16. Clean and dry all glassware, clean up all work areas, and return any borrowed items to the Stockroom.

Waste Disposal

Waste Chemical	Waste Container	Location
All Waste	Fluoride ISE	Near Cabinet

Data Analysis

1. The standard stock solution of Sodium Fluoride was approximately 2000 ppm. *Check the Stockroom posting for the actual concentration for your day in lab.* Using the true concentration given by the stockroom, calculate the concentration of Fluoride (in ppm) for each of your *four* calibration standards from the dilution factors you used to make each solution. The values should range ~0.2 ppm to 200 ppm.

2. Using the true concentration given by the stockroom, calculate the concentration of Fluoride, $[S]_i$, (in ppm) for your standard additions solution (~20 ppm) that you made in Step 8.

Calibration Curve

3. Construct *one* calibration curve (in ppm) by plotting the voltage *vs*. +log [fluoride concentration] using your four calibration standards. Find the line of best fit for the data. Show the equation and correlation coefficient (R^2) for each calibration curve on the graph.

4. Use the calibration curve's line of best fit to determine the concentration of fluoride in each of the *three unknown* samples (in ppm).

5. Calculate the average, standard deviations, and 95% and 98% confidence intervals for each unknown using an appropriate t-table.

Standard Additions Curve

6. Construct a standard additions curve (using a scatter plot) from equation 7. Plot the $V_{tot} \times 10^{E/m}$ vs (V_s), where V_{tot} is the total volume of the solution (10 mL), E is the measured potential of each standard solution, m is the slope from your calibration curve, and V_s is the volume of the standard. There will be a total of two standard

additions curves, one for the tap water unknown and one for the mouthwash. Each plot will have four points.

7. The x-intercept of the standard additions curve you generated in Step 4 can be used to determine the concentration of the unknown (this is discussed in your lecture notes in more detail).

   The equation of the line is given in the form y = mx + b from equation 7. The x-intercept is an extrapolation of the line of best fit to hit the x-axis. At the x-intercept of the line, y = 0 (no standard has been added); therefore, equation 7 can be rearranged to yield the following equation:

$$\text{x} - \text{intercept} = \frac{-[\text{X}]_\text{i}\,\text{V}_\text{X}}{[\text{S}]_\text{i}} \qquad (10)$$

   Where $[X]_i$ is the initial concentration of your unknown, V_x is the volume of your unknown, and $[S]_i$ is the initial concentration of your unknown. Plug in appropriate values of $[S]_i$ and V_x, then rearrange the above equation to solve for $[X]_i$.

8. Record the values for the initial fluoride concentration $[X]_i$ for both the tap water. As there is inherent error in using calibration curve data (m) to produce the standard additions curve, statistical analysis for the standard additions curve is not necessary.

9. The amount of fluoride in the mouthwash you used is reported as 0.01% (w/v). How does this value compare with your values obtained via the calibration curve as well as the standard additions method? What may account for any difference?

Discussion Questions

1. How do your results using calibration curves and standard additions differ? What may account for (if any) discrepancy between the two values?

2. How accurate do you think your fluoride ion concentration in bottled watter is? Why do you think this?

3. If an organic solvent was a contaminant in the solution, which method (calibration curve or standard additions) should be better to use in terms of data analysis? Why?

4. If TISAB was not added to your solutions prior to measurement, what would be the effect when measuring voltage on the multimeter?

5. Why must the solution be constantly and consistently swirled while making measurements? What would be the effect if the solution were not swirled in such a manner?

6. What other method can be used to determine fluoride content in water? What are the advantages/disadvantages to this other method?

Literature Review

Provide one peer-reviewed journal article that uses ion selective electrodes as means of quantifying any analyte. List the analyte and the sample medium. The article can be from any journal, (*J. Chem. Ed., J. Chem. Phys.*, etc.). Citation information and the title of the article is all that is required for this lab.

Conclusions

As part of your conclusions for this experiment, compare your experimental results for the fluoride content in tap water (calculated via calibration curve) with the levels in Austin city water, http://www.ci.austin.tx.us/water/waterreports.htm, and discuss whether your results seem reasonable with what one might expect to find in drinking water.

References

(1) Harris, D.C. *Quantitative Chemical Analysis*, W.H. Freeman, NY, 2003.

(2) Skoog, D.A.; Holler, F.J.; Crouch, S.R. *Principles of Instrumental Analysis*, 6th ed.; Brooks Cole: USA, 2006.

An Investigation of Electrochemistry, using Chronoamperometry & Cyclic Voltammetry

This experiment explores two controlled potential (*voltammetric*) analytical methods. *Chronoamperometry*, the change in current with time after making a step change in potential, will be used to determine the diffusion rate of the ferricyanide ion, an electro-active species. In addition, the basic principles of *cyclic voltammetry* (CV) will be introduced by qualitatively studying the oxidation and reduction of the ferricyanide ion (a simple, reversible, diffusion-controlled electron transfer process). You will also use CV to measure the diffusion rate of the ferricyanide ion. Lastly, CV will be used to qualitatively observe the electrochemistry of thyronine and the chemical processes occurring following its oxidation.

Required Reading

Quantitative Chemical Analysis by Daniel C. Harris

7th Edition

Introductory: Ch. 14 Fundamentals of Electrochemistry, Sect. 14-1 through 14-3, and Ch. 15 Electrodes and Potentiometry, Sect. 15-1 (Reference Electrode)

Theory/Data Analysis: Ch. 17 Electroanalytical Techniques, Sect. 17-1, 17-5 (Cyclic Voltammetry)

8th Edition

Introduction: Ch. 13 Fundamentals of Electrochemistry, Sect. 13-1 through 13-3; Ch. 14 Electrodes and Potentiometry, Sect. 14-1 (Reference electrode)

Theory/Data Analysis: Ch. 16 Electroanalytical Techniques, Sect. 16-1 and 16-5 (Cyclic Voltammetry)

Kissinger and Heineman, *J. Chem. Ed.*, **60** 9 (1983), 702-6. (Copies of this reference are on reserve in the library – it contains a good explanation of CV).

Pre-Lab

Written: (1) Draw a block diagram of a potentiostat and a 3-electrode cell (with all the major components identified and labeled). (2) Complete the pre-lab calculations described in Step #1 of the procedure. (3) Step-by-Step Procedure

Background

Electrochemistry is a broad term that describes an important field of chemistry that studies the relationship between chemical and electrical effects. Most electrochemical experiments involve either examining the chemical changes in a system due to the passage of an electrical current, or the production of energy as a result of chemical reaction. However, electrochemistry is used in a wide range of applications ranging from separations techniques to sensors. This experiment will introduce two common types of *voltammetry*: chronoamperometry and cyclic voltammetry. *Voltammetric* techniques measure the current flow when the potential applied to a working electrode is either low enough for reduction or

occur or high enough for oxidation to occur. For a *redox reaction*, the potential at which current begins to flow is related to the *standard reduction potential* for the pair of species being reduced or oxidized. The magnitude of the current resulting from the electron transfer reaction is related to the concentration of the species reacting at the electrode.

Chronoamerpometry

Chronoamperometry, the measure of current versus time after an instantaneous change in the potential of the working electrode (often called a *step change*, Figure 1*a*), is one of the more simple voltammetric methods. In the *chronoamperometry* portion of this experiment, an electrode will be immersed in a solution containing ferricyanide ions ($Fe(CN)_6^{3-}$). The electrode will be held at a potential higher than the ferricyanide reduction potential, where no reaction takes place (i.e., no current flows). By suddenly changing the electrode potential to a value significantly *lower* than the ferricyanide reduction potential, any $Fe(CN)_6^{3-}$ molecules that come into contact with the electrode will be instantly reduced to ferrocyanide ($Fe(CN)_6^{4-}$). If the solution is not stirred, there will be a high initial concentration of ferricyanide at the electrode, and a *high* current will be observed (due to the fast reaction rate). With time, the current will drop as the concentration of $Fe(CN)_6^{3-}$ near the electrode decreases, causing the reaction rate to slow (illustrated in Figure 1*b*).

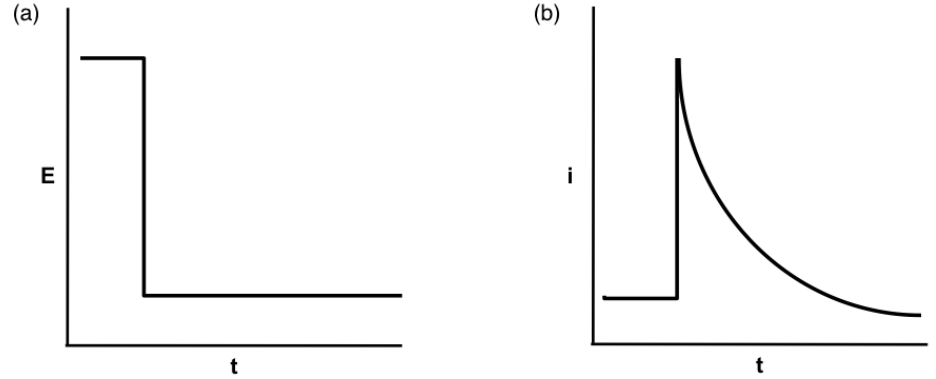


Figure 1: (a) Chronoamperometry Electrode Potential, (b) Chronoamperometry Current Response

This drop in current in the absence of stirring is described by the *Cottrell equation*, which is based on simple diffusion theory:

$$i = \frac{nFAc\sqrt{D_0}}{\sqrt{(\pi t)}} \quad (1)$$

where *i* is the current (amps, A), *n* is the number of electrons involved in the electrode reaction, F is the Faraday constant (96,484.6 C mol^{-1}), *A* is the area of the electrode (cm^2), *c* is the bulk concentration of the reacting species (mol L^{-1}), D_0 is the diffusion coefficient of the reacting species (cm^2 s^{-1}), and *t* is the time after the potential step change (seconds). A ***1.7 mm diameter platinum disk working electrode*** will be used for this experiment. *This equation predicts that current is proportional to the inverse of the square root of time. This type of theoretical behavior is only observed at times greater than a few milliseconds. At*

earlier times, electrode-charging current and reactions of species adsorbed on the surface of the electrode tend to increase the observed current.

Cyclic Voltammetry (CV)

A *cyclic voltammogram* is the electrochemical equivalent of an absorbance or emission spectrum. CV measures the current response of a solution over a *range* of applied potential. A cyclic voltammogram provides useful information about the identity and reactivity of the electroactive species in solution. In CV, by changing the potential of the working electrode at a constant rate while measuring the resulting current, a large potential range can be swept to determine the potentials where the electrode reactions occur for the analytes in a sample solution. After scanning through the potential region in which one or more electrode reactions take place, the direction of the *linear sweep* is reversed, and the electrode reactions of any intermediates and products formed during the forward scan can often be detected. The forward and backward scan may be repeated, and thus, this technique is cyclic (as shown in Figure 2*a*). An example of the current response to this cyclic potential scan can be seen below in the sample *cyclic voltammogram* (Figure 2*b*).

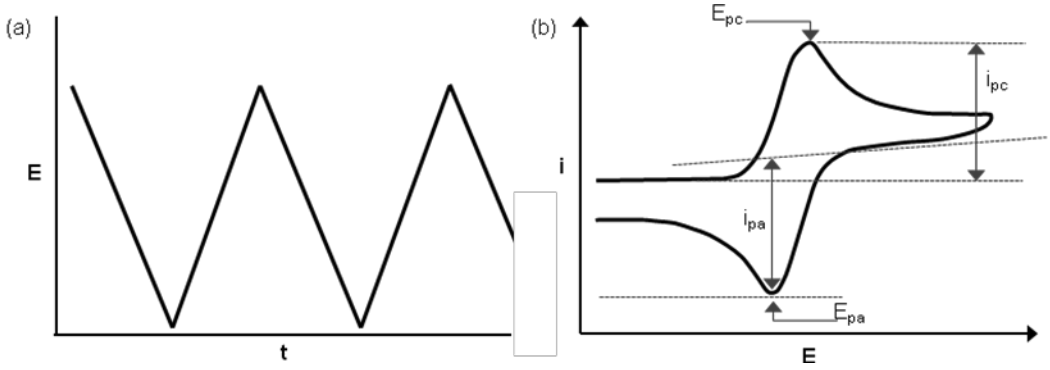


Figure 2: (a) CV Electrode Potential, (b) CV Current Response.

The CV portion of this experiment begins by examining the simple, recersible, diffusion-controlled, electron transfer reaction of a solution containing ferricyanide ions. The CV scan starts with the potential *above* the reduction potential of $Fe(CN)_6^{3-}$ and is linearly scanned toward a lower potential. No current will flow through the system until the potential is low enough to reduce $Fe(CN)_6^{3-}$ to $Fe(CN)_6^{4-}$ at the electrode surface. When the $Fe(CN)_6^{3-}$ begins reducing, the current rapidly increases (dependent on the electron transfer rate). The current continues to increase until it reaches the *peak cathodic current*, i_{pc}, observed at the E_{pc}, and drops sharply when the concentration of $Fe(CN)_6^{3-}$ at the electrode surface approaches zero. This peak in the current response results from the competition between two factors: 1) an increase in the $Fe(CN)_6^{3-}$ reduction rate as the potential continues to drop, and 2) the decrease in the amount of $Fe(CN)_6^{3-}$ close to the electrode surface. As a consequence, the cathodic current peaks at a potential slightly lower than the reduction potential of $Fe(CN)_6^{3-}$. The size of the peak cathodic current depends on a number of variables; including the area of the working electrode, the number of electrons involved in the electrode reaction, the concentration of the reacting species in solution, the diffusion coefficient of the reacting species, and the speed at which the potential is scanned.

After the cathodic current has peaked, while still scanning towards lower potential, the current drops back down due to depletion of $Fe(CN)_6^{3-}$ near the electrode surface. Now, the direction of the potential scan is *reversed* (from low toward more positive potential). Initially, the potential is still lower than the reduction potential, so there will still be cathodic current until the potential is high enough to oxidize the $Fe(CN)_6^{4-}$ back to $Fe(CN)_6^{3-}$. As the potential is increased, the rate of the oxidation reaction also increases until it reaches the maximum *anodic peak current*, i_{pa}, observed at the E_{pa}. The current drops rapidly as the reacting species, $Fe(CN)_6^{4-}$, is depleted at the electrode surface. Once the working electrode potential returns to the point at which the original cyclic voltammogram began, the cycle is complete.

In CV, the potentials at which the cathodic and anodic peaks are observed are independent of the scan rate. The potential halfway between the reduction and oxidation peaks is defined as the *half-wave potential*, $E_{1/2}$:

$$E_{1/2} = \frac{E_{pa} + E_{pc}}{2} \tag{2}$$

The *half-wave potential* is normally within a few mV of the *formal reduction potential*, E°, for the reversible redox reaction couple, provided that the reduced and oxidized forms have approximately equal diffusion coefficients.

The potential difference between the cathodic and anodic peaks of a cyclic voltammogram of a reversible redox reaction is a fixed quantity, and depends on the number of electrons transferred during the process, n:

$$E_{pa} - E_{pc} = \frac{2.22\ RT}{nF} = \frac{59.0\ mV}{n} \text{ at } 25°C \tag{3}$$

Therefore, a one-electron, reversible process should *ideally* exhibit a peak separation of 0.059 V at 25ºC.

Diffusion

An ion in solution can reach an electrode surface by means of three different methods: *convection* (stirring, temperature, or density gradients), *electromigration* (current flow), and *diffusion* (concentration gradient). In this experiment, <u>not</u> stirring the sample solution and keeping it at a constant temperature eliminate *convection*. *Electromigration* is reduced by using a *supporting electrolyte* (KCl) at a concentration much higher than that of the ferricyanide. The KCl carries the bulk current through the solution, decreasing the probability of ferricyanide traveling to/from the vicinity of the electroade via *electromigration*. By removing *convection* and *electromigration*, ferricyanide ions reach the electrode surface by *diffusion*, and the current is *diffusion-controlled*.

The cathodic peak current of the *first* forward pass of a *diffusion-controlled*, reversible cyclic voltammogram is described by the *Randles-Sevcik* equation:

$$i_{pc} = kAc\sqrt{(n^3 D_0 v} \text{ at } 25°C \quad (4)$$

where i_{pc} is the peak current (A), k is a constant (2.69×10^5 C mol^{-1} V$^{-1/2}$), A is the electrode area (cm^2), c is the bulk concentration of the reacting species (mol L^{-1}), n is the number of electrons involved in the electrode reaction, D_0 is the diffusion coefficient of the reacting species (cm^2 s^{-1}), and v is the potential sweep rate (V s^{-1}).

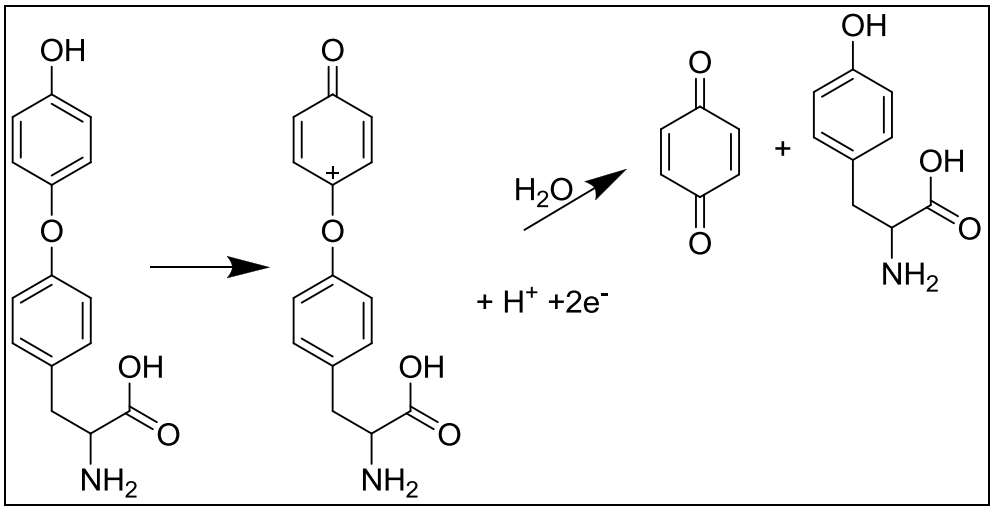
More Complex Systems

Once you have reached this point in the experiment you will already be familiar with a one-electron, reversible redox system. The ferricyanide CV reaction involved a fast rate constant, full availability of the required reagents, a peak-to-peak separation of ~59 mV, and a similarity in the appearance of the cathodic and anodic current peaks. Examples of analogous reversible systems include inorganic ions, metal complexes, and organic compounds capable of undergoing an electron transfer reaction *without making or breaking covalent bonds*.

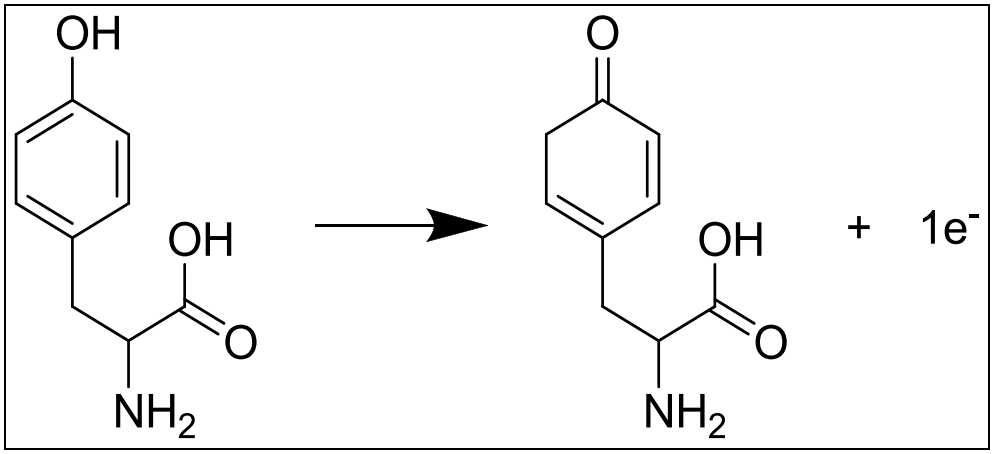
Electrochemical systems are rarely so simple. Frequently, the electron transfer step produces a species that quickly reacts with other components of the system, in a *quasi-reversible* or *irreversible* fashion. Additional chemical reactions directly affect the *available* concentration of both the reactants and products at the electrode surface, by altering the cyclic voltammogram. As a result, the cathodic and anodic peaks may be more spread out, less defined, or sometimes, nonexistent. In these cases, there is usually more than one product generated, causing several additional peaks in the voltammogram, with each characteristic of a different electrochemical reaction product. If the value of n (the number of electrons transferred) is larger than one for an overall reaction, the cyclic voltammogram will consist of overlapping one-electron processes, with the peak positions determined by the E^o values of each separate redox couple.

CV can be used to “untangle” such complex systems. It is possible to identify many side products following their generation at the electrode surface. One strength of CV is its ability to successively oxidize different molecules on different time scales. By changing the scan rate, it is possible to access different time domain reactions and elucidate some of the mysteries behind the mechanism of a reaction. Conversely, it is evident that this technique has limitations, as it is often needs to be used in conjunction with other techniques in order to *prove* a certain mechanistic pathway. One of the major drawbacks to this technique is that it is only capable of detecting electroactive species. However, one way to get around this drawback is to use electroactive species that bind to the non-electroactive side products of interest.

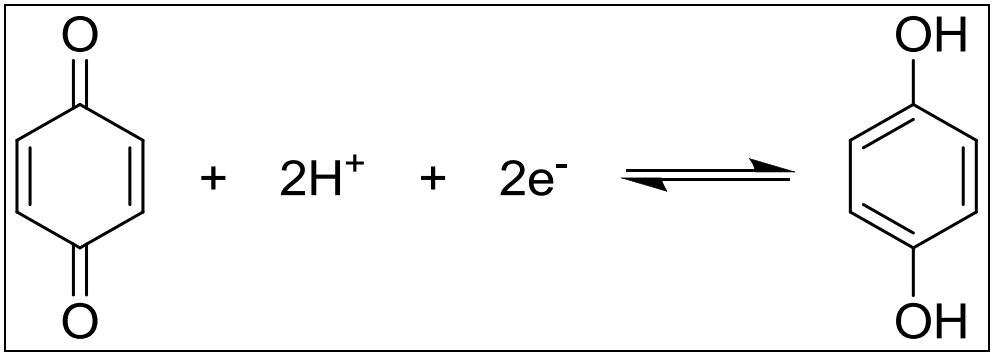
The final portion of this experiment investigates the electrochemical properties of thyronine, an analog of the thyroid hormone thyroxine. The molecular structures of thyronine and its degradation products, tyrosine and benzoquinone, along with their reduction or oxidation reactions, are shown below. By successively reducing and oxidizing these molecules with CV, you will examine these electrochemical reactions.



Thyronine: oxidation followed by a hydrolysis (making tyrosine and benzoquinone).



Tyrosine: oxidation (irreversible).



Benzoquinone: reduction **/** *p*-hydroquinone oxidation (reversible).

Instrumentation

Three electrodes will be used in this experimental setup: working, reference, and auxiliary. A *potentiostat* is used to control the potential at the surface of the *working electrode* relative to the potential of the *reference electrode*. The *reference electrode* is a reactive electrode and is non-polarizable (i.e., charge can be moved into and out of the electrode *without* changing its potential). A silver/silver chloride reference electrode will be used, consisting of a silver wire suspended in a saturated aqueous solution of potassium chloride and silver chloride. When a negative charge is applied to the electrode, the silver ions from AgCl are reduced to silver metal. Conversely, when a negative charge is removed from the electrode, silver metal is oxidized and produces silver ions. The potential of the working electrode is maintained at the desired absolute potential by constant comparison with the stable reference electrode. The amount of current passed through the reference electrode needs to be limited to avoid a shift in its potential; otherwise, the stability of the system would be disrupted. The *auxiliary electrode* is used to carry the majority of the current from the working electrode. This electrode is generally an inert, polarizable metal with a much greater surface area than the working electrode, so as not to limit the current. We will use a platinum wire as the *auxiliary electrode* in this experiment.

A good *working electrode* must be relatively unreactive (i.e., does not reduce or oxidize within the desired working potential range). Moreover, the *working electrode* must be polarizable; meaning that when charge is added to or removed from the electrode, its potential should change in a linear fashion. Carbon and relatively inert precious metals, such as platinum, mercury, and gold, are common examples of good working electrodes. It is important to note that not all materials make suitable electrodes for all electrochemical experiments. Different electrode surfaces do not have the same surface roughness, catalytic activity, potential range, and affinity – all factors that can have a considerable effect on the quality of the observed signal. For example, mercury electrodes are much smoother than other electrode surfaces, but are limited to a more negative potential window. Platinum is one of the most common electrode surfaces because it catalyzes many reactions and is not susceptible to oxide fouling. A **1.7 mm diameter *platinum disk*** working electrode will be used for the first part of this experiment (ferricyanide reactions). However, due to the limited working potential range for platinum (it does not extend positive enough), a glassy carbon electrode (GCE) will be used for the thyronine reactions. GCEs are often used with protein-like molecules because the electrode surface seems to naturally absorb these types of species. The GCE surface has an affinity for thyronine and its side products, but is susceptible to oxide fouling and requires a more lengthy cleaning procedure prior to using it in the experiment.

Chemicals & Their Location

Stockroom Kit	Lab Bench (Next to Instrument)
Alumina slurry (0.3 or 0.05 μm) *p*-Hydroquinone, DL-Thyronine, Tyrosine (in 25mL vol. flasks) Potassium chloride Potassium ferricyanide	Sulfuric acid, 1 M, made with ultrapure water Ultrapure water

Equipment & its Location

Stockroom Kit	Lab Bench (Next to Instrument)
Electrodes: Pt disk, glassy carbon, Pt wire, Ag/AgCl Polishing cloth Sample vial & electrode holder 25 mL volumetric flask (3) 50 mL volumetric flask Funnel Spatula Beakers	Computer Electrochemical analyzer Kimwipes Analytical Balance and weigh boats Sonicator

Safety Issues & Chemical Hazards

Chemicals	Health Hazards	Physical Hazards
Alumina	irritant	none
p-Hydroquinone	toxic	none
Thyronine	irritant	none
Tyrosine	irritant	none
Potassium chloride	irritant	**avoid contact with acids**
Potassium ferricyanide	highly toxic, carcinogen	corrosive
Sulfuric acid	irritant	none

Be very careful! *Contact of potassium ferricyanide with acid produces* ***LETHAL hydrogen cyanide gas****. Check all waste container labels before disposing of your waste. Sulfuric acid is highly corrosive. Wear gloves while working with these chemicals.*

Procedure

Wet chemicals are not allowed in the instrument area. All sample preparation must be done in the designated sample prep area.

There are various components to this procedure. The work should be divided amongst you so that the experiment can be completed more quickly: e.g, the solution can be prepared while the instrument is being setup.

Solution Preparation

Step #1 calculations should have been done as part of your pre-lab.

1. Calculate the amount of potassium ferricyanide and potassium chloride you will need to prepare 50 mL of a single aqueous solution that is ***both*** ~5 × 10^{-3} M potassium ferricyanide and 1 M potassium chloride.

2. Weigh out the amount of potassium ferricyanide and potassium chloride you calculated in Step #1 and record the exact weight of each chemical. Quantitatively transfer both chemicals to a clean, dry 50 mL volumetric flask (if you use a funnel, rinse the surface with ultrapure water to ensure a complete transfer). Using ultrapure water as your diluent, add ~25 mL and swirl periodically until both of the solids have dissolved. Dilute to the mark and mix well.

3. In the kit from the Stockroom, you were given *three* 25 mL volumetric flasks, labeled A, B, and C. Each contains a small amount of an unidentified solid. Do not make up these solutions yet (they degrade quickly).

Electrode Cleaning & Instrument Setup

Refer to the "Instrument Startup and Shutdown Guide" next to the instrument for all procedures to set up and run samples.

4. Identify the two electrodes from your kit (platinum disk and glassy carbon). *The platinum disk and glassy carbon working electrodes both look like black rods. To tell the difference between the two, remove the cap and examine the working end of the electrodes. The platinum disk electrode looks metallic, while the glassy carbon electrode looks like a shiny black surface. (A general diagram of a working electrode is shown below in Figure 3). If you are unable to tell the electrodes apart,* ***ask a TA for help****.*

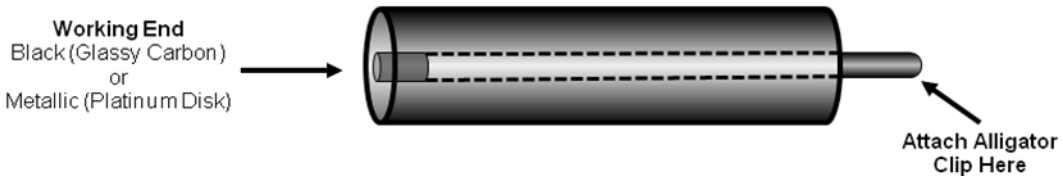


Figure 3: General Diagram of a Working Electrode

5. Polish the *platinum disk electrode ("Electrode Polishing" in Instrument Manual)*.

6. Rinse the sample vial thoroughly with ultrapure water. Fill the vial ~3/4 full with 1 M sulfuric acid and clamp it into position on top of the stir plate, and set up the *three-electrode cell*.

7. Clean the electrodes using the sulfuric acid and a potential sweep *("Cleaning the Pt working electrode" in Instrument Manual*). After cleaning, rinse the electrodes well using ultrapure water. Rinse the electrodes AND sample vial very well with ultrapure water, so that there is NO ACID remaining to react with the ferricyanide.

Ferricyanide Chronoamperometry

Refer to "Running Chronoamperometry" in Instrument Manual.

8. Fill the sample cell ~3/4 full with ferricyanide solution. Immerse the electrodes in the solution.

9. Run the chronoamperogram of the ferricyanide solution. *The data collected in this step should look like Figure 1b.*

Ferricyanide Cyclic Voltammetry

Refer to "Running Cyclic Voltammetry" in Instrument Manual.

10. Run the cyclic voltammogram of the ferricyanide solution. *The data collected in this step should look like Figure 2b.*

11. Determine the values of E_{pa}, i_{pa}, E_{pc}, and i_{pc} for *each* voltammogram, and record them in your notebook. Repeat for the multiple scan rates and sensitivity values listed in the Instrumental manual.

12. Once you have collected all of the ferricyanide voltammograms, **carefully** dispose of the ferricyanide waste in the appropriate waste containers. **Do not mix ferricyanide and acid!**

13. Rinse the sample vial and electrode several times, thoroughly, with ultrapure water.

Glassy Carbon Electrode Cleaning

14. Polish the glassy carbon electrode using alumina and a polishing cloth per the instructions in the "Instrument Startup and Shutdown Guide". *Note: the glassy carbon electrode must be cleaned **before each use** (i.e., for each of the three unknowns) because the surface is easily contaminated*.

Unknown Cyclic Voltammetry

15. Make up ***one* of the unknown solutions** (volumetric flasks labeled A, B, or C containing small amount of an unknown solid from the Stockroom) by diluting to volume with 1 M sulfuric acid. . If the solid does not dissolve with gentle swirling, dip the body of the flask in the sonicator (while holding the flask by the neck) to help the solid dissolve. *The unknowns decompose rather quickly in acidic media, so do not make these solutions until after all the ferrocyanide runs have been completed*.

16. Fill the sample vial with some 1 M sulfuric acid, and rinse the electrodes. Dispose of the acid and fill the sample vial ~3/4 full with the f*reshly made* unknown solution. *Note: These solutions decompose quickly in acidic media, so make the solutions and run them within a 15 minutes.*

17. Run the cyclic voltammogram of the first unknown. *Note: the data collected may not closely resemble Figure 2b.*

18. Determine the values of E_{pa}, i_{pa}, E_{pc}, and i_{pc} for the voltammogram, and record them in your notebook.

19. Dispose of the unknown sample. Rinse the electrodes and sample vial with sulfuric acid (to avoid cross-contamination of the next unknown solution). Repolish the glassy carbon electrode.

20. Make up the second unknown solution by diluting the second flask to volume with sulfuric acid, and use it to fill the sample vial ~3/4 full.

21. Collect the cyclic voltammogram of the second unknown. *If the voltammogram looks the same as the first unknown sample, then the electrodes are not clean enough. If this is the case, polish them again and rerun the cyclic voltammogram.*

22. Determine the values of E_{pa}, i_{pa}, E_{pc}, and i_{pc} for the voltammogram, and record them in your notebook.

23. Repeat Steps 19-22 for the remaining unknown solution.

Each unknown voltammogram should look different from each other. If any two appear the same, the electrodes need to be repolished, the sample vial needs to be cleaned again with sulfuric acid, and each sample needs to be run again.

Shutdown Procedures

24. Make sure that you have recorded *all* information about the sample you studied, the instrument parameters, and that each group member has the data saved.

25. After all runs have been completed, shut down the instrument according to the instructions provided in the “Instrument Startup and Shutdown Guide”. Disconnect the

electrodes and clean them by rinsing with ultrapure water. Return the Ag | AgCl reference electrode to its storage vial, and allow the other electrodes to air dry.

26. Put all waste in the *appropriate* waste containers (**do not mix ferricyanide and acid**). Clean and dry all glassware, and return all borrowed items to the Stockroom.

Waste Disposal

Waste Chemical	Waste Container	Location
All solutions containing ferricyanide	Ferricyanide	Lab Bench
All other solutions	Acidic Electrochem	Lab Bench

Data Analysis

1. Using the measured weights from Step #2, calculate the *actual* concentrations of the potassium ferricyanide and potassium chloride in your CV solution in both mol L^{-1} and mol cm^3 (same as mol mL^{-1}).

Ferricyanide Chronoamperometry

2. Open the chronoamperometry file using Excel. The first column of data is *time* (s) and the second column is *current* (A). Your first set of data corresponds to "Step 1" where the potential was held at 0.45 V. Approximately halfway down each column of values there is a message (text) indicating the start of "Step 2" where the potential was stepped to 0.1 V. Delete this line of text in the center of the data file.

3. Make a scatter plot to graph the current (A) *vs*. time (s). This graph should look similar to Figure 4.

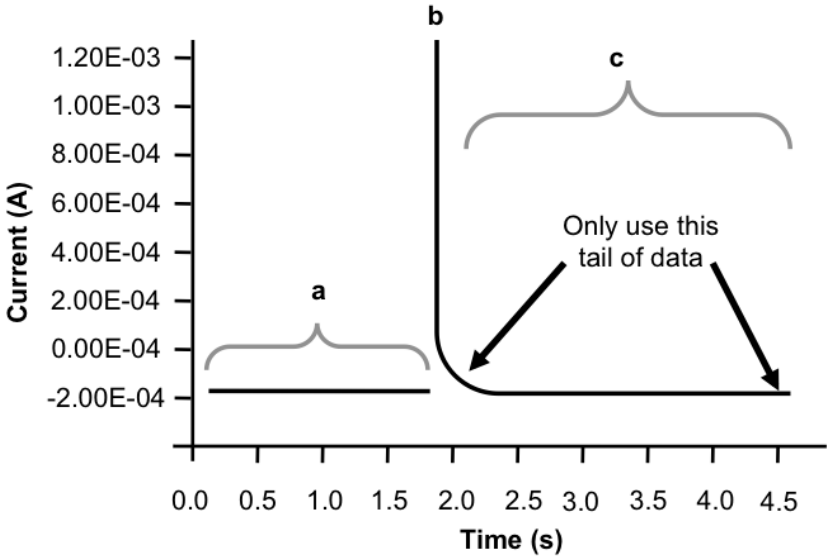


Figure 4: Chronoamperogram.

4. Only the data in the *diffusive tail* (points from later times toward the end of "Step 2", portion "c" in Figure 4) contains useful diffusion information. Copy the time and current columns of the "Step 2" data (the time range of 2.0 – 4.5 seconds in Figure 4). Paste the copied data into two new columns, and redefine the time when "Step 2" starts as t = 0 seconds, and adjust the remainder of the time scale accordingly by subtracting 2 seconds from each original time point. During the first several of hundred milliseconds, current is affected by other factors in addition to diffusion. Therefore, delete all data in your second data set for t < at least 400 ms, so that the only remaining data is the *tail* of the curve (due to diffusion and described by the Cottrell equation).

5. Create a new column and calculate $1/\sqrt{t}$ for each time point. Use a scatter plot and graph the current *vs*. $1/\sqrt{t}$. Find the line of best for your data, and show the equations and the correlation coefficient (R^2) on the graph.

6. Use the *slope* of the line of best fit for the graph in the previous step and the Cottrell equation (Eq. 1) to calculate the ferricyanicde diffusion coefficient ($cm^2\ s^{-1}$). (*Note - watch your units: 1 L = 10^3 m^3, 1 C = 1 A s, and concentration should be in mol cm^{-3} from Step #1).*

Ferricyanide Cyclic Voltammetry

7. Open the first ferricyanide CV data file. The first column of data is *potential* (V), the second column is *current* (A), and the third column is *charge* (C). Approximately halfway down each column of values there is a message (text) indicating the start of "Segment 2" begins. Delete this line of text in the center of the data file.

8. Make a line plot (not a scatter plot) to graph the current (A) *vs*. potential (V). Use the "reverse axes" option for the x-axis value to make the "duck" face to the right. The

graph should look similar to Figure 5 (below). *If you have extra data points at the beginning or end of the scan that are not part of the duck, you may delete them.*

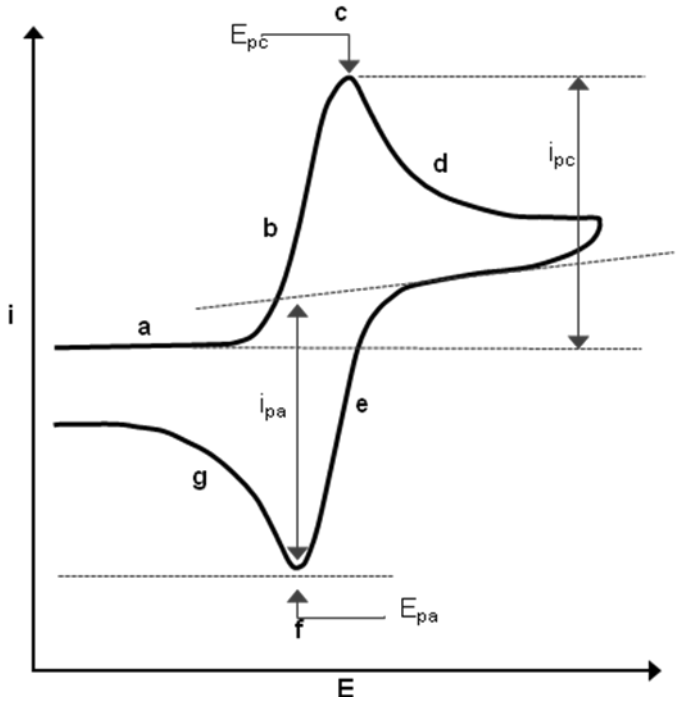


Figure 5: Cyclic Voltammogram.

9. Graph the rest of the ferricyanide voltammograms to make sure that all of them look okay. Only submit one representative ferricyanide cyclic voltammogram with your lab report.

10. On your representative ferricyanide cyclic voltammogram, label the location of the anodic and cathodic peaks, E_{pa}, i_{pa}, E_{pc}, and i_{pc}.

11. Create a table of the following parameters for the ferricyanide cyclic voltammogram for each scan rate investigated: scan rate (V/s), anodic peak current (A), cathodic peak current (A), anodic peak potential (V), and cathodic peak potential (V).

12. The *standard reduction potential* of a Ag | AgCl reference electrode (saturated KCl) is +0.197 V with respect to the *standard hydrogen electrode* (SHE) at 25ºC. The *formal reduction potential* of the ferricyanide reaction couple is ~0.470 V with respect to the SHE. This value can differ from the *standard reduction potential* due to non-standard reaction conditions (like the presence of KCl). Calculate your theoretical *formal reduction potential* for the ferricyanide in solution relative to the Ag | AgCl reference electrode.

13. From your measured values of the anodic and cathodic peak potentials for the four runs, calculate your average experimental half-wave potential, $E_{1/2}$, using Eq. 2, and compare it to the *formal reduction potential* (E^o).

14. Calculate the number of electrons (n) involved in this redox process from the anodic and cathodic peak potentials and Eq. 3. *Note: you can only have an integer number of electrons, so round your value to the nearest integer*.

15. Use a scatter plot (not a line plot) and graph the measured cathodic peak currents *vs*. the square root of the scan rate. Find the line of best for your data, and show the equations and the correlation coefficient (R^2) on the graph.

16. Use the slope of the line of best fit from the graph in the previous step and the Randles-Sevcik equation (Eq. 4) to calculate the diffusion coefficient of your reactive species. Watch your units!

Thyronine Cyclic Voltammetry

17. Open the three *unknown* CV files in Excel. For *each* unknown, plot the current (A) *vs*. potential (V). Use the "reverse axes" option for the x-axis value to show the starting potential at the far right of each graph. Determine the potentials of all anodic and cathodic peaks, and convert into *standard potentials* relative to the SHE.

Discussion Questions

1. (a) Explain what is happening in each region of the *ferricyanide chronoamperometry* scan (Figure 4, regions a-c). (b) Explain what is happening in each region of the *ferricyanide cyclic voltammogram* (Figure 5, regions a-g).

2. Is your experimentally predicted number of electrons (n) from Data Analysis Step #14 in agreement with the reactions that you attributed to the cathodic and anodic peaks?

3. (a) What is a diffusion coefficient? (b) What chemical species did you find the diffusion coefficient of? (c) Compare your diffusion coefficient values obtained from the two different techniques.

4. (a) Qualitatively explain the effect that stirring would have on the experimentally observed peak heights. (b) Why weren't the solutions stirred in this experiment?

5. The molecular structures of the three unknown chemicals (thyronine, tyrosine, and *p*-hydroquinone) and the reactions that occur involving these three chemicals are provided in the *background* portion of this experiment. (a) Using this information, and from evaluating the peak potentials and shapes of the three unknown voltammograms, determine the identity of each unknown solid. (b) Label each voltammogram with the unknown's identity. (c) Label all observed peaks with the balanced chemical reactions

that are occurring at each point. (d) Explain the reasoning that led you to match each chemical with that unknown voltammogram.

Literature Review

Provide one peer-reviewed journal article that uses cyclic voltammetry or chronoamperometry as means of determining a diffusion coefficient of any analyte. The article can be from any journal, (*J. Chem. Ed., J. Chem. Phys.*, etc.). Citation information and the title of the article are required. You must ALSO provide the name of the reactive species and the diffusion coefficient value.

Conclusions

As part of your conclusions for this experiment, compare your two diffusion coefficient values to a literature value for the ferricyanide diffusion coefficient (you will need to look this up, and cite your source). Discuss any observed differences between your two values and the literature value.

References

(1) Bard, A.J.; Faulkner, L.R. *Electrochemical Methods: Fundamentals and Applications*, 2nd ed.; John Wiley & Sons, Inc. NY, 2001.
(2) Harris, D.C. *Quantitative Chemical Analysis*, W.H. Freeman, NY, 2003.
(3) Kissinger and Heineman, *J. Chem. Ed.*, **60** 9 (1983), 702-6.
(4) Skoog, D.A.; Holler, F.J.; Crouch, S.R. *Principles of Instrumental Analysis*, 6th ed.; Brooks Cole: USA, 2006.

Gas Chromatography: Analysis of Recreational Alcohol

You will use *gas chromatography* (GC) to identify and quantify the alcoholic components of a recreational alcohol. The GC is equipped with a column specifically designed to separate polar compounds. An *internal standard* will be used in this experiment.

Required Reading

Quantitative Chemical Analysis by Daniel C. Harris

7th Edition

Introductory: Ch. 23 Introduction to Analytical Separations, Sect. 23-2 through 23-5
Technique: Ch. 2 Tools of the Trade, Sect. 2-6 (Micropipets)
Theory / Data Analysis: Ch. 24 Gas Chromatography, Sect. 24-1, 24-2, 24-3 (Flame Ionization Detector), and Ch. 5 Quality Assurance and Calibration Methods, Sect. 5-4.

8th Edition

Introduction: Ch. 22 Introduction to Analytical Separations, Sect. 22-2 through 22-5.
Technique: Ch. 2 Tools of the Trade, Sect. 2-6 (Micropipets).
Theory/Data Analysis: Ch. 23 Gas Chromatography, Sect. 23-1, 23-2, and 23-3 (Flame Ionization Detector); Ch. 5 5 Quality Assurance and Calibration Methods, Sect. 5-4.

Pre-Lab

Written: (1) Draw a block diagram of a GC (with all the major components identified and labeled). (2) Explain the purpose of an *internal standard* and how it will be used in this experiment. (3) Step-by-Step Procedure

Background

Gas chromatography (GC) is one of the most broadly used separations techniques. GC is extremely versatile and can be used for both qualitative and quantitative analysis of *volatile*, organic compounds. A liquid sample is injected and vaporized. The gaseous solution is then carried through the column by an inert, gaseous mobile phase. The stationary phase coating the column walls or packing material can either be a solid (gas-solid adsorption chromatography) or a liquid (gas-liquid chromatography). Quantitative analysis using GC has a number of advantages over other techniques, including its excellent sensitivity and wide range of applicability. Furthermore, chromatographic conditions can be easily manipulated to optimize the separation and offer potential freedom from interferences. GC is used for applications ranging from routine analyses to quality control of trace organic compounds, air, and water samples.

Instrumentation

You will use a GC with a capillary column. The fused-silica capillary column is coated with material designed for efficiently separating the alcohols present in whiskey. The temperature of the injection port, column, and detector are computer controlled. The column temperature can be varied over the course of the separation in a controlled manner (temperature programming). The instrumental parameters for this separation, including the flow rate, pressure of the helium carrier gas, and the injection port and detector temperatures, can be found in the "Instrument Startup and Shutdown Guide" next to the GC. As the separated components elute from the column, they are detected using a *flame ionization detector* (FID). In this experiment, the FID uses an air/hydrogen flame (FID schematic shown below in Figure 1).

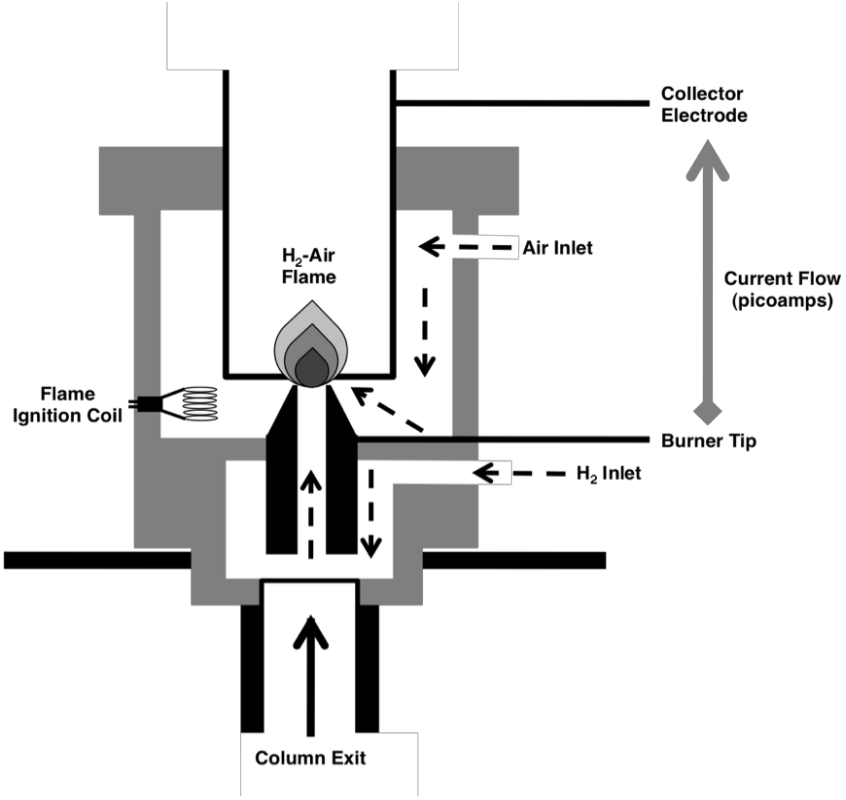


Figure 1: FID Schematic

Chemicals & Their Location

Stockroom Kit	Lab Bench (Next to Instrument)
1-butanol Ethanol, 40% aq sol'n. Ethyl acetate 2-methyl-1-butanol 3-methyl-1-butanol 2-methyl-1-propanol 1-propanol Whiskey sample	Air, compressed Helium, compressed Hydrogen, compressed

Equipment & its Location

Stockroom Kit	Lab Bench
25 µL automatic pipet Disposable pipet tips Injection syringe 50 mL volumetric flask (2) Assorted beakers	Gas Chromatograph Gas handling equipment

Safety Issues & Chemical Hazards

Chemical	Health Hazards	Physical Hazards
1-butanol	irritant	flammable
Ethanol	irritant	flammable
Ethyl acetate	irritant	flammable
Helium	none	compressed gas
Hydrogen	none	flammable, compressed gas
2-methyl-1-butanol	irritant	combustible
3-methyl-1-butanol	irritant	combustible
2-methyl-1-propanol	irritant	flammable
1-propanol	irritant	flammable

Many of these chemicals are flammable – keep them away from heat and ignition sources. Cylinders of compressed gas are potentially lethal weapons, and should be treated with respect. If the cylinder is punctured, the compressed gas will escape under high pressure and propel the cylinder at high velocities.

Procedure

Wet chemicals are <u>not allowed</u> in the instrument area. <u>All sample preparation must be done in the designated sample prep area</u>.

Once you have everything on the instrument set up, the standards and sample solutions are made, and you are running chromatograms, the only remaining tasks are injecting solutions. You are encouraged to talk about the discussion questions or work on other course-related materials. Students are not permitted to leave the lab for an extended period of time and just leave the experiment running – there are many things that could go wrong while you are absent.

Instrument Setup

Refer to the "Instrument Startup and Shutdown Guide" next to the instrument for all procedures to set up and run samples on the Gas Chromatograph.

The TA should have the instrument ready to run samples.

Preparing the Qualitative Sample

1. Put a small amount of 40% ethanol in a beaker. Hold the syringe between your thumb and fourth and fifth fingers (to hold it steady and prevent the needle from breaking). If held in this manner, your other two fingers will be free to raise and depress the plunger in a smooth motion. Rinse the injection syringe *three times* by filling it with ethanol and emptying it into a waste beaker.

2. Pour a small amount of the alcohol sample into a beaker. Rinse the injection syringe *three times* with the sample, discarding the waste.

3. *When you are ready to inject*, call your TA over to show your proper injection technique. You will be doing the sandwich method of injection (air – sample – air). *Carefully*, fill the syringe with 1.0 µL of air, then with 1.0 µL of sample, and finally with 1.0 µL of air. Do not fill the syringe until you (and the instrument) are ready for the injection.

4. Do not inject the sample without your TA present!!!! **Each student** in the group is required to complete an injection.

Running the Qualitative Sample

5. *Your TA will instruct you on proper injection technique*. Run a separation and collect the chromatogram for **1.0 µL** of the *qualitative* whiskey sample per the instructions in the "Instrument Startup and Shutdown Guide" next to the GC.

Each separation takes approximately 8 minutes. While you are waiting for the qualitative run to finish, move on to the next section and begin to make your standard mixture and sample solutions.

Standard Preparation

6. Clean and dry a 50.0 mL volumetric flask. Label it "STD" (standard). Rinse the flask with a small portion of 40% ethanol. Fill the flask ~half full with 40% ethanol.

7. Using a new pipet tip for each chemical, add 25.0 µL of each reagent listed below into the "STD" flask with the automated pipette provided. When adding each reagent, touch the tip to the flask's wall just above the liquid to ensure that all drops are transferred. Stopper the flask and up-end it to mix the added aliquot into the solution before it evaporates. After all of the chemicals have been added, mix well, dilute with 40% ethanol, stopper the flask, and up-end several times. *Leave the stopper in place at all times when you are not using the flask to prevent evaporation*.

1-butanol (internal standard)	3-methyl-1-butanol	ethyl acetate
2-methyl-1-propanol	2-methyl-1-butanol	1-propanol

Standard Separation

8. Run three quantitative separations of the standard mixture prepared in Step #6, injecting **0.5 µL** each time using the sandwich method. Prior to each injection, shake the standard solution to make sure it is well mixed before filling the injection syringe. The standard chromatogram should have one large off-scale peak (due to ethanol solvent), and six peaks, similar in size to each other, due to the six added components in the standard solution. All six peaks must be on-scale.

Quantitative Whiskey Sample Preparation

9. Clean and dry a 50.0 mL volumetric flask, label it "SAM" (sample). Rinse the flask with a small portion of the whiskey sample. Fill the flask ~half full with whiskey. Using a new pipette tip, add 25.0 µL of 1-butanol (internal standard) to the flask. Dilute to the mark with whiskey, and mix well. Leave the stopper in place when you are not using the flask.

Quantitative Whiskey Separation

10. Run the spike sample (whiskey + internal standard) three times, injecting **1.0 µL** using the sandwich method.

Peak Identification

11. The sample chromatogram displayed next to the instrument (and below in Figure 2) is a chromatogram of a scotch sample provided by the *column* manufacturer. The separation conditions used to collect this chromatogram are different from ours, so the retention times will not be the same. However, the *elution order* will be the same as our separation. The

manufacturer's chromatogram identifies more peaks than we have in our standard mixture. Additionally, you may have other peaks in your whiskey sample that are not seen in this chromatogram. *We will only be quantifying the alcohols in our standard mixture*. Make a note of their peak assignments here in your lab notebook. (Note: isobutanol = 2-methyl-1-propanol, active amyl alcohol = 2-methyl-1-butanol, and isoamyl alcohol = 3-methy-1-butanol).

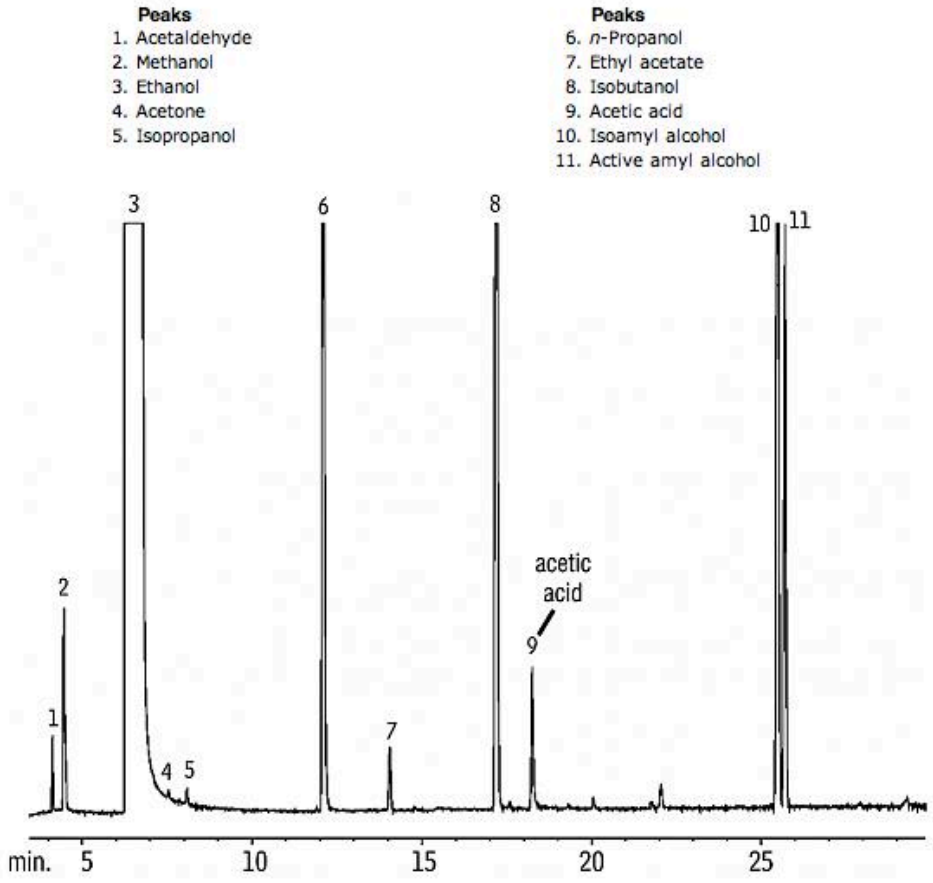


Figure 2: Sample Chromatogram (Scotch)
http://www.restek.com/chromatogram/view/GC_FF00112/scotch

Shutdown Procedures

1. Make sure that you have recorded *all* information about the sample you studied, including the manufacturer, brand name, and alcohol content from the bottle's label.

2. Make sure that you have recorded all of the instrument parameters and information, and that each group member has the data saved.

3. After all runs have been completed, shut down the instrument according to the instructions provided in the "Instrument Startup and Shutdown Guide" next to the GC.

4. Put all waste in the appropriate waste containers. Clean and dry all glassware, and return all borrowed items to the Stockroom.

Waste Disposal

Waste Chemical	Waste Container	Location
All chemical waste	GC	Lab Bench
Used pipet tips	Normal trash	Wet Lab

Data Analysis

1. Use the peak assignments to label the peaks in the chromatogram of the standard solution. (Recall: your *elution order* should be the same as the manufacturer's chromatogram.) Use the spiked samples to help identify the internal standard (1-butanol) peak.

2. Make a table listing each alcohol in the standard solution and its *average retention time*.

3. Use the table of the *average retention time* of each alcohol from the previous step to identify the peaks in the *spiked whiskey sample*. (There may be other peaks present in the whiskey chromatogram, associated with chemicals not included in your standard solution.)

4. Make a table of the area (<u>not the % area</u>) of all of the peaks corresponding to alcohols (6), <u>except for ethanol</u>, in your *standard chromatograms*.

5. The *detector response factor* of a specific analyte is a measure of the detector's sensitivity to that chemical. For this experiment, the *detector response factor* will be calculated for each alcohol *relative* to the detector's sensitivity to the *internal standard* (1-butanol).

$$\text{Detector Response Factor}_{\text{A}} = \frac{\text{Area of Peak}_{\text{A}} \text{ in Standard Mixture}}{\text{Area of Peak}_{\text{1-butanol}} \text{ in Standard Mixture}} \quad (1)$$

Note: Each alcohol in the standard solution is pure, and present in the same amount; so, no correction for relative concentrations is necessary.

Calculate the *detector response factor* for each alcohol in each of the chromatograms (3) of the standard solution. From these, calculate an *average detector response* for each analyte. If necessary, perform a Q-test to eliminate any outliers.

6. Tabulate the area (not the % area) for all of the peaks corresponding to alcohols (6), except for ethanol, in your *spiked whiskey chromatograms*. Use the *average detector response factor* and the following formula (below) to calculate the concentration of each of the six analytes in the whiskey sample. Report the average concentration of each alcohol in µL/L (ppm) with appropriate statistical analysis.

$$\frac{([1\text{-butanol}]\text{ in Sample}) \times (\text{Area of Alcohol}_A\text{ Peak in Sample})}{(\text{Average Detector Response Factor}_A) \times (\text{Area of 1-butanol Peak in Sample})} \quad (2)$$

Note: the final concentration of the internal standard (1-butanol) in each sample solution is 500 µL/L.

7. As discussed in your textbook, the *number of theoretical plates (N*) and the *height equivalent to a theoretical plate (HETP)* are parameters that can be used to characterize the efficiency of a separation. Use the following formulas to calculate (a) N, and (b) HETP for the six major components of the unknown whiskey sample:

$$N = 5.55\frac{t_r^2}{w_{1/2}^2} \qquad \text{HETP} = \frac{L}{N}$$

where $w_{1/2}$ is the full width of the peak at half its height, in units of time; and L is the length of the column.

Discussion Questions

1. (a) What *dual* purpose does the internal standard serve in this experiment? (b) What are some *ideal* characteristics an internal standard should possess? (c) The injection volume for the *spiked whiskey sample* was twice as much as the volume for the *standard solution.* Why doesn't this increased injection volume factor into any of the calculations to quantify the alcohols in the Data Analysis? (d) What is the purpose of the *qualitative whiskey sample*? (e) We could have used dilutions of the standard solution to make a calibration curve. Why we would *still* need to use an internal standard even if we made a calibration curve for this analysis?
2. You used expensive, GC-grade alcohols for the standard solution and spikes in this experiment. Why is the purity of the reagents so important for a GC analysis?

3. Compared to a packed column, what are the advantages and disadvantages of a capillary GC column?

4. You used a *flame ionization detector* (FID) in this experiment. (a) Explain how a FID detector works. (b) Another common detector for GC is a thermal conductivity detector (TCD). Describe how a TCD works.
(c) Both the FID and the TCD are classified as "bulk" detectors. What does this mean?
(d) What are the advantages and disadvantages of a FID and a TCD?

5. Mass spectrometry (MS) is another frequently used type of detector for GC. (a) Explain how a mass spectrometer can be used as a GC detector, including how the two instruments are interfaced. (b) What type of information does a MS detector provide? (c) What are some advantages and disadvantages of a MS detector over a FID?

6. A number of factors can contribute to the height of a theoretical plate in a separations experiment. The van Deemter equation describes how the linear flow rate influences the height equivalent of a theoretical plate in a separation. (a) Using your knowledge of the van Deemter *plot* (and equation), make an argument for which of the three terms has the largest effect on the separation efficiency *for GC.* (b) *Sketch* a qualitative van Deemter *plot* for this experiment, showing each of the three terms (this is only a rough sketch, it is not necessary to calculate A, B, and C from your data). (c) What physical parameters can be adjusted *for GC* to increase the efficiency of the separation (minimize HETP)? (d) Is it reasonable to expect that the modified parameters from part (c) would be optimal for all analytes? Why, or why not?

7. *If this is not your first separation experiment (HPLC, GC, CE)*: Compare your experimentally determined HETP from other separation experiments. Discuss the reasons for any differences.

Literature Review

Provide one peer-reviewed journal article that uses gas chromatography with a FID detector to quantify any hydrocarbon of your choice. The article can be from any journal, (*J. Chem. Ed., J. Chem. Phys.*, etc.). Citation information and the title of the article are required. You must ALSO provide the name of the internal standard (if any), the carrier gas, and the flow rate.

Conclusions

As part of your conclusions for this analysis, consider whether boiling point comparison can be used to predict GC elution order. Compare your peak assignments to the boiling point data provided in the table below. From your evaluation, discuss any differences between the experimental elution order and the theoretical elution order based on boiling point data.

Chemical	Boiling Point (˚C)
1-butanol	117
Ethanol	78.2
Ethyl acetate	77.1
Methanol	64.6
2-methyl-1-butanol	128
3-methyl-1-butanol	131
2-methyl-1-propanol	108
1-propanol	97.2

Boiling points taken from the *CRC Handbook of Chemistry and Physics*.

References

(1) Harris, D.C. *Quantitative Chemical Analysis*, W.H. Freeman, NY, 2003.

(2) Haynes, W.M., Ed. *CRC Handbook of Chemistry and Physics*, 91st Ed. (Internet Version 2011), CRC Press/Taylor and Francis, Boca Raton, FL.

(3) Restek Chromatography Products and Solutions Chromatogram Database,http://www.restek.com/chromatogram/view/GC_FF00112/scotch (accessed December 2010).

(4) Skoog, D.A.; Holler, F.J.; Crouch, S.R. *Principles of Instrumental Analysis*, 6th ed.; Brooks Cole: USA, 2006.

HPLC Analysis of Capsaicinoid Content in Hot Sauce

Capsainoids are a family of compounds naturally found in chili peppers, and are responsible for giving hot sauces their "heat". You will use *reverse phase high performance liquid chromatography* (HPLC) to determine how "hot" a pepper-based hot sauce is by separating and quantifying the capsaicinoid compounds from the sample. *Solid Phase Extraction* (SPE) will be used to prepare your sample prior to the separation. You will construct a *calibration curve* with capsaicinoid standards and use it to determine the overall capsaicin concentration in a hot sauce sample. You may bring your own sample to study.

Required Reading

Quantitative Chemical Analysis by Daniel C. Harris

7th Edition

Introduction: Ch. 23 Introduction to Analytical Separations, Sect. 23-2 through 23-5
Theory/Data Analysis: Ch. 25 High-Performance Liquid Chromatography, Sect. 25-1, 25-2 (through Spectrometric Detection), Ch. 28 Sample Preparation, Sect. 28-3 (Solid Phase Extraction), and Ch. 5 Quality Assurance and Calibration Methods, Sect. 5-1 (esp. spikes and spike recovery example)

8th Edition

Introduction: Ch. 22 Intro to Analytical Separations, Sect. 22-2 through 22-5
Theory/Data Analysis: Ch. 24 High Performance Liquid Chromatography, Sect. 24-1, 24-2 (Through Spectrometric Detection); Ch. 27 Sample Preparation, Sect. 27-3 (Solid Phase Extraction); Ch. 5 Quality Assurance and Calibration Methods, Sect. 5-1 (esp. spikes and spike recovery example)

Pre-Lab

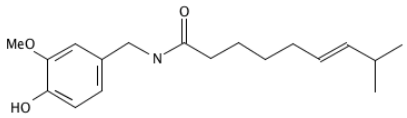
Written: (1) Draw a block diagram of a HPLC (with all of the major components identified and labeled). (2) Step-by-Step Procedure. (3) Assign tasks to team members—you will not finish in time if you wait till all samples are prepared before injecting your solutions.

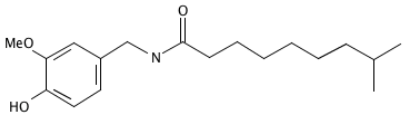
Background

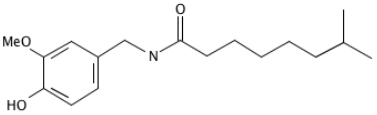
HPLC is a powerful analytical separation technique broadly used across numerous fields of science. HPLC is well suited for many types of analyses; and is particularly useful for studying compounds that are not easily volatilized, and hence, cannot be analyzed by gas chromatography in a straightforward manner. In HPLC, samples are carried through the *solid* stationary phase in a *liquid* mobile phase. While most stationary phases are made up of chemically coated polymer beads, there are many types available for the separation of specific compounds ranging from chiral molecules to ionic species. *Reverse phase*, the most widely used type of HPLC, with a hydrophobic stationary phase and a polar mobile phase, will

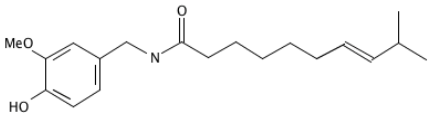
be employed in this experiment to separate and quantify the components responsible for giving chili peppers and hot sauce their “heat”.

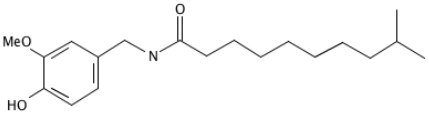
Capsaicinoids are the class of chemical compounds that give chili peppers, and hot sauces made from peppers, a “hot”, pungent taste. While many capsacinoids are present in peppers, capsaicin (N-[(4-hydroxy-3-methoxyphenyl)methyl]-8-methyl-6-nonenamide) and dihydrocapsaicin (N-[(4-hydroxy-3-methoxyphenyl)methyl]-8-methylnonanamide) account for over 90% of the heat in chili peppers. Additionally, different types of peppers can produce “heat” in different parts of the mouth (lips, tongue, back of the mouth…) due to differing proportions of capsaicinoids in the particular pepper. The five most common capsaicinoids found in peppers are as follows:

Capsaicin 

Dihydrocapsaicin 

Nordihydrocapsaicin 

Homocapsaicin 

Homodihydrocapsaicin 

The Scoville method is a commonly used *organoleptic* test to quantify the heat in hot peppers and sauces. The Scoville method involves trained pepper testers who determine the *relative* heat of peppers by *taste*, and assign each pepper a Scoville Pungency Value. As you might imagine, this technique has its hazards:

"The principal difficulties experienced in administering this procedure have been rapid tester fatigue and difficulty in continuing successful tester motivation. Tasters checked for reliability and test level reproducibility lost interest and became resentful or antagonistic on continued sample analysis at the frequency required for operation of a suitable quality control program." taken from *Food Technology*, Feb 1957, p 100.

Typical Scoville Ratings for peppers are as follows (gathered from a range of references, including cookbooks, *The Great Chile Book* by Mark Miller, and *Chile Pepper* magazine):

Pepper	Typical Scoville Rating (Scoville Units)
Red Savina™ Habañero	350,000 to 570,000
Habañero, Scotch Bonnet, Jamaican Hot	100,000 to 350,000
Chiltepin, Thai	50,000 to 100,000
Pequin, Cayenne, Tabasco, Rocoto, Aji Rojo	30,000 to 50,000
De Arbol, Japones	15,000 to 30,000
Serrano, Wax, Pasilla de Oaxaco	5,000 to 15,000
Jalapeño, Mirasol, Guajillo	2,500 to 5,000
Cascabel, Rocotillo, Chihuacle Negro	1,500 to 2,500
Ancho, Pasilla, Poblano, Negro	1,000 to 1,500
Anaheim, New Mexico, Mulato	500 to 1,000
Cherry, Pepperocini	100 to 500
Bell, Pimiento, Sweet Italian	0 to 100

The Scoville Threshold Pungency for five capsaicinoids has been determined by HPLC [*J. Food Sci.*, **42**, 660 (1977)]:

Capsaicinoid	Threshold Pungency (Scoville Units per % Weight)
Capsaicin	16.1×10^6
Dihydrocapsaicin	16.1×10^6
Nordihydrocapsaicin	9.3×10^6
Homocapsaicin	6.9×10^6
Homodihydrocapsaicin	9.2×10^6

In today's lab, you will determine a useful HPLC method to separate and quantify the capsaicin and dihydrocapsaicin in an unknown hot sauce sample by creating calibration curves for each from a series of dilutions from a supplied standard stock solution. You will then use the threshold pungency values (above) to convert your experimental results to a Scoville Rating for your unknown, and determine what type of peppers may have been used to make the hot sauce. You will only be quantifying capsaicin and dihydrocapsaicin in this experiment. *Solid Phase Extraction* (SPE) will be used during the hot sauce sample preparation.

Instrumentation

You will use a HPLC equipped with a C-18 nonpolar hydrocarbon reverse phase column (15 cm in length), a 20 µL injection loop, and a variable wavelength UV-Vis absorption detector. The parameters recommended for this experiment and the operating procedures are located next to the instrument in the "Instrument Startup and Shutdown Guide". These parameters include the components of the mobile phase solvents, the total flow rate and maximum back pressure, information about the separation column, and the UV-vis data for detection wavelength determination. All mobile phase solvents are degassed (to remove dissolved gases) and filtered prior to use to minimize the amount of particulate traveling through (and eventually clogging) the column. A guard column, filled with the same C-18 packing, is located in front of the separation column. The guard column protects the separation column, and is cheaper and easier to replace than a new analytical column.

Chemicals & Their Location

Stockroom	Lab Bench (Next to Instrument)
Acetonitrile 1% acetic acid in methanol Capsaicinoid standard, methanol soln. High purity water Methanol	Mobile phase (water/methanol)

Equipment & its Location

Stockroom Kit	
HPLC syringe	Sample vial (with caps) (6)
Assorted beakers	25 mL volumetric flask (1)
Mortar & Pestle	10 mL volumetric flask (5)
Solid phase extraction cartridges (2)	5 mL volumetric flask (2)
Glass filtering syringe	1 mL volumetric pipet (2)
0.2 µm luer-lock nylon filter (2)	2 mL volumetric pipet (2)
Glass graduated cylinder	4 mL volumetric pipet (1)
Glass funnel	5 mL volumetric pipet (1)
Lab Bench	
HPLC	

Safety Issues & Chemical Hazards

Chemical	Health Hazards	Physical Hazards
Acetic acid	lachrymator	corrosive, combustible
Acetonitrile	toxic, irritant	flammable
Capsaicin	*highly toxic*, severe irritant, sternutator	none
Dihydrocapsaicin	*highly toxic*	none
Helium	none	compressed gas
Methanol	toxic, irritant	flammable

Wear gloves *and take extreme care while working with these chemicals. Capsaicins are* ***highly toxic****. Do not touch your face or eyes with gloves on. Change your glove often*

Procedure

Wet chemicals are not allowed in the instrument area. All sample preparation needs to be done in the wet lab. Bring your samples into the instrument room in capped vials and only put chemicals in the designated areas

There are multiple components to this procedure. To make the most effective use of time, the work should be divided evenly among group members (i.e., – the standards can be prepared while the instrument is setup, and the hot sauce samples can be prepared while the standard chromatograms are collected. Though the work will be split up, each group member is

*responsible for understanding every step of the procedure **and** making an injection. In order to run all of the samples and finish on time, it is important to get your standard chromatograms running as soon as possible.*

Instrument Setup

Refer to the "Instrument Startup and Shutdown Guide" next to the instrument for all procedures to set up and run samples on the HPLC.

The TA should have the instrument ready to run samples.

1. Confirm with your TA that the system has been started, the lamps are on and ready (at least 15 minutes prior to your first run), and that the pumps are running (25% A, 75% B, 0.5 mL/min).

2. Confirm with your TA the column has been flowing for 10-15 minutes, and has equilibrated (see "Instrument Startup and Shutdown Guide" for how to do this in the software).

While the system equilibrates, prepare the capsaicin standard solutions.

Standards Preparation

Do not use plastic items other than the SPE cartridge and syringe filters in your sample preparation in this experiment. Components in most common plastic materials dissolve in acetonitrile, and could present interferences in your analysis. In particular, do not use plastic droppers, use glass Pasteur pipettes. Note: the SPE cartridge and syringe filters are plastic, but it is a high density plastic and only comes in brief contact with your sample. Thus, the plastic on the cartridge and filters will not significantly interfere with the analysis.

1. Record the concentration of the capsaicin stock solution (posted on the hallway bulletin board). The standard solution is diluted with methanol from a 65% capsaicin / 35% dihydrocapsaicin mixture. Use *volumetric pipets* to transfer 1-, 2-, and 5-mL aliquots of the stock solution into three separate 10 mL *volumetric flasks*. Dilute each of the three flasks to the 10 mL marking with methanol (NOT the *1% acetic acid in methanol*) and mix thoroughly.

2. Clean the sample vials and caps first by rinsing with distilled water and then with a small amount of methanol. Set the vials aside to dry.

3. Clean the filtering syringe by rinsing with distilled water and methanol.

4. After the rinses, remove the plunger from the syringe and screw the 0.2 µm filter (Figure 1) onto the end of the syringe. Transfer approximately 1-2 mL of the *least concentrated* standard into the syringe. Insert the plunger into the syringe, and push the aliquot through the filter. Discard this rinse into a waste beaker.

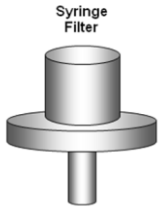


Figure 1: 0.2 μm Filter

5. Now, the syringe and filter are clean and ready to use with your standard solutions. Remove the plunger and pour ~2-3 mL of the *least concentrated* standard into the syringe. Insert the plunger and push the sample through the filter. *All solutions should be filtered into a clean, dry, labeled vial*. Once you have filtered the solution into a vial, cap it. You do not need to filter the entire 10 mL sample – you will only be injecting 50 µL into the HPLC for each run.

6. Repeat Steps 7 & 8 for the remaining two standard solutions. The same filter and syringe can be used for all of the standards as long as they are filtered in the order of *least* to *most* concentrated. It is **very** important to filter all of the solutions that will be injected into the HPLC. Large particles can clog the column.

7. *As soon as these three standard solutions are made, some of your group should begin to run them on the HPLC while the rest of the group prepares the unknown sample.* ***Refer to the "Instrument Startup and Shutdown Guide" procedures for the HPLC for proper injection technique.*** Make sure that your TA has demonstrated/explained proper injection technique to you prior to injecting your first sample.

Hot Sauce Sample (Unknown) Preparation – Liquid Extraction

8. Weigh ~10 g of your hot sauce sample in a weigh boat. *Carefully* transfer the hot sauce to the mortar. Do not throw out the weigh boat. Weigh the boat again *after* you transfer the hot sauce, and use the weigh by difference method to accurately determine the mass to the hot sauce transferred.

9. Using the graduated cylinder, add a 10 mL aliquot of acetonitrile to the unknown hot sauce sample. Use the pestle to fully grind and pulverize the sample for *at least* two minutes to maximize the efficiency of the extraction process.

10. Use a glass funnel and filter paper to filter the sample mixture into a 25 mL volumetric flask.

11. Rinse the mortar with an additional ~5 mL of acetonitrile, and filter this rinse into the 25 mL volumetric flask with the sample in the same manner.

12. Dilute the flask to the mark with acetonitrile and mix well. Allow the filtered sample to stand in the volumetric flask until any remaining particles settle to the bottom.

13. Transfer 2 mL aliquots of the filtered unknown mixture into two 10 mL volumetric flasks, labeled “UNKNOWN” and “SPIKED UNKNOWN”. To the “SPIKED UNKNOWN” flask, add 1 mL of the capsaicinoid stock solution. Dilute both flasks to the mark with *high purity water*, and mix well.

Solid Phase Extraction (SPE) Cartridge Conditioning

You will need to prepare TWO cartridges in this step.

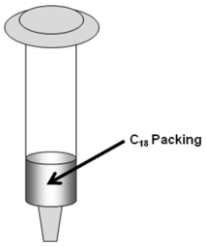


Figure 2: SPE Cartridge with C_{18} packing

14. Clamp each SPE cartridge (Figure 2) in an upright position and arrange a waste beaker beneath each cartridge

15. Condition the cartridge with the following solvents. The flow through the cartridge should be less than 5 mL per minute. You may use a graduated cylinder, as these are not quantitative aliquots.

    a. 6 mL methanol

    b. 6 mL acetonitrile

    c. 6 mL High purity water

16. Dispose of the conditioning waste in the proper container.

Solid Phase Extraction of Unknown Sample

17. Pass the entire 10 mL of your “UNKNOWN” solution through one of the conditioned SPE cartridges. You will not be able to pour all 10 mL into the cartridge at once. Collect the eluent in a waste beaker and discard once the whole 10 mL has passed through. Many components in the sample matrix, including the capsaicinoid analytes, will be retained in the cartridge’s C-18 stationary phase.

18. Wash the capsaicinoids and other retained compounds from the SPE column by first eluting with exactly 4 mL of methanol, followed by exactly 1 mL of the supplied methanol / 1% acetic acid solution. Collect the entire 5 mL of eluent in a 5 mL volumetric flask, labeled “UNKNOWN extract”. Use a Pasteur pipet to dilute to the mark with methanol, if needed..

19. Repeat Steps 20 & 21 with the second conditioned SPE cartridge for the "UNKNOWN SPIKED" sample, and collect in a 5 mL volumetric flask labeled "UNKNOWN SPIKED extract".

20. Filter both unknown solutions using the same procedure you did in Steps 6-7. Clean and use a new filter for your unknown sample, and then use the same filter for your spiked unknown mixture.

Running Samples on the HPLC

21. Clean the HPLC injection syringe *THREE* times with your first filtered sample, by filling it with your sample and emptying it into a waste beaker.

22. Fill the injection syringe (50 µL) with the sample to be injected by pumping the plunger back and forth a few times in the sample solution. Make sure you DO NOT have any air bubbles in the syringe. DO NOT inject air bubbles into the HPLC.

23. Please see the "Instrument Startup and Shutdown Guide" by the instrument for the procedure to run the samples on the HPLC.

Shutdown Procedures

1. See "Instrument Startup and Shutdown Guide" by the HPLC for instructions on how to turn off the instrument.

2. Make sure that you have recorded *all* of the instrument parameters and sample information (including manufacturer, band name…), and that each group member has the data saved.

3. Clean the injection syringe with fresh acetonitrile, making sure it is very clean before returning it to the HPLC Kit.

4. Put all waste in the appropriate waste containers.

5. Clean and dry all glassware, clean up all work areas, and return any borrowed items to the Stockroom.

Waste Disposal

Waste Chemical	Waste Container	Location
Mobile phase waste only	Mobile Phase Waste	Near Instrument
Solid waste (filters, filter paper, SPE cartridge…)	Normal Trash	
All other liquid waste	HPLC Waste	Near Instrument

Data Analysis

1. The standards stock solution provided contains ~250 ppm total capsaicinoids dissolved in methanol. *Check the hallway bulletin board posting for the actual concentration for your day in lab.* The total capsaicinoids in the solution are present in the proportion of 65% wt capsaicin / 35% wt dihydrocapsaicin. Calculate the amount of capsaicin and dihydrocapsaicin (in ppm) in each of your *three* calibration standards from the dilution factors you used to make each solution.

2. The *retention time* for a component is the amount of time between when the sample is injected onto the column and when the component reaches the detector. *Tabulate* the retention time and total peak area for the two capsaicinoid components in each standard solution. Label the capsaicin and dihydrocapsaicin peaks on the chromatograms. Make a table of these values, along with the concentrations calculated in Step #1, for each chemical in the standard solutions.

3. Construct *two* calibrations curves (one for capsaicin and the other for dihydrocapsaicin) by plotting the average peak area *vs*. concentration (in ppm). Find the line of best fit for the data. Show the equation and correlation coefficient (R^2) for each calibration curve on the graph.

4. Identify the capsaicinoid peaks in the chromatograms of your unknown and spiked unknown samples (use your standard chromatograms as a reference). Label the peaks on your unknown and spiked unknown chromatograms.

   While all of the chromatograms need to be included in the appendix of your lab report, it is only necessary to show a representative chromatogram for each sample in the body of your report.

5. Use your calibration curves to determine the concentration of the two capsaicinoids in your *unknown* sample (in ppm).

6. Recalling the extraction procedure for the unknown sample, take the dilution factors into account and convert the concentrations from Step #5 into *weight percent* of capsaicin and dihydrocapsaicin in the original hot sauce sample.

7. Use the *Threshold Pungency* values (listed in the *Background* portion of this experiment) to convert your weight percentages into Scoville Units using the following formula:

$$\text{Heat due to Component (Scoville Units)} = \frac{\text{Weight \% of Component}}{100} \times \text{Threshold Pungency of Component}$$

Add the Scoville Units due to capsaicin and dihydrocapsaicin to determine the *Total Heat Rating* in Scoville Units for your unknown hot sauce.

8. Use your calibration curves to determine the concentration (in ppm) of capsaicin and dihydrocapsaicin in your *spiked unknown* sample, and therefore, how many **mg** of each capsaicinoid you had in the 10 mL solution.

9. Repeat Step #8 for your unspiked sample.

10. Subtract the *mg of unspiked* (Step #9) from the *mg of spiked* (Step #8) for both capsaicin and dihydrocapsaicin to determine how much extra of the two capsaicinoids were present in the spiked sample.

11. Knowing that you added 1 mL of the supplied standard to the spiked sample, calculate how much capsaicin and dihydrocapsaicin you *actually* added. What % of the added spike was detected? (This is the *% recovery*).

12. Use the *% recovery* calculated in the previous step to correct the weight in mg of each analyte found in Step #8 for your *unknown* sample.

13. The *Harris* text discusses how the *number of theoretical plates* (*N*) and the *height equivalent to a theoretical plate* (*HETP*) are parameters used to characterize the efficiency of a separation. Use the following formulaes to calculate N and HETP for the two capsaicinoid components in your unknown hot sauce sample.

$$N = 5.55\frac{t_r^2}{w_{1/2}^2} \qquad\qquad HETP = \frac{L}{N}$$

where $w_{1/2}$ is the full width of the peak at half its height, in units of time; and L is the length of the column.

Discussion Questions

1. The SPE cartridge stationary phase contains the same type of C-18 packing as the reverse phase HPLC column. Explain how the SPE process works by describing what happens during the conditioning, extraction, and elution steps of the procedure.

2. Predict the elution order of the two capsaicinoids for a separation with a reverse phase HPLC column. *Provide reasoning for your predicted order*.

3. (a) Why was a spiked sample used in addition to the unknown hot sauce sample? (b) Explain what type of information can be gained from a spiked sample? (c) What do the results from *your* spiked sample indicate?

4. (a) Why is HPLC, used to separate and quantify the capsaicinoid content in hot sauce for this experiment? (b) Do you think that GC or CE would be more suitable for this analysis? (c) Why, or why not?

5. A number of factors can contribute to the height of a theoretical plate in a separations experiment. The van Deemter equation describes how the linear flow rate influences the height equivalent of a theoretical plate in a separation. (a) Using your knowledge of the van Deemter *plot* (and equation), make an argument for which of the three terms has the largest effect on the separation efficiency *for HPLC*. (b) *Sketch* a qualitative van Deemter *plot* for this experiment, showing each of the three terms (this is only a rough sketch, it is not necessary to calculate A, B, and C from your data). (c) What physical parameters can be adjusted *for HPLC* to increase the efficiency of the separation (minimize HETP)? (d) Is it reasonable to expect that the modified parameters from part (c) would be optimal for all analytes? Why, or why not?

6. *If this is not your first separation experiment (HPLC, GC, CE)*: Compare your experimentally determined HETP from other separation experiments. Discuss the reasons for any differences.

Literature Review

Provide one peer-reviewed journal article that uses HPLC to quantify any analyte of your choice. The article can be from any journal, (*J. Chem. Ed., J. Chem. Phys.*, etc.). Citation information and the title of the article are required. You must ALSO provide the description of the mobile phase (and percentages, if mixture), whether the experiment was carried out under isocratic or gradient elution, the method of quantification (calibration curve, standard additions, etc.), and the retention time of the analyte.

Conclusions

In your concluding remarks for this lab report, you should compare your experimental results for the Total Heat Rating for your unknown hot sauce sample to the Typical Scoville Ratings for different peppers to determine which type of pepper(s) may have been used to make the sauce. Does your prediction change when you account for the % recovery of each analyte?

References

(1) Harris, D.C. *Quantitative Chemical Analysis*, W.H. Freeman, NY, 2003.
(2) Miller, M.; Harrisson, J.; Frank, L.E. *The Great Chile Book*, Ten Speed Press: Berkeley, CA, 1991.
(3) Skoog, D.A.; Holler, F.J.; Crouch, S.R. *Principles of Instrumental Analysis*, 6th ed.; Brooks Cole: USA, 2006.

(4) Todd, P.H. JR; Bensinger, T.; Biftu, T. Determination of Pungency Due to Capsicum By Gas-Liquid Chromatography. *J. Food Sci.*, **42**: 660-5 (1977).

Gas Chromatography-Mass Spectroscopy: Determination of Caffeine in Coffee Using GC-MS

Caffeine analysis is routinely performed to meet regulatory standards under quality control procedures. Most methods of determining caffeine concentration in beverages involve labor-intensive methods that often involve the use of toxic organic solvents. Solid-phase micro-extraction when combined with GC-MS can eliminate many of these issues.

There are many different extraction techniques used in combination with GC-MS. The goal of the extraction step it to separate and concentrate the analyte from other chemicals or compounds present in the sample. Some commonly used extraction techniques are passive adsorption/thermal desorption, solvent extraction, headspace analysis, and solid-phase micro-extraction (SPME).

In this lab, you will determine the amount of caffeine in coffee under different brewing conditions. You will make a calibration curve using isotopically labeled caffeine as an internal standard. From this calibration curve, you will be able to determine the concentration of caffeine in these beverages using a GC-MS.

Required Reading

Quantitative Chemical Analysis by Daniel C. Harris

8th Edition

Introduction: Ch.22 Introduction to Analytical Separations, Sect. 22-1 through 22-4.
Theory: Ch. 23 Gas Chromatography, Sect. 23-1, 23-2 (Injection Types), 23-3 (Detectors), 23-4 (Sample Preparation)
Data Analysis: Ch. 4 Statistics, Sect. 4-7 through 4-9, and Ch. 5 Quality Assurance and Calibration Methods, Sect. 5-2, 5-3.

Pre-Lab

Written: (1) Complete the calculations required for preparing your calibration solutions. (2) Describe why a control step is necessary. (3) Step-by-Step Procedure for Solution preparation

Instrumentation

Gas chromatography-mass spectrometry (GC-MS) uses a GC for separation of volatile and semi-volatile analytes by elution through a capillary column. Separated analytes are then detected via mass spectrometry (MS). A mass spectrometer uses the difference in mass-to-charge ratio (m/z) of ionized atoms or molecules to separate them from each other. MS is therefore useful for determining chemical and structural information about molecules since they have distinctive fragmentation patterns that can be used to uniquely identify structural components. Our PolarisQ Ion Trap GC-MS uses electron impact (E.I.) to ionize and fragment the compounds separated by the GC and analyzes the fragment ions with a linear ion trap mass spectrometer. The advantages of the ion-trap MS include compact size and the ability to trap and accumulate ions to increase the signal-to-noise ratio of a measurement.

Electron Impact (EI) ionization bombards the sample with energetic electrons (70 eV) resulting in charge transfer and fragmentation. EI ionization results in very reproducible fragmentation patterns as long as the energy is held constant, so libraries of mass spectra using 70 eV EI ionization results have been compiled and can be used to identify thousands of compounds by their mass spectra. In addition, the fragmentation patterns can be interpreted for unknown compounds and used to deduce information about the original (parent) compound. You will be expected to use this method to analyze the MS of your analytes. See Harris, 6th Ed., Ch. 22-2 and Skoog, 5th Ed., Ch 20B-1, 20D for an overview. See also: Silverstein, Bassler and Morrill, *Spectrometric Identification of Organic Compounds*, Chapter 2 for a complete discussion of compound identification by mass spectrometry.

Full Scan or Selected Ion Monitoring (SIM) mode

In the full scan mode, the mass analyzer detects all ion masses in a given range (e.g., 50 – 200 amu) as they exit the GC, producing a total ion chromatogram (TIC). The TIC shows the sum of all ions exiting the GC at any given time. In this mode, the mass spectrometer also records the full mass spectrum at all times in the chromatogram. This way for any chromatographic peak (indicating a compound is being eluted), the mass spectrum for that compound may be displayed. The mass spectrum shows intensity vs. ion (fragment) mass. The acquired mass spectrum may then be compared to a library for compound identification or interpreted by the user to the same end. You will use the full scan mode to analyze your qualitative solution and identify the eluted compounds.

When the mass spectrometer is operated in SIM mode, it only detects the masses selected by the user. For example, if you know the mass of the major fragment ion (base peak) for a particular compound, you can selectively look for that mass. This significantly reduces the background of the chromatogram and improves S/N resulting in improved sensitivity for the compound of interest. You will analyze you qualitative solution in SIM mode to compare the S/N with the full scan mode. You will also use SIM mode to analyze all of your quantitative solutions.

Chemicals & Their Location

Stockroom Kit	Lab Bench (Next to Instrument)
~100 ppm ^{12}Caffeine in water ~1000 ppm ^{12}Caffeine in methanol ~100 ppm ^{13}Caffeine in methanol Methanol Ethyl Acetate Magnesium Sulfate (2) Coffee Samples	Helium (cylinder) Compressed air (cylinder)

Note: Isotopically labeled caffeine is extremely expensive, so please do NOT spill, waste, or lose any. You will only be given a small amount of this solution to complete your experiment

Equipment & its Location

Lab Bench	
Thermo-Finnigan PolarisQ GC-MS	
Kit	
5-50 µL autopipet and tips 100-1000µL autopipet and tips (7) plastic centrifuge tubes (disposable) Centrifuge tube rack 10 µL injection syringe, bevel tip Spatula (4) 10 mL volumetric flasks (11) 2 mL vials (7) 4 mL vials (1) 250 mL beaker (2) 50 mL beakers (1) small funnel	

Safety Issues and Chemical Hazards

Chemical	Physical hazards	Health hazards
Caffeine	None	Irritant, teratogen, toxic
^{13}C Caffiene	None	Irritant, teratogen, toxic
Methanol Ethyl Acetate Magnesium Sulfate	Flammable	Irritant

Procedure

Calibration Solution Preparation

1. Prepare a set of 4 standard solutions (10.0 mL each) with concentrations ranging from 25 to 250 ppm of your ^{12}C caffeine (m/z 194) stock solution. Dilute to volume with methanol. *You will be given a total volume of 30 mL of the ^{12}C caffeine solution to complete this lab.*

Calibration Curve Solutions: *In your pre-lab calculations*, choose what dilutions of the ~1000 ppm caffeine stock solution you will make to produce a four point calibration curve. All calibration curve solutions (and calculations) should be made to have a *final* volume of 10.0 mL, using methanol as the diluent. Include

the calculations in your notebook. Your TA must verify these calculations before you prepare these solutions.

2. Use an auto-pipetter to transfer 250 µL of each standard into a 2 mL vial. Add 50 µL of your internal standard (^{13}C caffeine (m/z 197)) to each vial.
3. Inject 1 µL of the prepared sample according to the instructions provided in the GC/MS instrument manual. Repeat the extraction/injection procedure for each standard.

Extraction of Caffeine from the Unknown Samples

1. Use an auto-pipette to transfer 4 mL of Ethyl Acetate and 1 mL of your unknown coffee sample into a centrifuge tube. Place the tube in the sonicator for exactly 2 minutes. Afterwards, place the tube in the centrifuge, balance the rotor, and centrifuge the sample for 30 seconds to separate the organic from the aqueous layer.

2. Transfer 2 mL (using the auto-pipette) of the organic layer into a 4 mL vial and add approximately 0.1 grams of anhydrous magnesium sulfate. Use the vortex mixer to agitate the vial for exactly two minutes. Afterwards, use an auto-pipette to transfer 250 µL of the organic solution into a 2 mL vial.

3. Use an auto-pipette to transfer 50 µL of the isotopically labeled caffeine solution provided into the same 2mL vial.

4. Inject 1 µL of the prepared sample according to the instructions provided in the GC/MS instrument manual. Repeat the extraction/injection procedure for each unknown a total of 3 times (6 extractions in total).

Control sample

1. Repeat the above procedure (extraction of coffee from the unknown samples) with 1 mL of a 100ppm caffeine in water solution instead of the 1 mL of coffee sample.

Instrumentation

1. You will run one method for this experiment. The method is named *(CH456_Caffeine.meth)* The GC-MS should be on when you arrive to lab. If it is not, consult your TA and Thermo-Finnigan PolarisQ GC-MS from Start-up to Shut-down guide.
2. Refer to "Starting Xcaliber and Setting up the Instrument" section of the

Thermo-Finnigan PolarisQ GC-MS from Start-up to Shut-down guide to get started and record the parameters of the method you will be using in this lab.

3. Refer to "**Preparing your Samples**" in the Thermo-Finnigan PolarisQ GC-MS from Start-up to Shut-down guide for extraction and injection procedures. You will need to record this information in your lab manual to put into your lab report.
4. Refer to "**Retrieving your Data**" for information about how to extract useful information from the GC chromatogram as well the mass spectrum. You will need a representative mass spectrum from both your qualitative and your quantitative runs (two in total for your lab report).

Running Calibration Solutions and Samples

Running a Sample:

1. Click on the entry for the solution you are ready to run (e.g., calibration soln) and then select Actions from the menu bar and select Run this Sample. Then make sure Run when Ready is selected and hit OK. You will see your sample information appear to the left and the instrument status will update as the GC and MS initialize and prepare for the first run.
2. When BOTH instruments are ready, the status will read "**Waiting for Contact Closure**", meaning the instrument is waiting for you to insert the syringe and press start.
3. Refer back to the Extraction Procedure before continuing past this point. Carefully insert the syringe into the GC inlet, wait for ~3 seconds while it heats up. Then press Start on the GC keypad and inject the sample. Both instruments should now read "running".
4. Select Real time plot to view your chromatogram and mass spectra as they are acquired.
5. While your samples are running, prepare your next solution for analysis. While your samples are running, analyze your data.

Shutdown Procedure

- Follow the Shut Down procedure in the Thermo-Finnigan PolarisQ GC-MS from Start-up to Shut-down guide .
- Put all waste in the appropriate waste containers.
- Clean all glassware.
- Clear up your work area.
- Return borrowed items to the Stockroom.
- Make sure you have recorded information on the sample you studied.

Waste Disposal

Waste chemical	Waste container	Location
All waste	GC/MS	Cabinet

Data Analysis

Qualitative Data Analysis (mass spectral interpretation)

1. The major fragment peaks for caffeine occur at a m/z ratio of 67, 82, 109, and 165. Identify these peaks and suggest a possible pathway of fragmentation. (You can write out the formulas as opposed to drawing the structures.)

Quantitative Data Analysis

2. Plot the ratio of the integrated peak area for both ^{12}C caffeine and ^{13}C caffeine (your internal standard) against ^{12}C caffeine concentration to generate a standard calibration curve.
3. Use the calibration curve to calculate the caffeine concentration in your unknown samples. Identify which unknown (if any) has the highest concentration of caffeine.
4. Take into account the dilution factor to determine the amount of caffeine present in your original sample.
5. Determine the extraction efficiency (percent recovery) for the coffee sample analysis: The Control solution (extracted 100ppm caffeine) may be used to evaluate the efficiency of the extraction process, since the amount of caffeine in the control solution is known. A truly quantitative extraction should result in a caffeine concentration equal to the amount originally contained in the control solution. However, most extraction processes are not truly quantitative; therefore, the percent recovery for the extraction must be determined. For the caffeine analysis, this is the percent of caffeine in the control sample that was transferred to the final sample you measured. Calculate the percent recovery for the control sample.
6. Assuming that your technique was consistent, each extraction of the unknowns should have the same efficiency. Therefore, use the percent recovery calculated from the control sample to correct the caffeine concentrations in your unknown samples.
7. Calculate the standard deviations and uncertainty in your measurements at the 95% confidence intervals for each unknown.

8. Use your calculated concentrations to determine how many mg of caffeine would be present in 8 fl oz. of each unknown.

Discussion Questions

1. What is the purpose of an internal standard?
2. What is the solvent delay MS parameter and why is it so important? Why are we

using a solvent delay in this lab?

3. There are two different modes to analyze sample (full mass scan and selected ion monitoring (SIM)). What is the difference? What advantages and disadvantages are there to running in each mode?
4. What are the main differences in using mass spectrometry (MS) vs. FID as a detector for GC? Under what experimental conditions would you prefer FID? How about MS?
5. How do the values you calculated for the caffeine concentration vary from what is usually reported for the "known" amount of caffeine in 8 fl oz of coffee? What may account for the discrepancy (if any)?

Literature Review

Provide one peer-reviewed journal article that uses GC/MS to quantify any analyte of your choice. The article can be from any journal, (*J. Chem. Ed., J. Chem. Phys.*, etc.). Citation information and the title of the article are required. What was the temperature profile of the run used to elute the analyte(s)? What type of injection was used (direct, headspace, SPME, split/splitless, etc.)? What type of mass spectrometer was used as the detector in the primary reference?

Conclusions

In your concluding remarks for this lab report, you should compare your experimental results for the caffeine content in coffee to values established in other reported literature. Are your results significantly changed by inclusion of the extraction efficiency? If so, what does this mean?

References

(1) Harris, D.C. *Quantitative Chemical Analysis*, W.H. Freeman, NY, 2003.

(2) Skoog, D.A.; Holler, F.J.; Crouch, S.R. *Principles of Instrumental Analysis*, 6th ed.; Brooks Cole: USA, 2006.