

LAB MANUAL: ANALYTICAL CHEMISTRY AT DUKE



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CHEM 401L Analytical Chemistry

Duke University

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Detailed Licensing

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1: ORIENTATION TO CHEM401L

Learning Objectives

By the end of this module, you'll be able to:

1. Access all course details in the syllabus, Canvas site, and this manual. This includes:
 - Finding instructions and resources for experimental modules.
 - Understanding guidelines and due dates for written assignments.
 - Accessing rubrics for every assignment.
 - Knowing where to find course policies and support.
2. Explain the specific policies for CHEM 401L.
3. Effectively plan and prepare for lab meetings and stay on top of course deadlines.
4. Submit assignments through Canvas.

 **Purpose:** The Orientation Module introduces you to the structure, expectations, and tools used in CHEM 401L. Before beginning experimental work, it's important that you understand how this course is organized, how you will communicate your findings, and what is expected of you as a member of a collaborative laboratory team. This module will prepare you to navigate the semester successfully.

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1.1: Pre-Semester Assignment

1.2: Welcome and Course Overview

1.3: Details about Meetings and Deadlines

1.4: Academic Integrity

1.5: Scientific Writing and Report Grading

1.6: Lab Performance and Professionalism

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1.1: Pre-Semester Assignment

Due: All items in Part I below must be completed before the first day of class (11:59 pm on the Sunday day prior to FDOC). Part II must be completed prior to the first Wet Lab meeting, and Part III should be completed as necessary.

Time: Plan at least three hours in the weeks prior to FDOC to complete Part I of this assignment. Parts II and III will take additional time.

Purpose: These pre-semester assignments are designed to help you start the semester prepared and confident. You will get familiar with the course structure, Canvas site, expectations, and resources.

Pre-Semester Assignment

Part I. Get Familiar with Course Tools and Policies

Complete the following written and preparation tasks before our first meeting:

1. Explore the Canvas Site
 - Log into Duke's Canvas platform.
 - Locate the CHEM 401L course site.
 - Carefully read the Course Syllabus (posted on Canvas).
 - Browse the Modules, Assignments, and Announcements sections.
2. Read the Orientation Materials on LibreText
 - Read the full "1: Orientation to CHEM401L" Module in the Lab Manual (this module).
 - Review the [Duke Chemistry Safety Manual](#) (linked in Canvas or the Lab Manual).
3. Complete the Orientation Assignment
 - Access and download the "Orientation Assignment" (at the end of the syllabus) from Canvas.
 - Complete the assignment carefully, following all instructions.
 - Submit your finished version as a PDF on Canvas before the first Dry Lab.
4. Download the full lab manual PDF in case of Canvas or LibreTexts outages.

Part II. Prepare for the First Week of Lab

1. Be Ready for Dry Lab (Monday)
 - Complete Part I above; Be prepared with any questions you may have about the Orientation materials.
 - Bring your computer to the first meeting.
2. Be Ready for Wet Lab (Sectional)
 - Complete the Pre-Lab Assignment for [Week 1, Module 2](#).
 - Know your assigned Wet Lab section and show up on time.
 - Dress appropriately: closed-toe shoes, clothes that cover your entire body from knees to elbows, your own eye protection.
 - Bring a lab notebook: you may use a physical notebook or electronic format (no template provided; use your training from CHEM 301L/310L).

Part III. Review Prerequisite Knowledge

You are expected to begin the course with working knowledge from prior chemistry and lab courses, especially:

1. General, Organic, and Physical Chemistry:
 - General chemical principles, units, and stoichiometry
 - Organic functional group reactivity
 - Thermodynamics, kinetics, and equilibrium
2. Physical Chemistry Laboratory ([CHEM301L](#) or [CHEM310L](#));
 - Error Analysis & Reporting:
 - Types of experimental error
 - Propagation of error
 - Basic statistical analysis

- Reporting significant figures and uncertainty
 - How to write a report in the format of a scientific manuscript
3. Electronic Absorption Spectroscopy:
- Beer-Lambert Law and calibration curves
 - Instrumental considerations (light source, detector, cuvettes)
 - Basic UV-vis theory

If you need to brush up on any of these areas, please revisit your prior course notes or request resources from the instructor.

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1.2: Welcome and Course Overview



Welcome to CHEM 401L!

This Advanced Analytical Chemistry Laboratory course is designed to simulate a research-intensive analytical chemistry experience. You will engage in authentic lab investigations, generate real data, and develop scientific arguments through writing. Success in this course requires engagement, reflection, and collaboration. Your instructors are excited to guide you through a semester of growth in both your technical and scientific communication skills.

Course Structure

The semester is divided into six parts:

1. Orientation Module (Pre-semester)
2. Calibration & Quantitation (2 weeks)
3. Project 1 (3 weeks), plus Week 4 for report revision
4. Project 2 (3 weeks)
5. Project 3 (3 weeks)
6. Final Reflection & Survey (Week 14)

Each week of the semester includes two meetings:

- Dry Lab (Mondays): Guided discussion, planning, and pre-lab work
 - Wet Lab (Sectional): Hands-on experimentation, data collection, and data analysis
-

Assignments and Grading

The course totals 524 points from pre-labs, notebook submissions, reports, peer review, lab citizenship, and feedback surveys. A detailed breakdown of points in each assignment is available in this [google spreadsheet \(Click\)](#).

Assignments each week | each module:

- **Weekly Pre-Lab:** Pre-labs are due before each Monday Dry Lab.
 - **Weekly Group Work:** At the end of each Dry Lab, your group will submit a group discussion report.
 - **Weekly Notebook:** At the end of each Wet Lab, you'll submit a notebook snapshot with a brief informal reflection.
 - **Each Module:** At the end of each Module, you will submit a formal report.
 - **Each Module:** At the end of each Module, you will complete a survey to provide feedback to your instructors about project design and learning support.
-

Promoting Diversity, Inclusion, and Avoiding Harassment



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All members of this course will foster an environment of listening and considering new ideas from a diverse group, with respect for all participants without regard to gender, race, ethnicity, color, sexual orientation, political orientation, age, religion, national origin, disability, or any other aspect of how we identify ourselves other than as fellow scientists.

There is zero tolerance in this course for any form of harassment or hate speech, during class meetings or outside of scheduled class time, which could include verbal or physical conduct that has the purpose or effect of interfering with anyone else's participation or performance, or of creating an intimidating, hostile, or offensive environment; any such harassment will result in dismissal from the course and will be reported to the college.

Duke University's Institutional Statement of Commitment to Diversity and Inclusion is found [here \(click\)](#):

"Duke aspires to create a community built on collaboration, innovation, creativity, and belonging. Our collective success depends on the robust exchange of ideas—an exchange that is best when the rich diversity of our perspectives, backgrounds, and experiences flourishes. To achieve this exchange, it is essential that all members of the community feel secure and welcome, that the contributions of all individuals are respected, and that all voices are heard. All members of our community have a responsibility to uphold these values."

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1.3: Details about Meetings and Deadlines

Where to find Information about this course

1. **Canvas:** Our course site is on Canvas. All course information is accessible through links on Canvas. All assignments will be submitted electronically on Canvas, and all rubrics are available in the assignments on Canvas.
2. **Lab Manual:** The lab manual lives on LibreTexts, and this is where you find daily tasks and agendas. You can download a copy of the lab manual at any time. Please note that the manual is a "living" document that we update regularly. The latest version is available on LibreTexts. We recommend that you download a pdf copy as a backup for times when you need to work offline. The Lab Manual will NOT be available in Wet Lab - you must have all procedures and plans written in your notebook ahead of time.
3. **Syllabus:** The syllabus contains additional details and policies that are not provided in the public manuals on LibreTexts, including instructor contact information. The detailed schedule of deadlines and assignments is also in the Syllabus document.

Deadlines

Submit your assignments and arrive to meetings on time to receive the maximum points for your work. See the schedule (syllabus) for a detailed schedule of all deadlines.

General Assignment Deadlines

- **Pre-Lab assignments:** these are due electronically by 10:00 am on the day of the "Dry Lab" meeting (Mondays).
- **Arrival to Lab:** Be ready to begin at the start time of each meeting. Arriving 5 minutes early is recommended.
- **Dry Lab Group Work:** The reporter from each group will submit a record of the group's work at the end of each dry lab session.
- **Notebook and Reflection:** A snapshot of your day's notebook and reflection is due at the end of each wet lab meeting.
- **Reports:** Report drafts are due at the third dry lab of each module; peer reviews are due by the end of that dry lab meeting. Final reports are due by the subsequent dry lab meeting. (see syllabus schedule)

Extensions and Late Assignments

This course is designed to keep you on track while also recognizing that real life happens. The policies below aim to balance accountability with flexibility, providing you reasonable options when things don't go as planned.

Policy on Extensions and Late Assignments

General Policy

- Deadlines are essential to ensure that you are prepared for lab work, receive timely feedback, and stay aligned with your team and the instructional schedule.
- Accommodations are always available for students facing serious or ongoing extenuating circumstances. If this applies to you, contact your academic dean to request a formal Dean's Excuse; your Dean will then reach out to your instructors so we can support you appropriately.
- The policies below apply to all students in the absence of a Dean's Excuse.

Late Assignments: If an assignment is not turned in by the deadline, the following point deductions will apply.

- **For Pre-Lab assignments:** Pre-labs are required preparation for Dry Lab and Wet Lab sessions. The policy for pre-labs is the most strict to protect your safety, the safety of expensive equipment, your team's success, and your ability to contribute meaningfully to lab work.
 - Pre-labs must be submitted before the Dry Lab session begins.
 - Late pre-labs will receive zero points.
 - If the pre-lab is still incomplete before your scheduled Wet Lab, you will not be allowed to participate in the experiment.
- **For All other assignments:** All other assignments can be submitted late but incur a deduction.
 - 10% deduction per 24-hour period, up to a maximum of 50% off.

Examples:

 - 5 minutes late → 10% off
 - 26 hours late → 20% off
 - More than 5 days late → 50% off
- **Deadline for All Late work:** Late work is only accepted up to 11:59 pm on LDOC (Last Day of Classes).

Extension Token: Each student may use one Extension Token during the semester — no explanation required.

- **The token allows you to submit:**
 - A scientific report up to two weeks late with no penalty, OR
 - Any other assignment up to two weeks late, as long as it doesn't impact safety, team grading, or lab scheduling.
- **⚠ Use It Wisely:**
 - This token is not a substitute for a Dean's Excuse. If you're dealing with a serious situation, ask your Dean or your instructional team for a different level of support.
 - Using the token may delay feedback or grading, which can impact your ability to perform on subsequent assignments. Do not use this as a license to procrastinate!

Final Deadline for Late Work

No late work will be accepted after 11:59 PM on the Last Day of Classes (LDOC).

Preparing for and Participating in Lab Meetings

This section outlines expectations for your preparation, participation, and behavior in both Dry Lab and Wet Lab sessions. These practices support your safety, efficiency, and success in a collaborative research-focused environment.

Before Lab: Preparation is Critical

- **Complete the Pre-Lab Assignment and submit it on Canvas BEFORE Dry Lab each week:** It is essential that each student be prepared for lab meetings by reading the appropriate module in this manual, and completing the specified pre-laboratory assignments. The pre-laboratory assignments are designed to prepare you to learn, operate efficiently, protect our equipment, and keep you safe during the experimental module. Each pre-lab assignment requires you read the entire module and write out the specified pre-lab assignments in your notebook. Be sure to have a work plan that makes efficient use of your time, and take notes that will help you during wet lab. Failure to do this will undoubtedly result in your failing to complete the laboratory in the allotted time (see "leaving the laboratory"), and therefore decrease the chance that you will earn desirable scores on your assignments.
 - The Pre-Lab is due before your Dry Lab; late pre-labs will receive a score of zero.
 - You will not be allowed to conduct experiments if the pre-lab assignment is not completed before your Wet Lab meeting.
 - If there is an extenuating circumstance that prevents your preparation, please inform your instructors as soon as possible.
- **Dress for Lab:** Dress appropriately for a laboratory environment. You must wear close-toes shoes and clothing must cover your body from your upper arms to your knees, including your torso. You should also have your own pair of protective eyewear, bring them to lab, and wear them the entire time you are in lab. [Safety eye wear can be purchased from the Graduate Chemistry Council \(GCC\)](#). If lab attire is not jiving with your wardrobe, keep a change of clothes on hand or purchase a lab coat and bring it with you so that you can cover up in lab. Students who are not dressed appropriately will be dismissed and

not allow to complete the experiment.

During Lab: Expectations for Wet Lab Sessions

- **Stay on task during the lab period:** Each lab module is designed so that a well-prepared student can complete the laboratory work within the scheduled laboratory meeting times. You will complete your work individually, and in some cases, you will share your results with your group members and analyze the data as a group. There will a dedicated TA to help you run the instruments, and answer questions. Lengthy sample preparation procedures should be started early in the lab period.
- **Record your work:** All students are expected to record laboratory observations and any numerical data in a dedicated laboratory notebook for this course; the notebook may be a physical bound notebook or an electronic notebook (eg OneNote or LabArchives). Each page should be titled, dated, and signed. You should have a way of collecting and organizing any instrument outputs (data), such as spectra and chromatograms. Every lab computer has internet access, and you may either email data to yourself or collect it using a usb drive. A snapshot of your notebook must be submitted at the end of each Wet Lab session.
- **Lab Etiquette:** During the lab period, you should actively maintain the shared spaces by cleaning up after yourself. Clean up spills immediately, brush powders off of the balances after every use, and put all trash or waste in the appropriate containers. At the end of each lab, you should leave the lab space, equipment, and instruments in good condition. Clean all glassware, remove any labels from the glassware, and return it to the appropriate location. Wipe down and clean the surfaces and equipment that you used and leave the lab better than as you found it.
- **Use the time with your instructors (and bring snacks to avoid hanger!):** The lab period is loooong for a reason! A big part of your work for this course includes data analysis and writing. There will be several occasions when your laboratory work is finished before the lab period ends. This "extra" time is deliberate - you should use this time to get your data off of the lab computers and discuss it with your instructors or your group. During scheduled lab hours is the best time to get help from your instructors! Do not squander the downtime during scheduled lab meetings! We recommend that you bring a snack to eat (Eat in the hallway or in conference room 1113)! A quick snack can help you have the energy to use the end of lab hours most effectively!
- **Leaving Lab:** In the interest of safety, we do not allow students in the lab after business hours (ie you do not have access to lab between ~ 6 pm - 9 am). Thus, you must leave the lab promptly at the end of the period. At 10 minutes prior to the end of the period, you must stop lab work and begin cleaning up (see lab etiquette). Your instructors must sign you out of lab before you leave for the day; they will record the time you leave (be sure they do this!) after checking that your space is tidy and all materials have been returned to the appropriate places.

Wet Lab Safety

The educational objectives of this course include learning about the hazards of materials, equipment and procedures in the chemical laboratory. This is very important because it determines the level of risk to which you and other students are exposed by your actions and the actions of others while you are in the laboratory. Through this type of training, you will gain the knowledge and experience to determine what precautions are necessary in order to limit risk to yourself and others from chemical hazards. We hope that you will make a serious effort at all times to ensure that your work in the laboratory is conducted as safely as possible. The following rules have been developed with your safety in mind:

- **SAFETY GLASSES MUST BE WORN AT ALL TIMES IN THE LABORATORY-NO EXCEPTIONS.** Contact lenses may be worn in the laboratory as long as you are also wearing safety glasses. You are required to bring your own safety eye wear. [Safety eye wear can be purchased on campus from the Graduate Chemistry Council \(GCC\).](#)
- A copy of the [Duke Chemistry Department Safety Manual](#) is available on the Chemistry web site. All students should read this manual prior to beginning laboratory work.
- No unauthorized experimentation will be allowed in the laboratory.
- Proper shoes are required in the laboratory - no bare feet, sandals or open-toe footwear.
- Never eat (including chewing gum), drink, or smoke in the laboratory.
- To minimize skin exposure to chemical hazards, skin must be covered from your shoulders and upper arms to your knees. Sleeved shirt and pants should be worn. We know it can get hot in Durham, so bring extra clothes with you to lab on hot days. Clothing that are not appropriate for lab include shorts, mini-skirts, crop tops, tank tops, low cut shirts, etc.
- Long hair should always be tied back while in the laboratory.
- Safety Data Sheets (SDS) and other safety notes for every chemical to be used in this laboratory course are kept in a ring binder in the lab and also online. Students should read these notes prior to beginning each experiment.
- Failure to observe safety regulations will result in substantial deduction in the lab grade and may result in ejection from the lab session. Persistent violation of safety regulations will result in permanent expulsion from the lab course.

- **Disposal of Solvents and Chemicals:** **Never** pour solvents or chemicals down a drain. Departmental waste disposal procedures are described in the safety manual. However, you should **ALWAYS** consult with a TA before attempting to dispose of any material.
- Precautions for individual experiments will be discussed with you by the TA's and are described in the lab manual.

Working with Your Instructional Team

- **Your instructors are here to help:** There is a team of instructors to help you, including the course instructor (a Ph.D. chemist) and Teaching Assistants (TA's) who are graduate students earning their PhD's in chemistry. There will be TA's in the lab with you each day, and at least one of them will be dedicated to helping you on each experiment. That dedicated TA will be present during the laboratory to assist you, offer tips, and advice. That same TA will be on the team of TAs that grades your lab report for that given experiment. A list of instructors and their contact information is posted on the syllabus.
- **Use lab time to get help:** You are strongly advised to consult with your TA about a given report before writing it. This consultation should happen during scheduled lab time when possible - do not leave early just because you have finished the experiment and cleaned up. If you complete the experiment in the first lab meeting, use the second lab meeting as a consultation with your TA. Use lab time wisely to work on your data and consult with your TA so they can help you during your time with them. The TA can show you the important points to be emphasized in evaluating your data and will discuss the grading scheme.
- **Email:** You may also email your TA at least 48 hours before your lab report is due to ask questions about an assignment; do NOT wait till the last moment to do this because you will not get help in time. Please be polite in your email correspondences. Please also recognize that questions about chemistry are often difficult to answer in an email, and a meeting may be much more effective. Please take advantage of lab time and office hours for this reason.
- **Office Hours:** The office hours schedule with your team of instructors is posted on the Syllabus.

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1.4: Academic Integrity

Academic Integrity vs Cheating

- **Academic integrity** means your work is an accurate representation of your own learning and understanding.
- **Cheating** or **academic dishonesty** occurs when your work misrepresents what you know, undermining your own learning and the trust that makes meaningful feedback possible.

Why Academic Integrity Matters in this Course

In this course, your written work is both a learning tool and an assessment tool.

- When you write, you articulate and refine your thinking.
- When we read your writing, your instructors and TAs see your reasoning, your decisions, and your conclusions. This allows us to give you feedback you can actually use to grow as a scientist.

You will also give and receive peer feedback. For that feedback to be meaningful — and for you to benefit from it — your work must reflect your own thinking.

Duke Community Standard

All students must adhere to the [Duke Community Standard \(DCS\)](#): Duke University is a community dedicated to scholarship, leadership, and service and to the principles of honesty, fairness, and accountability. Citizens of this community commit to reflect upon these principles in all academic and non-academic endeavors, and to protect and promote a culture of integrity.

To uphold the Duke Community Standard, students agree:

- will not lie, cheat, or steal in my academic endeavors;
- I will conduct myself honorably in all my endeavors;
- I will act if the Standard is compromised.

Regardless of course delivery format, it is the responsibility of all students to understand and follow all Duke policies, including academic integrity (e.g., completing one's own work, following proper citation of sources, adhering to guidance around group work projects, and more). Ignoring these requirements is a violation of the Duke Community Standard. Any questions and/or concerns regarding academic integrity can be directed to the Office of Student Conduct and Community Standards atconduct@duke.edu.

Using AI to Support Your Learning

AI tools (e.g., ChatGPT, Grammarly, Claude) can be powerful learning partners if you use them intentionally and transparently. But, if they are misused, these tools can undermine your learning.

Appropriate use of AI:

AI tools can play a valuable role in your learning process when used intentionally to coach you, or to help improve your writing.

You may use AI applications to:

- Improve organization and structure of your own writing using prompts like *“Revise this paragraph to improve clarity and structure, but keep the original meaning.”*
- Edit for grammar and clarity using prompts like *“Identify grammar errors and suggest fixed without changing the technical*

meaning of my writing.”

- Refine your phrasing or writing style. *“Make this explanation appropriate for an audience of scientists outside my specialty.”*
- Brainstorming ways to present an idea you’ve already developed. *“Suggest alternative ways to phrase this description of my method.”*

After using AI in any of these ways, you must review, edit, and verify the output yourself. Be aware that AI tools can change the meaning of your words, and AI can hallucinate and give test that is not based in reality. You are responsible for ensuring that your final writing is clear, correct, and accurate—and that it represents your own learning, thoughts, and understanding.

Inappropriate use of AI:

Using AI to generate substantive scientific content — such as designing methods, interpreting data, or drafting analysis you have not developed or do not fully understand — is a misuse of these tools.

Examples of inappropriate use of AI:

- Generating new sections or paragraphs you did not develop yourself. (e.g., “Write an introduction section for a scientific manuscript about ...”)
- Asking AI to analyze your data or draw conclusions that you cannot explain (e.g., “Interpret my results and write the discussion section.”)
- Rewriting entire drafts you did not author yourself.
- Fabricating sources, citations, or data.

Misusing AI in this way prevents your instructors and peers from providing meaningful feedback; it prevents you from learning and it wastes everyone’s time.

A Quick Test: The “Could I Explain This?” Rule

Before submitting any work that involved AI, ask yourself:

- Could I explain every choice, result, and statement in my own words?
- Could I recreate the reasoning or analysis without AI’s help?

If the answer is “no,” then the AI use is likely inappropriate for this course.

When in Doubt, ask.

If you are ever unsure about whether an AI tool is supporting your learning appropriately, please ask a member of the instructional team. We are here to help you learn how to use these tools thoughtfully, responsibly, and always in service of your authentic growth as a scientist and writer.

Acknowledgements

Whenever you receive outside help—whether from a friend, a textbook, or an AI tool—you must acknowledge it. To help your instructors understand what kinds of support you are using and where you may need additional guidance, we ask that you clearly document all sources of assistance for each assignment.

Example of an Acknowledgements statement:

This statement on the responsible use of AI tools was created by Kathryn Haas for Advanced Lab Courses at Duke University. The statement was drafted with the support of OpenAI’s ChatGPT. It draws inspiration from “AI with Intention: Principles and Action Steps for Teachers and School Leaders” by Tony Frontier and includes insights generously shared by Dr. Eliana Schonberg (Thompson Writing Program at Duke University).

1.5: Scientific Writing and Report Grading

Why Scientific Writing... in a Chemistry Lab Course?

Writing is equivalent to thinking. By writing about your experiments, you deepen your understanding of what you did and why it matters. Scientific writing is not just a course requirement — it's a central part of doing science. In this course, you will complete three "scientific-manuscript-style" reports using the skills and formats you learned in CHEM301L or 311L. Refer to the [Writing Support Materials](#) from CHEM301L for support.

Support for Writing

This course is designated as a [Writing \(W\)](#) course at Duke, which means that developing your scientific writing skills is a central learning goal. Dr. Haas has been teaching writing-intensive courses since 2014 and has worked closely with the Thompson Writing Program and the graduate TAs to ensure the entire instructional team can provide strong, consistent writing support.

In addition to feedback from your instructor and TAs, you will have access to the [Thompson Writing Studio](#) and an Embedded [Writing Consultant](#) (EWC) assigned to this course. EWCs are trained undergraduate peer tutors who will work with you to strengthen your writing process and help you communicate your science effectively.

You will meet with your EWC during the third Wet Lab session of each module, giving you the opportunity to get targeted feedback on your work in progress. The [Thompson Writing Studio](#) is also available for one-on-one appointments at any stage of your writing process.

Report Expectations

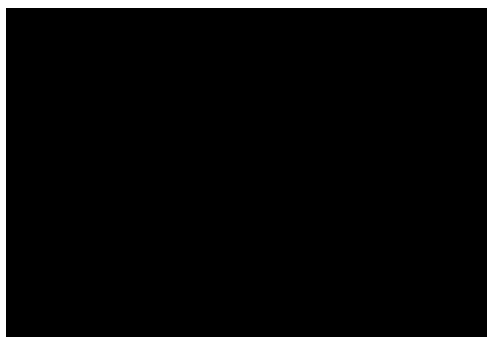
All reports (except for Module 2) must follow the structure of a scientific manuscript: Title, Introduction, Methods, Results, Discussion, Conclusions, and References. Use the formatting conventions you learned in prior lab courses (Refer to the [Writing Support Materials](#) from CHEM301L for support). Your goal is to present your analysis clearly, concisely, and accurately — not to write a perfect or lengthy document.

Submission Guidelines

Submit your report as one single pdf file on Canvas. Your report will be graded by a group of TA's. When TA's grade your report, they will not see your name (except on the title page) - this is to reduce any possible biases during point allotment.

Grading Criteria

Reports are scored using detailed rubrics developed by the instructional team. These are available in each Canvas assignment. A Generic version of the Rubric for a Report in the format of a scientific manuscript is given below. This generic rubric was developed with support from Dr. Shonberg of the Thompson Writing Program at Duke.



Writing Quality Policy ("B or Nothing")

Each section of your report must be B-quality ($\geq 80\%$) or better to earn points. If a section does not meet that standard, it receives zero points.

To support learning:

- Each Pre-lab assignment is designed to make progress, and for you to receive early feedback, on parts of your report.
- Embedded Writing Consultants will meet with you to give you individual advice on your writing.
- You may revise and resubmit the first report after receiving feedback from peers and from your instructors.
- Each report will have an early deadline, one round of peer review, followed by time for revisions, and a final submission at least one week later.
- You may submit one report late without penalty using your Extension Token.

Tips for Success in Report Writing

- Every assignment in this course — pre-labs, notebook entries, data analysis tasks — is designed to help you build your report piece by piece. Treat them as stepping stones toward your final manuscript.
- Use your time strategically:
 - Start drafting sections of your report during or right after lab, while your methods, observations, and insights are still fresh.
 - Create figures and plots as soon as you have your data, and review them with your TA to check for clarity and accuracy.
 - Use lab time for discussion—ask questions, clarify your interpretation, and get feedback before you start formal writing.
 - The sooner you start, the easier it will be to produce a clear, well-organized report without last-minute stress.

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1.6: Lab Performance and Professionalism

A portion of the total available points for this course are allotted to your performance in lab. This includes an expectation for preparation before the lab period, arriving on time, using time effectively, performing the tasks well, contributing to the culture of safety, and helping to maintain a clean and safe environment. Your performance and professionalism in the lab is assessed through **Lab Citizenship points**.

★ What does it mean to be a "Good Lab Citizen"?

Lab Citizenship refers to more than just following rules. It means self-regulating your behavior in a way that supports a safe, respectful, and productive lab environment—without needing constant reminders from instructors.

Your instructors are here to support your learning, not to act as the "lab police." We expect that you will take ownership of your space, your preparation, and your conduct. This includes not just compliance with safety policies, but also contributing positively to the shared environment, being considerate of others, and acting professionally.

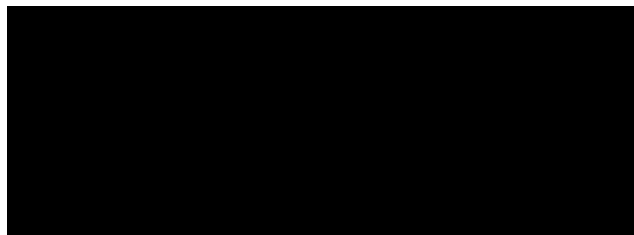
Good Lab citizens:

- Arrive on time and ready to work
- Wear proper lab attire and PPE at all times without reminders
- Turn notifications off on electronic devices used during meetings
- Clean up after themselves and leave shared spaces better than they found them
- Use lab time effectively and help their group stay on track
- Label solutions, handle chemicals safely, and follow disposal protocols
- Respect the physical space, equipment, and people in the lab

When you do these things consistently, it frees your instructors to focus on helping you learn and grow as scientists — not on enforcing rules. Your Lab Citizenship grade score reflects your ability to self-regulate and contribute to a positive lab culture.

Lab Citizenship Checklist

Each Wet Lab session, you can earn 1 Lab Citizenship point by demonstrating professional, safe, and respectful lab conduct. This point is awarded as an "all-or-nothing" score based on the following expectations.

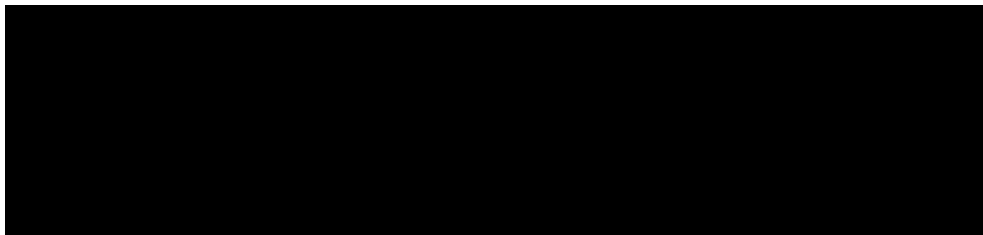


⚠ Students **will not receive partial credit**—failure to meet any of these expectations may result in 0 points for the day.
*Distraction by electronic devices (messages, social media) during lab time is considered a violation of safety and using time well. You are advised to turn off notifications on electronic devices used during meetings.

Lab Citizenship Penalties

While Lab Citizenship is typically graded on a 1-point-per-session basis, certain behaviors may result in point deductions from your lab report score. These behaviors compromise safety, professionalism, or lab functionality and require stronger consequences.

Repeated or serious infractions will result in direct point deductions from your lab report grade for the corresponding module. The instructional team will notify you if a deduction has been applied and explain why. Some, but not all, examples are shown below:



⚠ Repeated issues (e.g. forgetting goggles, being late, or poor cleanup) may also trigger **a conversation with the instructional team or department administrators** to resolve concerns and may result in failure of the course.

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2: CALIBRATION AND QUANTITATIVE TECHNIQUES

Learning Objectives

By the end of this module, you will be able to:

1. **Design and prepare a set of standard solutions** using appropriate volumetric techniques to support a quantitative analysis.
2. **Construct and interpret a calibration curve** using spectrophotometric data collected at a specific wavelength.
3. **Evaluate the quality of calibration data** using residuals and statistical evidence to justify regression model choice.
4. Apply a calibration model to **calculate the concentration of an unknown sample**, including propagation of error.
5. **Document experimental work clearly** and completely in a scientific notebook.
6. **Prepare publication-quality figures with captions.**
7. **Engage in safe and responsible laboratory practices**, demonstrating awareness of good lab citizenship.

The purpose of this introductory lab module is to practice using some of the basic quantitative techniques that you will use often in this course and in an analytical chemistry laboratory. These techniques include the use of an analytical balance, quantitative use of pipettes and volumetric glassware, quantitative solution preparation and dilution procedures. Although you may have used these basic equipment in previous laboratory courses, **analytical chemistry requires a *much higher* level of precision and accuracy than you may have experienced in general or organic chemistry courses.** One of the requirements for success in this course is the development of correct quantitative laboratory technique.

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[2.1: Introduction](#)

[2.2: Week 1](#)

[2.3: Week 2](#)

[2.4: Post-Lab](#)

[2.5: Week 2 - Individual vs Serial Dilution](#)

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2.1: Introduction

Introduction

In this two-session lab module, you will take on the role of a method developer. Your task is to evaluate how different calibration design choices influence the quality of analytical data. You will construct and compare calibration curves using visible-range absorbance measurements of a colored dye, bromocresol green.

Rather than simply following instructions, your goal is to generate, validate, and evaluate calibration curves using different tools and dilution strategies. You will also share your data with the class to support broader comparative analysis.

Bromocresol green

Bromocresol green ($pK_a = 4.66$) is an acid-base indicator dye that exhibits a pH-dependent absorption spectrum. The only structural difference between the **acid form (yellow)** and the **basic form (blue)** of this dye is the protonation of a phenolic oxygen. This experiment will be performed in 0.01 M sodium acetate; this weak base in solution will ensure that the pH is close to 8 and that bromocresol green dye is predominantly in its **basic form**.

A stock solution of bromocresol green will be provided to you in lab. You will use this *stock solution* to prepare a series of more dilute solutions. You will measure the absorbance of the diluted solutions at **612 nm (absorption maximum for the basic form of the dye)** and generate a *calibration curve* of absorbance vs concentration. You will then measure the absorbance of a bromocresol green solution of unknown concentration, and use Beer's law to determine its concentration.

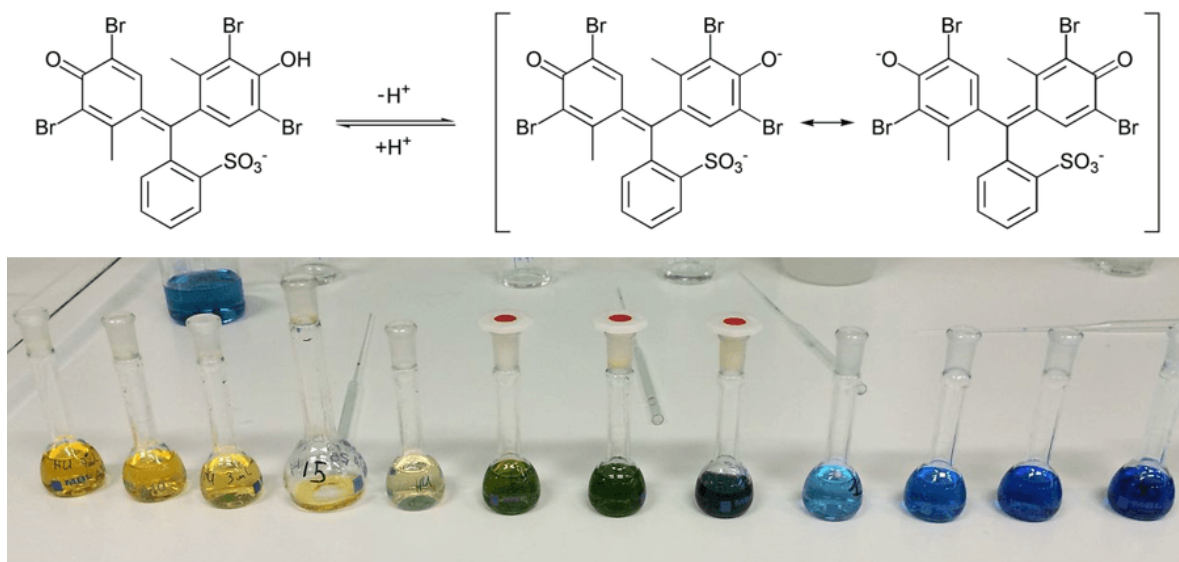


Figure 2.1.1: Top: acid dissociation of bromocresol green. Bottom, from left to right: five solutions of 0.1 M HCl with decreasing concentrations of bromocresol green (yellow); three buffered solutions at pH 3.78, pH 4.00, and pH 4.62 (shades of green) and four solutions of NaOH 0.1 M with increasing concentrations of bromocresol green (darker solutions are more concentrated). ([Natan Consigli](#), [Bromocresolo](#), crop, [CC BY-SA 4.0](#))

2.2: Week 1

Pre-Lab Assignment



Due: This pre-lab assignment is due prior to the first Wet Lab meeting, on the first week of classes.*

*The pre-lab assignments in this course are due prior to arriving at the Dry-Lab meetings — *except for this first module*. Due to the unique schedule in the first two weeks of the semester, the first two pre-labs will be due prior to arriving at Wet Lab meetings. Please note that starting the third week, all pre-lab assignments are due prior to the dry lab meeting (Monday)!



Purpose: In this module, you will plan, record, and analyze calibration data - this module is practice to get you warmed up for the three projects later in the semester. This pre-lab assignment will help you come prepared to measure solutions carefully and think about precision and accuracy.

Week 1 Pre-Lab Tasks

1. Read & Review

- Read Module 2 (this module) in the Lab Manual.
- Find the [Instrument Facilities Manual](#)
 - Read about how to use the [Analytical Equipment](#) (and watch the videos) - note the Tolerances (errors) of common equipment at the end of the page!
 - Read about [Solution Preparation](#) and practice the Error Propagation exercises.
 - Read about [Methods of Calibration](#): As you read, think about which type of calibration you will use in this module.*
**This is for your own reflection—no written response is required for submission or grading.*
 - Read about how to [Determine uncertainty from values determined from a Calibration Curve](#). Since we did not use calibration in P-chem lab, this may be completely new to you. Expect to see this new material in CHEM401L lecture!

2. Prepare your Notebook for Week 1 (Do the following in your notebook and turn it in on Canvas)

- Take notes** on the tools you'll use, solutions you'll make, and measurements you'll take.
 - Summarize the procedure in your own words.
 - Summarize the main points for proper use of volumetric pipets (with one t) and pipettes (with two t's).
 - Record the tolerances for all glassware and pipettes you'll use in this module!
- Get to know your analyte (Bromocresol Green)**
 - Draw the chemical structure of bromocresol green in its acidic and basic forms.
 - Note which form you will measure and the wavelength you'll use.
- Predict what you'll see & plan what you'll do**
 - Sketch a predicted calibration curve from solutions B-F (Absorbance vs. Concentration).
 - Label the standards (B-F), the blank, and the validation standard on your plot.
 - Show how you will calculate the concentration for at least one standard using $M_1V_1 = M_2V_2$.
- Prepare Data Tables in your notebook (see examples below).** Build a data table with the following:
 - Rows for standards B-F, control, unknown
 - Columns for 3 replicate absorbances, average, standard deviation

Example Notebook Tables

Prepare tables in your notebook for recording data in an organized way (see examples below). In the same table, provide space for summarizing propagated error in concentrations and the standard deviation of replicate absorbance measurements of each solution.

If you choose to record your data electronically, please use a shared document that your team can access and so you can collaborate on a shared file. Also, be sure to record a link or the file path to the electronic files in your notebook. There are example data tables for this module available in this [google sheet](#) (click). A screen shot of the example data tables are below.

Example table for Calibration 1, Trial 1:

Volumetric pipet Trial 1 data					Replicate measurements of absorbance (AU)					
Solution Label	Components*	Actual Concentration of Bromocresol	±	Propogated error	1	2	3	Average Absorbance	±	Standard deviation
Stock solution A	Stock solution* of Bromocresol Green (0.500 mM)	5.00E-04	±		NA	NA	NA	NA	±	NA
Calibration Standard B	1.000 mL of stock A, diluted* to 25.00 mL	2.00E-05	±						±	
Calibration Standard C1	15.00 mL of B, diluted* to 25.00 mL	1.20E-05	±						±	
Calibration Standard D1	15.00 mL of C, diluted* to 25.00 mL	7.20E-06	±						±	
Calibration Standard E1	15.00 mL of D, diluted* to 25.00 mL	4.32E-06	±						±	
Calibration Standard F1	15.00 mL of E, diluted* to 25.00 mL	2.59E-06	±						±	
Blank	0.01 M sodium acetate	0	±						±	
Validation Standard			±						±	
Sample			±						±	

*All samples are in a matrix of (and diluted with) 0.01 M Sodium acetate solution.

Example table for Calibration 2, Trial 1:

Micropipette Trial 1 data					Replicate measurements of absorbance (AU)					
Solution Label	Components*	Actual Concentration of Bromocresol	±	Propogated error	1	2	3	Average Absorbance	±	Standard deviation
Stock solution A	Stock solution* of Bromocresol Green (0.500 mM)	5.00E-04	±		NA	NA	NA	NA	±	NA
Calibration Standard B	1.000 mL of stock A, diluted* to 25.00 mL	2.00E-05	±						±	
Calibration Standard C2	1.80 mL of B, diluted* to 3.00 mL	1.20E-05	±						±	
Calibration Standard D2	1.80 mL of C, diluted* to 3.00 mL	7.20E-06	±						±	
Calibration Standard E2	1.80 mL of D, diluted* to 3.00 mL	4.32E-06	±						±	
Calibration Standard F2	1.80 mL of E, diluted* to 3.00 mL	2.59E-06	±						±	
Blank	0.01 M sodium acetate	0	±						±	
Validation Standard			±						±	
Sample			±						±	

*All samples are in a matrix of (and diluted with) 0.01 M Sodium acetate solution.

Dry Lab Agenda

This Dry Lab meeting is the first meeting of the semester.

1. **Course Welcome:** Brief course introduction and Q&A.
2. **Safety talk and Tour** of the Laboratory Space.
3. **Discussion about first Wet Lab session:** Come prepared with questions from your Pre-Lab.

Wet Lab, Week 1: Comparing Tools (Volumetric vs. Automatic pipettes)

Materials: Gather the following and inspect all equipment prior to use.

1. Bromocresol green solutions:

- **Solution "A",** a "stock solution" of 5.00×10^{-4} M bromocresol green in 0.1M sodium acetate (prepared by the instructional team by combining 0.0900 g of the sodium salt of bromocresol green (MW 720.02 g/mol) and 0.3402 g of sodium acetate trihydrate in a 250.00 mL volumetric flask, then dissolving in DI water and filling the flask to the t.c. volumetric mark). The *exact concentration should be written on the stock container*. Be sure to record the *actual concentration* in case it deviates from what is written in this manual.

- **Validation Standard** solution (aka "control solution") of known concentration. This is a solution of bromocresol green in 0.1 M sodium acetate. You will use this solution to **validate** your standard curve. *The exact concentration should be written on the control solution storage container.* Be sure to record the concentration in your lab notebook.
- **Sample** solution (a solution of bromocresol green of unknown concentration in a matrix of 0.1 M sodium acetate). You should use your validated calibration curve to determine the concentration of this sample.

2. **Matrix solution:**

- 125 mL of **0.01 M sodium acetate**: Prepared by the instructional team in advance by dissolving 2.7216 g of sodium acetate trihydrate (MW 136.08 g/mol) in 2.00 L of deionized water.

3. **Equipment:** each team will share the following equipment depending on availability.

- One **1000 μ L automatic micropipette**
- One **2.000 mL volumetric pipet** (optional for making solution B)
- At least one **plastic tip** for the micropipette
- One **25 mL volumetric flasks** (you could use several if available)
- One **50 mL volumetric flask** (optional for making solution B)
- Two disposable **transfer pipets** (to be reused throughout the solution preparation)
- Glass vials with screw cap lids for storage of solutions
- Two glass **cuvettes**
- **Spec 20 instrument**
- One **beaker** to collect waste

Important: Inspect your equipment

The function of the volumetric pipets and micropipettes depends on their condition! Carefully check their condition prior to use.

Volumetric (Class A glass) pipets:

- If the tip of the pipet is chipped, do not use it! Please alert your TA and get a new pipet.
- Visually inspect the pipet to be sure it appears clean.
- Rinse the pipet with your solution and drain it - visually inspect to ensure liquid is draining cleanly and there are no beads of solution on the inside of the pipette walls. If solution is beading up on the walls, either clean the pipette before use, or get a new one.

Micropipettes:

- Be sure that the plastic tip fits tightly onto the pipette barrel.
- Dial the pipette to 1000 microliters (**this looks like "100" on most of the p1000 pipettes**). Use a balance and a plastic weigh boat to test the pipette to be sure that it is delivering the correct volume. 1000 microliters = 1.000 mL = 1.000 grams. Consider the error expected when deciding whether to use the pipette.
- Repeat for 500 microliters.

Preparation of calibration standards

You will make a series of sequential or "serial" dilutions of bromocresol green and measure the absorbance of each solution in the series using a single-wavelength spectrometer. You will use these absorbance readings to construct a calibration curve and determine the concentration of an "unknown" solution. Each group (at least two people) should work together to create one validated calibration series using volumetric pipets, and one using automatic micropipettes, as described below. For groups of three and four teammates, do additional trials of each method depending on the number of additional people. Each person should produce at least one calibration set.

Note the tolerance of all equipment in your lab notebook so that you can propagate error in the concentration of each solution.

Calibration Standard "B"

You may collaborate and share this solution with your teammates or with other teams - however you are each individually responsible for the accuracy of this solution preparation and for recording how it was created in your notebook. **It is essential that the concentration of calibration standard B solution is accurate.**

- For convenience, use a micropipette and a volumetric flask: Use a **1.000 mL automatic micropipette** to quantitatively transfer 1.000 mL of stock solution to a **25.00 mL volumetric flask**. *It is critical that this transfer is precise and accurate — check the function of the pipette first, and then weigh your transferred liquid to be sure.* Then dilute with the matrix.
- When using the volumetric flasks, carefully dilute the solution up to the mark using **0.01 M sodium acetate solution** and mix well. Put some of this solution into a screw cap vial labeled "B" so that you can access the solution with a pipette in the next step. Each team will need at least ~20 mL of this solution or more. If you need to re-do the solution prep you will need even more. You can store the leftover solution B in a sealed vial.
- [Record triple replicate absorbance of Solution B](#) on at least two spectrometers (to be sure the spectrometers are giving consistent readings).

Calibration Standards C1-F1 — serial dilution, Class A volumetric glassware (Calibration Set 1)

One person in your team should create standard solutions for calibration set 1 (C1-F1) by serial dilution, using Class A volumetric glassware:

- Start with solution B. Prepare solution "C1" using a 15 mL volumetric pipette, quantitatively transfer 15 mL of solution B to a clean 25 mL volumetric flask. Carefully dilute the solution up to the mark using 0.01 M sodium acetate solution. Mix well by inverting 20 times. Transfer solution C1 to a labeled vial before the next step.
- In the same manner, perform 3 more serial dilutions to create solutions D1-F1 (see the example tables provided below).
 - Transfer and store each solution to a labeled vial and keep vials closed to avoid evaporation.
- Collect absorbance data using the "Data Collection" instructions below. Make sure to also collect the absorbance of the Blank, the Validation Standard, and the Sample.
- Use the data to determine if your calibration is valid. If it is not, troubleshoot with the help of your instructors, and try again to create the solutions.

Calibration Standards C2-F2 — serial dilution, automatic volumetric pipettes (pipetters)

One person in your team should create standard solutions for calibration set 2 (C2-F2) by serial dilution, using automatic pipettes:

- Prepare solution "C2" using a 1.000 mL volumetric pipette, quantitatively transfer 1.200 mL of sodium acetate matrix to a clean tube. Then, add 1.800 mL of solution B and mix well. These steps can be accomplished by dialing the pipette to half of the desired volume, and making two careful transfers to double the volume.

Important points:

- Use the pipette to gently mix this solution by slowly pipetting up and down, but be careful not to "shoot" the solution up into the barrel of the pipette by releasing the plunger too quickly. If the solution goes up too high, it will become contaminated, and the pipette should be cleaned - consult your instructor if this happens and start over.
 - Note that the error for all transfers must be propagated!
 - Due to the small volume of this solution, **record the absorbance of the C2 solution before creating the next one.** See "Data Collection" instructions below.
- Use the "Data Collection" instructions below to record the absorbance of solution C2. Then use solution C2 to create solution D2, and repeat this process for E2, and F2.
 - Use the pipette to mix each solution, then re-use your tip for the next solution.
 - Reserve a second tip exclusively for transferring the "clean" sodium acetate matrix.
 - Store all leftover solutions in labeled tubes and keep tubes closed to avoid evaporation.
 - Make sure to also collect the absorbance of the Blank, the Validation Standard, and the Sample.

3. Use the data to determine if your calibration is valid. If it is not, troubleshoot with the help of your instructors, and try again to create the solutions.

Data Collection using a visible spectrometer

Measure and record the absorbance of each solution at 612 nm using a Spec 20. Instructions for operating the Spec 20 are in the section, [Operating Instructions for Spec 20 Visible Spectrometers](#).

Procedure for data collection

1. Blank the Spec 20 using a cuvette filled with 0.01M sodium acetate. Keep this cuvette filled with sodium acetate for future use as a blank.
2. Fill a second cuvette with 0.01 M sodium acetate and measure its absorbance to be sure the second cuvette matches the first. The reading should be very close to zero. Record the triplicate absorbance measurement in your data table - this is absorbance at concentration zero.
3. Measure and record the absorbances of each solution in the serial dilution.
4. Measure and record the absorbance of the "control" solution.
5. Measure and record the absorbance of the "unknown" solution.
6. Keep your solutions until you have validated your calibration curve and determined that you do not need to repeat any of the absorbance readings.

Tips for data collection

- Use **one** cuvette (a "working cuvette") for all measurements and keep a second separate cuvette for all blanks ("blank cuvette"). The blank cuvette should be filled with 0.01 M sodium acetate solution.
 - After the instrument is zeroed with the blank cuvette, measure the absorbance of the working cuvette after filling it with the 0.01 M sodium acetate solution - this is your absorbance at zero concentration of analyte.
 - Be very sure that both cuvettes are clean before making these measurements.
- Measuring sequence and working with the cuvettes:
 - Use the same working cuvette for all measurements, and the same blank cuvette for all zeroing.
 - It is essential that residual solution from the previous measurement does not dilute or contaminate your next sample!
 - You can clean and dry the cuvette between measurements - but this is not recommended. It is tedious and ineffective unless you have access to a cuvette washer. Do not stuff towels into a cuvette to dry it, and **never blow compressed air into a cuvette**.
 - To prepare the cuvette for the next measurement:
 - i. Empty the cuvette and tap the cuvette gently upside down on a soft towel to remove as much liquid as possible.
 - ii. Rinse the cuvette with a very small amount (a few drops) of the next solution to be measured, and do this three times.
 - iii. Add the bulk of the next solution and make the measurement.
 - If you are measuring multiple solutions sequentially, work starting with the least concentrated solution and proceed in order of increasing concentration. However, this is not possible using the automatic pipette protocol due to limited volume.
 - The outside of the cuvette should always be clean and dry.
- Take three measurements for each solution (use same liquid for each measurement; take cuvette out and wipe down then replace in instrument) and calculate the average absorbance and standard deviation from three replicate readings for each solution. Usually, you should get exactly the same absorbance reading for all three replicate measurements. The three readings should not differ by more than ± 0.003 absorbance units or 1% of the absorbance (see uncertainty associated with the spectrometer readings described in the operating instructions.)
- Between each measurement, the sample cuvette should be emptied and then rinsed twice with small (ca. 1 mL or less) aliquots of the next solution to be assayed.
***Work carefully because you have limited quantities of each solution.

- Between measurements, check the stability of the instrument by inserting the blank and making sure it still reads zero absorbance.

For each calibration, measure the concentration of the following solutions:

1. **Blank** (sodium acetate matrix)
2. **Each calibration standard** (work from lowest to highest concentration F→B)
3. **Validation standard** (determine whether the calibration is validated)
4. **Sample** (determine and report its concentration)

Each solution's absorbance should be measured three replicate times and recorded in a table in your notebook. Store all solutions in sealed containers until the second week of this module and discard them only after you are certain you no longer need them.

Important Note

Measure each absorbance three times. If the standard deviation of the replicate absorbances is much greater than the uncertainty (tolerance) expected for the instrument (see [Operating Instructions for Spec 20 Visible Spectrometers](#)), there may be a technical issue with the spectrometer!

Notebook

Record detailed notes about your lab work in your notebook each time you are in lab. Also include the data analysis and responses to the reflection prompts*. Your notebook should be submitted on canvas prior to leaving Wet Lab each week - whatever state it is in at the end of lab is the state in which you should submit your work. If you are unable to complete data analysis and the reflection prompt within the period, it should be completed before the next lab period, and submitted with your next notebook submission.

Reflection Prompt Comparing Tools for Calibration

Your team has now completed calibration curves using two types of solution preparation tools: Class A volumetric glassware and automatic pipettes. Use your data and your experience to **compare these tools** in terms of both **analytical performance** and **usability**.

In your response, address the following:

- **Precision and Accuracy:** Which tool gave more precise and accurate results? Use the the following as evidence to support your answer:
 - **calibration statistics** from each calibration fit (correlation coefficient, error in slope, error in intercept)
 - **calculated error in solution concentrations** (from error propagation calculations).
- **Convenience and Practicality:** Which set of tools was faster or easier to use? Which was more prone to mistakes or required more careful technique? Use your direct experience from the lab session to support your reasoning.

Your reflection should demonstrate that you can use both **data** and **scientific reasoning** to justify your calibration strategy decisions.

*Reflection prompts should be addressed in your lab notebook and should be included with the lab notebook submission.

Shut down\cleanup

Only when you are absolutely sure that you are finished for the day, please do the following shut-down and cleaning tasks:

- Store solutions in screw-top containers. Add parafilm around the seal of the cap. Be sure all containers are labeled with your

name, date, section, and the contents of the container.

- Bromocresol green and sodium acetate waste should go in the **labeled waste container in the hood** dedicated for waste. Do not overfill the waste - notify a TA if the waste is above the shoulder of the bottle (where the bottle begins to narrow).
 - Volumetric pipetes should be rinsed with deionized water to remove excess reagent solution, and then placed in the **pipete washer with tips UP (toward the ceiling)**. The pipete washer is a huge white plastic tube with a screen at one end. It will be in the sink near the drawers where the pipettes are stored.
 - Rinse all other glassware 3 times with deionized water and remove all labels. Dry the glassware as well as possible and return it its storage location.
 - Check the sink and other areas where you worked to be sure you did not leave anything lying around.
 - Replace all automatic pipetters to their racks and place the tips nearby.
 - Wipe down the bench and sink area with a damp paper towel.
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Sources:

1. Skoog, D. A.; West, D. M.; Holler, F. J. Fundamentals of Analytical Chemistry, 7th edition, Saunders College Publishing, 1996.
2. Pickering, M. and Heiler, D. J. Chem. Ed., 1987, 64(1), 81.

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2.3: Week 2

Pre-Lab Assignment



Due: This pre-lab assignment is due prior to your Wet Lab meeting on the second week of classes.*

*The pre-lab assignments in this course are due prior to arriving at the Dry-Lab meetings — *except for this first module*. Due to the unique schedule in the first two weeks of the semester, the first two pre-labs will be due prior to arriving at Wet Lab meetings. Please note that starting the third week, all pre-lab assignments are due prior to the dry lab meeting (Monday)!



Purpose: The first part of this pre-lab is designed to help you reflect on your Week 1 and prepare you for experimental design in Week 2. This preparation will also support your ability to prepare a high quality post-lab report.

Week 2 Pre-Lab Tasks: Part 1

1. **Complete and submit any unfinished business from last week as part of this pre-lab submission.**
2. **Begin preparing your report:**
 - a. **Prepare a data table** that summarizes the concentration vs absorbance data for both calibrations.
 - Include propagated error for each standard's concentration. (Show at least one full worked example in your notebook).
 - Include average absorbance readings with standard deviation.
 - Do not include replicate absorbance readings - that belongs in your notebook, but not in a report.
 - b. **Prepare figures** to summarize your validated Session 1 data (the instructions here assume you have data to compare volumetric glassware to automatic pipettes).
 - Both curves plotted on the same axes (overlaid on the same plot).
 - Both regression models (lines) plotted.
 - Report the statistics for each linear regression.
 - c. **Discuss your results.**



Purpose: The second part of this pre-lab is to prepare you to engage in productive discussion and planning immediately upon arriving to the Week 2 Wet Lab session. You will need to discuss the plan with your teammates and come to consensus before beginning to work.

Week 2 Pre-Lab Tasks: Part 2

1. **Prepare data tables and a procedure for the following:**
 - **Priority 1:** Create a calibration using the opposite set of tools that you used last week. (Each person should try once with volumetric glassware and once with pipettes.)
 - **Priority 2:** Repeat your calibration from last week if you could not validate your curve.

Dry Lab Agenda

There is no Dry Lab meeting this week due to the Labor Day Holiday.

Wet Lab, Week 2: Comparing Strategies for Dilution (Serial vs. Individual)

Based on the results from part 1, and in consultation with your instructors, create new calibrations. Work with your team so that

each person contributes one additional calibration curve to the dataset.

Notebook

Record your plan, your lab work, and your response to the reflection prompt in your notebook. Your notebook should be submitted on canvas prior to leaving Wet Lab.

Reflection Prompt 2.3.2: Comparing Dilution Strategies for Calibration

Each person has now completed calibration curves using two different sets of tools, and as a group, you have more replicates on which to base your judgement: Use your calibration data and your lab experience to evaluate these two sets of tools.

1. **Calibration Quality:** Were all the calibration curves (from Weeks 1 and 2) validated using the control solution? Are there any trends in accuracy and precision depending on the type of tool used?
 - Include discussion of whether the validation standard (control) result was within the expected range for each tool set.
 - Discuss any differences in R^2 , error in slope, and error in intercept between the two strategies.
 - Discuss any differences in propagated errors.
2. **Convenience of micropipettes vs volumetric glassware:** Were either of the methods easier or more convenient?
3. **Overall Method Recommendation:** If you were asked to design a calibration for a future experiment, which tools and which dilution strategy would you choose, and why? What parameters (eg time constraints, requirement for reproducibility) might change your decision?

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2.4: Post-Lab

This assignment is designed to help you practice presenting scientific results: you will practice writing a results section and writing a discussion section—two essential skills you will use throughout this course and in your future work as a scientist. In this post-lab, you will interpret data, communicate findings through clear figures and statistics, and reflect on your experimental design choices. These are the same skills you will apply in the three major projects later this semester.

This assignment is graded and considered final—revisions are not accepted. Use it as an opportunity to check your understanding of calibration, error analysis, and data presentation before we move into larger projects. Completing this assignment thoughtfully will help you identify strengths and areas for improvement before those higher-stakes assignments.

Post-Module Assignment

1. **Results Section:** In your own words with data tables and plots created by you:
 - **Clear figures that show your calibration curves.**
 - Label each curve with the tool used (Class A glassware or automatic pipettes).
 - Overlay the curves for easy comparison.
 - **Clear Descriptions:** All figures should be introduced/described to the reader using text - just showing data is not sufficient.
 - State the key calibration statistics for each curve: For example, R^2 , slope $\pm$ error, intercept $\pm$ error.
 - State whether each calibration was validated using your control solution.
 - Report the calculated concentration of your unknown sample using each method.
2. **Discussion Section:** Write a short discussion (~300–500 words) that:
 - Compares the results: Which tools produced more accurate and precise data? Back this up with your statistics and your error propagation results.
 - Explains which method was easier, faster, or more practical for you in the lab.
 - States your recommendations to your future self: When would you choose Class A glassware vs automatic pipettes? What advice would you give yourself next time to design a calibration plan that works best for your goal?

Note on Writing and Format of Post-Lab Assignments

Beginning with this module, all post-lab report sections should be written in the style of a scientific manuscript. For Module 2, this means you are responsible for writing complete **Results** and **Discussion** sections, with clear headings, figures, and tables formatted appropriately. Later modules will expand to include additional manuscript sections (Introduction, Methods, Conclusions, etc.), but you should treat each post-lab from this point forward as a piece of a professional scientific manuscript.

Reminders and Tips:

- Review the expectations for a figure (PChem Lab!) to be sure your data plots, tables, and their captions are meeting expectations.
- Use correct units and report values with appropriate errors and certainty (sig. figs. and errors).
- Describe each piece of data using text in the results section. Figure captions and data alone are not sufficient for a results section.
- Proofread your work for clarity.
- Think of this as writing a small piece of a scientific report for your future research self.
- Think of this as practice for writing the results and discussion sections for future assignments in this course.

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2.5: Week 2 - Individual vs Serial Dilution

Pre-Lab Assignment



Due: This pre-lab assignment is due prior to your Wet Lab meeting on the second week of classes.*

*The pre-lab assignments in this course are due prior to arriving at the Dry-Lab meetings — *except for this first module*. Due to the unique schedule in the first two weeks of the semester, the first two pre-labs will be due prior to arriving at Wet Lab meetings. Please note that starting the third week, all pre-lab assignments are due prior to the dry lab meeting (Monday)!



Purpose: The first part of this pre-lab is designed to help you reflect on your Week 1 and prepare you for experimental design in Week 2. This preparation will also support your ability to prepare a high quality post-lab report.

Week 2 Pre-Lab Tasks: Part 1

1. **Complete and submit any unfinished business from last week.**
2. **Begin preparing your report:**
 - a. **Prepare a data table** that summarizes the concentration vs absorbance data for both calibrations.
 - Include propagated error for each standard's concentration. (Show at least one full worked example in your notebook).
 - Include average absorbance readings with standard deviation.
 - Do not include replicate absorbance readings - that belongs in your notebook, but not in a report.
 - b. **Prepare figures** to summarize your validated Session 1 data.
 - Both curves plotted on the same axes.
 - Both regression models plotted.
 - Report the statistics for each linear regression.
 - c. **Discuss your results.**



Purpose: The second part of this pre-lab is to prepare you to engage in productive discussion and planning immediately upon arriving to the Week 2 Wet Lab session. You will need to discuss the plan with your teammates and come to consensus before beginning to work.

Week 2 Pre-Lab Tasks: Part 2

1. **Design a New Dilution Plan** to create two new sets of calibration standards:
 - a. Serial dilution (can be similar to what you did last time)
 - b. Individually prepared standards
 - c. Show one worked example for each.
2. **Prepare a New Data Table**
 - a. Prepare new tables for Session 2 measurements.
3. **Reflection Prep:** Read the Session 2 Reflection Prompt.
 - a. Write 2-3 bullet points predicting what you expect your results will show.

Dry Lab Agenda

There is no Dry Lab meeting this week due to the Labor Day Holiday.

Wet Lab, Week 2: Comparing Strategies for Dilution (Serial vs. Individual)

Based on the results from part 1, and in consultation with your instructors, design a strategy to create two new calibrations. Use the same set of tools for both calibrations, but for one, use serial dilution, and for the other, use individual preparation from a stock. Work with your team so that each person contributes one calibration curve to the dataset.

Notebook

Record your plan, your lab work, and your response to the reflection prompt in your notebook. Your notebook should be submitted on canvas prior to leaving Wet Lab.

Reflection Prompt 2.5.2: Comparing Dilution Strategies for Calibration

You have now completed calibration curves using two different dilution strategies: A serial dilution (where each standard is made from the previous one), and a set of individually prepared standards (where each standard is made directly from a stock solution). Use your calibration data and your lab experience to evaluate these two approaches.

1. **Calibration Quality of Serial vs Individual Dilution:** Were both calibration curves (from Week 2) validated using your control solution? How do they compare in their accuracy and precision?
 - Include discussion of whether the validation standard (control) result was within the expected range for each method.
 - Discuss any differences in R^2 , error in slope, and error in intercept between the two strategies.
 - Discuss any differences in propagated errors.
2. **Convenience of Serial vs Individual Dilution:** Were either of the methods easier or more convenient?
3. **Overall Method Recommendation:** If you were asked to design a calibration for a future experiment, which tools and which dilution strategy would you choose, and why? What parameters (eg time constraints, requirement for reproducibility) might change your decision?

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3: WHAT'S ON YOUR MONEY? DESIGNING ANALYTICAL STRATEGIES FOR TRACE ORGANIC RESIDUES

Learning Objectives

- Students will **describe the molecular-level models** of chromatographic separation and mass spectrometric identification that are relevant to their work.
- Students will **use models to explain** how chromatography and mass spectrometry separate and specifically identify trace organic material.
- Students will **use evidence from the literature** to design a protocol for sample preparation and a method to perform qualitative and quantitative analysis in the context of detecting and quantifying trace organic material.
- Students will **demonstrate competence with instrumentation and instrument software** to obtain and analyze chromatographic and mass spectral data.
- Students will apply chemical knowledge to **analyze and interpret chromatographic and mass spectral data** in order to identify compounds and quantify illicit substances on currency.
- Students will **communicate the broad relevance, specific goals, methods, results, and analysis** of their work in the form of a scientific manuscript.

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[3.1: Introduction - Money](#)

[3.2: Week 1 - Money](#)

[3.3: Week 2 - Money](#)

[3.4: Week 3 - Money](#)

This page titled [3: What's on Your Money? Designing Analytical Strategies for Trace Organic Residues](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Kathryn Haas](#).

3.1: Introduction - Money

What's in your wallet? If you're holding paper currency, you may also be holding trace amounts of illicit drugs. Studies suggest that up to 80% of U.S. banknotes carry measurable levels of cocaine—and some are contaminated with methamphetamine, oxycodone, or other organic residues. These substances are likely transferred through indirect contact, such as with contaminated counting machines or storage environments.

In this experiment, you'll investigate what's on your money. You will be responsible for designing your own sample preparation procedure and calibration strategy to detect and quantify organic contaminants on paper currency. You'll choose what compounds to target—such as cocaine, methamphetamine, or other trace substances—and apply analytical principles to decide which tools and techniques are best suited for isolating and identifying them. Your team will extract material from bills, analyze it using a sensitive detection method that supports compound identification through fragmentation data, and interpret your findings based on comparison with known standards and spectral databases.

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3.2: Week 1 - Money

Pre-lab Assignment:

 **Due:** This pre-lab assignment is due prior to the Week 1 Dry Lab meeting.

 **Purpose:** The first two questions in this pre-lab assignment are designed to engage you in thinking about how organic compounds, such as cocaine, interact with paper currency at the molecular level. By analyzing the chemical structures of both the contaminant and the currency material, you will apply your understanding of intermolecular forces to explain why some organic substances adhere strongly and persistently to surfaces like paper. These foundational chemical principles will help you understand the behavior of trace organic compounds in real-world forensic and analytical contexts.

Questions:

1. **What molecular-level interactions contribute to the adsorption of cocaine onto paper currency?** In your response, include the chemical structures of cocaine and cellulose (the primary component of paper). Draw a representation of how cocaine may interact with the cellulose surface. Identify and explain the intermolecular forces (e.g., hydrogen bonding, van der Waals interactions, ionic interactions) that are likely responsible for the adsorption and retention of cocaine on the surface of currency.
2. **Make a prediction about what organic compounds you will find on your currency during this experiment,** using evidence to support your prediction. Use Web of Science or another literature database to research substances that contaminate currency, and might be found on your money.

 **Purpose:** The second group of questions in this pre-lab assignment will help you practice using scientific literature to justify key decisions in analytical method design. By exploring how researchers select instruments, prepare samples, and use internal standards, you will learn how to propose a method that balances accuracy, safety, cost, and practicality. These skills will support your ability to justify experimental choices in your scientific manuscript.

Questions:

Use a research database to access the articles cited below and add them to your Zotero Library. Use these papers as reference for selection of your analytical methods and sample preparation protocols. *You do not need to read these papers from start to finish. Read the sections of the papers that help you with this assignment.*

Papers:

- Parker, Patrick D., Brandon Beers, and Matthew J. Vergne. "What Is in Your Wallet? Quantitation of Drugs of Abuse on Paper Currency with a Rapid LC-MS/MS Method." *Journal of Chemical Education* 94, no. 10 (2017): 1522–26. <https://doi.org/10.1021/acs.jchemed.7b00196>
- Negrusz, A, JL Perry, and CM Moore. "Detection of Cocaine on Various Denominations of United States Currency." *Journal of Forensic Sciences* 1972-2005 43, no. 3 (1998): 626–29. <https://doi.org/10.1520/JFS16193J>.

1. **What instrument will you use?**
 1. What kind of instrument detector enables specific identification and quantitation of trace organic analytes?
 2. What kind of instrument component can separate compounds in a mixture so that they can be analyzed by the detector described in (a).
 3. Propose an instrument that you will use to detect and quantify trace organic compounds on your currency. Justify why this instrument is the right choice for specific identification and quantitation of very low concentrations of organic analytes.

2. **Suggest a procedure to prepare the sample;** the protocol (1) will extract organic material from currency, (2) is simple and low cost, (3) avoids the use of particularly hazardous solvents (like dichloromethane or chloroform), (4) takes less than 30 minutes to prepare a sample, and (5) does not destroy the currency.
3. In the two manuscripts above, **what internal standards were used** during calibration and quantitation?
4. **Will you use an internal standard?** Decide whether you think an internal standard is necessary. Justify your answer with evidence from the papers or from other reliable sources.

Dry-Lab Group Discussion:

In this discussion, you will collaborate with your research team to refine your understanding of how organic compounds interact with paper currency and you will refine your extraction protocol and analysis method. By drawing molecular interactions, evaluating extraction protocols, and discussing calibration strategies, you will apply chemical principles and literature evidence to make informed decisions about your experimental approach. This discussion should will be facilitated in small groups (2-4 students) by a member of the instructional team.

Instructions:

- Begin the session by grouping up and comparing the answers to your pre-lab questions in your group for ~5 minutes.
- Assign roles (scribe, reporter, spokesperson, time keeper)
 - The Time Keeper will start a timer and encourage group consensus.
 - The Scribe will record the group work.
 - The Spokesperson will summarize your group's discussion and questions to the instructors.
 - The reporter will submit the group record at the end of class.
- Write your work on the board and try to come to consensus as a group.
- If you have trouble reaching consensus, consult another group for advice, or consult an instructor when they are available.

Goals for Dry-Lab discussion:

1. Team consensus about method choice: Which instrument is best for this application, considering availability of instruments in the undergraduate learning labs?
2. Team consensus about extraction protocol: How will you prepare the sample?
3. Team consensus on calibration strategy: Will you use an internal standard?

Group Discussion Prompts

1. **Predict the relative amounts of cocaine and methamphetamine you will find on your currency.** Using your pre-lab response as a starting point, draw on the board:
 1. A molecular-level picture of how cocaine is adsorbed onto cellulose-based currency. Include chemical structures and show the intermolecular interactions.
 2. Do a similar analysis for methamphetamine and cellulose. Do you expect methamphetamine to absorb onto cellulose as well as cocaine does? Justify your prediction using the molecular structures as the basis.
 3. Predict the relative amounts of methamphetamine and cocaine on paper currency.
2. **What instrument will you use, and what are the appropriate settings?** Consider the instruments to which our course has access, and use the literature methods as a starting place. How will you need to modify instrument settings to see them maximum number of possible contaminants?
3. **Discuss your proposed extraction protocol with your group,** consider the limitations stated below, and finally consult your instructors. Discuss what are the costs and benefits of the suggested approaches and come to a group consensus about what protocol your team will use.
 1. Limitations of the analytical method: For example, if using gas chromatography, the sample should be dissolved in a solvent with a low boiling point so that the solvent elutes quickly and does not interfere with detection of the analyte ions. If using liquid chromatography, common mobile phases are water, methanol, and acetonitrile.
 2. Limitations of analytical solution preparation: If the solvent is too volatile, it will be challenging to transfer quantitatively, and difficult to prevent evaporation (which would affect concentration).

3. **Limitations imposed by purchased standards:** Since we cannot purchase these controlled substances without a license, the standards are purchased as dilute solutions. The standard solutions (cocaine, methamphetamine) are only available as dissolved in Methanol - this solvent is versatile and suitable for both GC and LC applications.
4. **Solubility:** The solutes must be soluble in the solvent.
5. **Safety:** Some solvents are not only flammable, but also particularly toxic. Do not use solvents that are particularly toxic, such as chlorinated solvents (chloroform and dichloromethane).
4. **Discuss your proposed calibration method with your group.** Specifically, discuss whether you think an internal standard should be used in calibration, and justify your response using evidence from analytical principles and the literature. Please consider the limitation that deuterated analytes are typically expensive.
5. **Write detailed protocols** for sample extraction and calibration strategy in your notebook to prepare for the wet-lab session.

Wet-Lab Agenda:

1. **Lessons and Demonstrations:** Your TA will give a short lesson about the instrument you will be using for analysis. Draw diagrams of the instruments and equipment in your notebook.
 - **Instrument:** Draw a diagram of the instrument components in your lab notebook. Include and label the autosampler, sample, injector, the components of the chromatography system, and the components of the mass spectrometer.
 - **Syringe-Driven Filter:** Draw the syringe driven filter device in your notebook.
 - **Autosampler vials:** Draw a diagram of the autosampler vial.
2. **Sample Preparation:** Confirm that all group members have the same plan for sample extraction. Record any observations and deviations from the planned protocol in your notebook and perform the sample extraction.
3. **Instrument method:** Choose or write an appropriate method for analysis of your samples. Record the details of the instrument settings in your notebook.
4. **Prepare sequence for sample injection.** Use the instructions in the instrument protocol to create a sequence file to inject the following:
 - A blank (the solvent matrix of your samples)
 - Sample(s)
 - A standard mixture
5. **Group discussion about electron ionization mass spectrometry.** (while waiting for samples to run)
 - Search for cocaine mass spectrum using the NIST WebBook.
 - Identify mass ion (303), and two most abundant fragment ions (82, 182) of cocaine.
 - Explain how electron ionization created the mass ion and the fragment ions (all are $z=1$).
6. **Prepare sequence for injection of calibration standards** to run overnight.
7. **Export qualitative data figures and report.**
 - Create a table that lists the compounds found, their mass ions, retention times, and confidence in the assignments. An example table is below.

Example Table:

Compound	Ions identified	Found r.t. in standard (min)	Found r.t. in sample (min)	Confidence in compound identification
exampleamine	42, 84	3.42	3.43	94%
examplone	54, 154, 187	5.43	5.42	85%
methylexample	56, 74	Not present	4.38	80%

Notebook

Record detailed notes about your lab work in your notebook each time you are in lab. Also include the data analysis and responses to the reflection prompts*. Your notebook should be submitted on canvas prior to leaving Wet Lab each week - whatever state it is in at the end of lab is the state in which you should submit your work. If you are unable to complete data analysis and the reflection prompt within the period, it should be completed before the next lab period, and submitted with your next notebook submission.

Reflection Prompt 3.2.

1. Briefly describe the extraction procedure your group developed. Explain the reasoning behind your choices, including how these choices can be justified using chemical principles and/or literature sources.
2. Justify your group's decision about whether to use an internal standard in your analysis. If you chose to use one, explain how its chemical properties make it appropriate for this experiment. Support your reasoning using relevant chemical principles (e.g., structural similarity, retention time, ionization efficiency, or extraction behavior).

*Reflection prompts should be addressed in your lab notebook and should be included with the lab notebook submission.

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3.3: Week 2 - Money

Pre-lab Assignment

 **Due:** This pre-lab assignment is due prior to the Week 2 Dry Lab meeting.

 **Purpose:** This pre-lab is designed to prepare you for a productive dry-lab discussion and upcoming data analysis. By organizing your qualitative results and drafting your Methods section now, you'll be ready to share work-in-progress and get early feedback to improve your report.

Follow up from Week 1

1. **Complete and submit any unfinished business from the last meeting.**
2. **Begin preparing your manuscript document:**
 - a. **Complete a draft of your Materials and Methods section.**
 - b. **Complete the Qualitative Results section, including description and figures that show the results of qualitative analysis.**
 - Prepare a table of compounds identified in your sample.
 - Prepare a figure that provides data to support the identification of components of your sample.
 - The table and figures should complement each other.

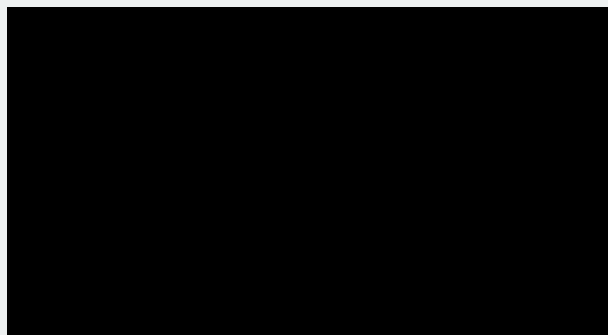
The exercise below uses example data to help you practice choosing between linear and nonlinear regression models using quantitative evidence. This practice will prepare you to analyze your own calibration data in lab this week and make informed, data-driven decisions. Completing it in advance will help you fully engage in the upcoming lesson on quantitative analysis of GC-MS results.

Questions

Practice evaluating regression models for calibration data.

3. **Examine the example data sets below and determine whether a linear or nonlinear regression model is more appropriate in each case.** For each data set:
 - Fit both a linear and a nonlinear model (e.g., quadratic or exponential, as appropriate)
 - Compare the quality of the fits using appropriate error metrics (e.g., R^2 , residual plots, standard error, or percent error in calculated concentrations)
 - Justify your choice of model using quantitative evidence, not just visual analysis.

What patterns in the data would indicate that a nonlinear model may be more appropriate than a linear one? How would an inappropriate regression model affect your interpretation of an unknown?



Hint available at the end of this section.

Dry Lab Group Discussion

In this session, you will work with your research team to improve your qualitative results section, and prepare for quantitative analysis of your calibration data. You will also share drafts of your Materials and Methods section to get early feedback from your peers. By analyzing example calibration data and refining your own data presentation, you will deepen your ability to justify decisions using chemical reasoning and statistical evidence. This discussion will take place with the whole team, and will be facilitated by a member of the instructional team. Please work with your team to follow the instructions below.

An instructor will come address questions as soon as they are available; if for some reason an instructor cannot help you during the session, we will address any questions reported out by your reporter before the end of the day.

Instructions:

- Assign the following roles, then follow the agenda below.
 - The recorder should record the conversation and questions on the board or in a shared electronic document.
 - The time keeper should set a timer with a loud noise to keep the group work on track.
 - The reporter will submit a photo of the white board or the shared document at the end of the period.

Agenda:

1. Discussion about how to interpret and show Qualitative Results - Use the Group Discussion Prompts to engage in discussion.
 - Time: **15 minutes**
 - Record: main points and questions that you have.
 - Prepare to discuss these points with your instructor when they become available.
2. Discussion about regression analysis - Use the Group Discussion Prompts to engage in discussion.
 1. Time: **15 minutes**
 2. Record: main points
 3. Prepare to discuss main points with your instructor.
3. Peer Review: Begin the session by peer-reviewing another person's document draft. Review the Materials and Methods Section and Qualitative Results section, adding constructive and critical feedback.
 - Time: **15 minutes**
 - Please be silent during this time so others can complete the assignment.
4. Discuss Materials and Methods - what questions came up as you reviewed other's work?
 - Time: **15 minutes**
 - Record: main points and questions on the board.

Group Discussion Prompts

Analysis of Qualitative Data:

1. **Confirmation Through Multiple Lines of Evidence**
 - If two compounds have similar retention times, what additional evidence can confirm identity?
 - How can you use both the retention time and fragmentation pattern to confidently assign identity to a peak?
2. **Library Search Validation:**
 - What does the library match score tell you, and how reliable is it?
 - How can comparing the MS of an unknown directly to the MS and retention time of a standard help confirm or challenge the library ID?
 - What should you do if a peak in your sample does not match any in your standard?
 - How would you communicate uncertainty in compound identification in your report?

Regression Analysis:

The form of a linear and a quadratic regression model is given below. Inspect these models:

$$\text{Straight Line: } y = mx + b \quad (3.3.1)$$

$$\text{Quadratic: } y = ax^2 + mx + b \quad (3.3.2)$$

The quadratic model includes more coefficients than a linear model, which gives it greater flexibility to fit the data—even if the underlying relationship is actually linear. This can lead to overfitting and the false impression that the quadratic model is "better." Even when a quadratic model is appropriate over a wide range of concentrations, a linear model might be more appropriate if your sample is at the lower end of the range, within a linear portion of the curve.

3. **Which statistical measures might mislead you into thinking a more complex model is better?**
4. **How can you know which to use?** What additional evidence can help you decide which model is truly more appropriate for your calibration data?

Goals for Dry Lab:

- Deepen your understanding of qualitative data:
 - Results section: Ensure your chromatograms and mass spectra are labeled and ready to use in your report, and that your description of the results is appropriate.
 - Discussion section: Prepare a strategy to write the Discussion of qualitative results.
- Improve your Materials and Methods section: Identify and address any gaps or unclear steps before you move forward with writing.
- Deepen your understanding of Calibration: Review how to evaluate regression model fit so you can apply those skills to your own calibration data later this week.

Wet Lab Agenda

1. **Access your data:** You initiated the calibration in Week 1. You will need to know your file names and in which directory you stored your data so you can locate the data and complete the analysis.
2. **Lessons and Demonstrations:** Your TA will give a short demonstration on how to perform quantitative analysis using the Agilent Mass Hunter Quantitative Analysis software
3. **Perform Quantitative Analysis:** You will work in groups to create a batch file and method for quantitative analysis
 - As a group, you will deliberate on the use of a straight line vs. quadratic regression model in order to most accurately represent your data. *In your lab notebook, make note of the results of any statistical or regression analysis used to come to a decision*
 - Produce a calibration curve using the regression model selected.
 - Perform quantitative analysis on your samples and standards. *Make sure to include error analysis!*
4. **Continue writing** the *Results* and *Discussion* sections of lab report (only after completing the reflection prompts below.)

Notebook

Record detailed notes about your lab work in your notebook each time you are in lab. Also include the data analysis and responses to the reflection prompts*. Your notebook should be submitted on canvas prior to leaving Wet Lab each week - whatever state it is in at the end of lab is the state in which you should submit your work. If you are unable to complete data analysis and the reflection prompt within the period, it should be completed before the next lab period, and submitted with your next notebook submission.

Reflection Prompt 3.3.

- **What regression model did you choose as the best fit for your calibration data?** Justify your choice using appropriate evidence.
- **In the sample data, how do the amounts of cocaine and methamphetamine compare to one another?** Rationalize this observation considering the molecular structures of cocaine, methamphetamine, and cellulose. You may also speculate about other factors that affect the presence of these substances on currency, as you see fit.

*Reflection prompts should be addressed in your lab notebook and should be included with the lab notebook submission.

Answers

Questions

An appropriate analysis of fit should include a plot of residuals. You've practiced this before in your P-Chem coursework—reviewing your residual plots from the FTIR of HCl and the Apples Lab (Matlab scripts) may help refresh your memory and guide your approach here.

3.4: Week 3 - Money

Pre-lab Assignment

 **Due:** This pre-lab assignment is due prior to the Week 3 Dry Lab meeting.

 **Purpose:** This pre-lab is designed to prepare you to share work-in-progress and get early feedback to improve your report.

Follow up from Week 2

1. **Complete and submit any unfinished business from the last meeting.**
2. **Prepare your manuscript document:**
 - a. **Update the Materials and Methods section** based on last week's peer feedback.
 - b. **Update the Qualitative Results section** based on last week's peer feedback.
 - c. **Write your Discussion of Qualitative Results.**
 - d. **Write the Quantitative Results section, including description and figures that show the results of quantitative analysis.**
 - Prepare a table that communicates the results of calibration and quantitation.
 - Prepare one or more figures that shows calibration data.
 - The table and figures should complement each other.
3. Bring a **printed copy** of your manuscript to the Dry Lab meeting.

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Dry Lab Group Discussion

In this session, you will peer review each other's reports and practice helping each other by giving impactful, actionable feedback.

Wet Lab Agenda

Record all work in your lab notebook as usual. If you are not doing wet-lab work in this session, please keep notes of your writing-related conversations in your lab notebook and complete the reflection prompts as usual.

1. **Revise Manuscript and Write Discussion, Conclusions, and Introduction Section.**
 2. **Meet with Writing Consultants.**
-

Notebook

Record detailed notes about your lab work in your notebook each time you are in lab. Also include the data analysis and responses to the reflection prompts*. Your notebook should be submitted on canvas prior to leaving Wet Lab each week - whatever state it is in at the end of lab is the state in which you should submit your work. If you are unable to complete data analysis and the reflection prompt within the period, it should be completed before the next lab period, and submitted with your next notebook submission.

Reflection Prompt

1. **How has writing about your lab experience supported your learning?**

2. **How has working with the EWC's supported your writing and learning?**
3. **What have you done that has supported your learning in this module? What could you do better in the future?**
4. **What were the ways in which your instructional team supported your learning? What could they do better in the future?**

4: QUANT-TEA-FY IT! HOW MUCH CAFFEINE IS IN YOUR DRINK?

Learning Objectives

After completion of this module you will be able to:

- **Describe and use molecular-level models** of electronic absorption spectroscopy and chromatographic separation to explain how analytes are detected and separated, including limitations of each method.
- **Apply chemical knowledge and instrumental theory to design, justify, and evaluate** quantitative analyses of mixtures using UV-vis spectroscopy and HPLC.
- **Demonstrate reproducible preparation of calibration standards and justify choices** of techniques that ensure accuracy in quantitative analysis.
- **Choose and evaluate calibration models**, using calibration statistics and estimated uncertainty to interpret results and assess method suitability.
- **Demonstrate competence with instrumentation and software** by selecting appropriate operating parameters, troubleshooting data collection, and critically analyzing spectroscopic and chromatographic output.
- **Interpret and integrate spectroscopic and chromatographic data** to identify analytes and determine their concentrations in complex mixtures.
- **Communicate** the significance, goals, methods, results, and interpretations of the work in a professional scientific manuscript format.

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[4.1: Introduction - Tea](#)

[4.2: Week 1 - Tea](#)

[4.3: Week 2 - Tea](#)

[4.4: Week 3 - Tea](#)

[4.5: HPLC procedure and instructions for data analysis](#)

[4.6: TA Notes](#)

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4.1: Introduction - Tea

Caffeine is a naturally occurring alkaloid found in coffee, tea, chocolate, and many energy drinks. It is one of the most widely consumed psychoactive substances in the world, valued for its stimulating effects on the central nervous system.^{1,2} While moderate caffeine intake is generally considered safe for most adults, excessive consumption can lead to health concerns such as insomnia, elevated heart rate, and increased blood pressure.³ Regulatory agencies, including the U.S. Food and Drug Administration, recommend labeling caffeine content for certain products, making accurate quantitation an important public health and consumer information goal.⁴

In this module, your team will design a method to quantitate the caffeine content of a variety of beverages.⁵ You will choose the samples, prepare and analyze calibration standards, validate your methods using control samples, and apply your results to determine caffeine concentrations. Along the way, you will connect molecular-level models of light absorption and chromatographic separation to the data you collect, gaining both conceptual understanding and practical skills in quantitative chemical analysis.

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This module was adapted from an experiment published on LibreTexts by University of California Davis ([CHE 115](#)); see page attributions below.

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4.2: Week 1 - Tea

Pre-Lab Assignment

 **Due:** This pre-lab assignment is due prior to the Week 1 Dry Lab meeting.

 **Purpose:** Prepare for the Week 1 Dry Lab meeting and Wet Lab session by selecting your beverage, planning your calibration strategy, and collecting background information for key parts of your scientific manuscript.

Pre-Lab Tasks for Week 1

1. Propose at least four beverage samples to examine, and research their caffeine content:

(This is just an initial proposal, you will refine it with your team during the Dry Lab meeting.)

- Select at least four beverage samples that you would be interested in examining.
- Research their approximate caffeine concentration using reputable sources (e.g., USDA FoodData Central, peer-reviewed publications, manufacturer data).
- Save the source(s) to your Zotero library and cite in ACS style.

2. Propose a method to quantitate caffeine content of your beverage sample.

- Be sure you propose methods that are accessible to your team.
- Justify your proposal with evidence from at least one peer-reviewed literature source.
- Cite sources in ACS Style.

3. Draft Introduction (partial)

- Write 1–2 paragraphs of background for your Introduction section. (This is just a first draft.)

Include:

- Brief explanation of caffeine's significance (chemical properties, psychoactive nature, health considerations), with citations.
- Why accurate caffeine quantitation matters (public health, labeling regulations, quality control), with citations.
- The structure of caffeine (create a figure using ChemDraw) and its chemical or physical properties that allow its detection by the proposed method.
- Include at least three references in ACS style.

4. Proposed plan to create calibration standards & to create diluted beverage samples

(This is just a first draft, you will refine it with your team during the Dry Lab meeting.)

- Select the tools (glassware, pipettes) and create a dilution plan for creating caffeine calibration standards in the *approximate* range **0.005 – 0.04 g/L** (the range does not need to be exact).
- Determine the dilution factor needed to bring your beverage sample into the middle of your calibration range. Keep in mind that your highest calibration point should be between 1.5 to 3 times more concentrated than that of your sample.
- Present your plan in a clear, stepwise outline or table.

5. Prepare a draft data table template

(This is just a first draft, you will refine it with your team during the Dry Lab meeting.)

- Create a table to record your UV-Vis absorbance readings for calibration standards and beverage samples.
- Include columns for:
 - Sample ID
 - Caffeine concentration (g/L)
 - Absorbance at λ_{max}
 - Replicate #
 - Notes/Observations

Submit your pre-lab assignment on Canvas, and be prepared to justify your plan for solution preparation to your team.

Dry Lab Group Discussion

The purpose of today's Dry Lab is to share and critique your individual pre-lab plans for calibration and sample preparation. By the end of this session, you should have considered multiple approaches, anticipated challenges, and refined your own plan for Wet Lab. You will also begin drafting sentences for the *Methods* section of your manuscript.

Instructions:

- Begin the session by grouping up and assigning roles (scribe, reporter, spokesperson, manager)
 - The Manager will start a timer and encourage group discussion and consensus.
 - The Scribe will record the group work.
 - The Spokesperson will summarize your group's discussion and questions to the instructors.
 - The reporter will submit the group record at the end of class.

Goals for Dry Lab:

1. Work as a team to refine your calibration plan and sample dilution plan so you can start preparing solutions right away in Wet Lab.
2. Work with your team to make a plan for dealing with technical issues during data collection.
3. Begin writing the Methods section of your manuscript.

Group Discussion Prompts

1. **Calibration Choices:** What choices did you each make when designing the plan for your calibration standards?
 - What choices did you each make when designing calibration standards (concentration range, number of points, dilution method)?
 - What tools did you select, and why?
 - How much of each standard will you each produce? Will your volumes provide enough material for UV-Vis (~3 mL), HPLC (~2 mL), and filtering?
 - Share your plans for creating the calibration standards. Is the strategy reasonable considering the necessary volumes and the tools available?
 - After discussion, what adjustments (if any) will you make?
2. **Sample:** Each group member should choose a unique sample. No two group members should use the same exact beverage.
 - Why do we dilute beverage samples instead of analyzing them directly?
 - Compare each member's dilution strategy for the chosen beverage:
 - Is it reasonable?
 - Is the anticipated diluted sample concentration within the calibration range?
 - What challenges might arise with your particular beverage (e.g., temperature, carbonation, particulates, solubility)?
3. **Validation & Troubleshooting**
 - What will you do if your standards give absorbances outside the quantitative range for UV-vis (0.2–0.8 au)?
 - Are shorter path-length cuvettes realistic to use, or is further dilution more practical? Do you need to collect UV-vis data on every standard to confirm accurate sample preparation?
 - Do you have enough solution to accommodate repeats or adjustments?
4. **Data Collection and Sharing**
 - Please see the canvas page describing the **CHEM401L Data Drive** (CHEM401L Data on Google Drive).
 - How will your group share data files (UV-Vis scans, calibration results) to make sure everyone has multiple examples for their reports?
 - What naming conventions or folders will you use to avoid losing or mixing up files?
5. **Writing:** While this discussion is fresh in your mind, write a few sentences that can support the Methods section of your manuscript.
 - Draft one sentence describing your calibration preparation.
 - Draft one sentence describing your beverage preparation.
 - Draft a one-sentence description of how you will validate your calibration.

Wet Lab Agenda

Goals: (1) Prepare a beverage sample and analyze it with your chosen method(s). (2) Prepare a set of caffeine calibration standards using your preferred method and tools, and validate your calibration solutions.

Do not throw away any solutions until the very end of this 3-week module! Plan to **store your solutions in sealed vials in the refrigerator for the entire 3 weeks.*

Prepare the instrument(s)

- Turn on the instruments and allow an appropriate warm up period.
 - If using a Cary UV-vis: [Validate](#) the instrument after warm-up, and before using it.
 - If using a Spec 20, be sure to "blank" often to avoid issues with lamp intensity drift.
 - If using HPLC, begin column equilibration and run two blanks after lamp warm up and before injecting sample.
 - If using GSCM, run two blanks before injecting sample.
 - If using another instrument, consult your instructor.

Prepare Caffeine Calibration Standards

Materials required:

- Caffeine (99% powder)
- Ultrapure water
- Analytical balance (no more than 0.5 mg uncertainty)
- Caffeine Validation Control solution (provided by TAs)
- Equipment for preparing solutions

You have access to:

- volumetric flasks to contain of 5, 10, 25, 50, 100, 250, 500, and 1000 mL
- volumetric pipette to deliver volumes of 1, 2, 3, 4, 5, 10, 15, 20, 25, 50, and 100 mL
- automatic pipetters to deliver between 100-5000 mL

A. Example protocol to prepare a stock solution of 0.1 g/L caffeine

1. *Quantitatively* weigh out between 8.0 to 10.0 mg of caffeine. The caffeine can be found on the shelf near the weigh station area.
2. *Quantitatively* transfer the caffeine into a clean 100 mL volumetric flask. Rinse the weigh boat with ultrapure water to be sure all powder is rinsed into the flask.
3. Dilute to the volumetric mark with ultrapure water. The stock solution will have a final concentration of ~ 0.1 g/L. Calculate the *true concentration* based on the actual mass of caffeine used.

Warning

(1) You must use *quantitative technique* to transfer the caffeine from the weigh paper to the receiving flask. After transferring the powder to the flask, rinse the weigh paper carefully with water, and capture the rinse with the flask to be sure that no trace powder is left behind.

(2) There is a very small amount of caffeine powder, but it takes some time to dissolve completely. Be sure that caffeine is completely dissolved in about 50 mL of water before diluting to the volumetric mark and before proceeding to the next step. There is a sonicator in the lab that you can use to help break up small caffeine particles and aid in dissolution.

B. Prepare calibration standards

Write out a specific plan for doing this before you get to lab (see pre-lab assignment). Carry out a serial dilution to obtain a series of calibration standard solutions that are approximately evenly spaced (in terms of their concentrations). The highest concentration should be about 0.04 ± 0.01 g/L, and the lowest concentration solution should be approximately 0.005

± 0.001 . Make enough of each solution so that you have *at least* 10 mL of each solution to play with. You should use ultrapure deionized water to make all solutions and dilutions. Ensure adequate mixing in each diluted solution before using it to create the next.

Prepare the Beverage Sample(s)

Each student is responsible for bring about 50 mL of at least one unique caffeinated beverage sample for testing. All together, the team is responsible for bringing four different (unique) beverage samples. Beverages used in lab should not be consumed.

Store beverage solutions at 4° C

Beverages and beverage samples must be stored in the refrigerator to avoid microbial growth. Warm all liquids to room temperature before measuring volumes!

Dilute the beverage samples to reach a concentration that can be quantitated by your calibration range (based on the expected caffeine content of the beverage, and within the range of the calibration standards).

Example beverage preparations:

1. **A Coffee, which is estimated at ~ 0.4 g/L caffeine.**
 - a. Pipette 5 mL of coffee into a clean and dry 100 mL volumetric flask and dilute to the mark with clean deionized water.
 - b. Filter the sample using the provided filter.
 - First, rinse the filter three times with 3 mL clean deionized water.
 - Rinse the filter with the sample solution by filtering the first 1-2mL of the sample into the waste beaker.
 - After rinsing the filter apparatus with sample solution, filter the sample directly into a clean vial and label the vial.
2. **A Tea, which is estimated at ~ 0.2 g/L caffeine.**
 - a. Pipette 10 mL of tea into a clean and Dry 100 mL volumetric flask and dilute to the mark with clean deionized water.
 - b. Filter the sample using the provided filter.
 - First, rinse the filter three times with 3 mL clean deionized water.
 - Rinse the filter with the sample solution by filtering the first 1-2mL of the sample into the waste beaker.
 - After rinsing the filter apparatus with sample solution, filter the sample directly into a clean vial and label the vial.
3. **A Soft Drink Sample, which is estimated at ~ 0.1 g/L caffeine.**
 - a. If the soft drink you selected is carbonated, decarbonate the soft drink by pouring it back and forth between two beakers until the bubbles cease.
 - b. Pipette 10 mL of the soft drink into a clean and Dry 50 mL volumetric flask and dilute to the mark with clean deionized water.
 - c. Filter the sample using the provided filter.
 - First, rinse the filter three times with 3 mL clean deionized water.
 - Rinse the filter with the sample solution by filtering the first 1-2mL of the sample into the waste beaker.
 - After rinsing the filter apparatus with sample solution, filter the sample directly into a clean vial and label the vial.

Analysis of caffeine sample

Use the method(s) of choice to analyze your sample and the control. After collecting this data, compare the sample to the control, and assess the utility of the method(s) for performing quantitative analysis of caffeine. Some things to consider:

- Are the features attributed to caffeine specific to caffeine, or are there matrix interferences that will prevent conclusive quantitation?

- If there are technical issues, what strategies can you use to overcome the problem?
- How confident are you that this method will be useful for quantitation of caffeine in this specific group of samples?
- Are other methods available that might give more reliable quantitation of caffeine in these samples? (Consider different methods on the same instrument, and methods using a different type of instrument.)

Validation of method and calibration standards

If you are confident that the method chosen is promising, then prepare to validate your method.

If possible, use the method to measure the response to the blank, the calibration standards, the validation control, and the samples. If possible during this lab period, construct your calibration curve, evaluate the quality of your data and the fit, and when you feel that you have a useful data set, use the data to determine if the method is valid. Otherwise, plan to make progress on this next week.

Keep in mind that one reason that a method fails validation is due to improperly prepared calibration standards. Prepare for this possibility, and the fact that you may need to prepare your solutions again if they are not appropriately prepared the first time.

Notebook

Record detailed notes about your lab work in your notebook each time you are in lab. Also include the data analysis and responses to the reflection prompts*. Your notebook should be submitted on canvas prior to leaving Wet Lab each week - whatever state it is in at the end of lab is the state in which you should submit your work. If you are unable to complete data analysis and the reflection prompt within the period, it should be completed before the next lab period, and submitted with your next notebook submission.

Reflection Prompt

1. **What have you learned from your experiments about the method(s), and will it (they) be sufficient for quantitation of caffeine in these samples?**
Justify your response with evidence.
2. **Dealing with unexpected challenges:** During this experiment, what unexpected challenges or technical issues did you experience with measuring the absorbances of your calibration solutions? What strategy did you use to address this issue, and was it effective?
3. **Considering the data you collected, what is the "best" wavelength for measuring caffeine concentration?** Justify your response based on at least two of the following:
 - Beer's Law
 - Sensitivity of the signal to changes in concentration
 - Signal to noise
 - Relative error in the absorption measurement

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4.3: Week 2 - Tea

Pre-lab Assignment

 **Due:** This pre-lab assignment is due prior to the Week 2 Dry Lab meeting.

 **Purpose:** This pre-lab is designed to prepare you for a productive dry-lab discussion and upcoming data analysis. By organizing your results from Week 1 and drafting your Methods section now, you'll be ready to share work-in-progress and get early feedback to improve your report.

Follow up from Week 1

1. **Complete and submit any unfinished business from the last meeting.**
2. **Begin preparing your manuscript document:**
 - a. Complete a draft of your **Materials and Methods**, including sub-sections for **Calibration Solution Preparation**, **Sample Preparation**, and **Instrument Methods**.
 - b. Create a draft of the **Results** section, including the following elements in your manuscript draft (Results section):
 - i. **Sample Analysis and Comparison to Caffeine Standard (or control)**
Show evidence that your sample can be analyzed using this method, that caffeine can be detected in the sample, and that this method is promising for the current application (quantitation of caffeine in the specific group of samples).
 - ii. **Calibration Spectra or Chromatograms**
Show the data (overlaid spectra or chromatograms) that demonstrate that the instrument responds to increasing caffeine in a predictable way.
 - iii. **Calibration Curve Figure**
 - Plot absorbance vs. concentration with best-fit line, equation, and R^2 .
 - Include slope, intercept, and their errors.
 - Apply same figure formatting standards as above.
 - iv. **Calibration Validation**
 - Compare measured value for validation control solution to expected value.
 - Perform error analysis and state whether your curve is validated.
 - If not validated, use a literature molar extinction coefficient for caffeine to re-determine solution concentrations, and explain discrepancies.
 - v. **Some things to keep in mind while preparing figures.** (This is still a draft, but make your figures as well as possible at this time, within reason.)
 - Indicate λ_{max} on spectra with an annotation or in the figure caption.
 - Indicate relevant retention times on chromatograms with an annotation or in the figure caption.
 - Apply formatting standards you learned from previous modules.
 - All figures and tables must have a descriptive title & unique number, in consecutive order, formatted per ACS guidelines.
 - All figures must have axis labels with units.
 - All figures must have a caption that describes what is shown and why it matters.
 - Use legend, line types, and color scheme to make results figures accessible.
 - Use instrument software, python, or another approved plotting tool to create publication-quality figures.
3. **Justifying the method:** Write a short paragraph for your manuscript explaining why the method of choice is valid for quantitation of caffeine in the specific group of beverage samples. Use evidence (data) to support your justification.
4. **Use the Instrument Manual and the HPLC Simulation described below to prepare for a dry lab discussion about the following:**
 - In an isocratic HPLC method, what is the effect of **Solvent B percentage** on peak shape, separation of peaks, and overall retention of the compounds?
 - What is the effect of a **gradient method (from 10%-90% B) compared to an isocratic method at 50% B** on peak shape, separation of peaks, and overall retention of the compounds?

- What method conditions could you use to isolate caffeine from other compounds and obtain an ideal peak shape? What conditions would not work?

Submit your assignment on Canvas and be prepared to discuss your responses with the team during Dry Lab.

Dry Lab Agenda

In this Dry Lab, you will use the HPLC simulator and group discussion to guide your decision-making about which HPLC method is best for analyzing caffeine in beverage samples.

 **Purpose:** To connect your simulator exploration to real-world method design choices. To compare isocratic and gradient methods in terms of peak shape, separation, and practicality. To prepare a justification for the method you will use in your manuscript.

Instructions

1. **Warm-Up (5 minutes, in groups of two or three):** Share your pre-lab justification with your group. Why do we need HPLC analysis after UV-vis calibration?
2. **Small Group Work (25–30 minutes, in groups of two to three):** Discuss the questions below with your group. Use your simulator observations to support your answers. Record your group's responses in notes, including sketches or comparison tables.
3. **Team Discussion (20 minutes):** Groups will report out. We will compare results and weigh trade-offs between gradient and isocratic methods.
4. **Writing Connection (5 minutes):** Draft 1–2 sentences for your manuscript's Methods section that justify the HPLC method choice.
5. **Start Data Collection!**
 - If you are in the Tuesday Lab section, go to lab now, choose the appropriate method in your sequence template and ask your TA to hit "go" as soon as the samples are at room temperature.
 - If you are in Wednesday or Thursday Lab section, arrange a time to add the method to your sequence file and arrange for your samples to be run overnight.
 - If there is not time to run samples before your lab meeting, you will run them during the lab period this week.

Group Discussion Prompts

Simulator Observations

1. In an isocratic method, how does the percentage of Solvent B affect:
 - Peak shape?
 - Separation of peaks?
 - Overall retention time?
2. In a gradient method (10% → 90% B) compared to an isocratic method at 50% B, how do: Peak shape, Separation, and Retention times differ?
3. Based on your observations, which conditions gave you the clearest caffeine peak?

Method Design Decisions

4. If the best possible separation were your only goal, which method would you choose? Why?
5. If the most practical method for repeated beverage analyses were your priority, which method would you choose? Why? (*Consider this:* between sample injections for gradient methods, the column must be re-equilibrated at the starting %B — this usually *doubles* the time required to run samples using a gradient method!)
6. What are the trade-offs of gradient vs. isocratic methods?

Connecting to Practice

7. Why is a gradient method usually superior for peak shape and separation?
8. Despite that gradient methods are superior in some aspects, why might someone choose an isocratic method?
9. Draft a justification statement for your manuscript that explains why the chosen method is appropriate.

Wet Lab Agenda

Note

There are several UV-vis instruments and several HPLC instruments available to reduce waiting time. In addition, there are some extra computers with the UV-vis software and HPLC software so that students can use the instruments for data collection while other students complete data analysis on a separate computer. Please let your team know if you are waiting to use the instrument or the software.

1. **Repeat your solution preparation as necessary** if your solutions from Week 1 failed to create a valid calibration curve.
2. **Create or finish creating a sequence for running your injections on the HPLC.** Apply the method you chose in the Dry Lab meeting. Your sequence should include injections for the following:
 - Always start a sequence with 2 blank injections.
 - Run *All* beverage samples from your group first if possible (each group member should have a unique type of beverage sample). If all samples are not prepared at the time you create your sequence, communicate with your group members so you can access the sample runs from their data collection. The samples can only be quantitated using your calibration curve if they were run using the same *method* and on the same exact instrument.
 - Another blank to be sure no residue from samples is left on the column.
 - Calibration blank
 - All of your calibration standards — start with the most dilute sample first.
 - Another blank to wash the column after the highest concentration standard.
 - Validation control
3. **Analyze the Sample Data:** Collect the data from at least three unique beverage samples (see above). The instructions for data analysis are in the [Instrument Facilities Manual](#). Be sure to capture the chromatograms of each sample, the peak retention times, peak height, and peak area (from the integration of peaks). It is your choice to collect data for all peaks, or just for caffeine.
4. **Analyze the calibration data:** The instructions for data analysis are in the [Instrument Facilities Manual](#). You should record the retention times, peak height, and peak integration of the caffeine peak in each calibration standard and the validation control. Also figures that would support your results section. The calibration data should be processed using a software of your choice (we do not recommend trying to perform calibration and quantitation with the instrument software).
5. **Continue writing** the *Results* and *Discussion* sections of lab report (only after completing the reflection prompts below.)

Notebook

Record detailed notes about your lab work in your notebook each time you are in lab. Also include the data analysis and responses to the reflection prompts*. Your notebook should be submitted on canvas prior to leaving Wet Lab each week - whatever state it is in at the end of lab is the state in which you should submit your work. If you are unable to complete data analysis and the reflection prompt within the period, it should be completed before the next lab period, and submitted with your next notebook submission.

Reflection Prompt 4.3.0

- **If you were not able to collect HPLC data:**
 - What did you do differently this time when creating calibration standards compared to last time?
 - Did your revised strategy work?
 - What did you learn from additional practice at solution preparation?
- **If you were able to collect HPLC Data:**

- How did the real data compare to your expectations? Support your response using appropriate evidence.
- Was the HPLC validation consistent with expectations based on the UV-vis results?
- You are to include the quantitation of at least three samples in your report (your own sample plus two other unique beverages); How do the measured caffeine amounts compare to literature values.
- **What data will be most useful to support your results section?**

*Reflection prompts should be addressed in your lab notebook and should be included with the lab notebook submission.

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4.4: Week 3 - Tea

Pre-lab Assignment

 **Due:** This pre-lab assignment is due prior to the Week 3 Dry Lab meeting.

 **Purpose:** This pre-lab is designed to prepare you to share work-in-progress and get early feedback to improve your report.

Follow up from Week 2

1. **Complete and submit any unfinished business from the last meeting.**
2. **Prepare your manuscript document:**
 - a. **Update the Materials and Methods section** based on last week's work.
 - b. **Update the Qualitative Results section** based on last week's work.
 - c. **Write your Discussion of Qualitative Results.**
 - d. **Write the Quantitative Results section, including description and figures that show the results of quantitative analysis.**
 - Prepare a table that communicates the results of calibration and quantitation.
 - Prepare one or more figures that shows calibration data.
 - The table and figures should complement each other.
3. Bring a **printed copy** of your manuscript to the Dry Lab meeting.

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Dry Lab Group Discussion

In this session, you will peer review each other's reports and practice helping each other by giving impactful, actionable feedback.

Wet Lab Agenda

Record all work in your lab notebook as usual. If you are not doing wet-lab work in this session, please keep notes of your writing-related conversations in your lab notebook and complete the reflection prompts as usual.

1. **Revise Manuscript and Write Discussion, Conclusions, and Introduction Section.**
 2. **Meet with Writing Consultants.**
-

Notebook

Record detailed notes about your lab work in your notebook each time you are in lab. Also include the data analysis and responses to the reflection prompts*. Your notebook should be submitted on canvas prior to leaving Wet Lab each week - whatever state it is in at the end of lab is the state in which you should submit your work. If you are unable to complete data analysis and the reflection prompt within the period, it should be completed before the next lab period, and submitted with your next notebook submission.

Reflection Prompt

1. **How has writing about your lab experience supported your learning?**
2. **How has working with the EWC's supported your writing and learning?**
3. **What have you done that has supported your learning in this module? What could you do better in the future?**
4. **What were the ways in which your instructional team supported your learning? What could they do better in the future?**

4.5: HPLC procedure and instructions for data analysis

Analysis of Caffeine in Beverages

1.

Treatment of Data

The general criteria for writing and submitting lab reports are found in the [orientation module](#). Specific additional expectations for this module are given below.

1. You can print the reports after each run or after all runs are complete. You can find the data reports by clicking the “data analysis” tab in the bottom left toolbar of the instrument control software window.
2. Before completing the lab, confirm that you have peaks for each of your runs. If you notice any issues with your data, talk with your TA.
3. Create a calibration curve using the caffeine standard peak height or area versus the concentration. This can be done using the Chemstation Software or using the calibration template that you created earlier this semester.
 - a. To create a calibration curve in *Chemstation*, start by clicking the “data analysis” tab in the bottom left corner of the window. This will open the HPLC (offline) window.
 - b. Find your data in the file navigator on the left panel, and highlight it.
 - c. Double click the first standard run in the sequence window.
 - d. Find the “Calibration” tab in the menu bar and select “New Calibration Table.”
 - e. A new window “Calibrate: HPLC1” will appear and select “Automatic setup.”
 - f. Set the “level” to 1 and put the concentration of the first run in the “Default Amount.” Press “OK.”
 - g. Double click the second run and go to the “Calibration” menu bar and click “Add Level.”
 - h. Set the “Level” to 2 and enter the second run’s concentration in the “Default Amount.” Press “OK.”
 - i. Repeat steps g and h for each of the remaining standard solutions.
 - j. The Calibration Table and the Calibration Curve can be viewed in the bottom of the window. Confirm that all the points are there.
4. Validate your calibration using a control sample provided by your instructors.
5. Calculate the concentrations of caffeine in each beverage sample. *Note that each beverage was diluted differently.

Post-Lab Questions

Questions for Part 1: Separating a Paraben Mixture

****Include ALL Elution Data Reports****

1. How many compounds are observed in the paraben mixture? Identify each peak on the gradient chromatogram.
2. How would you experimentally confirm that your peak identifications are correct?
3. Compare the results from the isocratic runs to the results from the gradient run.
4. What advantages does the gradient elution offer?
5. What disadvantages does the gradient elution have compared to the isocratic elution?
6. The molar response factor of a given analyte is defined as (Peak area/moles of analyte injected). Why do the response factors of analytes change depending on the wavelength used by the UV detector? How would you experimentally determine what wavelength to use with a certain analyte in order to obtain spectra with the greatest response factor?

Questions for Part 2: Analysis of Caffeine in Beverages

1. Using the calibration curve, determine the concentration of caffeine for each beverage in g/L.
 - a. Describe whether you used peak height, peak area, or both to estimate the concentration. Discuss the advantages to each method.
 - b. Make sure you take into account the dilutions you made when preparing the samples.
2. Discuss the errors potentially made during this experiment.
3. Discuss how retention times depend on methanol and the pH of the mobile phase. What factors contribute to the choice of mobile phase composition and pH in the present analysis.

References

1. Engeli RT, Rohrer SR, Vuorinen A, et al. Interference of Paraben Compounds with Estrogen Metabolism by Inhibition of 17 β -Hydroxysteroid Dehydrogenases. *Int J Mol Sci.* 2017;18(9):2007. [pdf](#)
2. Larsson K, Ljung Björklund K, Palm B, et al. Exposure determinants of phthalates, parabens, bisphenol A and triclosan in Swedish mothers and their children. *Environ Int.* 2014;73:323-33. [pdf](#)
3. McDevitt, V. L.; Rodriguez, A.; Williams, K. R. Analysis of Soft Drinks: UV Spectrophotometry, Liquid Chromatography, and Capillary Electrophoresis. *J. Chem. Ed.* 1998, 75, 625-629. [pdf](#)
4. Skoog, D., Holler, F. J., & Crouch, S. R. (2017). *Principles of Instrumental Analysis* (Seventh ed.). Boston, MA: Cengage Learning.

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4.6: TA Notes

This page is directed toward laboratory teaching assistants, but is deliberately left open for students to read.

TA Notes

After completion of this module students will be able to:

1. Use analytical techniques to create a serial dilution starting from pure, solid material. (Use an analytical balance, volumetric glassware, and pipettes)
2. Use a full-spectrum UV-vis to measure the absorbance of a series of solutions.
3. Determine the best wavelength to monitor the absorbance of an analyte.
4. Validate a series of standard solutions using a control.
5. Explain the principles of liquid-liquid partitioning.
6. Explain the effect of solvent polarity on retention times.
7. Explain the effects of isocratic and gradient methods on retention times and peak shape.
8. Interpret chromatographic data to identify components of a mixture and determine their concentration.

Pre-Semester Preparations

Do the following during the TA training and orientation week(s)

1. Check that the materials listed below are available and sufficient for all students to use them. If they are running low, please create more or inform the instructor.
 - **Caffeine, 99%:** There should be at least ~25 g of caffeine powder available. If not, please inform the lab director.
 - **Volumetric Glassware:** Be sure that there is sufficient glassware for four students to complete the experiment simultaneously. Please inspect the glassware for chips or obvious contamination. Clean or dispose of glassware as needed and inform the instructor if more glassware should be added to the lab space.
 - **HPLC Autosampler vials:** About 6 vials per student
 - **Syringes and syringe-driven filters:** One each per student
 - Access to UV-vis and at least two quartz cuvettes for each student (Glass cuvettes will not work! Students can take turns.)
2. Complete instrument training with Dr. Haas.
3. Perform the entire experiment as if you were a student.
4. Write and submit a report as if you were a student.
5. Practice grading your and your partner's report using the grading rubric.

Pre-Lab Preparations

When you are on TA duty, arrive at least 20-30 minutes early to prepare and ready the following materials for students.

- Check to be sure that caffeine powder is in its place (notify the instructor if we should order more).
- Check to be sure there are cuvettes available for students to use. They should be **quartz** cuvettes, not glass.
- Remind students to sign into lab
- Check that the student's pre-lab is complete
 - Check their proposed calibration points and ensure that the control solution is approximately in the middle of their range.
- Tell students to gather their glassware as soon as they arrive and get set up on a bench space so that they can make solutions.

- They should clean their glassware prior to use.
- The Cary 100 UV-vis instruments should be turned on at the beginning of lab period. Let the students be involved in this.
- Check that all HPLC modules are powered on (please do not touch the buttons on the bottom components!). The degasser (top unit) is the only one that should possibly be turned off at the button on the instrument.
- Check solvent reservoirs and refill if they are less than half full.
- Show students how to open the software and turn the instrument components on from the software.
- Show students how to start a method running to equilibrate the column to the starting conditions of their first run. Be sure that the pressure is reasonable and the instrument is performing as expected. Otherwise, get help.

Helping your students

During the laboratory session, you are expected to be present and available for the entire 4.5 hours. Your job is to help students to complete the experiment safely and successfully, while upholding expectations and academic standards for the students.

Creating Standard Solutions and Analysis by UV-Vis

In this first laboratory session, please provide explicit instruction on the following:

- **Weighing solid material:** They can begin by weighing their caffeine when their glassware is clean. Please show students how to use the balances correctly and remind them that there is error associated with the measurement. (refer to P-chem materials...we assume that it is in the last digit of the reported number.) Show them how to quantitatively transfer their material into a volumetric flask, rinsing the weighing container into the flask with water.
- **For using volumetric flasks:** Remind them to use good technique. We can assume students know how to use these, but.....It takes a while to dissolve the solid material. **DON'T LET THEM MOVE ON TOO QUICKLY!** With powder in flask, fill it about 1/2 to 3/4 with solvent, swirl and sonicate as necessary to dissolve the solid before filling to the line.

Help students to analyze data immediately upon collection. **They should validate their standard curve (UV-vis) using a control solution.** The control solution is provided, and it should be within the concentration range of their calibration curve. If the control is outside their calibration range, a new control should be created. The students should treat the control as a sample in their calibration template, then compare their derived concentration to the known concentration. If the values are the same, then the curve is validated. If not, the students should re-create the standards using correct analytical technique. The students should proceed to the HPLC module only after the calibration is validated.

Encourage students to proceed to the next step and begin creating methods for the HPLC if they have validated their curve and there is additional time in the period.

Analysis of Caffeine by HPLC

Be available to answer questions and troubleshoot any issues as students build HPLC methods and sequence files. Please discuss the following with them:

- General principles of reverse phase chromatography.
- How we predict relative retention of molecules based on their structure and the type of chromatography.
- General expectations for isocratic vs gradient methods.
- Selection of the appropriate wavelength for monitoring caffeine.

During data collection, check to be sure the report is giving peak integrations. If not, please show the students how to manually integrate the peaks and harvest their data electronically.

Remind students to use the control solution to validate their calibration. In this case, if the UV-vis calibration is valid, we expect the HPLC to also be validated. However, if this is not the case, please discuss with the instructor. If the UV-vis calibration was validated, we have confidence that the solutions were made correctly, and there must be another reason that the HPLC calibration has failed.

Shut down/ clean up

15 minutes prior to the end of the period, students should stop lab work and clean up. They are to leave the room in clean condition prior to the end of the period (17:55).

- Remind students when it is time to clean up.
- Remind students to save their samples!
- Turn off the UV-vis
- Remind students that all glassware should be returned to the storage locations.
- Any samples that are stored should be labeled clearly with the student's name and section.
- Remind students to sign out with the time they leave and get your signature.
- Check the TA checklist for additional items to be completed.

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5: TAINTED TAP! WHAT'S IN YOUR WATER?

Learning Objectives

After completing this module students will be able to:

- **Design and justify a calibration strategy** for quantitative elemental analysis, considering when external calibration or the method of standard additions is most appropriate.
- **Analyze and evaluate the influence of matrix effects** on instrument response, and propose strategies to overcome these effects.
- **Apply ICP-OES data to detect and quantify Pb in water**, and assess the results relative to regulatory standards.
- **Interpret differences between data from** unfiltered, tainted, and filtered **samples** to evaluate the effectiveness of a commercial water filter.
- **Integrate scientific literature and regulatory guidelines** to place experimental findings in an environmental and public health context.
- **Communicate research findings** in the format of a scientific manuscript.

5.1: Introduction - Water

5.2: Week 1 - Water

5.3: Week 2 - Water

5.4: Week 3 - Water

5.5: ICP-OES Procedure and instructions for treatment of data

5.6: TA Notes

5.1: Introduction - Water

Access to clean, safe drinking water is a critical public health issue. Even at very low concentrations, toxic metals such as lead (Pb) can pose serious health risks, particularly for children.^{1,2} While water in the U.S. is regulated, problems such as the Flint water crisis^{3,4} and other college campus-related discoveries of Pb in drinking water⁵ show that contamination can still occur. Many people turn to consumer water filters as a solution — but how well do they work to remove Pb and other contaminants from water?

In this module, you will investigate these issues by analyzing drinking water samples with inductively coupled plasma–optical emission spectroscopy (ICP-OES). You will design and justify a calibration strategy, assess matrix effects, and evaluate whether a commercial filter removes Pb and other elements effectively. Finally, you will interpret your results in the context of regulatory standards and communicate your findings in the style of a scientific manuscript.

You will work with your group to address these research questions:

- A. Are there detectable amounts of toxic Pb in the water sources across Duke's campus?
 - B. What other metal contaminants are in your drinking water?
 - C. How well does a commercial water filter remove Pb and other metal ions from drinking water?
-

This module was adapted from Gouger, Ashley R., and Jaime E. Mirowsky. “Using Project-Based Learning to Assess the Effectiveness of Water Filtration Devices in Removing Chemical Analytes in an Undergraduate Analytical Chemistry Laboratory.” *Journal of Chemical Education* 99, no. 12 (2022): 4016–23. <https://doi.org/10.1021/acs.jchemed.2c00338>.

References

1. CDC. “About Lead in Drinking Water.” Childhood Lead Poisoning Prevention, August 20, 2025. <https://www.cdc.gov/lead-prevention/prevention/drinking-water.html>.
2. US EPA, OW. “Basic Information about Lead in Drinking Water.” Overviews and Factsheets. February 2, 2016. <https://www.epa.gov/ground-water-and-drinking-water>.
3. Wikipedia. “Flint water crisis.” August 10, 2025. <https://en.wikipedia.org/w/index.php?did=1305206269>.
4. CDC. “CDC Newsroom.” January 1, 2016. https://archive.cdc.gov/www_cdc_gov/ater-lead.html.
5. The Daily Tar Heel. “Three Hamilton Hall Drinking Fountains Found to Have Detectable Levels of Lead.” Accessed August 27, 2025. <https://www.dailytarheel.com/article...all-lead-short>.
6. Wikipedia. “Lead and Copper Rule.” July 16, 2025. <https://en.wikipedia.org/w/index.php?did=1300860169>.

5.2: Week 1 - Water

Pre-lab Assignment:

 **Due:** This pre-lab assignment is due prior to the Week 1 Dry Lab meeting.

 **Purpose:** Collect background information that can support the introduction section of your report, and prepare to make team decisions about Pb calibration and targets for qualitative analysis. You'll read, calculate, and then propose a plan you can defend to your group.

Pre-Lab Tasks

Read (and add references to Zotero)

- Review the [Methods of Calibration](#).
- Read about how [ICP-OES](#) works, and what you can detect using this method.
- Investigate the EPA's **Lead and Copper Rule**.
 - US EPA, OW. "Lead and Copper Rule." Other Policies and Guidance. October 13, 2015. <https://www.epa.gov/dwreginfo/lead-and-copper-rule>.
 - Wikipedia. "Lead and Copper Rule." July 16, 2025. <https://en.wikipedia.org/w/index.php...did=1300860169>.
- Read [this article](#) about Pb detected in the water on UNC's campus! <https://www.dailytarheel.com/article/2022/09/university-hamilton-hall-lead-short>
- Find at least one reliable source on the health impacts of lead exposure (e.g. CDC, EPA, or peer-reviewed article) and write at least one sentence about the health effects of Pb. Cite your source(s).
- Use reliable sources to identify at least two additional contaminants that could be in your drinking water and that can be detected using ICP-OES. Cite your sources of information.
- Bring your water sample to WET lab (1.0 L of water from a tap at Duke — Due at the start of Wet Lab - collect the sample prior to 1:25 pm)

Pre-Lab Questions

1. According to the EPA:
 - What is the *safe* level of Pb in drinking water?
 - What is the EPA action level for Pb, and how does it shape your expectations for this analysis?
2. Given the EPA action level, what hypotheses do you form about our campus's water, and how will these hypotheses guide your calibration strategy?
3. Design a strategy for preparing the tainted water sample so that it will have 25 ppb Pb. Show your calculations and justify why this concentration is appropriate for the ICP-OES method.
4. Given the choice between **external calibration** and **standard additions**, what factors should your team consider in deciding which to use for this experiment? Describe how matrix effects might influence your decision.
5. Based on your predictions about the water samples and possible matrix effects, which calibration method should your team adopt? Justify your choice by comparing the advantages and limitations of each method in this context. Be prepared to defend your proposal to your team in Dry Lab.
6. If your team adopts the calibration method you proposed, what specific standards will you need to prepare? Show one example calculation and explain why these standards are appropriate for detecting ~25 ppb Pb with ICP-OES.
7. Suppose one team member argues for external calibration because it is simpler and faster, while another argues for standard additions because of possible matrix effects. How would you lead your team to consensus on which method to use?

Water Sample

Due: Collect 1.0 L of water sample prior to the Week 1 WET Lab meeting. Bottles are available for sample collection; they are in the cabinets underneath the ICP-OES in 1112 FFSC.

Each group member should collect one unique water sample. You may take water from unique locations on campus or from the same locations at a different time of day. (See where other groups have collected samples on [google maps](#)). Each sample should be approximately 1 L of water, and collected using a clean metal-free container. The water should be chosen from a drinking water source on Duke's campus.*

Caution

If you are using your own container to collect drinking water for analysis, it must be rinsed with nitric acid to remove residual metals from the inside of the container. Be sure to use a metal-free sample collection vessel.

*If you would prefer to analyze water sources from your home or another place of interest, you are welcome to do so. We can perform qualitative analysis for almost any element - feel free to follow your interests! If you would like to do qualitative analysis on additional samples, they should be collected in smaller 50 mL tubes or in your own containers. You could choose to collect water from a filtered bottle filling station - but some contaminants may be removed already by the filling station filter.

Dry-Lab Group Discussion:

In this discussion, you will practice making scientific decisions as a team. Instead of simply following a procedure, you will weigh evidence, anticipate challenges such as matrix effects, and reach consensus on an analysis plan. By the end of this session, your group will have chosen a calibration strategy and identified additional elements for qualitative analysis. These decisions will prepare you to enter Wet Lab with a clear plan, so that your calibration standards and water samples can be prepared efficiently and consistently. This work also develops transferable skills in collaborative problem-solving, justifying methodological choices, and documenting group decisions—skills that are essential for authentic scientific research.

 **Goals:** By the end of today's discussion, your team will:

- Choose one calibration strategy (all members use the same).
- Finalize a Pb calibration set (levels, glassware plan, who prepares what).
- Select ≥ 4 qualitative targets beyond Pb and record your rationale (how they help interpret filter performance and matrix effects).

Instructions:

- Begin the session by grouping up and assigning roles (scribe, reporter, spokesperson, time keeper)
 - The Time Keeper will start a timer and encourage group consensus.
 - The Scribe will record the group work.
 - The Spokesperson will summarize your group's decisions to the class in 2 minutes at the end of discussion.
 - The reporter will submit the group record at the end of class.
- Work on the discussion questions together in your group for ~25 minutes.
- Report out
 - What are the elements chosen for qualitative analysis?
 - Describe your calibration strategy and justify this choice.
- As a group, make a detailed plan for creating calibration standards so that you can begin solution preparations immediately at the start of wet lab.

Group Discussion Prompts

1. Elements for Qualitative Analysis

- Each group member brings at least two candidate elements from the pre-lab. As a group, discuss:
 - Which elements are most likely to be present in drinking water at detectable levels?
 - Decision:** Select at least three elements for qualitative analysis (beyond Pb).
 - Action:** Record your group's list with a short justification for each.

2. In this module, we are addressing the question "How well does a water filter work?". Considering this, **explain why we are creating a tainted water sample** instead of just working with the plain drinking water sample.

3. Calibration Strategy for Quantitative Analysis of Pb

- Share your individual pre-lab calibration proposals. What evidence or reasoning did each of you use?
- If you use external calibration, how can you validate your calibration and determine whether there are matrix effects?
- In the context of this project, what are the pro's and con's of calibrating with the method of standard additions instead of external calibration?
- Decision Point:** Which calibration method will your group use?
- Action:** Write down your choice and the main reasons (bullet points are fine).

4. Protocol for Standard Preparation: Review your pre-lab calculations for tainting and standard preparation.

- Is the range of standards appropriate for measuring ~25 ppb Pb? Explain your reasoning.
- Decision Point:** Finalize the set of calibration standards that your group will prepare in Wet Lab. Your entire group should agree on the range and concentrations of each standard. Each group member will prepare their own calibration replicate(s).
- Action:** Write down the agreed concentrations and required glassware/pipettes. Create a group protocol for preparing the standards.

5. In the context of analyzing drinking water with ICP-OES, what kinds of questions can we answer using qualitative analysis, and what questions require quantitative analysis? Give at least one example for each from your group's water project.

6. How might the choices your group made today appear in the Introduction or Methods section of your manuscript? Draft one sentence that could appear in your Methods section to describe your calibration strategy.

Wet-Lab Work:

 **Goals for Wet Lab:** By the end of Week 1 Wet Lab, aim to accomplish:

- Prepare three samples for QUAL analysis (~10-15 mL each).
- Build a group method file for QUAL analysis.
- Run all samples using the QUAL method and harvest data in a form that is useful for your report.
- Make progress toward calibration of the instrument for QUANT analysis.

Agenda for Wet Lab

- Prepare containers** (remove trace metals)
- Prepare samples**
 - source (untainted) samples
 - tainted water samples
 - filtered water samples
- Collect qualitative data on the samples.**
- Prepare calibration standards and spiked recovery samples**
- Collect calibration and sample data** to determine the Pb concentration (if time allows)
 - Create a calibration curve
 - Use your calibration to determine the amount of Pb in your water samples.
 - Validate your method by performing a spiked recovery if applicable - If you cannot validate your method, revise your strategy and try again.
- Store all samples** in sealed containers and clean up.

Instructions for Completing Wet Lab Work

1. Prepare your containers

Remove trace metals from any glassware or reusable sample containers!

All sample containers and glassware must be rinsed well with dilute nitric acid (0.1 M) to dissolve and remove any trace metal ions that are bound to the interior surface of the container. Expose the interior of the container to 0.1 M nitric acid (HNO_3) in deionized water for 10 minutes, then rinse with deionized water.

2. Sample Preparation

Each group member will prepare three types of samples for this analysis using their unique source water. Prepare each sample for analysis by filling an autosampler tube about 4/5 full with the sample:

1. **Source water sample:** This is the water directly from the source tap (aka "untainted water"). Reserve approximately 100 mL of your source water sample for direct analysis using the ICP-OES. In other words, save some and don't taint it or filter it so we can measure its "original" contents. Fill an autosampler tube with about 10-12 mL of this water for QUAL analysis. You will use the remaining ~90 mL for your QUANT calibration standards or validation.
2. **Tainted water sample:** We *really* hope, and expect, that you won't find much, if any, toxic metal in the water on our campus! So...to give you some Pb to work with, you will use a certified element standard to contaminate **500 mL of water** sample to a final concentration of **25.0 ppb Pb**. This is your "tainted" water sample. After you prepare the tainted water solution, prepare a sample for analysis by putting about 10-12 mL in a labeled 15 mL tube. Reserve some of this tainted water for calibration or validation.
 - The [detection limit of ICP-OES is close to 5 ppb for Pb](#) under some conditions. Adding a known amount of Pb to your sample will give you a better chance to measure any trace amounts less than the limit of detection that are in the water sample.
3. **Filtered water:** You will use a Brita water filter cartridge to filter a portion of your tainted water and then measure the Pb concentration of the filtered water.
 - **Steps to prepare the filtered water:**
 - a. Start by rinsing the filter and pitcher with [deionized water](#) three times.
 - b. Remove all water from the pitcher and gently shake the pitcher to remove excess water from the entire filter unit.
 - c. Prepare the filter: Filter a small portion of your tainted water three times, discard the filtered water each time into a waste container.
 - d. Filter a fourth portion of the tainted water, and collect the filtrate into a clean container. Collect enough filtered sample for the Qual analysis, as well as for calibration or validation. Transfer 10-12 mL of the filtered water to an autosampler tube. This is the filtered water sample for analysis.

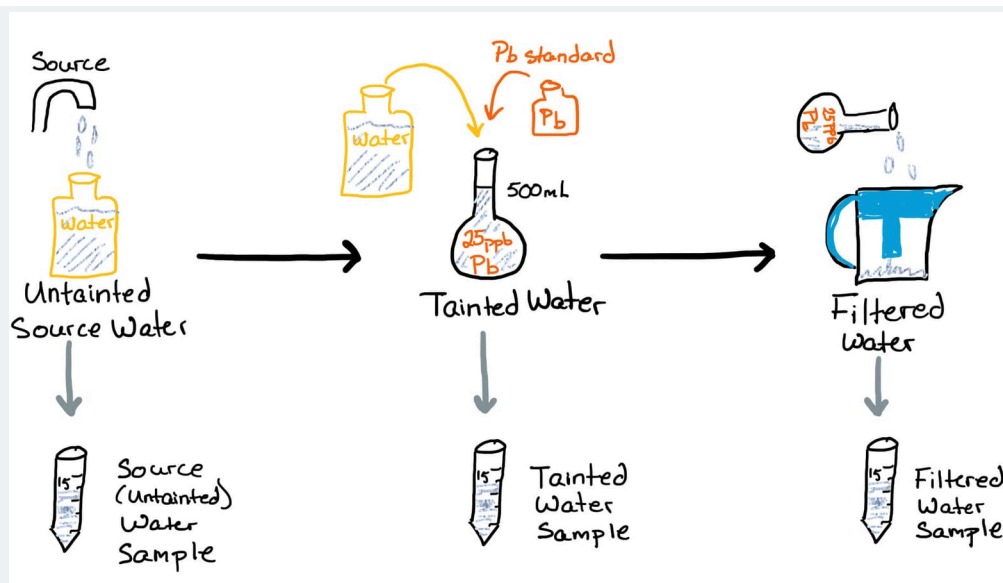


Figure 5.2.1: Scheme for sample preparation (CC BY SA; Kathryn Haas)

3. Qualitative Analysis of samples (what's in this water?)

As a group, create an ICP-OES instrument method and analyze all of your group's samples (3 samples per person). Your qualitative analysis method should include **Pb** and all the **elements that your team chose** for analysis (from your Dry Lab decisions).

- Instructions for **creating a method** for qualitative analysis are in the Instrument Facilities Manual ([click here](#)).
- Instructions for **operating the instrument** are in the Instrument Facilities Manual ([click here](#)).
- Instructions for **viewing and exporting qualitative data** are in the Instrument Facilities Manual ([click here](#)).

Your Data Checklist

To prepare for analysis and manuscript writing, be sure to export and save the following items to your own computer or cloud storage before you leave the lab:

- **For each element and each water source:** Overlaid emission spectra of that element in the blank, in the source water, the tainted water, and the filtered water. (If there were two unique water sources and three elements, this would be six separate figure panels).
- **Export the emission peak values** (the results table) for all samples and all elements, including the blank

4. Prepare Calibration Standards

The calibration method is up to you! Review the different methods of calibration ([single vs multi-point](#), [external calibration](#), [standard addition](#)) that you have or will learn about in the complimentary Analytical Chemistry Lecture Course. You should choose a method of multi-point calibration and plan how you will create the standard solutions necessary to calibrate the instrument. The goal is to accurately measure a solution of approximately 25 ppb Pb. Your group will be provided with the following materials:

- Up to **250 mL** of a certified Pb standard that contains 1.000 ± 0.002 mg/L (ppm) Pb.
- deionized water
- 0.1 M nitric acid in deionized water
- any flasks and containers available in the lab
- digital pipettes (for delivering volumes less than 1 mL, or odd volumes <5 mL for which volumetric pipets are unavailable)

- volumetric pipets (for most volumes > 1 mL)

You will be given a certified Pb standard that you can use to create the standard solutions or spikes necessary to calibrate the method. You should use volumetric glassware and techniques as you've practiced earlier in this course. If you need to transfer a volume of <5 mL that does not correspond to a typical capacity of a volumetric pipet, a digital pipette can be used to transfer volumes between 10-5000 μL . Take note of the errors associated with different types and sizes of pipet(te)s (see orientation module). Make sure that all the glassware is cleaned well to remove any trace contamination (see instructions above).

5. Quantitative Analysis of Pb (how much?)

As a group, create an ICP-OES instrument method and analyze all of your group's calibration standards and samples. Your quantitative analysis method should include only **Pb** for elemental analysis and calibration.

- Instructions for **creating a method** for quantitative analysis are in the Instrument Facilities Manual ([click here](#)).
- Instructions for **operating the instrument** are in the Instrument Facilities Manual ([click here](#)).
- Instructions for **viewing and exporting quantitative data** are in the Instrument Facilities Manual ([click here](#)).

The analysis of all samples will take a while, and can be automated to run while you are away. While these samples are running, work on harvesting qualitative data.

Notebook

Record detailed notes about your lab work in your notebook each time you are in lab. Also include the data analysis and responses to the reflection prompts*. Your notebook should be submitted on canvas prior to leaving Wet Lab each week - whatever state it is in at the end of lab is the state in which you should submit your work. If you are unable to complete data analysis and the reflection prompt within the period, it should be completed before the next lab period, and submitted with your next notebook submission.

Reflection Prompt 5.2.0

We assume you haven't gotten a chance to fully collect or analyze calibration data at this point, so these questions are focused on *qualitative* data.

1. **Qualitative Analysis Results:** What elements did you detect in your source water, tainted water, and filtered water? Which of these findings were expected based on your pre-lab predictions, and which were surprising?
2. **Matrix Effects and Calibration Choices:** Based on your results, what evidence do you see of possible matrix effects? How would you evaluate whether your calibration strategy (external vs. standard additions) is an effective choice?
3. **Filter Effectiveness:** How did filtration affect the signals for Pb and your team's additional elements? Did the filter perform as you expected? What evidence supports your conclusion?
4. **Reproducibility:** How reproducible are your results based on replicate measurements or repeated scans? If variation differs by signal intensity, what might explain that trend?
5. **Data Presentation & Communication:** Include one overlaid spectrum (at least one analyte across source/tainted/filtered).
 - Provide a short figure caption that communicates what the spectrum shows and why it matters.
 - Write 2-3 sentences explaining how you might present these results in the Results section of a scientific manuscript.

5.3: Week 2 - Water

Pre-lab Assignment

 **Due:** This pre-lab assignment is due prior to the Week 2 Dry Lab meeting.

 **Purpose:** This pre-lab is designed to prepare you for a productive dry-lab discussion and to prime you to reasonably interpret your data. By organizing your qualitative results and drafting your Methods section now, you'll be ready to share work-in-progress and get early feedback to improve your report.

Follow up from Week 1

1. **Complete and submit any unfinished business from the last meeting.**
2. **Begin preparing your manuscript document:**
 - a. **Complete a draft of your Materials and Methods section.**
 - b. **Complete the Qualitative Results section, including description and figures that show the results of qualitative analysis.**
 - Prepare a table of the elements you searched for in qualitative analysis for all samples, and that indicates their presence in your sample.
 - Prepare a figure or multiple figures that shows the results of qualitative analysis.
 - The table and figures should complement each other.
 - c. **Draft your Discussion of qualitative results:** Analyze and interpret the qualitative results for all the samples from your group:
 - **For all source water samples:** Explain whether each element is present or absent in detectable amounts in each sample, using evidence from your results section to justify your analysis.
 - **Compare source water to tainted:** Does the addition of Pb to the matrix (tainting) affect the signals of other elements? Support your assertions using evidence from your results section. Explain the meaning of these results in the context of matrix results.
 - **Compare tainted sample to filtered sample:** What effect does filtration have on the intensity of the signals from each element? Support your assertions using evidence from your results section. Explain the meaning of these results in the context of matrix effects.

Many people assume that signal intensity reflects the amount of an element present, but this is not always true! Matrix effects can make signals appear stronger or weaker without a real change in concentration. In this pre-lab, you will read about Brita's claims for filtration, make predictions about how well the filter should work for Pb and other elements, and consider why signals might increase after filtration. This work will prepare you to identify when a signal change is likely due to matrix effects versus true contamination, and to defend your interpretation during group discussion.

Pre-Lab Questions

Read the claims about the efficiency of Brita water filters for removal of substances from drinking water. (<https://www.brita.com/why-brita/better-water/>). We use a *standard* Brita pitcher filter in this experiment.

1. **Compare Predictions to Evidence:** After reading Brita's claims, what efficiency do you expect for Pb removal? What efficiency do you expect for your group's additional elements? State your predictions clearly so you can compare them to your qualitative results now, and your quantitative results later this week.
2. **Signals that Increase After Filtration:** Sometimes, the intensity of an element's signal *increases* after filtration. What are two possible explanations for this observation — one related to matrix effects and one related to an actual change in sample composition? Which explanation would you expect to be more common, and why?

3. **Responding to Unexpected Results:** Suppose your group observes higher intensity signal for an element in the filtered sample than in the tainted sample. How would you decide whether this is a real contamination event (e.g., improper filter preparation) versus a matrix effect? What additional evidence or checks could help you distinguish between these possibilities?

Dry Lab Group Discussion

 **Goals:** By the end of today's discussion, your Team will:

- Compare qualitative results across all Groups to look for evidence of matrix effects.
- Identify which elements increased or decreased after filtration, and debate possible explanations.
- Distinguish between matrix effects and true contamination events using shared evidence.
- Draft clear sentences for the Results and Discussion sections of your manuscript that reflect both Group and Team-level evidence.

Instructions

1. Step 1 — Group Check-In (10 minutes)

Within your 4-person Group:

- a. Compare your pre-lab predictions to your Group's actual qualitative results.
- b. Decide: Did we observe any element with an increased signal after filtration?
- c. Nominate one spokesperson to report back to the Team.

2. Step 2 — Team Data Pooling (15 minutes)

Each Group's spokesperson shares findings with the Team (3 short reports).

- a. As a Team, nominate two scribes; one to write on the whiteboard, and another to record in a shared google sheet.
- b. Create a combined table (on the whiteboard and in the shared document)
 - Columns = Elements tested
 - Rows = Group A, Group B, Group C
 - Record whether signals after filtration were ↑ (increased), ↓ (decreased), or = (no change).

3. Step 3 — Team Interpretation (15 minutes)

As a Team, address the following:

- a. Which elements showed signal increases in at least one Group's data?
- b. Are these more consistent with matrix effects or true contamination? What evidence supports your conclusion?
- c. If Groups disagree, what additional checks or information could help resolve the discrepancy?

4. Step 4 — Group Drafting (20 minutes)

- a. Re-group with the members of your section and set a timer for 10 minutes: Each person in the group should spend 10 minutes revising the Discussion draft that they wrote for their pre-lab assignment. Focus on
 - At least one results sentence describing a shared observation (e.g., "In all filtered samples, signals for Cu decreased relative to tainted water.").
 - At least one discussion sentence interpreting it (e.g., "This decrease is consistent with decrease in Cu contamination in the sample as a result of filtration.").
- b. Take turns reading your revised sentences aloud, and giving feedback to each other. Spend the remaining time in dry lab to refine your Discussion section — even though you are each writing your own manuscript, your group should reach consensus on the interpretation of your qualitative evidence as a whole.

Wet Lab Agenda

If you were not able to initiate quantitative data collection in week 1, please prioritize the solution preparation and data collection. Then proceed with the steps below.

1. **Access your data:** You will need to know your file names and in which directory you stored your data so you can locate the data and complete the analysis.
2. **Perform Quantitative Analysis:** Depending on how you calibrated your samples, you will work with your group or individually to process and export the quantitative data.

- Produce a calibration curve using the appropriate regression model.
- Assess the validity of your calibration.
- Perform quantitative analysis on your samples.
- *Make sure to include error analysis!*

3. **Continue writing** the *Results* and *Discussion* sections of lab report (only after completing the reflection prompts below.)

Your Data Checklist

To prepare for analysis and manuscript writing, be sure to export and save the following items to your own computer or cloud storage before you leave the lab:

Calibration Standards Data:

- Overlaid emission spectra for all Pb calibration standards.
- Pb emission peak values for each standard exported in a format that you can process using your favorite software.
- A calibration curve for Pb (plotted using your favorite software), including best-fit line and regression statistics
- Documentation of your calibration strategy (external vs. standard additions) and its validation (e.g., spiked recovery).

Sample Data:

- Overlaid emission spectra of Pb in all water samples (source, tainted, filtered) run at the same time as your calibration data.
- Pb emission peak values for each sample
- A table of Pb concentrations in each water sample, with error estimates.

Evidence for Notebook Snapshot

- Any exported files or screen captures that show results, with notes to show your understanding of their relevance and meaning.

Notebook

Record detailed notes about your lab work in your notebook each time you are in lab. Also include the data analysis and responses to the reflection prompts*. Your notebook should be submitted on canvas prior to leaving Wet Lab each week - whatever state it is in at the end of lab is the state in which you should submit your work. If you are unable to complete data analysis and the reflection prompt within the period, it should be completed before the next lab period, and submitted with your next notebook submission.

Reflection Prompt 5.3.0

In your notebook submission, respond to the following:

1. **Results:** Briefly summarize what you understand about your quantitative results.
2. **Filter Effectiveness:** Based on your quantitative results, how effective was the filter at removing Pb from tainted water? Do you know if the filter remove other elements? What evidence supports your conclusion?
3. **Validation:** Did your calibration strategy pass validation? If not, what does this mean for your interpretation of filter effectiveness, and what changes must you make in your calibration approach before repeating the analysis?

5.4: Week 3 - Water

Pre-lab Assignment

 **Due:** This pre-lab assignment is due prior to the Week 3 Dry Lab meeting.

 **Purpose:** This pre-lab is designed to prepare you to share work-in-progress and get early feedback to improve your report.

Follow up from Week 2

1. **Complete and submit any unfinished business from the last meeting.**
2. **Prepare your manuscript document:**
 - a. **Update the Materials and Methods section** based on last week's peer feedback.
 - b. **Update the Qualitative Results section** based on last week's peer feedback.
 - c. **Write your Discussion of Qualitative Results.**
 - d. **Write the Quantitative Results section, including description and figures that show the results of quantitative analysis.**
 - Prepare a table that communicates the results of calibration and quantitation.
 - Prepare one or more figures that shows calibration data.
 - The table and figures should complement each other.
3. Bring a **printed copy** of your manuscript to the Dry Lab meeting.

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Dry Lab Group Discussion

In this session, you will peer review each other's reports and practice helping each other by giving impactful, actionable feedback.

Wet Lab Agenda

Record all work in your lab notebook as usual. If you are not doing wet-lab work in this session, please keep notes of your writing-related conversations in your lab notebook and complete the reflection prompts as usual.

1. **Revise Manuscript and Write Discussion, Conclusions, and Introduction Section.**
 2. **Meet with Writing Consultants.**
-

Notebook

Record detailed notes about your lab work in your notebook each time you are in lab. Also include the data analysis and responses to the reflection prompts*. Your notebook should be submitted on canvas prior to leaving Wet Lab each week - whatever state it is in at the end of lab is the state in which you should submit your work. If you are unable to complete data analysis and the reflection prompt within the period, it should be completed before the next lab period, and submitted with your next notebook submission.

Reflection Prompt

1. **How has writing about your lab experience supported your learning?**

2. **How has working with the EWC's supported your writing and learning?**
3. **What have you done that has supported your learning in this module? What could you do better in the future?**
4. **What were the ways in which your instructional team supported your learning? What could they do better in the future?**

5.5: ICP-OES Procedure and instructions for treatment of data

Data Collection

Qualitative Analysis (Qual-What's in it?)

You can choose to measure for the presence of any analytes you wish (aside from Pb) in the water samples. Each team member should choose at least one unique analyte for qualitative analysis (see the pre-lab assignment) and you should measure for that analyte in the three samples (source water, tainted water, filtered water).

Quantitative Analysis (Quant -How much Pb?)

Determine the Pb concentration of the source (untainted) water samples, the tainted water samples, and the filtered water samples.

- Create a calibration curve
 - Use your calibration to determine the amount of Pb in your water samples.
 - Validate your method by performing a spiked recovery. If you cannot validate your method, revise your strategy and try again.
-

Treatment of Data

The general criteria for writing and submitting lab reports are found in the [orientation module](#). Specific additional expectations for this module are given below.

More detailed instructions for viewing and exporting data using the software are found in the [Instructions for Viewing and Exporting Results using Syngistix Software](#).

Qualitative analysis:

- State what analytes your group chose for qualitative analysis, and justify each choice with a pithy statement in your lab report introduction.
- Determine whether you have detected the presence of your chosen analytes in your source water, tainted water, and filtered water.
- Show the overlaid spectra of each analyte in the water samples (Similar to Figure 5.5.2).
- Discuss whether the analyte concentrations are changed before and after tainting and filtration, and explain how you would quantitate the analytes to determine if there are matrix effects.

Quantitative analysis:

Include the following in your report:

An example figure is given below in Figure 5.5.2. This was created by putting two screen captures in the same powerpoint slide, annotating the panels, and taking a screen shot.

- Description of calibration strategy and its validation (include data on spiked recovery).
- Overlaid spectra of **Pb emission from the calibration**. You can create such a figure using the Examine Spectra and then use a screen capture or printing the active screen (ball menu, see Figure 5.5.1).

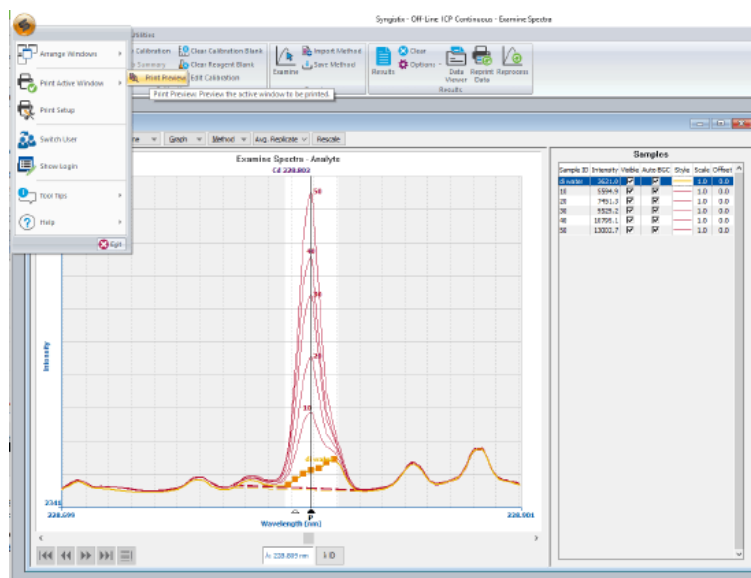


Figure 5.5.1: Spectra can be viewed using the Examine button in the Results Tab, and then printed to a pdf using the ball menu (top right) and then choose Print Active Window. Alternatively a screen shot can be taken using Shift + Windows + s. (CC-BY-SA; Kathryn Haas)

If you'd like to create multi-panel figures, or do more customized labeling of emission spectra, one way is to copy and past each figure into a powerpoint slide and arrange the plots, add annotations, etc (see example below in Figure 5.5.2). Then take a screen shot of your figure and paste it in a word document.

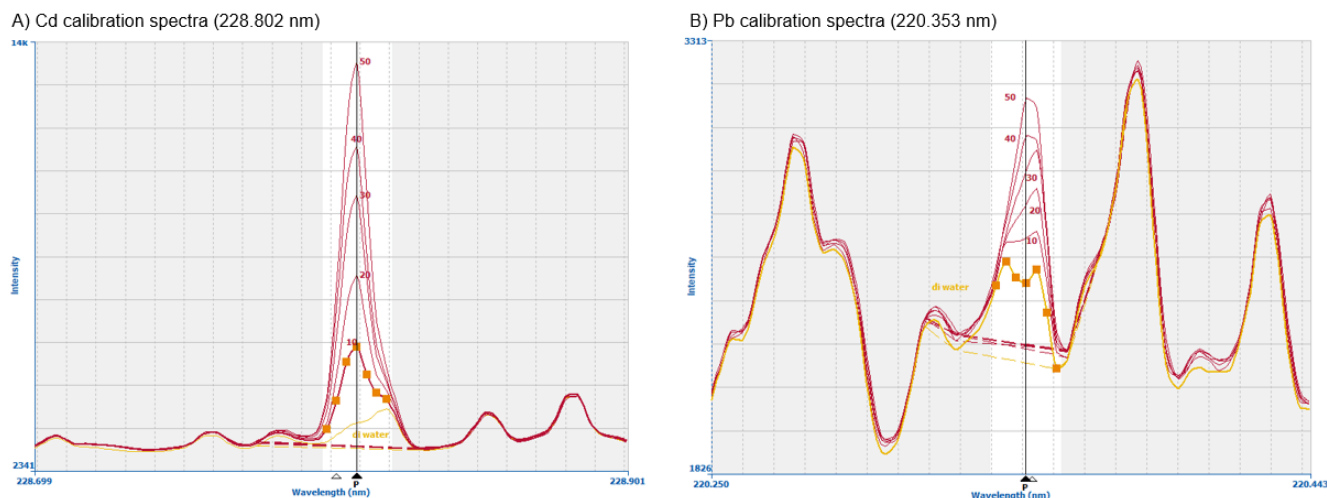


Figure 5.5.2: Emission spectra from Cd and Pb in calibration standards detected by ICP-OES, monitored at 228.802 and 220.353 nm, respectively. Spectra from deionized water (di water) are shown in yellow. Standards that contain 10, 20, 30, 40, and 50 ppb of Pb and Cd are shown in red and indicated using red labels (units in ppb = $\mu\text{g/L}$).

- Plots of calibration curve for Pb including a best fit line, R, and error in slope and intercept. These plots should be generated by you, not by the software's automatic analysis.
- Overlaid spectra of Pb in water samples (similar to Figure 5.5.1) .
- Quantitative determination of Pb and Cd in each water sample, including error in the derived concentrations.
- In the discussion, compare the Pb concentration in each of the samples and determine (1) the concentration of Pb in the source, (2) the concentration of Pb in the tainted water, and (3) the concentration of Pb in the filtered water. Comparison of (2) and (3) will indicate the efficacy of the filter for removing each metal.

5.6: TA Notes

This page is directed toward laboratory teaching assistants, but is deliberately left open for students to read.

TA Notes

After completing this module your students will be able to...

- describe external calibration and the method of standard additions.
- describe the pros and cons to using different calibration methods.
- describe how to validate a calibration using a spike.
- explain what a matrix effect is, and how to recognize it.

Pre-Semester Preparations

Do the following during the TA training and orientation week(s)

1. Check that the materials listed below are available and sufficient for all students to use them. If they are running low, please create more or inform the instructor.
 - **0.1 M Nitric Acid** should be prepared by dissolving 66 mL of 70% nitric acid (15.8 M) in 10 L of ultrapure water.

Caution

This should only be done by a chemist trained to safely handle concentrated nitric acid. 70% nitric acid penetrates standard lab gloves and acid-resistant 5 mil neoprene gloves should be worn.

- **Pb standard solution, 1000 ppm is provided.** This solution is much too concentrated for the students to use directly in their calibration. Thus, a more dilute solution should be made first. A solution of 1 ppm = 1000 ppb is more suitable as a working solution.

Caution

Pb is very toxic to humans and other living organisms. Wear PPE and handle carefully.

- **Calibration solutions:** A five-point calibration (including the blank) is typical, and a calibration curve should range between 0 and 2-3 times the concentration of the analyte in the samples. TAs should carefully prepare excess external calibration solutions as they complete the module, and have them on hand for instrument performance checks during the semester.
 - 1g/L Na solution should also be prepared or available to run a sodium bullet test.
2. Complete instrument training with Dr. Haas.
 3. Perform the entire experiment as if you were a student.
 4. Read this: [Guided-Inquiry Experiment for Teaching the Calibration Method of Standard Addition in the Analysis of Lead with Graphite Furnace Atomic Absorption Spectroscopy | Journal of Chemical Education \(acs.org\)](#)
 5. Write and submit a report as if you were a student.
 6. Practice grading your and your partner's report using the grading rubric.

Pre-Lab Preparations

When you are on TA duty, arrive at least 20-30 minutes early to prepare and ready the following materials for students.

- **0.1 M nitric acid:** this should already be prepared using metal-free nitric acid by the lab director, and excess solution should be available in a large carboy in the hood or prep room. As students arrive to lab, remind them that they should rinse their glassware with this solution to remove all residual metal from the glass.
- **1 L of drinking water** collected by students: As your students arrive on the first day of the module, check that they have collected water or ask them to do it immediately.
- **Pb standard solution, 1000 ppm:** This is a solution purchased and used as received. If it is running low please inform the instructor.
- **Deionized water:** Please check that the ultrapure water is running and there are no leaks in the system under the sink.
- **Brita filter:** Please check that the Brita filter is available and begin rinsing the filter with ultrapure water in preparation for the students to use it.
- Remind students to sign into lab
- Check that the student's pre-lab is complete

Helping your students

During the laboratory session, you are expected to be present and available for the entire 4.5 hours. Your job is to help students successfully complete the experiment while upholding expectations and academic standards for the students.

In this particular laboratory session, you should let students choose their calibration strategy. You can discuss the pros and cons of different strategies, but it should ultimately be the decision of the student group as to what method they choose to calibrate the instrument. You should also discuss validation with them, but it is their choice as to how they validate their method or whether they do the validation at all.

This lab module is meant to be open-ended and also to demonstrate matrix effects. Please discuss matrix effects with the students and make them aware of this as a possible explanation of their results. The students can, and should, use lab time to test any hypotheses they develop to explain their results. For example, if the signal changes after filtration, and the students think there is a matrix effect, that hypothesis can be tested by the method of standard additions or by a simple spike to each of the samples in question. Alternatively, if students believe the filter is adding metals to their filtered sample, that hypothesis can be tested by measuring the metal signal of ultrapure water before and after exposure to the filter.

Encourage students to use *at least* two full lab periods (assuming a 3-period module) to perform lab work. Ideally, data is actively processed during collection and students are able to finalize data collection and begin writing their reports during the third week of lab.

Shut down/ clean up

15 minutes prior to the end of the period, students should stop lab work and clean up. They are to leave the room in clean condition prior to the end of the period (17:55).

- Remind students when it is time to clean up.
- Remind students that all glassware should be returned to the storage locations.
- Any samples that are stored should be labeled clearly with the student's name and section.
- Remind students to sign out with the time they leave and get your signature.
- Check the TA checklist for additional items to be completed.

6: UNDER CONSTRUCTION EXPERIMENTS

6.1: Ultraviolet-visible spectroscopy (UV-vis)

6.1.1: Methods of Calibration

6.1.2: Data analysis

6.1.3: Pre-lab assignment

6.1.4: Absorbance and Beer's Law

6.1.5: UV/Vis Instrumentation

6.1.6: UV-vis multicomponent analysis procedure

6.1.7: Data analysis

6.2: Cyclic Voltammetry

6.3: Fourier Transform Infrared Spectroscopy (FTIR)

6.4: Molecular Fluorescence

6.5: Capillary Electrophoresis

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6.7: Vocabulary

6.8: GC-MS Procedure and instructions for data analysis

6.9: Instructions for Manual Operation of the Agilent GCMS

6.10: Caffeine serial dilution and analysis using (UV-vis) spectroscopy

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6.11: Determination of cocaine on bills using Gas Chromatography, Mass Spectrometry (GC-MS)

6.11.1: GC-MS Pre-Lab Assignment

6.11.2: GC-MS Procedure and instructions for data analysis

6.11.3: TA Notes

6.11.4: Archive of GCMS procedure

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Glossary

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This page titled [6.1: Ultraviolet-visible spectroscopy \(UV-vis\)](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Kathryn Haas](#).

6.1.1: Methods of Calibration

Introduction

Suppose we need to determine the concentration of two analytes, X and Y , that are mixed in a sample. If each analyte has a wavelength where the other analyte does not absorb, then we can proceed using Beer's Law to determine the concentration. Unfortunately, UV/Vis absorption bands are so broad that it is sometimes impossible to find suitable wavelengths. Because Beer's law is additive the mixture's absorbance, A_{mix} , at a given wavelength will be a sum of the absorbance of each analyte:

$$(A_{mix})_{\lambda_1} = (\varepsilon_X)_{\lambda_1} bC_X + (\varepsilon_Y)_{\lambda_1} bC_Y \quad (6.1.1.1)$$

where λ_1 is the wavelength at which we measure the absorbance. Because Equation 6.1.1.1 includes terms for the concentration of both X and Y , the absorbance at one wavelength does not provide enough information to determine either C_X or C_Y . If we measure the absorbance at a second wavelength, λ_2

$$(A_{mix})_{\lambda_2} = (\varepsilon_X)_{\lambda_2} bC_X + (\varepsilon_Y)_{\lambda_2} bC_Y \quad (6.1.1.2)$$

then we can determine C_X and C_Y by solving simultaneously Equation 6.1.1.1 and Equation 6.1.1.2. Of course, we also must determine the value for ε_X and ε_Y at each wavelength. For a mixture of n components, we must measure the absorbance at n different wavelengths.

Example 6.1.1.1

The concentrations of Fe^{3+} and Cu^{2+} in a mixture are determined following their reaction with hexacyanoruthenate (II), $\text{Ru}(\text{CN})_6^{4-}$, which forms a purple-blue complex with Fe^{3+} ($\lambda_{\text{max}} = 550 \text{ nm}$) and a pale-green complex with Cu^{2+} ($\lambda_{\text{max}} = 396 \text{ nm}$) [DiTusa, M. R.; Schlitt, A. A. *J. Chem. Educ.* **1985**, 62, 541–542]. The molar absorptivities ($\text{M}^{-1} \text{cm}^{-1}$) for the solutions of the individual metal complexes at the two wavelengths are summarized in the following table.

analyte	ε_{550}	ε_{396}
Fe^{3+} standard	9970	84
Cu^{2+} standard	34	856

When a sample that contains Fe^{3+} and Cu^{2+} is analyzed in a cell with a pathlength of 1.00 cm, the absorbance at 550 nm is 0.183 and the absorbance at 396 nm is 0.109. What are the molar concentrations of Fe^{3+} and Cu^{2+} in the sample?

Solution

Substituting known values into Equation 6.1.1.1 and Equation 6.1.1.2 gives

$$\begin{aligned} A_{550} = 0.183 &= 9970C_{\text{Fe}} + 34C_{\text{Cu}} \\ A_{396} = 0.109 &= 84C_{\text{Fe}} + 856C_{\text{Cu}} \end{aligned}$$

To determine C_{Fe} and C_{Cu} we solve the first equation for C_{Cu}

$$C_{\text{Cu}} = \frac{0.183 - 9970C_{\text{Fe}}}{34}$$

and substitute the result into the second equation.

$$0.109 = 84C_{\text{Fe}} + 856 \times \frac{0.183 - 9970C_{\text{Fe}}}{34}$$

$$= 4.607 - (2.51 \times 10^5) C_{\text{Fe}}$$

Solving for C_{Fe} gives the concentration of Fe^{3+} as 1.8×10^{-5} M. Substituting this concentration back into the equation for the mixture's absorbance at 396 nm gives the concentration of Cu^{2+} as 1.3×10^{-4} M.

Let's consider the example of a mixed Co and Cr solution (similar to the unknown solutions you will analyze in this lab module). To obtain results with good accuracy and precision **the two wavelengths should be selected so that $\epsilon_X > \epsilon_Y$ at one wavelength and $\epsilon_X < \epsilon_Y$ at the other wavelength**. It is easy to appreciate why this is true. Because the absorbance at each wavelength is dominated by one analyte, any uncertainty in the concentration of the other analyte has less of an impact. Figure 6.1.1.1 shows the spectra of a Cr standard and a Co standard, as well as a spectrum of the mixture. From the spectra of the standards, we can see that 400 nm is a reasonable choice for one of the wavelengths because it is a point of maximum absorption for Cr^{3+} , and $\epsilon_{\text{Cr}} > \epsilon_{\text{Co}}$. A reasonable choice for a second wavelength is 505 nm; it is a point of maximum absorption for Co^{2+} where $\epsilon_{\text{Co}} > \epsilon_{\text{Cr}}$. These two wavelengths are used in Practice Exercise 6.1.1.1 (below). When the choice of wavelengths is not obvious, one method for locating the optimum wavelengths is to plot ϵ_X/ϵ_Y as function of wavelength, and determine the wavelengths where ϵ_X/ϵ_Y reaches maximum and minimum values [Mehra, M. C.; Rioux, J. J. *Chem. Educ.* **1982**, 59, 688–689].

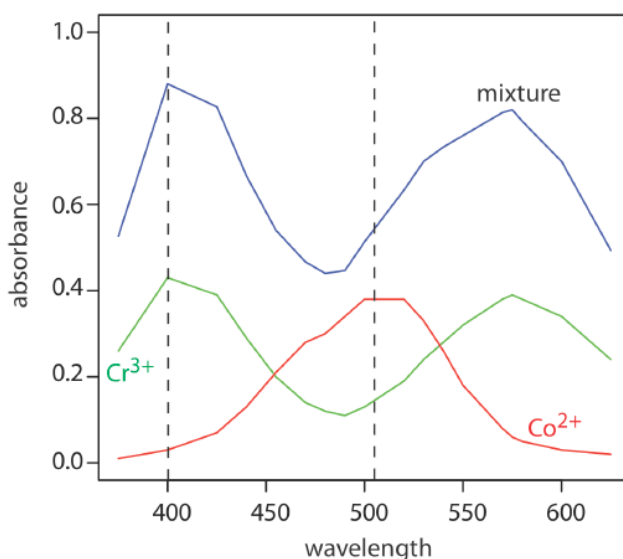


Figure 6.1.1.1. Visible absorption spectra for 0.0250 M Cr^{3+} , 0.0750 M Co^{2+} , and for a mixture of Cr^{3+} and Co^{2+} . The two wavelengths used to analyze the mixture of Cr^{3+} and Co^{2+} are shown by the dashed lines. The data for the two standards are from Brewer, S. *Solving Problems in Analytical Chemistry*, John Wiley & Sons: New York, 1980.

Exercise 6.1.1.1

The absorbance spectra for Cr^{3+} and Co^{2+} overlap significantly. To determine the concentration of these analytes in a mixture, its absorbance is measured at 400 nm and at 505 nm, yielding values of 0.336 and 0.187, respectively. The individual molar absorptivities ($\text{M}^{-1} \text{cm}^{-1}$) for Cr^{3+} are 15.2 at 400 nm and 0.533 at 505 nm; the values for Co^{2+} are 5.60 at 400 nm and 5.07 at 505 nm.

Answer available at the end of this section.

Limitations to Single-point calibration

The example immediately above used a single point standardization of [Co] and [Cr]. A single-point standardization is the least desirable approach for standardizing a method. There are two reasons for this. First, any error in our determination of ϵ_X and

ε_Y carries over into our calculation of C_X and C_Y . Second, our experimental value for each ε is based on a single concentration of analyte. To extend this value of ε to other concentrations of analyte requires that we assume a linear relationship between the signal and the analyte's concentration, an assumption that often is not true [Cardone, M. J.; Palmero, P. J.; Sybrandt, L. B. *Anal. Chem.* **1980**, 52, 1187–1191]. Figure 6.1.1.1 shows how assuming a constant value of ε leads to a determinate error in C_A concentration if ε becomes smaller at higher concentrations of analyte. Despite these limitations, single-point standardizations find routine use when the expected range for the analyte's concentrations is small.

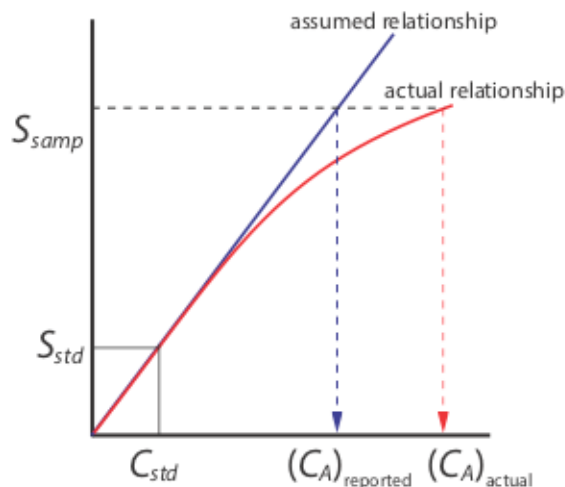


Figure 6.1.1.1 : Example showing how a single-point standardization leads to a determinate error in an analyte's reported concentration if we incorrectly assume that k_A is constant. The assumed relationship between S_{samp} and C_A is based on a single standard and is a straight-line; the actual relationship between S_{samp} and C_A becomes curved for larger concentrations of analyte.

The better way to standardize a method is to prepare a series of standards, each of which contains a different concentration of analyte. Standards are chosen such that they bracket the expected range for the analyte's concentration. A **multiple-point standardization** should include at least three standards, although more are preferable. A plot of S_{std} versus C_{std} is called a **calibration curve**. The exact standardization, or calibration relationship, is determined by an appropriate curve-fitting algorithm. There are two advantages to a multiple-point standardization. First, although a determinate error in one standard introduces a determinate error, its effect is minimized by the remaining standards. Second, because we measure the signal for several concentrations of analyte, we no longer must assume that the response (ε in this case) is independent of the analyte's concentration. Instead, we can construct a calibration curve similar to the "actual relationship" indicated in Figure 6.1.1.1 .

External Calibration using Multiple External Standards

The most common method of standardization uses one or more **external standards**, each of which contains a known concentration of analyte. We call these standards "external" because they are prepared and analyzed separate from the samples.

Figure 6.1.1.2 shows a typical **multiple-point external standardization**. The volumetric flask on the left contains a reagent blank and the remaining volumetric flasks contain increasing concentrations of Cu^{2+} . Shown below the volumetric flasks is the resulting calibration curve. Because this is the most common method of standardization, the resulting relationship is called a **normal calibration curve**.

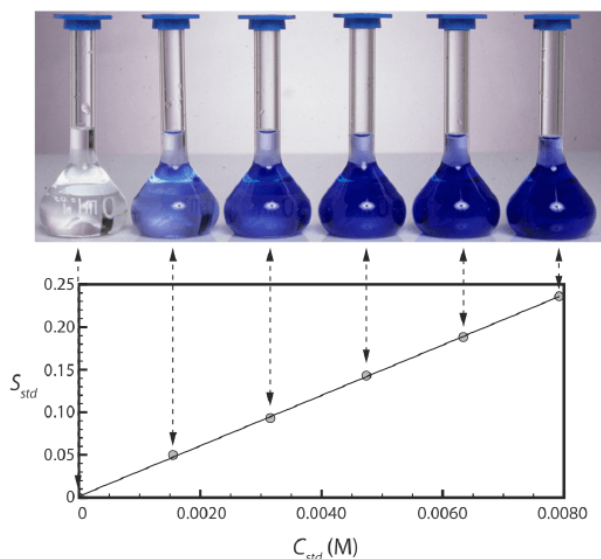


Figure 6.1.1.2 : The photo at the top of the figure shows a reagent blank (far left) and a set of five external standards for Cu^{2+} with concentrations that increase from left-to-right. Shown below the external standards is the resulting normal calibration curve. The absorbance of each standard, S_{std} , is shown by the filled circles.

When a calibration curve is a straight-line, as it is in Figure 6.1.1.2, the slope of the line gives the value of $b\epsilon$ (where b is path length). This is the most desirable situation because the method's sensitivity remains constant throughout the analyte's concentration range. When the calibration curve is not a straight-line, the method's sensitivity is a function of the analyte's concentration. In Figure 6.1.1.1, for example, the value of $b\epsilon$ is greatest when the analyte's concentration is small and it decreases continuously for higher concentrations of analyte. The value of $b\epsilon$ at any point along the calibration curve in Figure 6.1.1.1 is the slope at that point. In either case, a calibration curve allows us to relate S_{samp} to the analyte's concentration.

Limitations to External Calibration

There is a serious limitation, however, to an external standardization. When we determine an analyte's response (ϵ) in this case) using external calibration, the analyte is present in the external standard's matrix; that is usually a much simpler matrix than that of "real" samples. When we use an external standardization we assume the matrix does not affect the value of the response (ϵ). **However, if the matrix does affect the response, we introduce a proportional determinate error into our analysis.** This is not the case in Figure 6.1.1.3, for instance, where we show calibration curves for an analyte in the sample's matrix and in the standard's matrix. In this case, using the calibration curve for the external standards leads to a negative determinate error in analyte's reported concentration. If we expect that matrix effects are important, then we try to match the standard's matrix to that of the sample, a process known as **matrix matching**. If we are unsure of the sample's matrix, then we must show that matrix effects are negligible or use an alternative method of standardization. Both approaches are discussed in the following section.

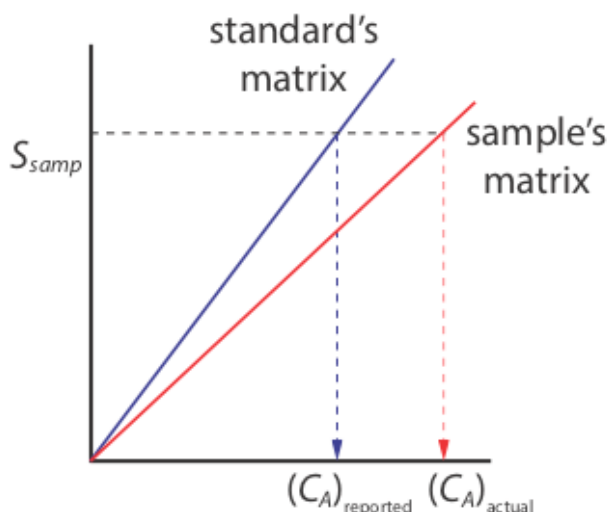


Figure 6.1.1.3 : Calibration curves for an analyte in the standard's matrix and in the sample's matrix. If the matrix affects the value of k_A , as is the case here, then we introduce a proportional determinate error into our analysis if we use a normal calibration curve.

Standard Additions

We can avoid the complication of matching the matrix of the standards to the matrix of the sample if we carry out the standardization *in the sample*. This is known as the **method of standard additions**.

There are several methods of standard addition, and some are described in detail in **5.3: Determining the Sensitivity** by David Harvey. We will focus on standard addition by adding standard analyte solution directly to the sample, measuring the signal both before and after the spike (Figure 6.1.1.5). In this case the final volume after the standard addition is $V_o + V_{std}$ and the relationship between the absorbance and concentration of a analyte "X" becomes (the standard is a standard solution of the analyte X):

$$A_{sample} = \epsilon b C_X$$

$$A_{spiked} = \epsilon b \left(C_X \frac{V_o}{V_o + V_{std}} + C_{std} \frac{V_{std}}{V_o + V_{std}} \right) \quad (6.1.1.3)$$

$$\frac{A_{sample}}{C_X} = \frac{A_{spiked}}{C_X \frac{V_o}{V_o + V_{std}} + C_{std} \frac{V_{std}}{V_o + V_{std}}} \quad (6.1.1.4)$$

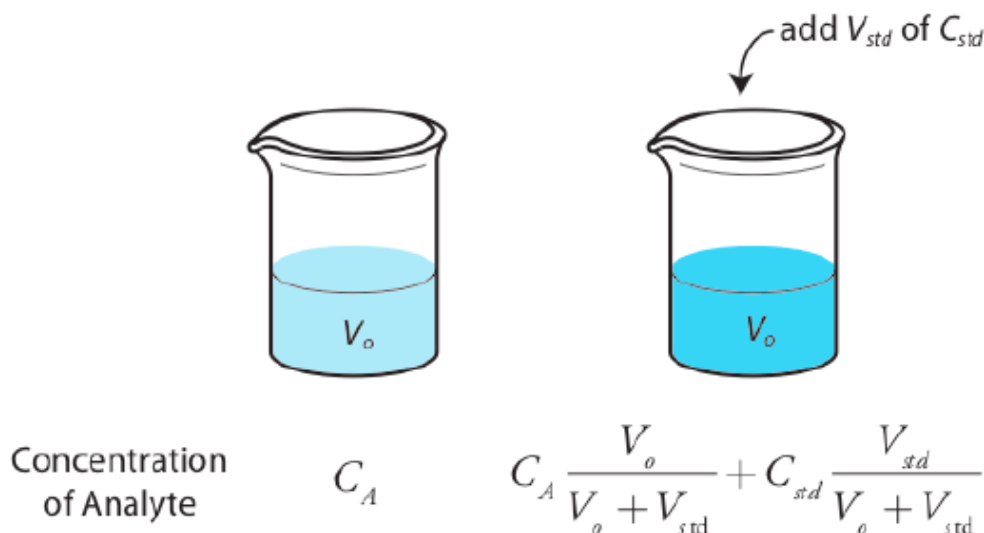


Figure 6.1.1.5 : Illustration showing an alternative form of the method of standard additions. In this case we add the spike of external standard directly to the sample without any further adjust in the volume.

Multiple Standard Additions

We can adapt a single-point standard addition into a multiple-point standard addition by preparing a series of samples that contain increasing amounts of the external standard. Figure 6.1.1.6 shows two ways to plot a standard addition calibration curve based on equation 6.1.1.3. In Figure 6.1.1.6, plot (a) we plot A_{spike} (or more generally S_{spike}) against the volume of the spikes, V_{std} . In plot (b) we plot $C_{std} \times \frac{V_{std}}{V_f}$ on the x axis instead (where $V_f = V_o + V_{std}$ is total volume after the spike). If k_A is constant, then the calibration curve is a straight line in both cases. It is easy to show that the slope is related to the response of the analyte in the given matrix (k_A and that x-intercept is related to the concentration of the analyte C_A ; the x-intercept is equivalent to $-\frac{C_A V_o}{C_{std}}$ in the case of plot (a) or $-\frac{C_A V_o}{V_f}$ in the case of plot (b) (see Figure 6.1.1.6).

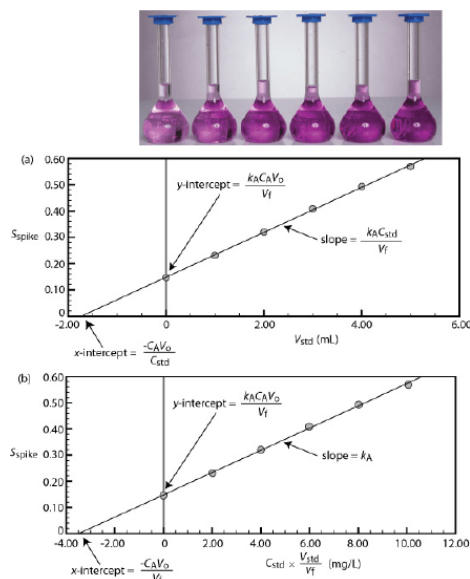


Figure 6.1.1.6 : Shown at the top of the figure is a set of six standard additions for the determination of Mn^{2+} . The flask on the left is a 25.00 mL sample diluted to 50.00 mL with water. The remaining flasks contain 25.00 mL of sample and, from left-to-right, 1.00, 2.00, 3.00, 4.00, and 5.00 mL spikes of an external standard that is 100.6 mg/L Mn^{2+} . Shown below are two ways to plot the standard additions calibration curve. The absorbance for each standard addition, S_{spike} , is shown by the filled circles.

For Mixtures with overlapping signals: The two-analyte Generalized Standard Addition Method (GSAM)

We can extend the standard addition method to quantitate multiple components of a mixture using the **Generalized Standard Addition Method (GSAM)**. This is the preferred method of calibration when quantitation of multiple components are necessary and matrix effects are suspected. This is the method you will use to quantitate two components of a mixture (eg Co and Cr) that have overlapping spectral features (see Figure 3.4.1) and when matrix effects are suspected. For a sample with components X and Y, the initial absorbance of the sample at λ_1 is:

$$A_{\lambda_1}^0 = \varepsilon_{X_{\lambda_1}} b C_X^0 + \varepsilon_{Y_{\lambda_1}} b C_Y^0 \quad (6.1.1.5)$$

Where $\varepsilon_{X_{\lambda_1}}$ is the response factor (aka sensitivity, molar extinction coefficient, or the molar absorptivity) for component X at λ_1 , and $\varepsilon_{Y_{\lambda_1}}$ is the response factor for component Y at λ_1 .

In this method, the volume of total sample will change as standard solution of X or Y is added to the sample to create the standard addition calibration for X and Y. When volume changes, concentrations are not additive. But we can correct for this inconvenient truth by multiplying by total volume, V_t . When C is multiplied by V, the result is total moles (n) of each analyte; this value is additive. Multiplying by V gives the equation below.

$$V^0 A_{\lambda_1}^0 = \varepsilon_{X_{\lambda_1}} b n_X^0 + \varepsilon_{Y_{\lambda_1}} b n_Y^0 \quad (6.1.1.6)$$

As standards are added to the sample to create the calibration, the moles of analyte will change by Δn_x and Δn_y , and the new volume-corrected absorption for any point on the calibration would be:

$$V^i A_{\lambda_1}^i = \varepsilon_{X_{\lambda_1}} b (\Delta n_x + n_X^0) + \varepsilon_{Y_{\lambda_1}} b (\Delta n_Y + n_Y^0) \quad (6.1.1.7)$$

where Δn_X is the moles of X added and n_X^0 is the initial amount of analyte in the sample. The same follows for analyte Y.

The initial volume-corrected absorption, $V^0 A^0$ (Equation 6.1.1.6) can be factored from Equation 6.1.1.7 to give the change in absorbance upon standard addition:

$$\Delta(V A_{\lambda_1}) = V^i A_{\lambda_1}^i - V^0 A_{\lambda_1}^0 = \varepsilon_{X_{\lambda_1}} b (\Delta n_X) + \varepsilon_{Y_{\lambda_1}} b (\Delta n_Y) \quad (6.1.1.8)$$

For a mixture of two analytes, absorbance would be measured at two wavelengths as described above. The same model described here for λ_1 , also applies for λ_2 :

$$\Delta(V A_{\lambda_2}) = V^i A_{\lambda_2}^i - V^0 A_{\lambda_2}^0 = \varepsilon_{X_{\lambda_2}} b (\Delta n_X) + \varepsilon_{Y_{\lambda_2}} b (\Delta n_Y) \quad (6.1.1.9)$$

Using a known volume of the sample, we can add known amounts of one analyte to the solution to create a calibration line with at least 3 points. For example, suppose we have a sample containing two analytes, X, and Y in a complex matrix where matrix effects are unknown; and suppose we want to determine the concentrations of [X] and [Y] in the sample. This is a problem that can be solved by GSAM. First, we would obtain standard solutions of X and Y, collect their absorption spectra, and select appropriate wavelengths for analysis. Then, we would collect a spectrum of the analyte solution containing unknown amounts of X and Y.

To create a standard curve for X, we could add a standard solution of X into a known quantity of the sample while monitoring the solution mixture at the two wavelengths. Under these conditions, Δn_Y is zero, and so equation 6.1.1.8 is simplified to a $\Delta(V A_{\lambda_1}) = \varepsilon_{X_{\lambda_1}} b (\Delta n_X)$; this is the form of a linear equation with the slope that is $\varepsilon_{X_{\lambda_1}} b$. By plotting $\Delta(V A_{\lambda_1})$ at one wavelength vs Δn_X we can determine the molar response factor of X at that wavelength ($\varepsilon_{X_{\lambda_1}}$). And, by plotting $\Delta(V A_{\lambda_2})$ at the second wavelength vs Δn_X we can determine the molar response factor of X at the second wavelength ($\varepsilon_{X_{\lambda_2}}$).

Then, using the same sample as above, we can add two aliquots of Y and follow a similar strategy plotting Δn_Y against $\Delta(V A_{\lambda_1})$ and $\Delta(V A_{\lambda_2})$ to find the response factors of Y at the two wavelengths.

Once the response factors of both analytes X and Y are determined at the two different wavelengths, the concentrations of each analyte in the original sample can be determined by simultaneously solving equation 6.1.1.5 and the analogous equation at λ_2 :

$$A_{\lambda_1}^0 = \varepsilon_{X_{\lambda_1}} b C_X^0 + \varepsilon_{Y_{\lambda_1}} b C_Y^0 \quad \text{and} \quad A_{\lambda_2}^0 = \varepsilon_{X_{\lambda_2}} b C_X^0 + \varepsilon_{Y_{\lambda_2}} b C_Y^0 \quad (6.1.1.10)$$

Answers

Substituting into Equation 6.1.1.1 and Equation 6.1.1.2 gives

$$A_{400} = 0.336 = 15.2C_{\text{Cr}} + 5.60C_{\text{Co}}$$

$$A_{400} = 0.187 = 0.533C_{\text{Cr}} + 5.07C_{\text{Co}}$$

To determine C_{Cr} and C_{Co} we solve the first equation for C_{Co}

$$C_{\text{Co}} = \frac{0.336 - 15.2C_{\text{Cr}}}{5.60}$$

and substitute the result into the second equation.

$$0.187 = 0.533C_{\text{Cr}} + 5.07 \times \frac{0.336 - 15.2C_{\text{Cr}}}{5.60}$$

$$0.187 = 0.3042 - 13.23C_{\text{Cr}}$$

Solving for C_{Cr} gives the concentration of Cr^{3+} as $8.86 \times 10^{-3} \text{ M}$. Substituting this concentration back into the equation for the mixture's absorbance at 400 nm gives the concentration of Co^{2+} as $3.60 \times 10^{-2} \text{ M}$.

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6.1.2: Data analysis

1. Calculate the standard deviation of each of your average absorbance measurements at each of the two wavelengths.
2. Calculate $\Delta(VA)$ at each of the two wavelengths (Equations 3.4.8 and 3.4.9). In other words, calculate $\Delta(VA_{\lambda_1})$ and $\Delta(VA_{\lambda_2})$ for each addition. (Hint: $\Delta(VA_{\lambda_1})^0$ and $\Delta(VA_{\lambda_2})^0 = 0$)
3. After each addition, calculate the total cumulative amount (moles) of each standard that has been added; Δn_X (eg cobalt nitrate = X) and Δn_Y (eg chromium nitrate = Y).
4. Generate a table of $\Delta(VA)$ and Δn values (example shown in Table 1). For the purposes of this data analysis, it is important that λ_1 correspond to a λ_{max} for component X, and that λ_2 correspond to a λ_{max} for component Y.
5. Calculate the number of moles of analyte, n_{Co}^0 (eg cobalt nitrate) and n_{Cr}^0 (eg chromium nitrate), in the unknown solution, as well as the concentration of each. The moles of analyte may be calculated (a) manually or (b) using matrix algebra and the Matlab program (instructions below). You should use both methods to analyze your first set of data. You may choose either method for analysis of your second data set and state your reasons for making that choice.

Table 1: Data arranged in preparation for matrix analysis

Solution	Total Vol (L)	Ave Abs λ_1	ΔVA_{λ_1} (component X)	Ave Abs λ_2	ΔVA_{λ_2} (component Y)	Δn_X	Δn_Y
(0) sol. A	0.01		$\Delta VA_{\lambda_1}^0 = 0$		$\Delta VA_{\lambda_2}^0 = 0$	$\Delta n_X^0 =$	$\Delta n_Y^0 =$
(2) A+B(Co)	0.015		$\Delta VA_{\lambda_1}^2 =$		$\Delta VA_{\lambda_2}^2 =$	$\Delta n_X^2 =$	$\Delta n_Y^2 =$
(3) A+2B(Co)	0.02		$\Delta VA_{\lambda_1}^3 =$		$\Delta VA_{\lambda_2}^3 =$	$\Delta n_X^3 =$	$\Delta n_Y^3 =$
(4) A+2B+C(Cr)	0.025		$\Delta VA_{\lambda_1}^4 =$		$\Delta VA_{\lambda_2}^4 =$	$\Delta n_X^4 =$	$\Delta n_Y^4 =$
(5) A+2B+2C(Cr)	0.03		$\Delta VA_{\lambda_1}^5 =$		$\Delta VA_{\lambda_2}^5 =$	$\Delta n_X^5 =$	$\Delta n_Y^5 =$

MANUAL DATA CALCULATION

The ϵ values for X and Y and λ_1 and λ_2 that are described in Section 3.4 (see GSAM) may be determined from a plot of Δn vs ΔVA (four sets of plotted values). The slopes of each plot would give the value of ϵb for each respective analyte and wavelength. For example, the plot of Δn_{Co} vs ΔVA_{λ_1} would yield a linear plot with slope of $\epsilon_{\lambda_1 Co}$, and the plot of Δn_{Co} vs ΔVA_{λ_2} would yield a linear plot with slope of $\epsilon_{\lambda_2 Co}$.

Note: Use only the portion of each plot where the slope is >0 to derive each value of ϵ . The initial analyte amounts may then be calculated from the initial absorbance readings, A^0 , using Equations 3.4.10.

DATA ANALYSIS USING MATLAB

1. Open Matlab.
2. Prepare to record your data analysis work so that you can show your work to your TA and troubleshoot later if necessary. You can do this by using the editor window to record your work, and then saving your work as a script (eg **uv_data_analysis.m**). Alternatively, you can use the command window and type **diary uv_data_analysis** so that all of your on-screen work (with the exception of graphs) will be recorded in the file "**uv_data_analysis**". Either of these files can be printed as a pdf later and submitted as an appendix to your report.
3. Use matrix algebra to derive the ϵ values.
Equation (3.4.8) may be rewritten as

$$[VA] = [K][N] \quad (6.1.2.1)$$

where $[VA]$ is a 2 by 5 matrix of volume corrected absorbances for each solution at two wavelengths; $[K]$ is a 2 by 2 matrix of the response factor (aka sensitivity or $k = \varepsilon b$) values for each analyte-wavelength pair; and $[N]$ is a 2 by 5 matrix of the amounts of standard added, Δn :

$$\begin{pmatrix} \Delta VA_{\lambda_1}^0 & \Delta VA_{\lambda_1}^2 & \Delta VA_{\lambda_1}^3 & \Delta VA_{\lambda_1}^4 & \Delta VA_{\lambda_1}^5 \\ \Delta VA_{\lambda_2}^0 & \Delta VA_{\lambda_2}^2 & \Delta VA_{\lambda_2}^3 & \Delta VA_{\lambda_2}^4 & \Delta VA_{\lambda_2}^5 \end{pmatrix} = \begin{pmatrix} k_{\lambda_1 X} & k_{\lambda_1 Y} \\ k_{\lambda_2 X} & k_{\lambda_2 Y} \end{pmatrix} \begin{pmatrix} \Delta n_X^0 & \Delta n_X^2 & \Delta n_X^3 & \Delta n_X^4 & \Delta n_X^5 \\ \Delta n_Y^0 & \Delta n_Y^2 & \Delta n_Y^3 & \Delta n_Y^4 & \Delta n_Y^5 \end{pmatrix} \quad (6.1.2.2)$$

- a. First, enter the matrices $[VA]$ and $[N]$ into the Matlab workspace using the data from Table 1, and following directions from your instructor.

`VA = [enter values in matrix form] (use spaces between numbers and a semicolon between
N = [enter values in matrix form]`

- b. Using Equation (3.6.2), determine the 2 by 2 matrix, $[K]$, by entering the following command:

`K = VA/N`

- c. From the k values, calculate the molar absorptivities (ε) (in $\text{cm}^{-1}\text{M}^{-1}$) of cobalt nitrate and chromium nitrate at the two selected wavelengths ($k = \varepsilon b$).
4. Calculate the initial moles of Co and Cr using matrix algebra.
From Equations (3.4.10),

$$\begin{pmatrix} \Delta VA_{\lambda_1}^0 \\ \Delta VA_{\lambda_2}^0 \end{pmatrix} = \begin{pmatrix} k_{\lambda_1 X} & k_{\lambda_1 Y} \\ k_{\lambda_2 X} & k_{\lambda_2 Y} \end{pmatrix} \begin{pmatrix} n_X^0 \\ n_Y^0 \end{pmatrix} \quad (6.1.2.3)$$

which may be rewritten as:

$$[VA^0] = [K][n_i] \quad (6.1.2.4)$$

Therefore

$$[K]^{-1}[VA^0] = [n_i] \quad (6.1.2.5)$$

- a. First, create the matrix VAi for two initial values of VA : Using the initial absorbance readings $A_{\lambda_1}^0$ and $A_{\lambda_2}^0$, and the initial volume (0.01 L), to calculate $VA_{\lambda_1}^0$ and $VA_{\lambda_2}^0$. Enter the $VA_{\lambda_1}^0$ and $VA_{\lambda_2}^0$ values into a matrix called VAi . To do this with matlab, enter the following, but substitute the values you calculated into the matrix:

`VAi = [ VA10 ; VA20 ]`

- b. Use the K matrix (with ε values) calculated in step (3) above, Equation (3.6.5), and Matlab to determine the initial analyte amounts n_X^0 and n_Y^0 as follows:

$$n_i = \text{inv}(K) * VA^0 \quad (6.1.2.6)$$

where n_i is the Matlab label given to $[n_i^0]$ and VAi is the label given to $[VA^0]$.

In Matlab, enter the following:

`ni = inv(K)*VAi`

The matrix n_i is a 2 by 1 matrix containing the initial analyte amounts (moles) n_X^0 and n_Y^0 .

5. For each of your two data sets, report the unknown cobalt nitrate and chromium nitrate concentrations in units of moles/liter (molarity). Calculate the weight/volume percent of each component.

$$\text{concentration of solute in } \left(\frac{w}{v} \right) = 100 \times \frac{\text{mass of solute (g)}}{\text{volume of solution (mL)}} \quad (6.1.2.7)$$

6. When you have completed the analysis using Matlab, save your variables as follows:

`save uv_data_analysis_1`

7. To obtain a printout of your diary, you must first exit Matlab and then enter the print command:

```
diary off
```

```
exit
```

8. If you plotted graphs following the instructions below you saved copies of your graphs. Print them using the usual method of printing graphs from Matlab (see the [Matlab chapter](#) if you need help).

GRAPHICAL ANALYSIS USING MATLAB

(Optional. Plots made using excel or other software are ok too.) **NOTE: You must show the least squares line on each of these plots.**

1. To determine $k_{\lambda_1 X}$ from the slope of the plot of Δn_1 vs ΔVA_{λ_1} .

Plot the graph as follows and determine the slope ($k_{\lambda_1 X}$) of the non-vertical portion:

```
plot(N(1,:),VA(1,:), 'o'), title('[N1] VS [VA1]')  
xlabel('[N1]'), ylabel('[VA1]')
```

Save this graph.

```
lsq(N(1,1:3),VA(1,1:3))
```

2. To determine k21 from the slope of the plot of Δn_1 vs ΔVA_2 .

Plot the graph as follows and determine the slope (k21) of the non-vertical portion:

```
plot(N(1,:),VA(2,:), 'o'), title('[N1] VS [VA2]')  
xlabel('[N1]'), ylabel('[VA2]')
```

Save this graph.

```
lsq(N(1,1:3),VA(2,1:3))
```

3. To determine k12 from the slope of the plot of Δn_2 vs ΔVA_1 .

Plot the graph as follows and determine the slope (k12) of the non-vertical portion:

```
plot(N(2,:),VA(1,:), 'o'), title('[N2] VS [VA1]')  
xlabel('[N2]'), ylabel('[VA1]')
```

Save this graph.

```
lsq(N(2,3:5),VA(1,3:5))
```

4. To determine k22 from the slope of the plot of Δn_2 vs ΔVA_2 .

Plot the graph as follows and determine the slope (k22) of the non-vertical portion:

```
plot(N(2,:),VA(2,:), 'o'), title('[N2] VS [VA2]')  
xlabel('[N2]'), ylabel('[VA2]')
```

Save this graph.

```
lsq(N(2,3:5),VA(2,3:5))
```

6.1.3: Pre-lab assignment

Please read this entire module on UV-vis, paying particular attention to the explanation of [Quantitative Analysis of Mixtures](#) and [The two-analyte Generalized Standard Addition Method \(GSAM\)](#). Let the questions below guide your attention.

Please complete the following in your lab notebook and be prepared to discuss these points with your TA:

1. In writing, please explain the advantages to using **multi-point calibration** compared to **single-point calibration**.
2. In writing, please explain the principle of **standard addition** and describe how it is different than a method using **external calibration**.
3. Describe the context in which standard addition should be used as a calibration method and discuss the advantages of standard addition over other methods of analysis.
4. Describe how you should choose appropriate wavelengths in the following scenarios:
 - a. to quantitate a single component in a solution.
 - b. to quantitate two components of a multi-component mixture.
5. Describe GSAM and why it is useful to analyze a mixture of Co and Cr ions in an uncharacterized matrix.
6. Prepare a Table in your notebook to record data (see the [Procedure](#) for an example).
7. You were able to validate your calibration curve for the QUANT lab using a standard solution of known concentration. Is it possible to validate your GSAM calibration using known standards? If so, how?

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6.1.4: Absorbance and Beer's Law

Transmittance and Absorbance

As light passes through a sample, its power decreases as some of it is absorbed. This attenuation of radiation is described quantitatively by two separate, but related terms: transmittance and absorbance. As shown in Figure 6.1.4.1 a, transmittance is the ratio of the source radiation's power as it exits the sample, P_T , to that incident on the sample, P_0 .

$$T = \frac{P_T}{P_0} \quad (6.1.4.1)$$

Multiplying the transmittance by 100 gives the percent transmittance, %T, which varies between 100% (no absorption) and 0% (complete absorption). All methods of detecting photons—including the human eye and modern photoelectric transducers—measure the transmittance of electromagnetic radiation.

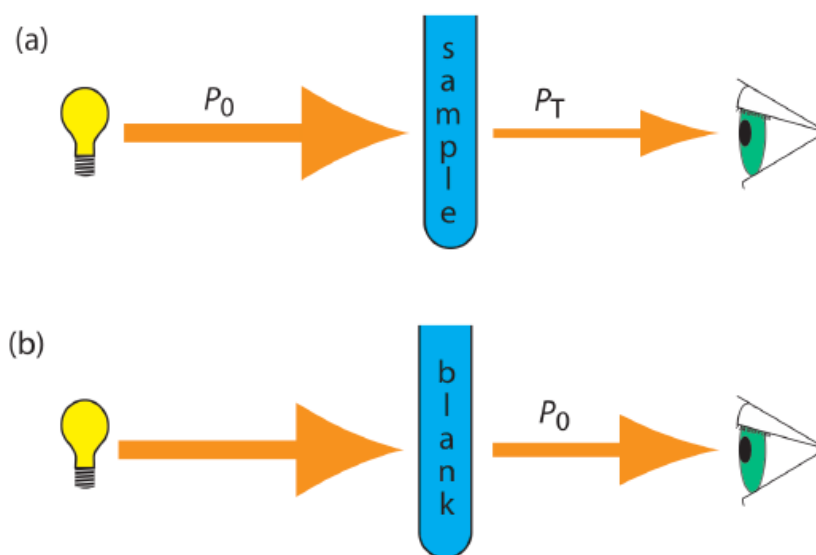


Figure 6.1.4.1 . (a) Schematic diagram showing the attenuation of radiation passing through a sample; P_0 is the source's radiant power and P_T is the radiant power transmitted by the sample. (b) Schematic diagram showing how we redefine P_0 as the radiant power transmitted by the blank. Redefining P_0 in this way corrects the transmittance in (a) for the loss of radiation due to scattering, reflection, absorption by the sample's container, and absorption by the sample's matrix.

Equation 6.1.4.1 does not distinguish between different mechanisms that prevent a photon emitted by the source from reaching the detector. In addition to absorption by the analyte, several additional phenomena contribute to the attenuation of radiation, including reflection and absorption by the sample's container, absorption by other components in the sample's matrix, and the scattering of radiation. To compensate for this loss of the radiation's power, we use a method blank. As shown in Figure 6.1.4.7 b, we redefine P_0 as the power exiting the method blank.

An alternative method for expressing the attenuation of electromagnetic radiation is absorbance, A , which we define as

$$A = -\log T = -\log \frac{P_T}{P_0} \quad (6.1.4.2)$$

Absorbance is the more common unit for expressing the attenuation of radiation because it is a linear function of the analyte's concentration.

We will show that this is true in the next section when we introduce Beer's law.

Example 6.1.4.1

A sample has a percent transmittance of 50%. What is its absorbance?

Solution

A percent transmittance of 50.0% is the same as a transmittance of 0.500. Substituting into Equation 6.1.4.2 gives

$$A = -\log T = -\log(0.500) = 0.301$$

Exercise 6.1.4.1

What is the %T for a sample if its absorbance is 1.27?

Answer available at the end of this section.

Equation 6.1.4.1 has an important consequence for atomic absorption. As we learned from Figure 6.1.4.6, atomic absorption lines are very narrow. Even with a high quality monochromator, the effective bandwidth for a continuum source is $100 - 1000 \times$ greater than the width of an atomic absorption line. As a result, little radiation from a continuum source is absorbed when it passes through a sample of atoms; because $P_0 \approx P_T$ the measured absorbance effectively is zero. For this reason, atomic absorption requires that we use a line source instead of a continuum source.

Absorbance and Concentration: Beer's Law

When monochromatic electromagnetic radiation passes through an infinitesimally thin layer of sample of thickness dx , it experiences a decrease in its power of dP (Figure 6.1.4.2).

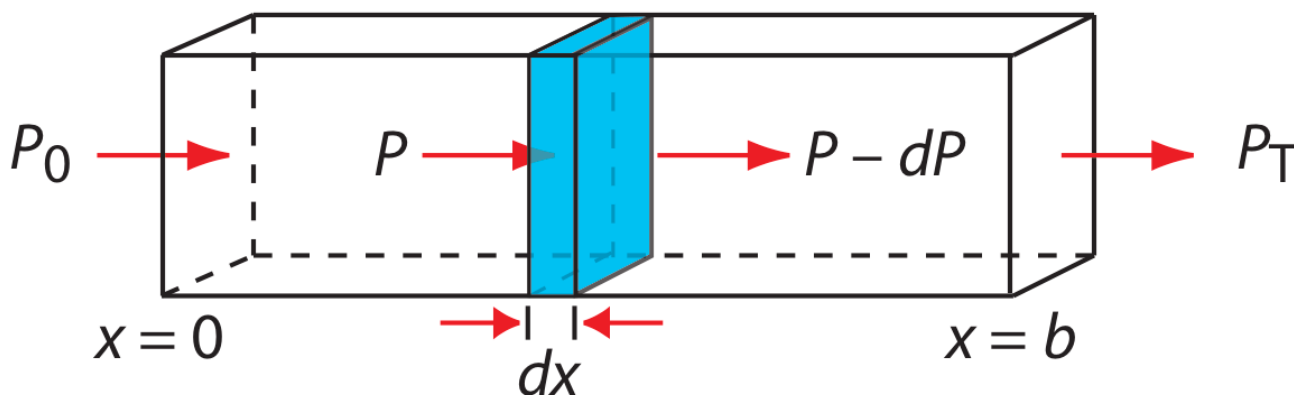


Figure 6.1.4.2. Factors used to derive the Beer's law.

This fractional decrease in power is proportional to the sample's thickness and to the analyte's concentration, C ; thus

$$-\frac{dP}{P} = \alpha C dx \quad (6.1.4.3)$$

where P is the power incident on the thin layer of sample and α is a proportionality constant. Integrating the left side of Equation 6.1.4.3 over the sample's full thickness

$$-\int_{P=P_0}^{P=P_t} \frac{dP}{P} = \alpha C \int_{x=0}^{x=b} dx$$

$$\ln \frac{P_0}{P_T} = \alpha b C$$

converting from ln to log, and substituting into Equation 6.1.4.2, gives

$$A = abC \quad (6.1.4.4)$$

where a is the analyte's **absorptivity** with units of $\text{cm}^{-1} \text{conc}^{-1}$. If we express the concentration using molarity, then we replace a with the **molar absorptivity**, ϵ , which has units of $\text{cm}^{-1} \text{M}^{-1}$.

$$A = \epsilon b C \quad (6.1.4.5)$$

The absorptivity and the molar absorptivity are proportional to the probability that the analyte absorbs a photon of a given energy. As a result, values for both a and ϵ depend on the wavelength of the absorbed photon.

Example 6.1.4.2

A $5.00 \times 10^{-4} \text{ M}$ solution of analyte is placed in a sample cell that has a pathlength of 1.00 cm. At a wavelength of 490 nm, the solution's absorbance is 0.338. What is the analyte's molar absorptivity at this wavelength?

Solution

Solving Equation 6.1.4.5 for ϵ and making appropriate substitutions gives

$$\epsilon = \frac{A}{bC} = \frac{0.338}{(1.00 \text{ cm})(5.00 \times 10^{-4} \text{ M})} = 676 \text{ cm}^{-1} \text{ M}^{-1}$$

Exercise 6.1.4.2

A solution of the analyte from Example 6.1.4.2 has an absorbance of 0.228 in a 1.00-cm sample cell. What is the analyte's concentration?

Answer available at the end of this section.

Equation 6.1.4.4 and Equation 6.1.4.5, which establish the linear relationship between absorbance and concentration, are known as Beer's law. Calibration curves based on Beer's law are common in quantitative analyses.

As is often the case, the formulation of a law is more complicated than its name suggests. This is the case, for example, with Beer's law, which also is known as the Beer-Lambert law or the Beer-Lambert-Bouguer law. Pierre Bouguer, in 1729, and Johann Lambert, in 1760, noted that the transmittance of light decreases exponentially with an increase in the sample's thickness.

$$T \propto e^{-b}$$

Later, in 1852, August Beer noted that the transmittance of light decreases exponentially as the concentration of the absorbing species increases.

$$T \propto e^{-C}$$

Together, and when written in terms of absorbance instead of transmittance, these two relationships make up what we know as Beer's law.

Beer's Law and Multicomponent Samples

We can extend Beer's law to a sample that contains several absorbing components. If there are no interactions between the components, then the individual absorbances, A_i , are additive. For a two-component mixture of analyte's X and Y , the total absorbance, A_{tot} , is

$$A_{tot} = A_X + A_Y = \epsilon_X b C_X + \epsilon_Y b C_Y$$

Generalizing, the absorbance for a mixture of n components, A_{mix} , is

$$A_{mix} = \sum_{i=1}^n A_i = \sum_{i=1}^n \epsilon_i b C_i \quad (6.1.4.6)$$

Limitations to Beer's Law

Beer's law suggests that a plot of absorbance vs. concentration—we will call this a Beer's law plot—is a straight line with a y -intercept of zero and a slope of ab or ϵb . In some cases a Beer's law plot deviates from this ideal behavior (see Figure 6.1.4.3), and such deviations from linearity are divided into three categories: fundamental, chemical, and instrumental.

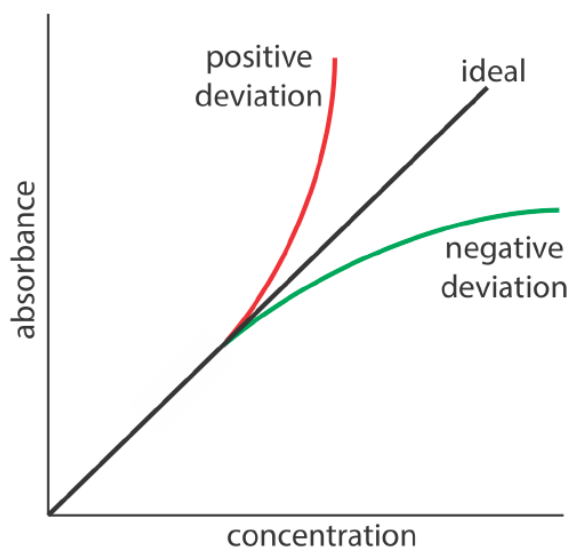


Figure 6.1.4.3 . Plots of absorbance vs. concentration showing positive and negative deviations from the ideal Beer's law relationship, which is a straight line.

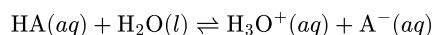
Fundamental Limitations to Beer's Law

Beer's law is a limiting law that is valid only for low concentrations of analyte. There are two contributions to this fundamental limitation to Beer's law. At higher concentrations the individual particles of analyte no longer are independent of each other. The resulting interaction between particles of analyte may change the analyte's absorptivity. A second contribution is that an analyte's absorptivity depends on the solution's refractive index. Because a solution's refractive index varies with the analyte's concentration, values of a and ϵ may change. For sufficiently low concentrations of analyte, the refractive index essentially is constant and a Beer's law plot is linear.

Chemical Limitations to Beer's Law

A chemical deviation from Beer's law may occur if the analyte is involved in an equilibrium reaction. Consider, for example, the weak acid, HA . To construct a Beer's law plot we prepare a series of standard solutions—each of which contains a known total concentration of HA —and then measure each solution's absorbance at the same wavelength. Because HA is a weak acid, it is in equilibrium with its conjugate weak base, A^- .

In the equations that follow, the conjugate weak base A^- is written as A as it is easy to mistake the symbol for anionic charge as a minus sign.



If both HA and A^- absorb at the selected wavelength, then Beer's law is

$$A = \varepsilon_{HA} b C_{HA} + \varepsilon_A b C_A \quad (6.1.4.7)$$

Because the weak acid's total concentration, C_{total} , is

$$C_{total} = C_{HA} + C_A$$

we can write the concentrations of HA and A^- as

$$C_{HA} = \alpha_{HA} C_{total} \quad (6.1.4.8)$$

$$C_A = (1 - \alpha_{HA}) C_{total} \quad (6.1.4.9)$$

where α_{HA} is the fraction of weak acid present as HA . Substituting Equation 6.1.4.8 and Equation 6.1.4.9 into Equation 6.1.4.7 and rearranging, gives

$$A = (\varepsilon_{HA} \alpha_{HA} + \varepsilon_A - \varepsilon_A \alpha_{HA}) b C_{total} \quad (6.1.4.10)$$

To obtain a linear Beer's law plot, we must satisfy one of two conditions. If ε_{HA} and ε_A have the same value at the selected wavelength, then Equation 6.1.4.10 simplifies to

$$A = \varepsilon_A b C_{total} = \varepsilon_{HA} b C_{total}$$

Alternatively, if α_{HA} has the same value for all standard solutions, then each term within the parentheses of Equation 6.1.4.10 is constant—which we replace with k —and a linear calibration curve is obtained at any wavelength.

$$A = k b C_{total}$$

Because HA is a weak acid, the value of α_{HA} varies with pH. To hold α_{HA} constant we buffer each standard solution to the same pH. Depending on the relative values of α_{HA} and α_A , the calibration curve has a positive or a negative deviation from Beer's law if we do not buffer the standards to the same pH.

Instrumental Limitations to Beer's Law

There are two principal instrumental limitations to Beer's law. The first limitation is that Beer's law assumes that radiation reaching the sample is of a single wavelength—that is, it assumes a purely monochromatic source of radiation. Even the best wavelength selector passes radiation with a small, but finite effective bandwidth (See Chapter 10.1 of *Harvey's Analytical Chemistry*, in Figure 10.1.10). Polychromatic radiation always gives a negative deviation from Beer's law, but the effect is smaller if the value of ε essentially is constant over the wavelength range passed by the wavelength selector. For this reason, as shown in Figure 6.1.4.4, it is better to make absorbance measurements at the top of a broad absorption peak. In addition, the deviation from Beer's law is less serious if the source's effective bandwidth is less than one-tenth of the absorbing species' natural bandwidth [(a) Strong, F. C., III *Anal. Chem.* **1984**, 56, 16A–34A; Gilbert, D. D. *J. Chem. Educ.* **1991**, 68, A278–A281]. When measurements must be made on a slope, linearity is improved by using a narrower effective bandwidth.

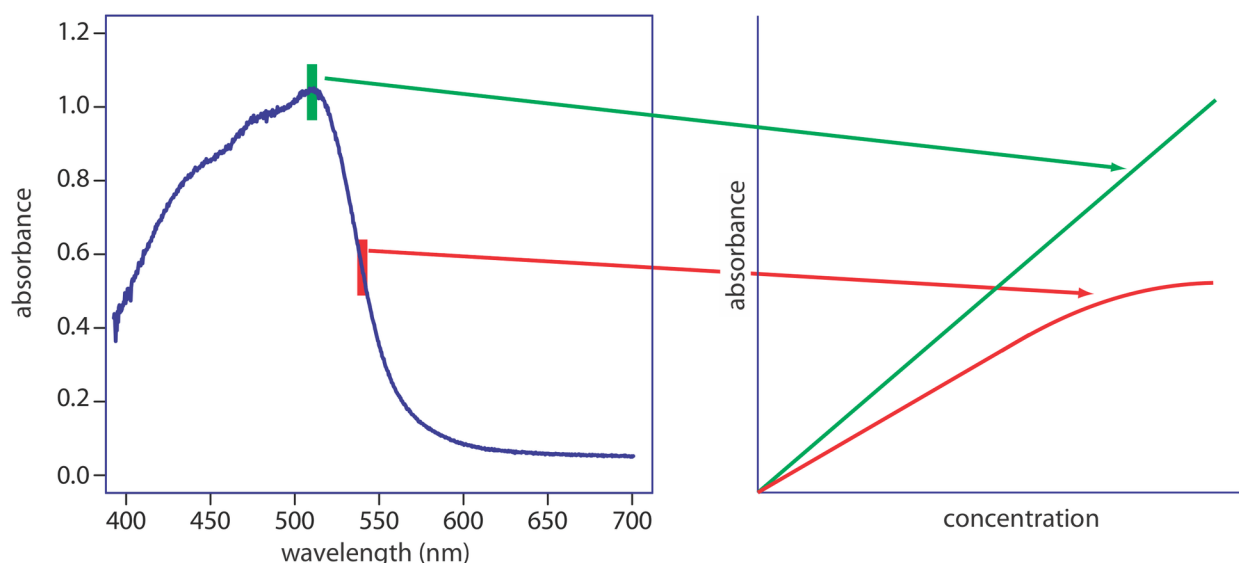


Figure 6.1.4.4 . Effect of wavelength selection on the linearity of a Beer's law plot. Another reason for measuring absorbance at the top of an absorbance peak is that it provides for a more sensitive analysis. Note that the green Beer's law plot has a steeper slope—and, therefore, a greater sensitivity—than the red Beer's law plot. A Beer's law plot, of course, is equivalent to a calibration curve.

Stray radiation is the second contribution to instrumental deviations from Beer's law. Stray radiation arises from imperfections in the wavelength selector that allow light to enter the instrument and to reach the detector without passing through the sample. Stray radiation adds an additional contribution, P_{stray} , to the radiant power that reaches the detector; thus

$$A = -\log \frac{P_T + P_{\text{stray}}}{P_0 + P_{\text{stray}}}$$

For a small concentration of analyte, P_{stray} is significantly smaller than P_0 and P_T , and the absorbance is unaffected by the stray radiation. For higher concentrations of analyte, less light passes through the sample and P_T and P_{stray} become similar in magnitude. This results in an absorbance that is smaller than expected, and a negative deviation from Beer's law.

Answers

To find the transmittance, T , we begin by noting that

$$A = 1.27 = -\log T$$

Solving for T

$$\begin{aligned} -1.27 &= \log T \\ 10^{-1.27} &= T \end{aligned}$$

gives a transmittance of 0.054, or a %T of 5.4%.

Making appropriate substitutions into Beer's law

$$A = 0.228 = \epsilon b C = (676 \text{ M}^{-1} \text{ cm}^{-1})(1 \text{ cm})C$$

and solving for C gives a concentration of $3.37 \times 10^{-4} \text{ M}$.

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6.1.5: UV/Vis Instrumentation

In this section we examine several different instruments for UV-vis absorption spectroscopy, with an emphasis on the specific instruments used in our teaching labs at Duke, and their advantages and limitations. Methods of sample introduction also are covered in this section. For more information about absorption spectroscopy, or for a description of the clinical, environmental, forensic, and other applications of absorption spectroscopy, see [10.3: UV/Vis and IR Spectroscopy](#) by David Harvey.

Instrument Designs for Molecular UV/Vis Absorption

Single-Beam Spectrophotometer

An instrument that uses a monochromator for wavelength selection is called a **spectrophotometer**. The simplest spectrophotometer is a single-beam instrument equipped with a fixed-wavelength monochromator (Figure 6.1.5.2). Single-beam spectrophotometers are calibrated and used in the same manner as a photometer. One example of a single-beam spectrophotometer is Thermo Scientific's Spectronic 20D+, which is shown in the photographic insert to Figure 6.1.5.2 . *This is similar to the [Genesys Spec 20](#) that you used during the QUANT experiment.*

The Spectronic 20D+ has a wavelength range of 340–625 nm (950 nm when using a red-sensitive detector), and a fixed effective bandwidth of 20 nm. Battery-operated, hand-held single-beam spectrophotometers are available, which are easy to transport into the field. Other single-beam spectrophotometers also are available with effective bandwidths of 2–8 nm. Fixed wavelength single-beam spectrophotometers are not practical for recording spectra (in contrast to a single wavelength) because manually adjusting the wavelength and recalibrating the spectrophotometer is awkward and time-consuming. The accuracy of a single-beam spectrophotometer is limited by the stability of its source and detector over time.

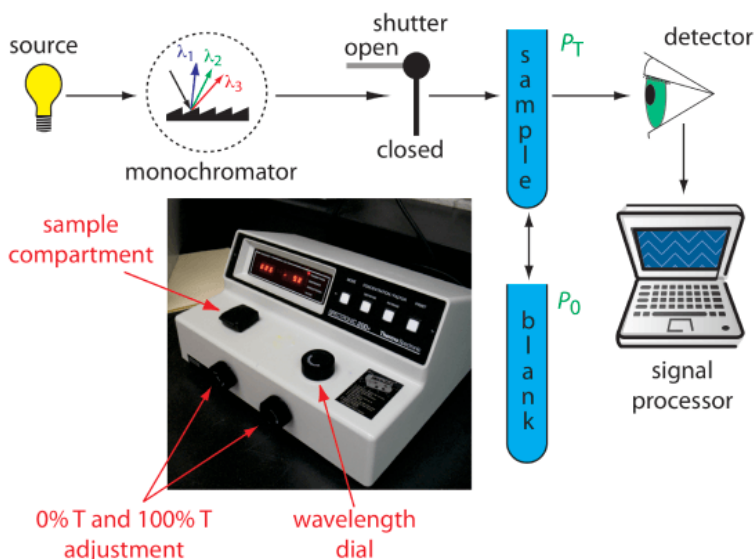


Figure 6.1.5.2 . Schematic diagram of a fixed-wavelength, single-beam spectrophotometer. The photographic insert shows a typical instrument.

The shutter remains closed until the sample or blank is placed in the sample compartment. The analyst manually selects the wavelength by adjusting the wavelength dial. Inset photo modified from: Adi (www.commons.Wikipedia.org).

Double-Beam Spectrophotometer

The limitations of a fixed-wavelength, single-beam spectrophotometer is minimized by using a **double-beam** spectrophotometer (Figure 6.1.5.3). A chopper controls the radiation's path, alternating it between the sample, the blank, and a shutter. The signal processor uses the chopper's speed of rotation to resolve the signal that reaches the detector into the transmission of the blank, P_0 , and the sample, P_T . By including an opaque surface as a shutter, it also is possible to continuously adjust 0%T. The effective

bandwidth of a double-beam spectrophotometer is controlled by adjusting the monochromator's entrance and exit slits. Effective bandwidths of 0.2–3.0 nm are common. A scanning monochromator allows for the automated recording of spectra. Double-beam instruments are more versatile than single-beam instruments, being useful for both quantitative and qualitative analyses, but also are more expensive and not particularly portable. **You will use a double beam spectrometer in the UV-vis module in this course, and the instrument you will use is the same as that pictured in Figure 6.1.5.3 ; it is a Cary UV-vis double-beam spectrometer.**

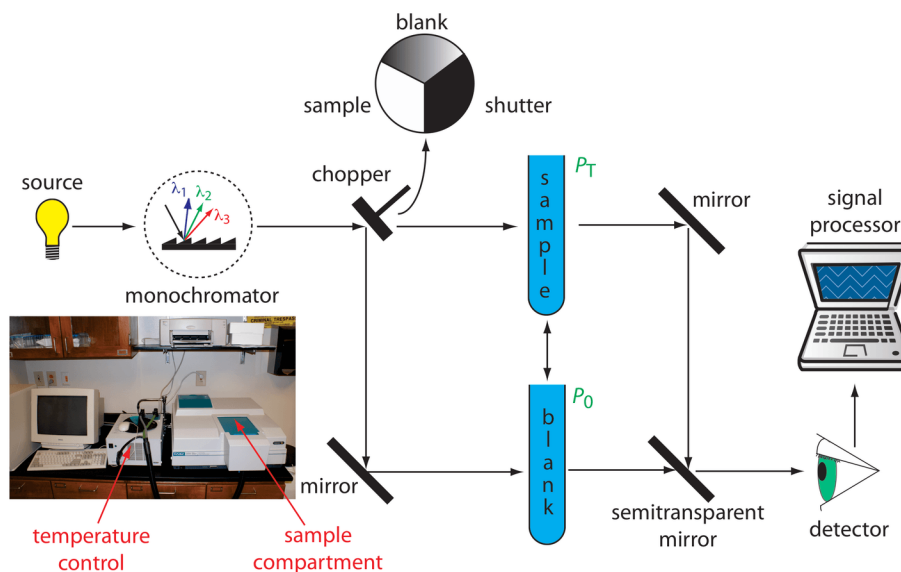


Figure 6.1.5.3 . Schematic diagram of a scanning, double-beam spectrophotometer. A chopper directs the source's radiation, using a transparent window to pass radiation to the sample and a mirror to reflect radiation to the blank. The chopper's opaque surface serves as a shutter, which allows for a constant adjustment of the spectrophotometer's %T. The photographic insert shows a typical instrument. The module in the middle of the photo is a temperature control unit that makes it possible to heat or cool the sample to a constant temperature.

Diode Array Spectrometer

An instrument with a single detector can monitor only one wavelength at a time. If we replace a single photomultiplier with an array of photodiodes, we can use the resulting detector to record a full spectrum in as little as 0.1 s. In a diode array spectrometer the source radiation passes through the sample and is dispersed by a grating (Figure 6.1.5.4). The photodiode array detector is situated at the grating's focal plane, with each diode recording the radiant power over a narrow range of wavelengths. Because we replace a full monochromator with just a grating, a diode array spectrometer is small and compact. This is the kind of spectrometer that you will use during the HPLC experiment; **a photodiode array detector (PDA) is integrated into the HPLC system as an in-line detector.**

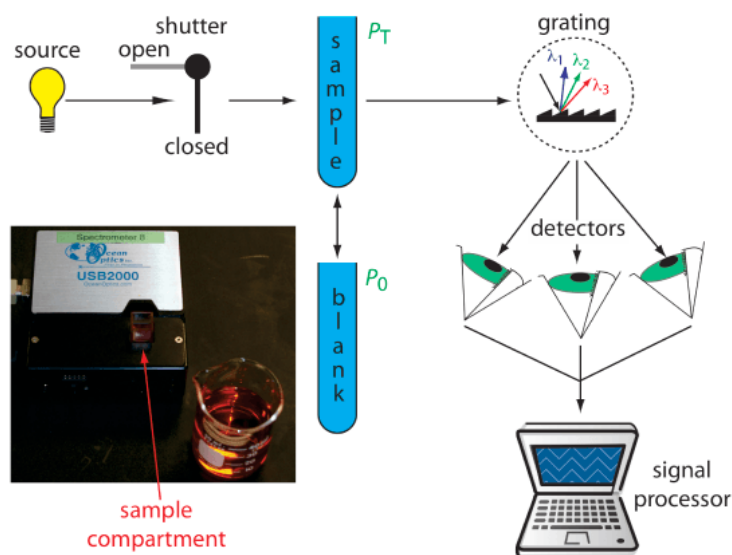


Figure 6.1.5.4 . Schematic diagram of a diode array spectrophotometer. The photographic insert shows a typical instrument. Note that the 50-mL beaker provides a sense of scale. Because the spectrometer is small and compact, it is easy to transport into the field.

One advantage of a diode array spectrometer is the speed of data acquisition, which allows us to collect multiple spectra for a single sample. Individual spectra are added and averaged to obtain the final spectrum. This **signal averaging** improves a spectrum's signal-to-noise ratio. If we add together n spectra, the sum of the signal at any point, x , increases as nS_x , where S_x is the signal. The noise at any point, N_x , is a random event, which increases as $\sqrt{n}N_x$ when we add together n spectra. The **signal-to-noise ratio** after n scans, $(S/N)_n$ is

$$\left(\frac{S}{N}\right)_n = \frac{nS_x}{\sqrt{n}N_x} = \sqrt{n}\frac{S_x}{N_x}$$

where S_x/N_x is the signal-to-noise ratio for a single scan. The impact of signal averaging is shown in Figure 6.1.5.5 . The first spectrum shows the signal after one scan, which consists of a single, noisy peak. Signal averaging using 4 scans and 16 scans decreases the noise and improves the signal-to-noise ratio. One disadvantage of a photodiode array is that the effective bandwidth per diode is roughly an order of magnitude larger than that for a high quality monochromator.

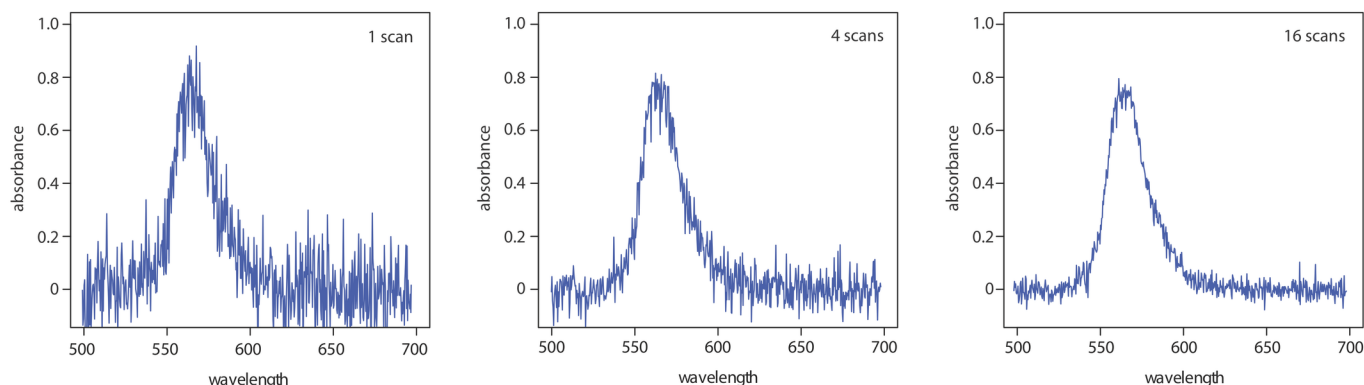


Figure 6.1.5.5 . Effect of signal averaging on a spectrum's signal-to-noise ratio. From top to bottom: spectrum for a single scan; average spectrum after four scans; and average spectrum after adding 16 scans.

For more details on signals and noise, see Introduction to Signals and Noise by Steven Petrovic, an on-line resource that is part of the Analytical Sciences Digital Library.

Sample Cells (cuvettes)

Samples that are in a liquid or solution state are placed in cells constructed with materials that are transparent to UV and/or Visible light. Common cuvette materials are quartz, glass, and plastic (Figure 6.1.5.6). Glass and plastic are useful for samples that require Vis transparency, although glass is not transparent to UV light below ~380 nm. Some plastics are also not transparent to UV light. Quartz is transparent to all UV and Vis wavelengths, but quartz is expensive. A quartz cell is required when analysis is necessary at wavelength <380 nm is necessary because other materials show a significant absorption in the UV region.

By far, the most common pathlength is 1 cm (10 mm), as is the case for the "standard" size cuvette pictured at the far left of Figure 6.1.5.6 . Cells with shorter (as little as 0.1 cm) and longer pathlengths (up to 10 cm) are available. Longer pathlength cells are useful when analyzing a very dilute solution or for gas samples. The highest quality cells allow the radiation to strike a flat surface at a 90° angle, minimizing the loss of radiation to reflection.

Regular glass test tubes are often used as a sample cell with simple, single-beam instruments, although because the glass varies in thickness along the test tube walls, differences in the cell's pathlength and optical properties add an additional source of error to the analysis. Use of test tubes is not recommended when high precision and accuracy are priorities.



Figure 6.1.5.6 . Examples of sample cells for UV/Vis spectroscopy. From left to right (with path lengths in parentheses): rectangular plastic cuvette (10.0 mm), rectangular quartz cuvette (5.000 mm), rectangular quartz cuvette (1.000 mm), cylindrical quartz cuvette (10.00 mm), cylindrical glass cuvette with quartz windows (100.0 mm). Cells often are available as a matched pair, which is important when using a double-beam instrument.

If we need to monitor an analyte's concentration over time, it may not be possible to remove samples for analysis. This often is the case, for example, when monitoring an industrial production line or waste line, when monitoring a patient's blood, or when monitoring an environmental system, such as stream. With a **fiber-optic probe** we can analyze samples *in situ*. An example of a remote sensing fiber-optic probe is shown in Figure 6.1.5.7 . The probe consists of two bundles of fiber-optic cable. One bundle transmits radiation from the source to the probe's tip, which is designed to allow the sample to flow through the sample cell. Radiation from the source passes through the solution and is reflected back by a mirror. The second bundle of fiber-optic cable transmits the nonabsorbed radiation to the wavelength selector. Another design replaces the flow cell shown in Figure 6.1.5.7 with a membrane that contains a reagent that reacts with the analyte. When the analyte diffuses into the membrane it reacts with the reagent, producing a product that absorbs UV or visible radiation. The nonabsorbed radiation from the source is reflected or scattered back to the detector. Fiber optic probes that show chemical selectivity are called optrodes [(a) Seitz, W. R. *Anal. Chem.* **1984**, 56, 16A–34A; (b) Angel, S. M. *Spectroscopy* **1987**, 2(2), 38–48].

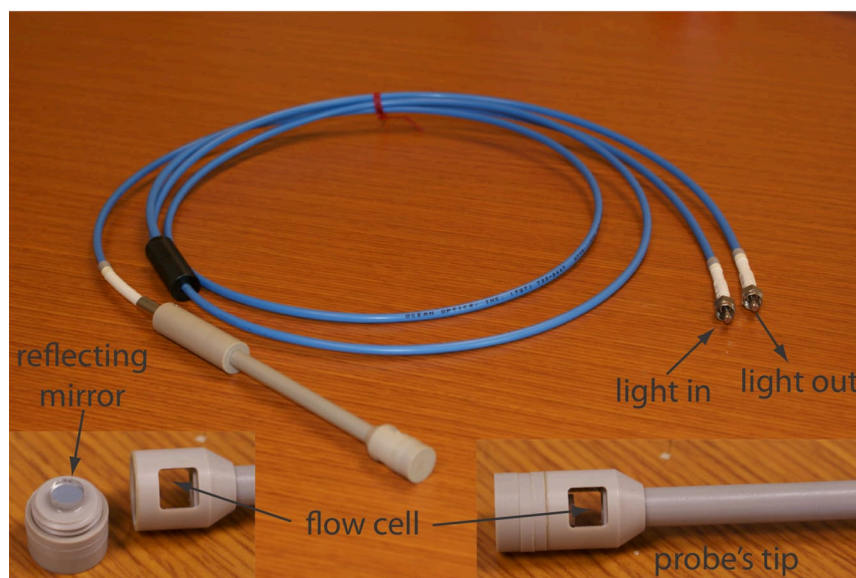


Figure 6.1.5.7 . Example of a fiber-optic probe. The inset photographs at the bottom of the figure provide close-up views of the probe's flow cell and the reflecting mirror.

Evaluation of UV/Vis and IR Spectroscopy

Accuracy

Under normal conditions a relative error of 1–5% is easy to obtained with UV/Vis absorption. Accuracy usually is limited by the quality of the blank. Examples of the type of problems that are encountered include the presence of particulates in the sample that scatter radiation, and the presence of interferents that react with analytical reagents. In the latter case the interferant may react to form an absorbing species, which leads to a positive determinate error. Interferents also may prevent the analyte from reacting, which leads to a negative determinate error. With care, it is possible to improve the accuracy of an analysis by as much as an order of magnitude.

Precision

In absorption spectroscopy, precision is limited by indeterminate errors—primarily instrumental noise—which are introduced when we measure absorbance. Precision generally is worse for low absorbances where $P_0 \approx P_T$, and for high absorbances where P_T approaches 0. We might expect, therefore, that precision will vary with transmittance.

We can derive an expression between precision and transmittance by applying the propagation of uncertainty as described in Chapter 4. To do so we rewrite Beer's law as

$$C = -\frac{1}{\epsilon b} \log T \quad (6.1.5.1)$$

Table 4.3.1 in Chapter 4 helps us complete the propagation of uncertainty for Equation 6.1.5.1; thus, the absolute uncertainty in the concentration, s_C , is

$$s_c = -\frac{0.4343}{\epsilon b} \times \frac{s_T}{T} \quad (6.1.5.2)$$

where s_T is the absolute uncertainty in the transmittance. Dividing Equation 6.1.5.2 by Equation 6.1.5.1 gives the relative uncertainty in concentration, s_C/C , as

$$\frac{s_c}{C} = \frac{0.4343 s_T}{T \log T}$$

If we know the transmittance's absolute uncertainty, then we can determine the relative uncertainty in concentration for any

measured transmittance.

Determining the relative uncertainty in concentration is complicated because s_T is a function of the transmittance. As shown in Table 6.1.5.3, three categories of indeterminate instrumental error are observed [Rothman, L. D.; Crouch, S. R.; Ingle, J. D. Jr. *Anal. Chem.* **1975**, 47, 1226-1233]. A constant s_T is observed for the uncertainty associated with reading %T on a meter's analog or digital scale. Typical values are ± 0.2 – 0.3% (a k_1 of ± 0.002 – 0.003) for an analog scale and $\pm 0.001\%$ (a k_1 of ± 0.00001) for a digital scale.

Table 6.1.5.3 . Effect of Indeterminate Errors on Relative Uncertainty in Concentration

category	sources of indeterminate error	relative uncertainty in concentration
$s_T = k_1$	%T readout resolution noise in thermal detectors	$\frac{s_C}{C} = \frac{0.4343k_1}{T \log T}$
$s_T = k_2 \sqrt{T^2 + T}$	noise in photon detectors	$\frac{s_C}{C} = \frac{0.4343k_2}{\log T} \sqrt{1 + \frac{1}{T}}$
$s_T = k_3 T$	positioning of sample cell fluctuations in source intensity	$\frac{s_C}{C} = \frac{0.4343k_3}{\log T}$

The effect of a constant s_T on the relative uncertainty in concentration is shown by curve A in Figure 6.1.5.16. Note that the relative uncertainty is very large for both high absorbances and low absorbances, reaching a minimum when the absorbance is 0.4343. This source of indeterminate error is important for inexpensive UV/Vis spectrophotometers. **To obtain a relative uncertainty in concentration of ± 1 – 2% , the absorbance is kept within the range 0.1–1.**

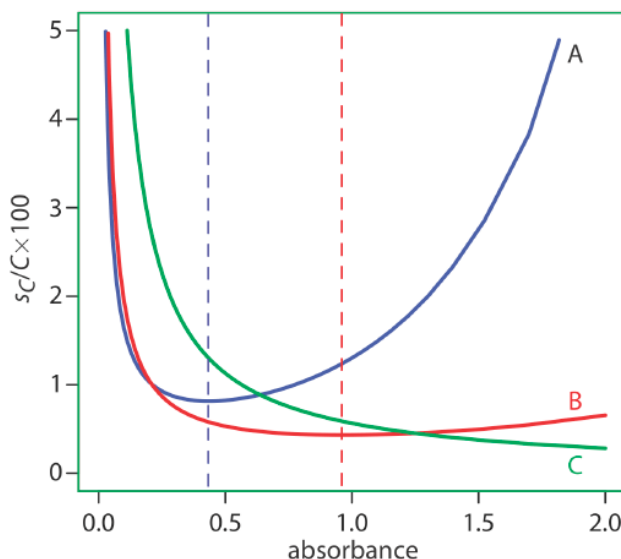


Figure 6.1.5.16 . Percent relative uncertainty in concentration as a function of absorbance for the categories of indeterminate errors in Table 6.1.5.10.3. A: $k_1 = \pm 0.0030$; B: $k_2 = \pm 0.0030$; and C: $k_3 = \pm 0.0130$. The dashed lines correspond to the minimum uncertainty for curve A (absorbance of 0.4343) and for curve B (absorbance of 0.963).

Values of s_T are a complex function of transmittance when indeterminate errors are dominated by the noise associated with photon detectors. Curve B in Figure 6.1.5.16 shows that the relative uncertainty in concentration is very large for low absorbances, but is smaller at higher absorbances. Although the relative uncertainty reaches a minimum when the absorbance is 0.963, there is little change in the relative uncertainty for absorbances between 0.5 and 2. This source of indeterminate error generally limits the precision of high quality UV/Vis spectrophotometers for mid-to-high absorbances.

Finally, the value of s_T is directly proportional to transmittance for indeterminate errors that result from fluctuations in the source's intensity and from uncertainty in positioning the sample within the spectrometer. The latter is particularly important because the optical properties of a sample cell are not uniform. As a result, **repositioning the sample cell in a different orientation may lead to a change in the intensity of transmitted radiation**. As shown by curve C in Figure 6.1.5.16, the effect is important only at low absorbances. This source of indeterminate errors usually is the limiting factor for high quality UV/Vis spectrophotometers when the absorbance is relatively small.

When the relative uncertainty in concentration is limited by the %T readout resolution, it is possible to improve the precision of the analysis by redefining 100% T and 0% T. Normally 100% T is established using a blank and 0% T is established while preventing the source's radiation from reaching the detector. If the absorbance is too high, precision is improved by resetting 100% T using a standard solution of analyte whose concentration is less than that of the sample (Figure 6.1.5.17 a). For a sample whose absorbance is too low, precision is improved by redefining 0% T using a standard solution of the analyte whose concentration is greater than that of the analyte (Figure 6.1.5.17 b). In this case a calibration curve is required because a linear relationship between absorbance and concentration no longer exists. Precision is further increased by combining these two methods (Figure 6.1.5.17 c). Again, a calibration curve is necessary since the relationship between absorbance and concentration is no longer linear.

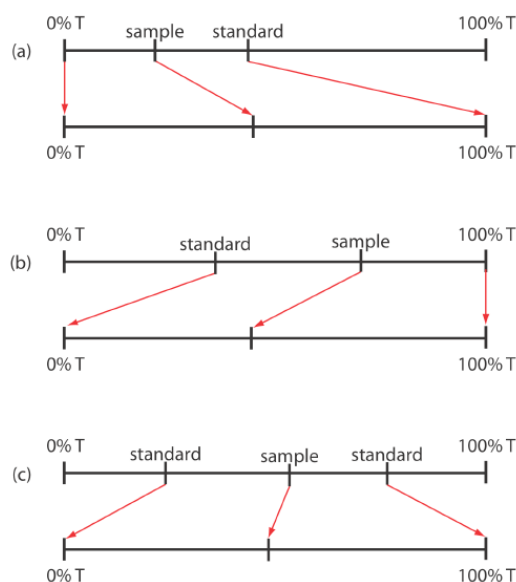


Figure 6.1.5.17 . Methods for improving the precision of absorption methods: (a) high-absorbance method; (b) low-absorbance method; (c) maximum precision method.

Sensitivity

The sensitivity of a molecular absorption method, which is the slope of a Beer's law calibration curve, is the product of the analyte's absorptivity and the pathlength of the sample cell (ϵb). **You can improve a method's sensitivity by selecting a wavelength where absorbance is at a maximum** or by increasing pathlength.

See Figure 3.2.4 for an example of how the choice of wavelength affects a calibration curve's sensitivity.

Selectivity

In many cases it is possible to find a wavelength where the analyte is the only absorbing species. In those cases, selectivity is not a problem. However, when two or more species do contribute to the measured absorbance, a multicomponent analysis is still possible, as discussed in the [next section](#).

Time, Cost, and Equipment

The analysis of a sample by molecular absorption spectroscopy is relatively rapid, although additional time is required if we need to convert a nonabsorbing analyte into an absorbing form. The cost of UV/Vis instrumentation ranges from several hundred dollars for a simple filter photometer, to more than \$50,000 for a computer-controlled, high-resolution double-beam instrument equipped with variable slit widths, and operating over an extended range of wavelengths.

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6.1.6: UV-vis multicomponent analysis procedure

Readying and validating the UV-vis

Turn on

1. Make sure that the sample compartment door is closed tightly, then turn the UV-vis on. The power switch is located on the lower left side of the front of the instrument. Green light will be lit when instrument is on.
2. Warm up the instrument for about 30 minutes (during which you should prepare samples as instructed below).

Validate

Validate the instrument after it has been on for 30 minutes.

1. Open the "Analytical Lab" folder on the desktop and double click on the "Validate" icon. This program will test if the instrument is working properly.
2. Click on the "Tests" button. You will see a text box labeled "Test Order". Using the red X button on the right (not the bottom), deselect the top three tests until only "Wavelength Accuracy - Ho₂O₃" remains and click "OK".
3. Open the sample chamber, and insert the Holmium Oxide filter into the front cuvette holder (make sure the back holder is empty).
4. Click the "Start" button at the top of the screen, and click "OK" in the pop-up screen to affirm that the Holmium Oxide filter is in place.
5. If you see "Holmium Oxide Method - PASSED" in the report at the end of the scan, you may proceed to the next step. If not, get help from your TA.
6. Click the "Print" button at the bottom of the screen.
7. Close the Validate Program (OK to close without saving data) and double click on "Scan" icon in the "Analytical Lab" folder on the desktop.

Procedure

Hazards

- **Chromium(III)nitrate nonahydrate (CAS No: 7789-02-8):** Chromium(III) compounds are considered toxic. ORL-RAT LD50: 3250 mg/kg. The compound is harmful if inhaled or swallowed. Use gloves at all times when handling this material or its solutions.
- **Cobalt(II)nitrate hexahydrate (CAS No: 10026-22-9):** This compound is harmful if inhaled or swallowed. ORL-RAT LD50: 691 mg/kg.

Use gloves when handling all solutions in this experiment.

Check the SDS safety information by entering the CAS Number at <https://chemicalsafety.com>, or any other SDS site.

Materials

- 0.09 M [Cr(NO₃)₃] solution - confirm the exact concentration with your TA.
- 0.4 M [Co(NO₃)₂] solution - confirm the exact concentration with your TA.
- Deionized water (solvent)
- 15 mL of an "unknown" solution containing a mixture of chromium nitrate and cobalt nitrate.
- 2 x 1 cm glass or quartz cuvettes (transparent in the visible region; cut-off at ~340 nm)
- 3 x 10 mL pipettes
- 2 x 5 mL pipettes
- 2 x 25 mL volumetric flasks
- 2 x 50 mL Erlenmeyer flasks

- 2 x 50 mL beakers
- Transfer pipettes

Prepare Standard Solutions, Measure Absorbance Spectra, and Select Wavelengths

**Please handle cuvettes with care. A cuvette should never be set directly on the bench top - it should always be placed in a rack when not in use.

Prepare Standard (B and C)

1. Quantitatively dilute 10.00 mL of the 0.4 M $[\text{Co}(\text{NO}_3)_2]$ to 25 mL using DI water in a 25.00 mL volumetric flask. This is your working solution B. Calculate the molarity of cobalt nitrate in this solution (B).
2. Quantitatively dilute 10.00 mL of the 0.09 M $[\text{Cr}(\text{NO}_3)_3]$ solution to 25.00 mL using deionized water. This is your working solution C. Calculate the molarity of chromium nitrate in this solution (C).
3. Measure the absorbance spectrum of each solution using the Cary UV-vis

Use the Cary UV-vis to measure absorbance spectra from 350 - 820 nm

1. Open the Scan software.
2. Click on "Setup". Under "X Mode" heading (on "Cary" tab), set "Mode" to Nanometers, and set the wavelength range to 340-820 nm.
3. Click on the "Options" tab and set the "SBW" to 1.0 and the "Beam Mode" to "Double". This allows you to measure the reference and sample cuvettes simultaneously. Under the same tab, set "Source" to "UV-Vis" and set "Source Changeover" to 350 nm. Set "Display options" to "Individual data".
4. Click "OK".
5. Open the sample chamber. Insert a cuvette of DI water into the rear sample holder (reference beam), and insert a cuvette of solution B into the front sample holder (sample beam).
6. Click the "Start" button at the top of the screen.
7. In the "Save As" window, create a file in the "Cary WinUV" folder named UVstXX (with your student number in place of the XX) and click "Save".
8. In the "Sample Name" window, name your sample "Solution B" and click "OK".
9. At the end of the scan, note the wavelength(s) of maximum absorbance, and click the "Print" button at the bottom of the screen.
10. Replace the solution B cuvette with a cuvette of solution C. If you are using a single cuvette for both solutions, be sure to rinse and dry the cuvette before adding the next solution.
11. Click the "Start" button. Your previous file name (UVstXX) should appear again in the "Save As" window, so click "Save" and say "OK" to overwriting the file. Name this sample "Solution C" and click "OK".
12. At the end of the scan, note the wavelength(s) of maximum absorbance, and click the "Print" button at the bottom of the screen.
13. Click on "Graph" and select "Trace Preferences". Check both Solution B and Solution C from the list and click "OK".
 - You can take a screen shot or copy/paste this spectrum into a word document if you plan to include the spectra in your report.

Select wavelengths

Using the two absorbance spectra collected for Solutions B and C, select two wavelengths that you will use to perform the standard addition.

Consult your TA about selecting 2 wavelengths for the remainder of the experiment.

Standard Addition

1. Quantitatively pipette 10.00 mL of solution A (unknown) into a clean and dry 50.00 mL Erlenmeyer flask. This is your sample for analysis, and the entire contents of the Erlenmeyer flask must be preserved.
2. Transfer a portion of the sample from the Erlenmeyer flask to the cuvette.

Quantitative transfer is critical!

- The Results of your Analysis will depend upon quantitative transfer of your solution. Any loss of solution during transfer steps will result in large error in your results.
- When you have completed the transfer, save the transfer pipette in the Erlenmeyer flask - use this same transfer pipette for every transfer and for mixing and rinsing.
- Use the same cuvette for every absorbance reading - do not clean or dry the cuvette, but rinse it well with the solution to be measured.

3. Measure the absorbance of the solution.

Use the Cary UV-vis to measure at discrete wavelengths

1. Double click the "Simple Reads" icon (in the Analytical Lab folder).
 2. Click "Setup" and set the "Read at wavelength" to record the 2 wavelengths you selected with your TA. (must type a semicolon between the two wavelengths) Click "OK".
 3. Ensure that the external faces of the cuvette are clean and dry and insert into the spectrophotometer.
 4. Press "Start". Absorbances at the selected wavelengths will be displayed in a table on the screen. Remove the cuvette, wipe down the sides of the cuvette, and measure again. Repeat one more time so you have 3 readings for each solution at each wavelength.
4. Quantitatively return the cuvette solution to the Erlenmeyer flask *using the pipette*. Do not rinse the cuvette or the Pasteur pipette with water. Do not dry the cuvette.
 5. Quantitatively transfer 5.00 mL of solution B (the working cobalt nitrate solution) into the Erlenmeyer flask and mix thoroughly. Rinse the cuvette and the Pasteur pipette by transferring small portions of the sample solution back and forth from the flask to the cuvette at least three times.
 6. Transfer a portion of the well-mixed solution to the cuvette. Insert the cuvette into the cell holder in exactly the same orientation as before (a small black mark near the outside top may help). Measure and record the absorbance three times at the two wavelengths as before. Then return the sample from the cuvette to the flask.
 7. Repeat the last two steps using another addition of a 5.00 mL aliquot of solution B, then measuring the absorbance of the new solution.
 8. Using 5.00 mL aliquots of the chromium nitrate (working solution C) repeat the last 2 steps adding solution C as the standard to the sample solution of A + B.

Cleanup

1. Rinse all of the cuvettes thoroughly with deionized water and dab gently with a wipe to dry. Place partially-dried cuvettes upside down in a rack to finish drying, and return the rack to the shelves.

Treatment of data

The general criteria for writing and submitting lab reports are found in the [orientation module](#). Specific additional expectations for this module are given below.

Record the absorbance values in a table in your notebook. It is also a good idea to prepare a table like this in excel. An example table is given below:

Example Data Table											
Solution	λ_1				λ_2				Total Volume (L)	Δn_{Co}	Δn_{Cr}
	Absorbance				Absorbance						
	1	2	3	Ave	1	2	3	Ave			
A									0.01000		
A+B									0.01500		
A+2B									0.02000		

A+2B+C									0.02500		
A+2B+2C									0.03000		

6.1.7: Data analysis

1. Calculate the standard deviation of each of your average absorbance measurements at each of the two wavelengths.
2. Calculate $\Delta(VA)$ at each of the two wavelengths (Equations 3.4.8 and 3.4.9). In other words, calculate $\Delta(VA_{\lambda_1})$ and $\Delta(VA_{\lambda_2})$ for each addition. (Hint: $\Delta(VA_{\lambda_1})^0$ and $\Delta(VA_{\lambda_2})^0 = 0$)
3. After each addition, calculate the total cumulative amount (moles) of each standard that has been added; Δn_X (eg cobalt nitrate = X) and Δn_Y (eg chromium nitrate = Y).
4. Generate a table of $\Delta(VA)$ and Δn values (example shown in Table 1). For the purposes of this data analysis, it is important that λ_1 correspond to a λ_{max} for component X, and that λ_2 correspond to a λ_{max} for component Y.
5. Calculate the number of moles of analyte, n_{Co}^0 (eg cobalt nitrate) and n_{Cr}^0 (eg chromium nitrate), in the unknown solution, as well as the concentration of each. The moles of analyte may be calculated (a) manually or (b) using matrix algebra and the Matlab program (instructions below). You should use both methods to analyze your first set of data. You may choose either method for analysis of your second data set and state your reasons for making that choice.

Table 1: Data arranged in preparation for matrix analysis

Solution	Total Vol (L)	Ave Abs λ_1	ΔVA_{λ_1} (component X)	Ave Abs λ_2	ΔVA_{λ_2} (component Y)	Δn_X	Δn_Y
(0) sol. A	0.01		$\Delta VA_{\lambda_1}^0 = 0$		$\Delta VA_{\lambda_2}^0 = 0$	$\Delta n_X^0 =$	$\Delta n_Y^0 =$
(2) A+B(Co)	0.015		$\Delta VA_{\lambda_1}^2 =$		$\Delta VA_{\lambda_2}^2 =$	$\Delta n_X^2 =$	$\Delta n_Y^2 =$
(3) A+2B(Co)	0.02		$\Delta VA_{\lambda_1}^3 =$		$\Delta VA_{\lambda_2}^3 =$	$\Delta n_X^3 =$	$\Delta n_Y^3 =$
(4) A+2B+C(Cr)	0.025		$\Delta VA_{\lambda_1}^4 =$		$\Delta VA_{\lambda_2}^4 =$	$\Delta n_X^4 =$	$\Delta n_Y^4 =$
(5) A+2B+2C(Cr)	0.03		$\Delta VA_{\lambda_1}^5 =$		$\Delta VA_{\lambda_2}^5 =$	$\Delta n_X^5 =$	$\Delta n_Y^5 =$

MANUAL DATA CALCULATION

The ϵ values for X and Y and λ_1 and λ_2 that are described in Section 3.4 (see GSAM) may be determined from a plot of Δn vs ΔVA (four sets of plotted values). The slopes of each plot would give the value of ϵb for each respective analyte and wavelength. For example, the plot of Δn_{Co} vs ΔVA_{λ_1} would yield a linear plot with slope of $\epsilon_{\lambda_1 Co}$, and the plot of Δn_{Co} vs ΔVA_{λ_2} would yield a linear plot with slope of $\epsilon_{\lambda_2 Co}$.

Note: Use only the portion of each plot where the slope is >0 to derive each value of ϵ . The initial analyte amounts may then be calculated from the initial absorbance readings, A^0 , using Equations 3.4.10.

DATA ANALYSIS USING MATLAB

1. Open Matlab.
2. Prepare to record your data analysis work so that you can show your work to your TA and troubleshoot later if necessary. You can do this by using the editor window to record your work, and then saving your work as a script (eg **uv_data_analysis.m**). Alternatively, you can use the command window and type **diary uv_data_analysis** so that all of your on-screen work (with the exception of graphs) will be recorded in the file "**uv_data_analysis**". Either of these files can be printed as a pdf later and submitted as an appendix to your report.
3. Use matrix algebra to derive the ϵ values.
Equation (3.4.8) may be rewritten as

$$[VA] = [K][N] \quad (6.1.7.1)$$

where $[VA]$ is a 2 by 5 matrix of volume corrected absorbances for each solution at two wavelengths; $[K]$ is a 2 by 2 matrix of the response factor (aka sensitivity or $k = \varepsilon b$) values for each analyte-wavelength pair; and $[N]$ is a 2 by 5 matrix of the amounts of standard added, Δn :

$$\begin{pmatrix} \Delta VA_{\lambda_1}^0 & \Delta VA_{\lambda_1}^2 & \Delta VA_{\lambda_1}^3 & \Delta VA_{\lambda_1}^4 & \Delta VA_{\lambda_1}^5 \\ \Delta VA_{\lambda_2}^0 & \Delta VA_{\lambda_2}^2 & \Delta VA_{\lambda_2}^3 & \Delta VA_{\lambda_2}^4 & \Delta VA_{\lambda_2}^5 \end{pmatrix} = \begin{pmatrix} k_{\lambda_1 X} & k_{\lambda_1 Y} \\ k_{\lambda_2 X} & k_{\lambda_2 Y} \end{pmatrix} \begin{pmatrix} \Delta n_X^0 & \Delta n_X^2 & \Delta n_X^3 & \Delta n_X^4 & \Delta n_X^5 \\ \Delta n_Y^0 & \Delta n_Y^2 & \Delta n_Y^3 & \Delta n_Y^4 & \Delta n_Y^5 \end{pmatrix} \quad (6.1.7.2)$$

- a. First, enter the matrices $[VA]$ and $[N]$ into the Matlab workspace using the data from Table 1, and following directions from your instructor.

`VA = [enter values in matrix form] (use spaces between numbers and a semicolon between
N = [enter values in matrix form]`

- b. Using Equation (3.6.2), determine the 2 by 2 matrix, $[K]$, by entering the following command:

`K = VA/N`

- c. From the k values, calculate the molar absorptivities (ε) (in $\text{cm}^{-1}\text{M}^{-1}$) of cobalt nitrate and chromium nitrate at the two selected wavelengths ($k = \varepsilon b$).
4. Calculate the initial moles of Co and Cr using matrix algebra.
From Equations (3.4.10),

$$\begin{pmatrix} \Delta VA_{\lambda_1}^0 \\ \Delta VA_{\lambda_2}^0 \end{pmatrix} = \begin{pmatrix} k_{\lambda_1 X} & k_{\lambda_1 Y} \\ k_{\lambda_2 X} & k_{\lambda_2 Y} \end{pmatrix} \begin{pmatrix} n_X^0 \\ n_Y^0 \end{pmatrix} \quad (6.1.7.3)$$

which may be rewritten as:

$$[VA^0] = [K][n_i] \quad (6.1.7.4)$$

Therefore

$$[K]^{-1}[VA^0] = [n_i] \quad (6.1.7.5)$$

- a. First, create the matrix VAi for two initial values of VA : Using the initial absorbance readings $A_{\lambda_1}^0$ and $A_{\lambda_2}^0$, and the initial volume (0.01 L), to calculate $VA_{\lambda_1}^0$ and $VA_{\lambda_2}^0$. Enter the $VA_{\lambda_1}^0$ and $VA_{\lambda_2}^0$ values into a matrix called VAi . To do this with matlab, enter the following, but substitute the values you calculated into the matrix:

`VAi = [ VA10 ; VA20 ]`

- b. Use the K matrix (with ε values) calculated in step (3) above, Equation (3.6.5), and Matlab to determine the initial analyte amounts n_X^0 and n_Y^0 as follows:

$$n_i = \text{inv}(K) * VA^0 \quad (6.1.7.6)$$

where n_i is the Matlab label given to $[n_i^0]$ and VAi is the label given to $[VA^0]$.

In Matlab, enter the following:

`ni = inv(K)*VAi`

The matrix n_i is a 2 by 1 matrix containing the initial analyte amounts (moles) n_X^0 and n_Y^0 .

5. For each of your two data sets, report the unknown cobalt nitrate and chromium nitrate concentrations in units of moles/liter (molarity). Calculate the weight/volume percent of each component.

$$\text{concentration of solute in } \left(\frac{w}{v} \right) = 100 \times \frac{\text{mass of solute (g)}}{\text{volume of solution (mL)}} \quad (6.1.7.7)$$

6. When you have completed the analysis using Matlab, save your variables as follows:

`save uv_data_analysis_1`

7. To obtain a printout of your diary, you must first exit Matlab and then enter the print command:

```
diary off
```

```
exit
```

8. If you plotted graphs following the instructions below you saved copies of your graphs. Print them using the usual method of printing graphs from Matlab (see the [Matlab chapter](#) if you need help).

GRAPHICAL ANALYSIS USING MATLAB

(Optional. Plots made using excel or other software are ok too.) **NOTE: You must show the least squares line on each of these plots.**

1. To determine $k_{\lambda_1 X}$ from the slope of the plot of Δn_1 vs $\Delta V A_{\lambda_1}$.

Plot the graph as follows and determine the slope ($k_{\lambda_1 X}$) of the non-vertical portion:

```
plot(N(1,:),VA(1,:), 'o'), title('[N1] VS [VA1]')  
xlabel('[N1]'), ylabel('[VA1]')
```

Save this graph.

```
lsq(N(1,1:3),VA(1,1:3))
```

2. To determine k21 from the slope of the plot of Δn_1 vs $\Delta V A_2$.

Plot the graph as follows and determine the slope (k21) of the non-vertical portion:

```
plot(N(1,:),VA(2,:), 'o'), title('[N1] VS [VA2]')  
xlabel('[N1]'), ylabel('[VA2]')
```

Save this graph.

```
lsq(N(1,1:3),VA(2,1:3))
```

3. To determine k12 from the slope of the plot of Δn_2 vs $\Delta V A_1$.

Plot the graph as follows and determine the slope (k12) of the non-vertical portion:

```
plot(N(2,:),VA(1,:), 'o'), title('[N2] VS [VA1]')  
xlabel('[N2]'), ylabel('[VA1]')
```

Save this graph.

```
lsq(N(2,3:5),VA(1,3:5))
```

4. To determine k22 from the slope of the plot of Δn_2 vs $\Delta V A_2$.

Plot the graph as follows and determine the slope (k22) of the non-vertical portion:

```
plot(N(2,:),VA(2,:), 'o'), title('[N2] VS [VA2]')  
xlabel('[N2]'), ylabel('[VA2]')
```

Save this graph.

```
lsq(N(2,3:5),VA(2,3:5))
```

6.2: Cyclic Voltammetry

GOALS

1. Students should be able to optimize the experimental condition to produce the best waveform.
2. Students should be able to extract key information from a CV waveform. (i.e. peak current and peak potential)
3. Students should be able to use the extracted information for practical application. (i.e. determining the unknown concentration of a compound)

Introduction

Cyclic voltammetry (CV) is a technique used to study reaction mechanisms that involve the transferring of electrons. The method involves linearly varying an electrode potential between two limits at a specific rate while monitoring the current that develops in an electrochemical cell. This experiment is performed under conditions where voltage is in excess of that predicted by the **Nernst equation** (Equation 6.2.1).¹ Although CV is best at providing qualitative information about reaction mechanisms, several quantitative properties of the charge transfer reaction can also be determined.

$$E = E^{\circ} - \frac{RT}{nF} \ln Q \quad (6.2.1)$$

Cyclic voltammetry involves applying a voltage to an electrode immersed in an electrolyte solution, and seeing how the system responds. In CV, a linear sweeping voltage is applied to an aqueous solution containing the compound of interest. A linear sweeping voltage is defined by the voltage (or potential) being varied linearly at the speed of the scan rate. The variation of the voltage can be seen in **Figure 1.1**. The voltage is initially given by Equation 6.2.4 (see below). After the voltage reaches a certain maximum value, the potential is reversed and the sign of v_t reverses and E_i becomes the maximum voltage, E_{λ} . The switch takes place at the peak which can be seen in **Figure 1.1**. The process can then be repeated in a periodic, or cyclic manner. The voltage after the potential sweep direction is switched is given by Equation 6.2.5.

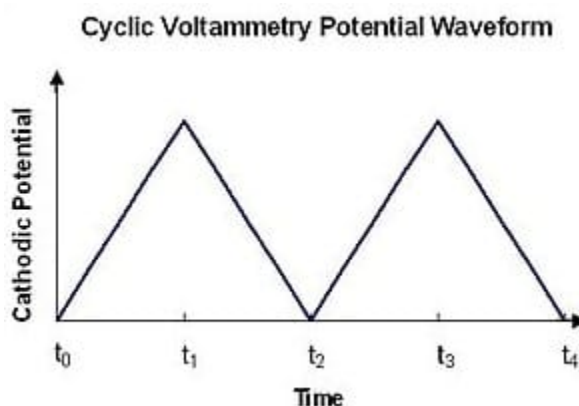


Figure 1.1: Potential versus time program for cyclic voltammetry showing the forward and reversed linear potential ramp.

As an important tool for studying mechanisms and rates of oxidation and reduction processes, CV provides the capability for generating a species during the forward scan and then probing its fate with the reverse scan or subsequent cycles. This process can occur within a few seconds. There is a unique aspect of cyclic voltammetry: three electrodes used. The electrodes are a working electrode, a reference electrode, and a counter electrode. The working electrode can be seen as a medium whose reductive or oxidative power can be externally adjusted by the magnitude of the applied potential. As the potential is increased or decreased linearly versus time, the working electrode becomes a stronger oxidant or reductant, respectively. Therefore, the working electrode, which typically consists of a chemically inert conductive material such as platinum, acts as a donor or acceptor of electrons. The general reaction written in Reaction 6.2.2 refers to the addition of electrons to the oxidized electrode and the electrode transforming to its most reduced form.



The reference electrode, typically AgCl or calomel, keeps the potential between itself and the working electrode constant. The potential is measured between the reference and working electrodes, and the current is measured between the working and counter electrodes. A counter electrode is employed to allow for accurate measurements to be made between the working and reference electrodes. The counter electrode's role is to ensure that the current does not run through the reference electrode since such a flow would change the reference electrodes potential. A voltage sweep from E_i (initial voltage) to E_f (final voltage) is produced using a signal generator. The voltage is applied to the working electrode using a potentiostat. A potentiostat is an external power source. By sweeping the voltage slowly, information may be extracted from a graph of potential versus current going through the sample. **Polarography** utilizes this method of analysis where the limited current arising from a redox process in the solution during the sweep. This information is used to quantitatively determine the concentration of species that are electrochemically active in solution.

CV differs from polarography in two important ways. Firstly, the working electrode at which the reactions of interest occur has a constant area, not one which changes with time as in classical polarography. This electrode may be a solid such as graphite or platinum with a small surface area, or a stationary or hanging mercury drop. The latter type of electrode may have its surface renewed periodically. The second difference is that the potential of the working electrode is scanned rapidly over a wide potential range and then returned to its initial value using an applied potential signal which varies linearly with time between the initial value and the final value at the limit of the forward scan. Normally, this technique is applied so that currents due to **reduction** processes are observed during the forward scan and those due to **oxidation** on the reverse scan. The resulting cyclic voltammogram from a typical potential against time profile is shown in **Figure 1.2**.

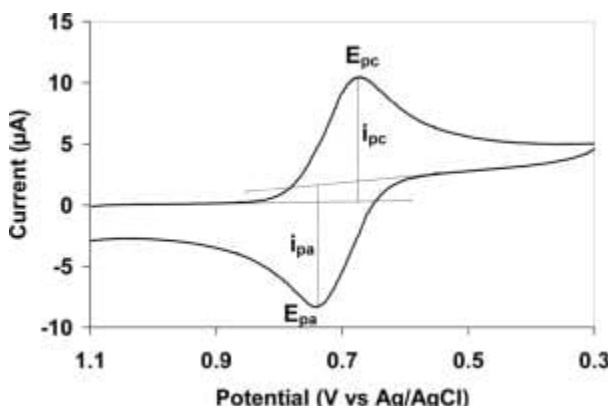


Figure 1.2: Cyclic Voltammogram of measured current versus applied potential. This diagram also shows the points where the y-values of peak anodic current (i_{pa}) and peak cathodic current (i_{pc}) as well as the x-values of peak anodic potential (E_{pa}) and peak cathodic potential (E_{pc}).

Guided Example

Consider the response of the electron transfer reaction



to the application of an electrode potential which is varying linearly with time. The electrode potential is given by the equation

$$E = E_i - vt \quad (6.2.4)$$

where E_i is the initial potential, v is the potential sweep rate (in volts s^{-1}) and t is time after the start of voltage sweep. After reaching some time, λ , the direction of the potential sweep is switched, and the equation describing the electrode potential becomes:

$$E = E_\lambda + v(t - \lambda) \quad (6.2.5)$$

where E_λ is the value of E at the switching point. Considering that the initial sweep is in the negative direction where reduction reactions are expected, it is clear that, if the sweep rate is sufficiently slow, the current against potential curve approaches that

obtained by steady-state measurements. However, as v is increased, a peak develops on the i - E curve which becomes increasingly prominent (Figure 1.3). The peak is produced from the combined effects of high mass transfer rates in the non-steady state followed by the progressive depletion of the reactant in the diffusion layer.

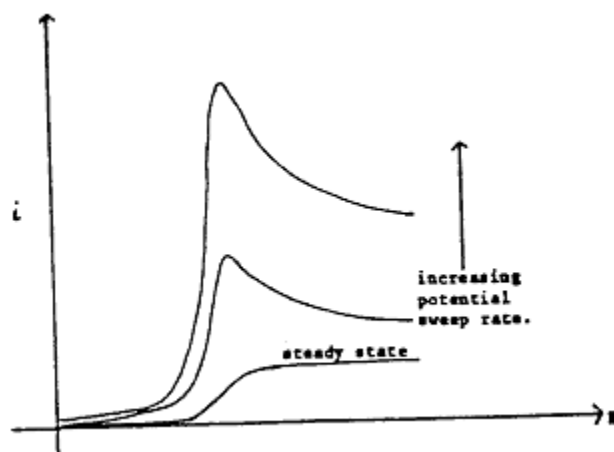


Figure 1.3. Effect of potential sweep rate on the PE curves in a linear potential sweep experiment adjacent to the electrode.

It should be noted that since E is a linear function of t , the potential axis is also a time axis.

In order to relate the observed current to the reactant concentration c_A , one must know how c_A varies with distance from the electrode, x , and with time, t . This variation is described by **Fick's Law** for mass transfer by diffusion:

$$\frac{\partial c_A}{\partial t} = D_A \frac{\partial^2 c_A}{\partial x^2} \quad (6.2.6)$$

where D_A is the diffusion coefficient of reactant A (units: cm^2/s). The solution of this second order partial differential equation requires specification of boundary and initial conditions and is described in textbooks on electroanalytical chemistry^{1,2}. If the electron transfer reaction is sufficiently fast in Reaction 6.2.3 to maintain a Nernstian equilibrium at the electrode surface (i.e., the reversible case), then the peak current, i_p for a negative sweep is given by Equation 6.2.7. The variables are described in **Table 1.1**.

$$i_p = (2.69 \times 10^5) n^{3/2} S D_A^{1/2} v^{1/2} C_A, \quad (6.2.7)$$

Table 1.1: Variables of the modified Fick's Law, Equation 6.2.7

Variable	Description	Units
i_p	peak current	A
n	number of electrons transferred	-
S	Surface Area	cm^2
D_A	Diffusion coefficient	cm^2/s
v	scan rate	V/s
C_A	the concentration of compound A in the bulk solution	mole/cm^3

You may notice that the peak potential is *independent* of sweep rate and is related to the half-wave potential ($E_{1/2}$) by

$$E_p = E_{1/2} - \frac{0.0285}{n} \quad (6.2.8)$$

Furthermore, the shape of the peak is defined by the potential difference between the peak and the position when the current is **one half** of that at the peak:

$$E_p = E_{p/2} - \frac{0.0565}{n} \quad (6.2.9)$$

Thus, using the measured values of i_p , E_p , and $E_{p/2}$, one can determine n and D_A for a given electrode area and sweep rate. If the electrode reaction is slow so that the surface concentrations of A and B are no longer related by the [Nernst equation](#) at a given sweep rate, then the peak characteristics change such that

$$E_p - E_{p/2} > \frac{0.0565}{n} \quad (6.2.10)$$

and the peak potential now depends on the sweep rate. Using the appropriate boundary conditions to describe the rate of Reaction 6.2.3 in the forward direction, Equation 6.2.6 may be solved to obtain expressions for i_p and E_p which are now much more complicated.

After the direction of the potential sweep is reversed, a second current peak is observed corresponding to oxidation of the product B . When Reaction 6.2.3 is reversible, implying that B is stable, the height of this peak is equal to that observed on reduction, but with the current flowing in the opposite direction. The method of estimating peak currents is illustrated in [Figure 1.4](#), where i_{pc} is the peak potential in the cathodic sweep.

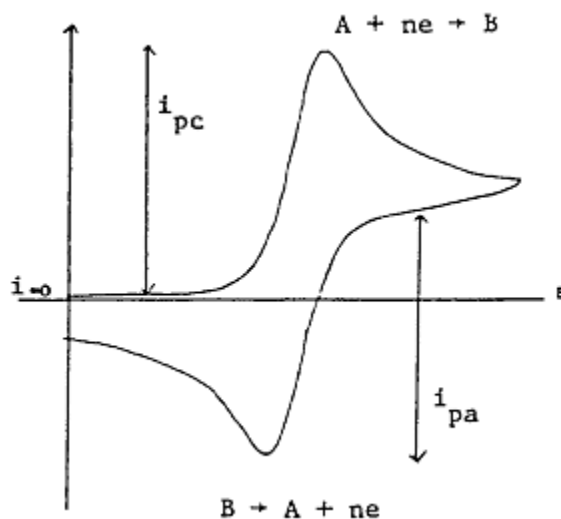


Figure 1.4: CV Waveform of Reaction 6.2.3

The CV waveform in the above figure for the process Reaction \ (6.2.3\) and assumes that only A is initially present in the solution. ΔE_p is then defined as

$$\Delta E_p = |E_{pc} - E_{pa}|. \quad (6.2.11)$$

When the electrode process is **reversible** and it **independent** of sweep speed and is described by Equation 6.2.12.

$$\Delta E_p = \frac{0.0565}{n} \quad (6.2.12)$$

As v is increased to the stage at which Nernstian equilibrium for Reaction 6.2.3 cannot be maintained, ΔE_p increases with increasing sweep rate, and the shape and position of the peaks depend on both v and the kinetic parameters of the electrode reaction.

Reaction Mechanisms

One of the major uses of cyclic voltammetry is in the rapid qualitative identification of electrode [reaction mechanisms](#). Organic molecules often undergo a rapid chemical reaction with the solvent or some other constituent of the solution after the electron transfer process. The resulting reaction scheme, referred to as the **ECE (electrode-chemical-electrode) mechanism**, can be written



where Z is the solvent or some other species. The first and third reactions are labeled E since they involve the electrode, and the second step (or any other chemical step) is labeled C . Hence, the above three-step mechanism is referred to as the ECE reaction mechanism. It is possible to garner information about the (non-electrode-dependent) rate constant for step 2 (Reaction 6.2.14) via cyclic voltammetry.

The standard potential for Reaction 6.2.13 is generally different from that for Reaction 6.2.15. A typical current-potential curve for such a system is shown in [Figure 1.5](#). The current on the reverse sweep will depend on the sweep rate and the rate constant for Reaction 6.2.14, which is assumed to take place under [pseudo first-order](#) conditions ($c_Z \gg c_B$). For very fast sweep rates, very little B will react to form C , and the CV waveform will have the same appearance as the reversible case, with reduction and oxidation peaks at I and II, respectively. As v is decreased, peak II diminishes more rapidly and peak I less rapidly than the usual. The dependence $v^{1/2}$ would predict this because the chemical step removing species B becomes important and peak I has a contribution from Reaction 6.2.15. In addition, a peak develops at III due to oxidation of D .

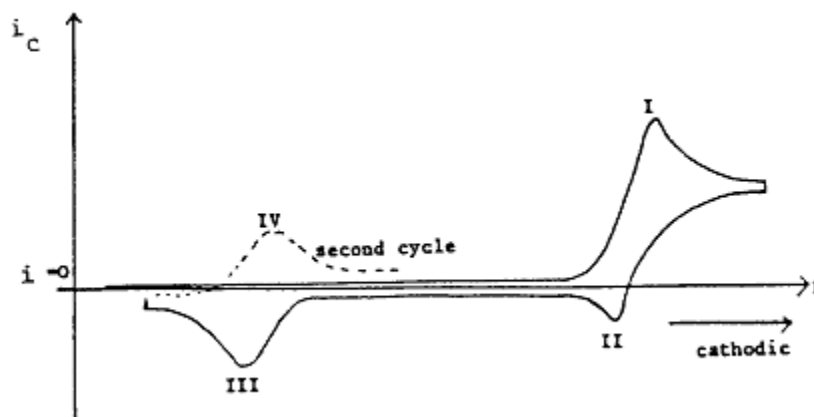


Figure 1.5: Cyclic voltammogram at an intermediate sweep rate for a system with an ECE mechanism.

It should also be noted that the current-potential curves on the second and successive sweeps are not the same as that observed on the first. At very slow sweep rates, peak II disappears completely and peak I then corresponds to the process



The variation of peak I with sweep rate is shown in [Figure 1.6](#) for the case that $n = m = 2$.

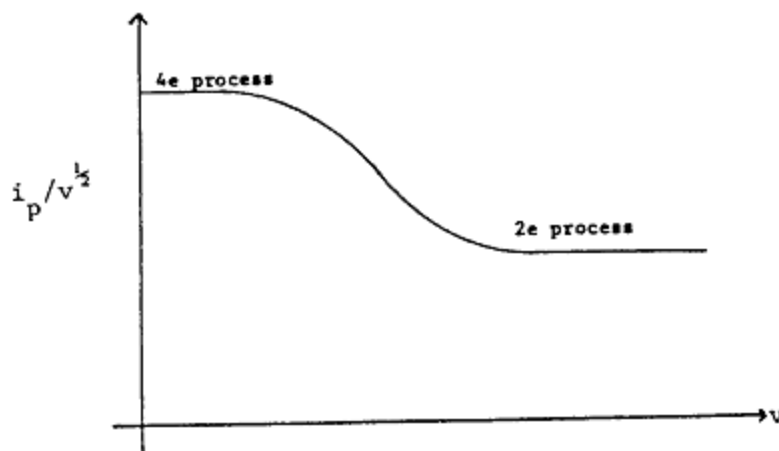


Figure 1.6: Variation of measured current for peak I from Figure 1.4 vs. potential scan rate, v .

The rate constant k for the chemical Reaction 6.2.16 can be obtained from an analysis of data obtained in the transition region of Figure 1.6 or from the ratio of peaks I and II at intermediate sweep rates in Figure 1.5.

Cyclic voltammetry can be applied to the analysis of many other reaction mechanisms including those with dimerization of the product of electron transfer, with preceding chemical steps, catalytic processes, etc.

The Instrument

The instrument used for this experiment is the BAS Epsilon potentiostat. It is controlled from a computer running Windows. A number of different electrochemical techniques are available in the Epsilon software, including cyclic voltammetry (current vs. potential for a linear potential sweep), chronoamperometry, time base, bulk electrolysis (current vs. time at a constant potential), and chronopotentiometry (potential vs. time at a fixed current).

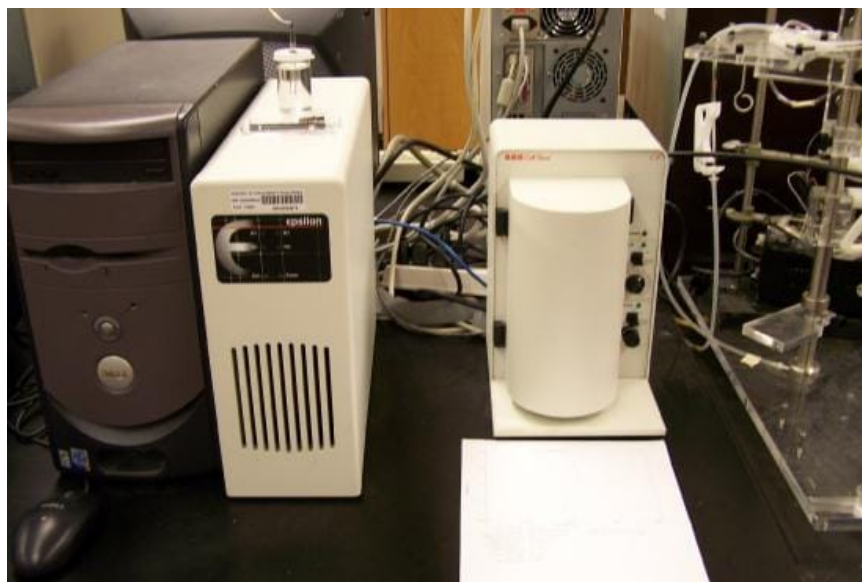




Figure 1.7: UCD cyclic voltammetry Instrument (including potentiostat)

To begin an experiment, make sure that the Epsilon unit is turned on, and doubleclick the Epsilon icon on the desktop. Select **New** from the File menu or click the New icon. This will generate a menu that lists the available techniques. (This list can also be generated by selecting Select NEW Experiment from the Experiment menu or by using the F2 key.)

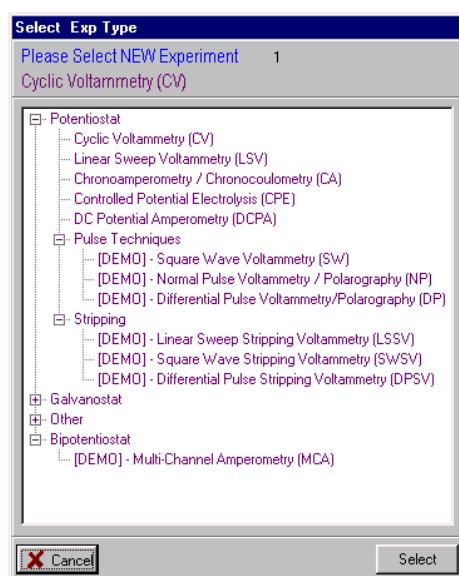


Figure 1.8: Computer Program

Highlight Cyclic Voltammetry (CV), and click Select to confirm the selection. An experiment window containing an empty axis set is displayed (Figure 1.9), and the appropriate parameters are set in the various dialog boxes.

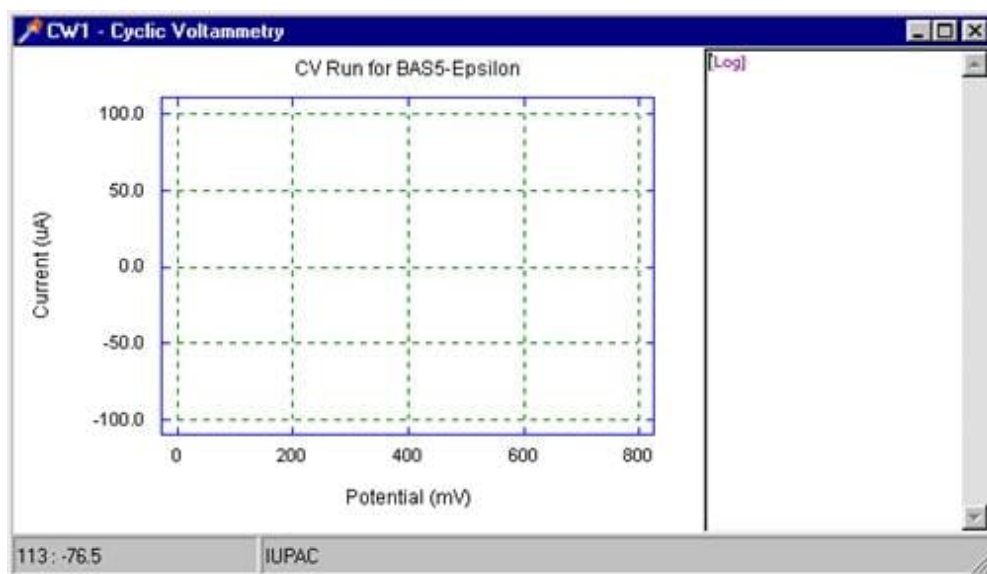


Figure 1.9: Computer Program

The potential limits and the scan rate for CV are set using the Change Parameters dialog box (Figure 1.10) in either the Experiment menu or the pop-up menu (the pop-up menu is accessed with the right mouse button).

Figure 1.10: Change Parameters dialog box for cyclic voltammetry.

1. Potential values are entered in mV, and the **Scan Rate** in mV/s.
2. If the **Apply Open Circuit Potential for Initial E** box is checked, then the open circuit potential will automatically be measured and used as the Initial Potential.
3. When the experiment is started, the cell is held at the **Initial Potential** for the number of seconds defined by the **Quiet Time**.
4. There are two gain stages for the current-to-voltage converter. The default values of these stages that are used for a given current **Full-Scale** value are determined by the software. However, they can be adjusted manually using the Filter / F.S. dialog box. This dialog box is also used to change the analog **Noise Filter Value** settings from the default values set by the software. The **Full-Scale** value should be at the 10 mA/V setting at the start of the experiment, and then adjusted to a more convenient range depending on the maximum current observed in the experiment. Ask your TA about scale values to better observe your results.
5. The default condition of the cell is that the cell is **On** (i.e., the electronics are connected to the electrodes) during the experiment, and is **Off** between experiments. **THIS OPTION SHOULD NOT BE CHANGED SINCE CONNECTING OR**

DISCONNECTING THE ELECTRODES WHEN THE CELL IS ON CAN RESULT IN DAMAGE TO THE POTENTIOSTAT, THE CELL, AND/OR THE USER!

6. Clicking the **IR-COMP** button activates the iR compensation option (compensates for the drop in voltage due to the resistance of the solution).
7. Clicking **Exit** will exit the dialog box without saving any changes made to the parameter values. Any changes can be saved by clicking **Apply** before exiting.
8. Range of allowed parameter values:
 - **Potential** = -3275 - +3275 mV
 - **Scan Rate** = 1 - 10,000 mV/s (also see below)
 - **Quiet Time** = 0 - 100 s
 - The **# of Segments** is limited by the total number of data points that can be stored (32,000) (note that in this initial version, the potential resolution of the current measurement is fixed at 1 mV).
10. Once the parameters have been set, the experiment can be started by clicking **Run** (either in this dialog box, in the **Experiment** menu, in the pop-up menu, on the Tool Bar, or using the **F5** key).

Experimental Procedure

Part 1: Reduction of the Ferricyanide Anion

In this portion of the experiment, you will be investigating the reduction of the ferricyanide anion. The purpose of this part of this experiment is to discover and develop an understanding of the properties of this reaction. This reaction can be important in the field of soil science because Iron is a common element in soil. Iron can bind to cyanide ligands to make them less bioavailable in natural systems. The reduction of the Iron (III) complex can be represented in the following balanced half-reaction.



Four conditions will be measured in this part in order to observe the effects of concentration and counter-ion selection on the voltammogram. To begin the experiment, take note of the solutions already prepared for you. These solutions can be found in the cabinet directly under the instrument. There should be solutions of 4 mM ferricyanide in 1 M KNO₃, 1 M KNO₃, and 4 mM ferricyanide in 1 M Na₂SO₄. If these solutions are empty or missing, notify your TA.

The four conditions in this part of the experiment will use the electrodes listed in **Table 1.2**. The electrodes can be found in a drawer. Do not attach the electrodes until your first solution is ready.

important

For the Platinum disc electrode, note the diameter of the electrode in your lab notebook; this value will be important for your post-lab calculations.

Table 1.2: Electrodes used for Part 1 of the Experiment.

Electrode Type	Electrode Material	Wire Color
Reference	Saturated Calomel Electrode (SCE)	White
Working	Platinum Disc	Black
Counter	Platinum Wire	Red

When using these electrodes, it is important that they are clean. If the electrodes are not clean, the CV waveform produced will not be accurate. Before beginning the experiment, have your TA demonstrate the cleaning procedure for the working electrode. **The cleaning procedure is as follows:** Put a few drops of the cleaning solvent on a cloth. Gently rub the working electrode on the damp cloth.

The initial settings will be the same for all four conditions. These settings are listed in **Table 1.3**. It is important to note that the “full scale” setting can be adjusted to view your peak more clearly. An important setting not listed in **Table 1.3** is the scan rate. The scan rates used will be given in the procedure sections of each condition.

Table 1.3: Initial Settings for Part 1.

Initial Potential	+700 mV
Switching Potential	-200 mV
Final Potential	+700 mV
# of Segments	2
Scan Rate	250 mV/s
Quiet Time	10 seconds
Full Scale	10 mA/V

Condition 1 – 4 mM Ferricyanide in 1 M KNO₃

1. Fill the cell with 4 mM Ferricyanide with 1 M KNO₃.
2. Deoxygenate the solution for 5-10 minutes using the gas tank provided. Your TA will set the pressure gauge as needed.
3. While the solution is deoxygenating, clean the electrodes, set the initial conditions in the computer software using the conditions from **Table 1.2** (The first scan rate will be **250 mV/s**), and prepare the dilution you will use in Condition 2 (the necessary dilution can be found in the Condition 2 section).
4. Once the solution has been deoxygenated, assemble the cell, place a small stir bar into the cell, and lower the electrodes into the cell.
5. Perform the scan using the procedure described in the Instrument Operation section.
6. After the scan (the scan will only take a few seconds), lift the electrodes out of the solution and stir the solution for 10 seconds or until the bubbles are removed. After stirring let the solution rest for 1 minute. **DO NOT stir the solution while electrodes are lowered or the scans are running, the stir bar can break the electrodes.**
7. Use the procedure in the Data Acquisition section to secure your data files.
8. Repeat steps 5-7 at scan rates of **160, 100, 50, and 20 mV/s**. Make sure to stir the solution between each scan.
9. After completing your scans, ask the TA if your results look reasonable. If they do not, the electrodes may need to be cleaned using the cleaning procedure.
10. Dispose of your solution in the specified waste container.

Condition 2 – 2 mM Ferricyanide in 1 M KNO₃

1. Dilute the 4 mM Ferricyanide with 1 M KNO₃ to 2 mM Ferricyanide with 1 M KNO₃. Perform the necessary dilution calculation in your laboratory notebook. This dilution needs to be done with the 1 M KNO₃ solution, otherwise, the KNO₃ concentration would be altered.
2. Deoxygenate the solution for 5-10 minutes using the gas tank provided. Deoxygenation can begin while the scans for Condition 1 are being performed.
3. Repeat steps 4-10 for this in the Condition 1 procedure for this condition running a scan with rates of **250, 160, 100, 50, and 20 mV/s**.

Condition 3 – 1 M KNO₃

1. Fill the cell with the 1 M KNO₃ solution.

2. Deoxygenate the solution for 5-10 minutes using the gas tank provided. Deoxygenation can begin while the scans for Condition 2 are being performed.
3. Assemble the cell with the 1 M KNO₃ solution, place a small stir bar into the cell, and lower the electrodes into the cell.
4. Perform the scan (**scan rate = 10 mV/s**) using the procedure described in the Instrument Operation section. Begin deoxygenating the solution in Condition 3.
5. Do not stir the solution, no other scans will be performed for Condition 3.
6. Use the procedure in the Data Acquisition section to secure your data files.
7. Look at your results and try to conclude if your scan looks reasonable. If you are having a difficult time determining this, ask your TA for help.

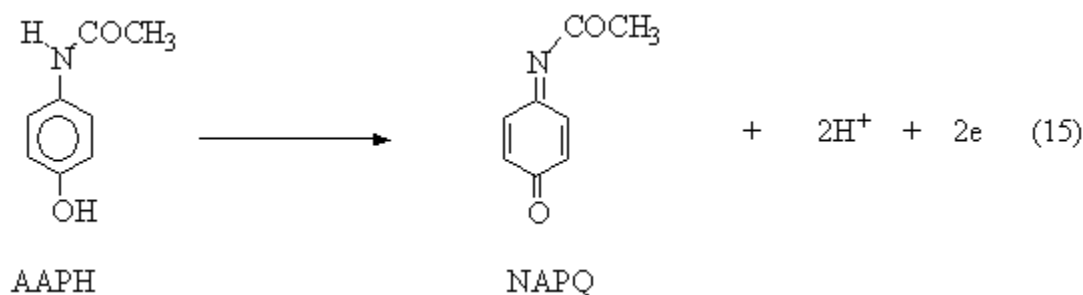
Condition 4 – 4 mM Ferricyanide in 1 M Na₂SO₄

1. Fill the cell with the 4 mM Ferricyanide in 1 M Na₂SO₄ solution.
2. Deoxygenate the solution for 5-10 minutes using the gas tank provided. Deoxygenation can begin as soon as the deoxygenation of Condition 3 is complete.
3. Repeat steps 4-10 in condition 1's procedure for scan rates of **250, 100, and 50 mV/s**.
4. After completing the last scan, disassemble the cell, rinse the electrodes with DI water and immerse them in a cell filled with DI water. Dry the electrodes and put them away into their respective cases.

When you are finished, rinse all three electrodes with deionized water and immerse them in the cell filled with deionized water.

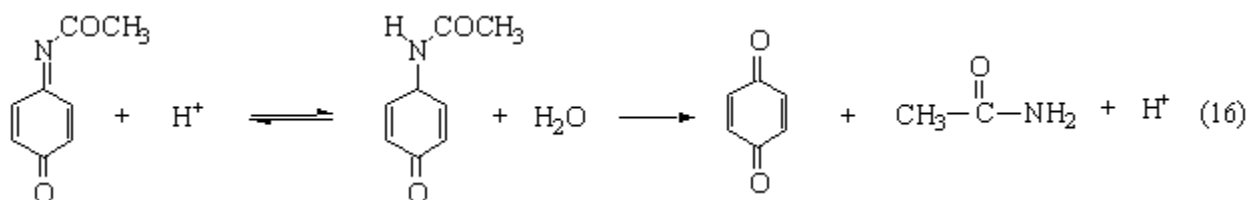
Part 2: The Oxidation of Acetaminophen

Tylenol is an over-the-counter pain relief medication that is commonly used in the United States. The reason that Tylenol work is because it contains the active ingredient Acetaminophen (AAPH). When someone takes Acetaminophen, also known as Paracetamol, the molecule undergoes a complete oxidation process in an aqueous solution. This oxidation reaction is the beginning of a reaction series that is demonstrated below. This is a pH-dependent process. The extent to which reaction 6.2.20 occurs is dependent on the rate of reaction 6.2.19.



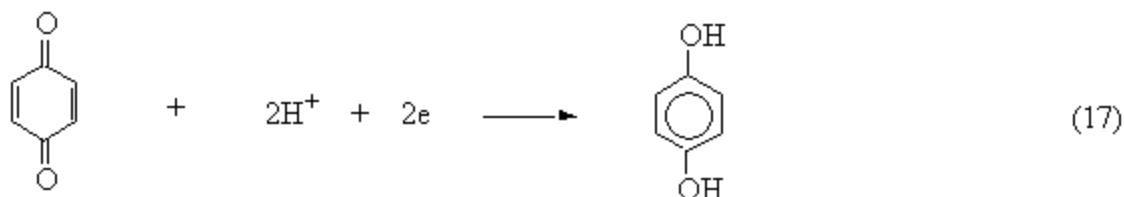
(6.2.18)

The product can be protonated, and then undergo the elimination of acetamide to form benzoquinone (BQ):



(6.2.19)

The rate of the first reaction is obviously pH-dependent. Finally, benzoquinone may be reduced to hydroquinone:



(6.2.20)

In this portion of the experiment, the goal is to demonstrate this mechanism and determine the best pH conditions for this reaction. After you have determined the best conditions, you will determine the concentration of AAPH in a Tylenol tablet. The electrodes used in this part are listed in **Table 1.4** and the initial settings are listed in **Table 1.5**.

Table 1.4: Electrodes used for Part 2 of the Experiment.

Electrode Type	Electrode Material	Wire Color
Reference	Silver/Silver Chloride (Ag/AgCl)	White
Working	Glassy Carbon	Black
Counter	Platinum Wire	Red

Table 1.5: Initial Settings for Part 2.

Initial Potential	0 mV
Switching Potential	+1000 mV
Final Potential	-200 mV
# of Segments	2
Quiet Time	10 seconds
Full Scale	10 mA/V

Begin this part by preparing the solutions for conditions 1, 2, and 3 of pH 6, 2.2, and 1.8 respectively. Start by checking to see that there is an AAPH solution made for you. It may be in the refrigerator or in the same cabinet as the ferricyanide solution and counter-ion solutions. Once you have located the AAPH solution and three 100 mL volumetric flasks, begin by adding 5 mL of the AAPH solution to each of the volumetric flasks. The final concentration of AAPH should be 3.5 mM. Fill one flask with pH 6 buffer to the line. Fill the next solution using pH 2.2 buffer and fill the last flask using 1.8 M Sulfuric Acid.

Condition 1 - pH 6

1. Deoxygenate the pH 6 buffer solution for 5-10 minutes. Your TA will reset the pressure gauge if this part is performed on the second day.
2. While the solution is deoxygenating, **clean the electrodes with the help of your TA** and set the initial conditions in the computer software using the conditions from **Table 1.5** (The first scan rate will be **250 mV/s**).
3. Assemble the cell with the electrodes from **Table 1.4** and the necessary solution. Place a small stir bar into the cell and lower the electrodes into the cell.
4. Perform the scan (**scan rate = 250 mV/s**) using the procedure described in the Instrument Operation section. Begin deoxygenating the solution in Condition 3.

5. After the scan (the scan will only take a few seconds), lift the electrodes out of the solution and stir the solution for 10 seconds or until the bubbles are removed. After stirring let the solution rest for 1 minute. **DO NOT stir the solution while electrodes are lowered or the scans are running, the stir bar can break the electrodes.**
6. Use the procedure in the Data Acquisition section to secure your data files.
7. Repeat steps 4-6 at scan rates of 100 and 40 mV/s. Make sure to stir the solution between each scan.
8. Look at your results and try to conclude if your scans look reasonable. If you are having a difficult time determining this, ask your TA for help. The electrode may need to be cleaned again.
9. Dispose of your solution in the specified waste container.

Condition 2 – pH 2.2

1. Deoxygenate the pH 2.2 buffer solution for 5-10 minutes. This process can begin as soon as the deoxygenation of Condition 1 is complete.
2. Repeat steps 3-9 in the Condition 1 section of Part 2.

Condition 3 – 1.8 M Sulfuric acid

1. Deoxygenate the 1.8 M Sulfuric Acid solution for 5-10 minutes. This process can begin as soon as the deoxygenation of Condition 2 is complete.
2. Repeat steps 3-9 in the Condition 1 section of Part 2.
3. Do not rinse all of the electrodes and put them away, they will be used in the determination of the AAPH in the Tylenol tablet.
4. As a group, determine the most appropriate condition pH condition for this reaction and the best scan rate. The best scan rate can be determined by seeing which scan gives you the clearest data. The best pH condition can be determined by picking the condition that gave the sharpest peaks.

Condition 4 – Tylenol Tablet in with the best conditions

1. Weigh the Tylenol Tablet.
2. Crush the tablet with a mortar and pestle into a fine powder. Transfer the fine powder into a 250 mL volumetric flask.
3. Fill the volumetric flask with the appropriate pH condition that you selected.
4. Deoxygenate the solution for 5-10 minutes.
5. Run the scan at your chosen scan rate using the initial settings listed in **Table 1.5**.
6. Perform the procedure in the Data Acquisition section.
7. Compare the peak height in this trial to the peak height of the scan performed with the same pH condition and scan rate. Use this information to determine the approximate concentration. Use this information to develop a concentration range that can be used to develop a calibration curve.
8. Dispose of your solution in the specified waste container.

Condition 5 – Calibration Curve (Solution #1)

1. Make the dilution using the standard AAPH solution used in conditions 1,2, and 3 in a 100 mL volumetric flask. Fill the flask to the line with your chosen pH condition.
2. Deoxygenate the solution for 5-10 minutes.
3. Run the scan at the chosen scan rate using the initial settings listed in **Table 1.5**.
4. Perform the procedure in the Data Acquisition section.

Condition 6 – Calibration Curve (Solution #2)

1. Repeat the procedure from Condition 5 for the second concentration used for the calibration curve.
2. After completing the last scan, disassemble the cell, rinse the electrodes with DI water and immerse them in a cell filled with DI water. Dry the electrodes and put them away into their respective cases.

Post-Lab Questions

Part 1: Reduction of the Ferricyanide Anion

****INCLUDE ALL CV WAVEFORMS****

Condition 1 – 4 mM Ferricyanide in 1 M KNO₃

1. Tabulate the measured values of i_{pc} , i_{pa} , E_{pc} , E_{pa} , and $E_{pc/2}$ for each scan rate.
2. Determine the Diffusion Coefficient (D_A) for each scan rate using Equation 6.2.7.
3. Find the average, standard deviation, and relative deviation of D_A for the Condition 1 scans. Discuss potential errors that could account for this deviation.
4. Calculate $E_{1/2}$ for each scan rate using Equation 6.2.8.
5. Test the reversibility of each scan rate using Equations 6.2.11 and 6.2.12. Is this reaction reversible?

Condition 2 – 2 mM Ferricyanide in 1 M KNO₃

1. Tabulate the measured values of i_{pc} , i_{pa} , E_{pc} , E_{pa} , and $E_{pc/2}$ for each scan rate.
2. Plot i_{pc} and i_{pa} versus $v^{1/2}$ for each scan rate. Make one plot i_{pc} versus $v^{1/2}$ that includes a series from the 2 mM data and a series from the 4 mM data. Make a second plot of i_{pa} versus $v^{1/2}$ that includes a series from the 2 mM data and a series from the 4 mM data.
3. Determine the Diffusion Coefficient (D_A) for each scan rate using Equation 6.2.7.
4. Find the average, standard deviation, and relative deviation of D_A for the Condition 2 scans. Discuss protentional errors that could account for this deviation.
5. Calculate $E_{1/2}$ for each scan rate using Equation 6.2.8.
6. Test the reversibility of each scan rate using Equations 6.2.11 and 6.2.12. Is this reaction reversible?

Condition 3 – 1 M KNO₃

1. Is there any current flowing through this cell? Explain.

Condition 4 – 1 M Na₂SO₄

1. Compare your voltammograms to the voltammograms that you acquired in Condition 1. Discuss the differences.
2. Does the charge of the supporting electrolyte affect the reaction?

Part 2: The Oxidation of Acetaminophen

****INCLUDE ALL CV WAVEFORMS****

1. Plot i_p versus $v^{1/2}$. On the plot, have three data series. The series one should be the data from Condition 1, the second series is the data from Condition 2, and the third series is the data from Condition 3.
2. Which system is the appropriate buffer system? Explain.

Determining the Unknown AAPH in Tylenol

1. Report the mass of the Tylenol Tablet.
2. Develop a calibration curve using your solutions from Condition 5 and 6. Use a third data point that uses the same pH condition and scan rate. This data point will come from the data gathered in either Condition 1, 2, or 3.
3. Use the calibration curve to determine the concentration of AAPH and report this value.
4. Calculate and report the mass of AAPH in the tablet.
5. Calculate and report the mass percent. Discuss any error in scans or in the determination for this value.

Outside Links

- <http://www.chem.uoa.gr/applets/Apple...LDiffus2.html>
- <http://pubs.acs.org/doi/abs/10.1021/j100661a017>

References

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4. Skoog, D., Holler, F. J., & Crouch, S. R. (2017). *Principles of Instrumental Analysis* (Seventh ed.). Boston, MA: Cengage Learning.
5. Elgrishi, N.; Rountree, K.J.; Brian D. McCarthy, B.D.; et al. A Practical Beginner's Guide to Cyclic Voltammetry. *J. Chem, Ed.* **2018** 95 (2), 197-206.

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6.3: Fourier Transform Infrared Spectroscopy (FTIR)

Goals

1. Students should develop an understanding of molecular symmetry and vibrational modes.
2. Students should be able to use symmetry elements to predict which vibrational modes are IR active.
3. Student's should be able to use a solve the pollution mystery by determining the air pollutant and the source of the air pollutant.

Introduction

The procedure of this lab does not follow the normal format. In this lab, you have been hired by an environmental testing company to monitor air pollutants. By working through a series of exercises, you will gain important information to determine the air pollutant in an air sample and therefore determine the source of the pollution. The air sample was taken in a location 20 m away from an open farm field where strawberries were growing. In the field on one side, there were cows grazing; on the other side, there was a natural gas pumping station. Across the street, there was a gas station with an auto repair shop specializing in air conditioner repair and a dry cleaner. This information narrows the list of suspected chemicals to six. Test your air sample using an infrared spectrometer and determine the culprit.

The **FTIR / Vibrational** spectroscopy experiment is an adaptation of "Pollution Police" by Profs. Jodye Selco and Janet Beery at the University of Redlands, which was presented at the Division of Chemical Education Regional ACS meeting in Ontario, CA 1999. Many students currently in CHE 115 have already taken CHE 124A which focuses on symmetry, molecular vibrations, and point groups. For students that have taken CHE 124A, use this lab as an opportunity to use your knowledge and assist your lab partners that have not taken the class yet.

There are resources available to further an understanding of FTIR. The theory of FTIR spectrometer operation is discussed in SHN Chapters 16 and 17. The group theory and vibrational quantum mechanics are discussed in McQuarrie and Simon (Chem 110B text) Chapters 12 and 13. Principles of Infrared Spectrometry and its application can be found in Skoog, Holler, and Crouch (the textbook for CHE 115) in chapters 16 and 17.

The FTIR exercise follows the format of a detective story involving solving a series of problems rather than the normal lab format. The experimental portion of the exercises are problems #9 and #10. The computer in room 3475 will be used to complete problems #2, 7, and 8. The computers are set up to run HyperChem, Gaussian, and Spartan.

Setting the Scene

You have been hired by an environmental testing company to monitor air pollutants. Air pollutants can be released from many types of sources. A few examples of sources are: factories, cars, or cattle. Sometimes, volatile chemicals can evaporate from agricultural fields. Since most air pollutant chemicals can absorb infrared light, we are able to detect them with an infrared spectrometer. Some of the molecules commonly found in the atmosphere include those in Table 3.1. Water and small amounts of carbon dioxide, methane, and sulfur trioxide are found in "clean" air samples. Large amounts of any of the chemicals other than water usually indicate atmospheric pollution.

Table 3.1: List of compounds commonly found in the air.

Chemical Name	Chemical Formula	Description
Water	H ₂ O	Ubiquitous in the atmosphere, even on dry days.
Carbon Dioxide	CO ₂	Produced by the combustion of fuels (including the food we eat) and is a major source of greenhouse warming in the atmosphere.
Methyl halides	CH ₃ X	Used as pesticides that are sprayed on agricultural fields. They currently are under strict EPA controls and are partially responsible for the ozone hole.

Chemical Name	Chemical Formula	Description
Dichlorodifluoromethane	CCl_2F_2	refrigerant that is partially responsible for the ozone hole.
Ethylene	C_2H_4	A component of natural gas. Is used to ripen bananas.
Acetylene	C_2H_2	Fuel for high-temperature torches.
Sulfur trioxide	SO_3	Originates from the burning of sulfur-containing fuels such as coal. Reacts with water to form acid rain.
Benzene	C_6H_6	A component of gasoline; is carcinogenic.
Dichloroethylenes (DCE)	$\text{C}_2\text{H}_2\text{Cl}_2$	Used in the dry-cleaning and polymer industries.
Perchloroethylene (PCE)	C_2Cl_4	Used in the dry-cleaning and polymer industries.
Hydrogen Peroxide	HOOH	A component of smog .

When testing, a test sample is first obtained by taking an evacuated cell to the target location, opening a valve, and allowing the ambient air to fill the cell. An FTIR – [Fourier Transform Infrared Spectrometer](#) will be used to perform Infrared Spectrometry. The final output from the spectrometer called an infrared spectrum ([Figure 3.1](#)), is a plot of the intensity of light reaching the detector divided by the initial intensity of light, as a function of frequency (%Transmittance= I/I_0 vs. frequency). The goal of this project is to gain a better understanding of group theory and to identify atmospheric pollutants from their infrared spectra.

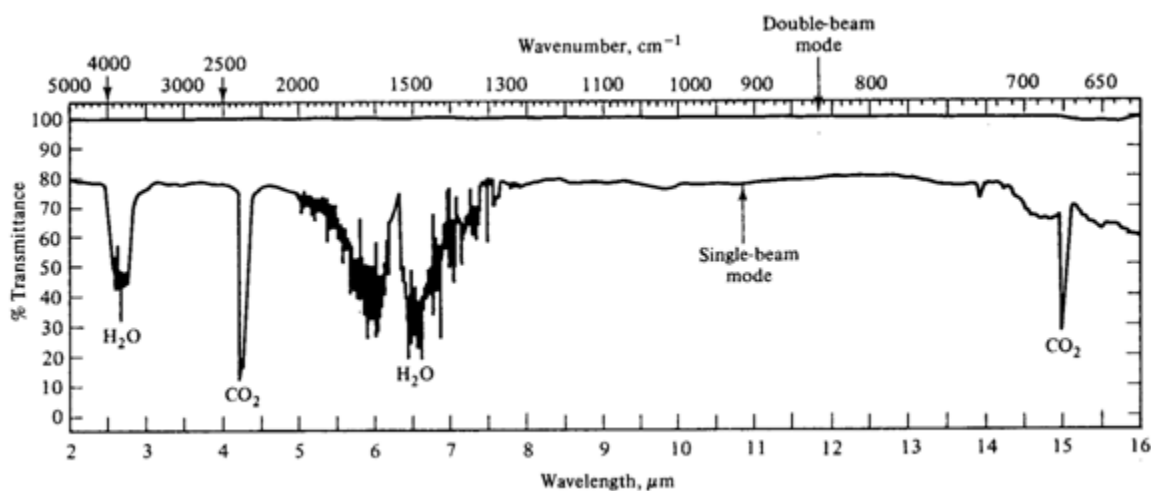


Figure 3.1: An infrared spectrum of air. The lower, single-beam trace clearly shows the absorption of atmospheric gases. The top, double-beam trace shows that the reference beam compensates nearly perfectly for the air absorption, producing a stable 100% T.

For the molecules listed in Table 4.1, examine the three-dimensional ball-and-stick models for these molecules and compare them with the two-dimensional representations of the molecules in Figure 3.2. In the drawings in Figure 3.2, straight lines represent bonds that lie in the plane of the paper; two lines between a pair of atoms represent a double bond; a filled arrowhead indicates a single bond that is angled out of the plane of the paper toward you; and the dashed arrowhead indicates a bond angled into the plane of the paper away from you. Carbon dioxide is an example of a linear molecule; water, ethylene, sulfur trioxide, and benzene are planar molecules.

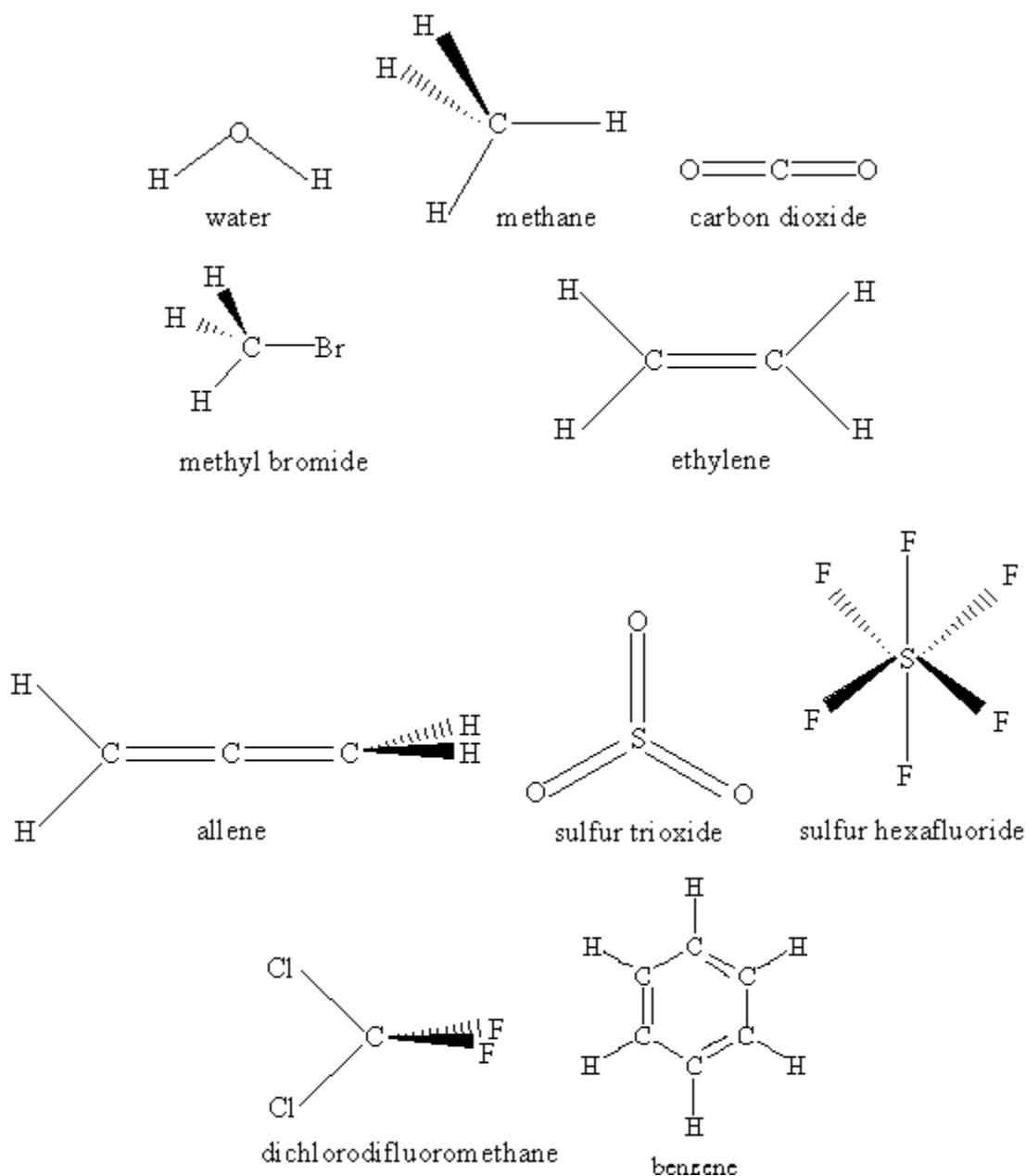


Figure 3.2: Molecules of Study originally listed in Table 4.1.

Problem #1: Center-of-mass and Coordinate Axes

In order to accomplish our goal of identifying the chemical pollutant you are investigating from its infrared spectrum, we must know which infrared frequencies, if any, the molecule absorbs. To decide which molecules absorb infrared radiation and at which frequencies, we will need to examine various properties of the molecules themselves, including their centers of mass, their Cartesian coordinate axes, their vibrations, and their symmetries. Specifically, we will compare the actions of the symmetry operations of a molecule on its Cartesian coordinate axes with the actions of the symmetry operations on its vibrations.

When a molecule absorbs infrared radiation of a given frequency, this energy causes the molecule to vibrate in a specific way; the atoms bounce against each other much like balls connected by a spring. The vibrational motions of a molecule that absorb infrared radiation are the ones that exhibit the same behavior as do the Cartesian coordinate axes of the molecule when the atoms of the molecule are permuted in certain ways. This is a result of the orthogonal interaction between the electromagnetic field of the light and the electric field of the molecule itself. Therefore, you will begin by drawing in a three-dimensional coordinate

system for each of the molecules in the set assigned (see Table 3.2). The convention for molecules is that the origin of the axis system is placed at the center of mass of the molecule. (This is the weighted average of the positions of the atoms.) First, determine approximately where this should be. (You might want to reexamine the ball-and-stick models.) Remember, the masses of the different atoms are different. To find out how much each atom weighs; consult a periodic table of the elements. The mass number for each type of atom appears at the bottom of the square in which the atomic symbol appears.

By convention, **the z-axis is the unique axis**, if there is one. This axis is also called the molecular axis, or axis of highest symmetry. In a linear molecule, it corresponds to the line formed by the molecule; this is true for carbon dioxide. For benzene, the z-axis is the out-of-plane axis since it is the unique axis. If there doesn't seem to be a unique axis, then place the heaviest atoms in the molecule along the z-axis (often, there is more than one way to do this). Once the z-axis is assigned, the in-plane axis usually is the y-axis and the out-of-plane axis is the x-axis. In addition, axes should be placed along the molecular bonds whenever possible.

Example 3.1

The y- and z-axes for water and carbon dioxide are shown in Figure 3.3. The x-axis is out-of-plane from the origin (just below the O atom and pointing straight out at you for water, but centered in the C atom and pointing directly away from you for carbon dioxide).

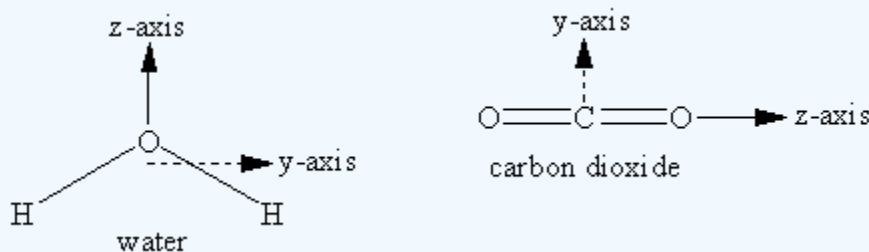


Figure 3.3: Cartesian coordinate axes for water and carbon dioxide

Assignment

Draw in the three Cartesian coordinate axes in a picture of each molecule assigned to you in Table 3.2 as outlined in the steps below. (If you do not assign them correctly now, you will have a chance later to re-label them.)

- Estimate, by eye, the center of mass for each molecule, keeping in mind the atomic masses for each type of atom.
- Draw in the z-axis using the rules above.
- Draw in the two remaining axes using the rules above.

Table 3.2: Assigned molecules for each group

Group Letter	Assigned Molecules
A and E	dichlorodifluoromethane, hydrogen peroxide, nitrous oxide, sulfur trioxide
B and F	<i>trans</i> -1,2-dichloroethylene, ethylene, nitrous oxide, methyl bromide
C and G	<i>gem</i> -1,1-dichloroethylene, dinitrogen tetroxide, nitrous oxide, ammonia
D and H	<i>cis</i> -1,2-dichloroethylene, perchloroethylene, nitrous oxide, methyl iodide

Problem #2: Molecular Vibrations

The types of molecular vibrations a molecule has determine whether or not it absorbs infrared light. Hence, you need to determine the types of vibrations your molecules make. To ensure that all of them have been identified, we need to know how many are

possible. Consider a molecule that is a collection of N atoms connected together in a specific way by chemical bonds. In order to describe the motions of the molecule, we need to consider the motions of each individual atom. This means that we need 3 degrees of freedom for every atom within the molecule for a total of $3N$ degrees of freedom. However, the atoms within the molecule have a specific geometric relationship to the other atoms in the molecule; this results in a redistribution of the number of independent degrees of freedom. The motion through space of the molecule uses three degrees of freedom, reducing the $3N$ degrees of freedom to $3N - 3$. Since the molecule also can rotate (like a spinning baton or Frisbee), we require two degrees of freedom to describe the coordinates about which a linear molecule can spin and three degrees of freedom for a non-linear molecule (for a linear molecule there is no concerted rotation about the molecular axis (z-axis)). This leaves $3N - 5$ degrees of freedom for the linear molecule and $3N - 6$ for the non-linear molecule still unaccounted for; each of the remaining degrees of freedom describes a distinct coordinated internal motion, or vibration, of the atoms within the molecule.

For example, when there are only two atoms in the molecule (e.g. O_2 , N_2 , or CO), there is only one vibrational motion: $3(2) - 5 = 1$. In the case of benzene (C_6H_6) there are 12 atoms and $3(12) - 6 = 30$ vibrational motions possible! As it turns out, not all of these vibrations are capable of absorbing infrared radiation. For the simplest molecules, such as water, it is easy to draw pictures representing the vibrational motions.

Example 3.2

Water has $3(3)-6=3$ vibrations and carbon dioxide has $3(3)-5=4$ vibrations as shown in Figures 3.4 and 3.5.

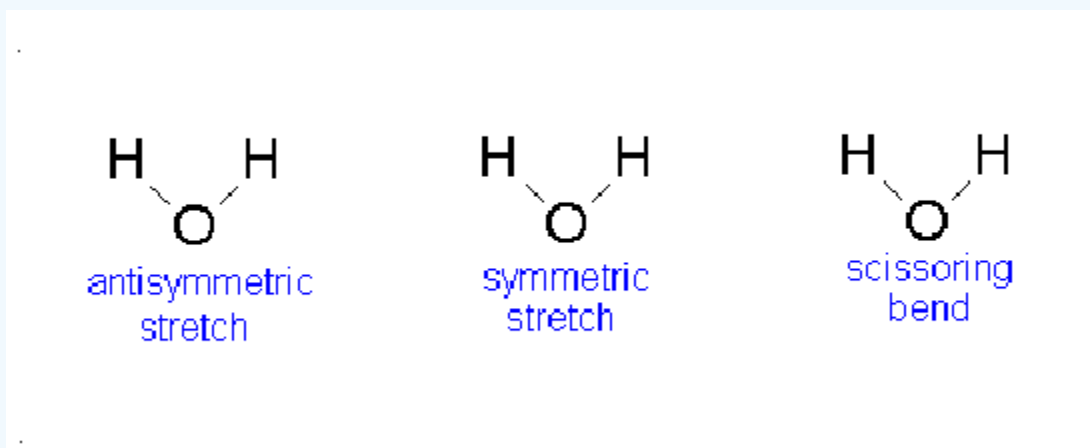


Figure 3.4: Vibrations of water

Consider the water molecule as it undergoes the asymmetric stretch; as it reaches its most extreme position, it has one “arm” extended and the other “arm” contracted. The bend is a change largely in angle and not inter-atom distances.

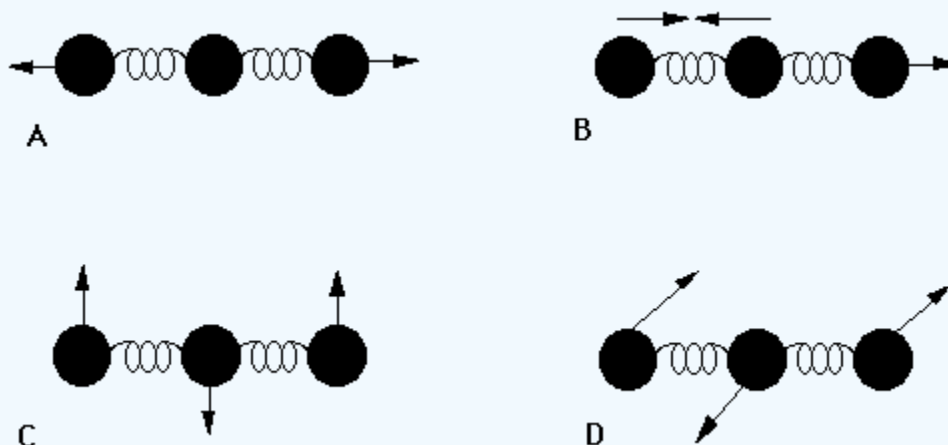


Figure 3.5: Vibrations of carbon dioxide

The four vibrations for CO₂ consist of two stretches (one symmetric and one asymmetric) and two bends, that are degenerate (with the same energy) but involve perpendicular motions.

Assignment

For each molecule, calculate the number of vibrational motions using the formula given above. Then, use a computer program to view the vibrational motions for water, carbon dioxide and the other molecules assigned. Record the motions of the atoms in the molecule using the symbols from the examples above, and record the frequency calculated for each vibration. Note that some of these motions are out-of-plane motions. Be sure to rotate the molecules on the computer screen so that you examine the motions from many different angles. (Many of the programs calculate the frequencies in cm⁻¹; this is actually the frequency, in s⁻¹, divided by the speed of light (3.00×10^{10} cm/s). In this exercise, make note of the frequencies in cm⁻¹ given by the HyperChem program.) Again, you are to complete the following steps for the molecules assigned to you in Table 3.2. This assignment will require a lot of space in your lab notebook due to all of the vibrations you will be drawing.

- Determine the number of vibrational motions, $3N - 6$ or $3N - 5$, to make sure that you know how many vibrational motions to record.
- Record the vibrational motions for your molecules, following the notation used in the examples above.
- Record the frequency for each of these vibrations.

'16 Operation

- Double-click "PC Spartan '16" on desktop. The Spartan computer is located in the TA area of 3475 and has a bright orange name tag.
- Click the new page icon in the top left corner. Atoms with various bond choices will appear. If you prefer a more advanced setting, click the Expert tab.
- Draw your molecule by clicking on the atom with the correct number of bonds needed. To join two atoms, repeat this process by touching the mouse arrow to the open bond.
- Once you are done drawing your molecule, click the Glasses ("View") icon located just below the Geometry scroll-down. This will finalize your drawing.
- Next, go to the Setup scroll-down and select Calculations. The following will be your entries:
 - Calculate: **Equilibrium Geometry with Hartree-Fock 3-21G(*)**
 - Compute: **IR**
 - Print: **Vibrational Modes**
- Go to Setup and scroll down to Submit. Make a new folder with your group's letter in the Chem 115 folder. Label within your folder as you see fit.
- After you save the file you will be prompted twice that Spartan has started and completed. Press OK both times.
- Go to Display and scroll to Spectra. Here you will find the frequencies associated with the different vibrations of your molecule. Click on any of the checkboxes to view the animation for the vibration associated with that particular frequency.

Problem #3: Symmetry Elements and Symmetry Operations

Because the molecules we are examining are very small, the rules of quantum mechanics govern the processes in which we are interested in. According to quantum mechanics, not all light absorption processes are allowed; many are "forbidden" by symmetry. If we want to determine which molecular vibrations absorb infrared light, we need to examine the actions of the symmetries of the molecules on the coordinate axes and on the molecular vibrations. We begin by determining the symmetry elements that each molecule possesses.

A symmetry operation on a molecule is an action that moves the molecule into a position that is indistinguishable from the starting position. A symmetry element of a molecule is a geometric feature of the molecule about which a symmetry operation is performed. Symmetry elements include planes and axes; symmetry operations include reflections across planes and rotations about axes. In the case of water, 180° rotation about the z-axis is a symmetry operation, denoted C₂; while the z-axis itself is a

symmetry element, a C_2 axis. The symbol C_3 indicates a three-fold axis of symmetry, a symmetry element; while C_3 indicates a 120° rotation about a C_3 axis, a symmetry operation. Rotation by 240° about a C_3 axis is denoted C_3^2 . Rotation by 360° about a C_3 axis is equivalent to doing nothing; that is, $C_3^3 = \hat{E}$, where $\hat{E}$ is the identity operation.

Table 3.3: A list of all symmetry elements and operations

Symmetry Element		Symmetry Operation	
Symbol	Description	Symbol	Description
E	Identity	$\hat{E}$	No change
C_n	n-fold axis of symmetry	$\hat{C}_n$	Rotation about the axis by $360^\circ/n$
σ	Plane of symmetry	$\hat{\sigma}$	Reflection through the plane
i	Center of Inversion	$\hat{i}$	Reflection through the center
S_n	n-fold rotation-reflection axis of symmetry, also called improper rotation axis	$\hat{S}_n$	Rotation about the axis by $360^\circ/n$ followed by a reflection through a plane perpendicular to that axis

The plane of symmetry, σ , is also referred to as a reflection plane or mirror plane. The symbol σ_v is used to denote a “vertical” plane of symmetry that is parallel to an axis of highest symmetry (z-axis, or C_n with largest n), while the symbol σ_h is used to denote a “horizontal” plane of symmetry that is perpendicular to the axis of highest symmetry (taken as z-axis). The symbol σ_d denotes a “dihedral” plane of symmetry that bisects an angle between atoms.

Example 3.3

Figures 3.6 and 3.7 illustrate several of the symmetry elements listed in Table 3.3.

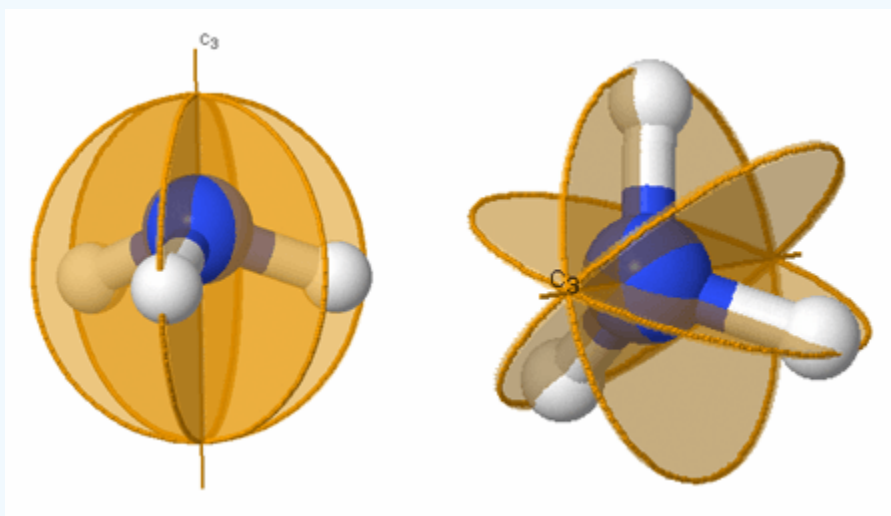


Figure 3.6: Symmetry elements for two views of ammonia (NH_3). The C_3 axis is labeled and the three mirror planes, σ_v , σ_v' , σ_v'' are labeled as gold circles.

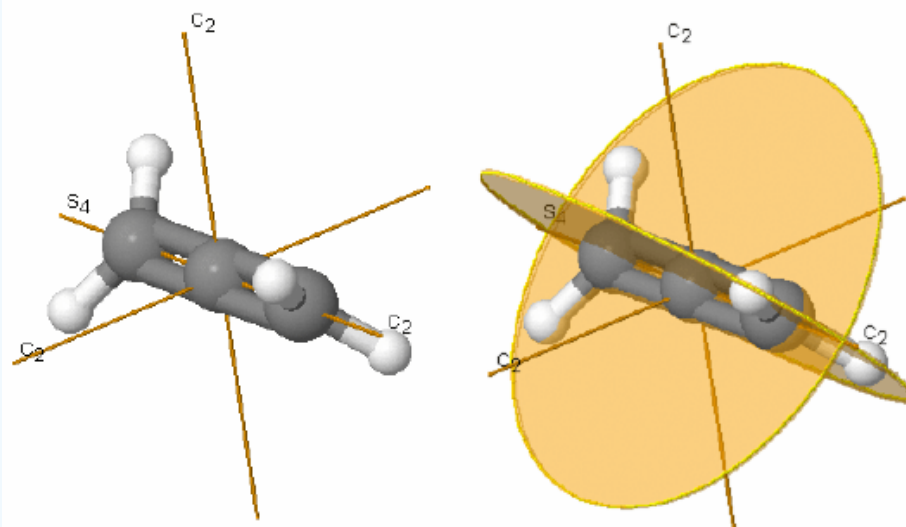


Figure 3.7: Symmetry elements for allene (C_3H_2). The C_2 and S_4 axis is labeled and the two mirror planes, σ_d , σ_d' are labeled as gold circles.

Example 3.4

Figures 3.8 and 3.9 show the symmetry elements and operations of water and carbon dioxide, respectively.

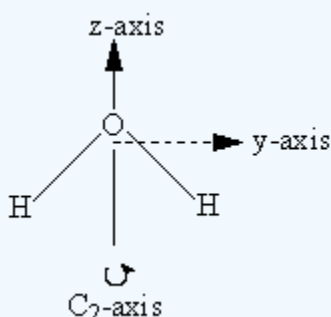
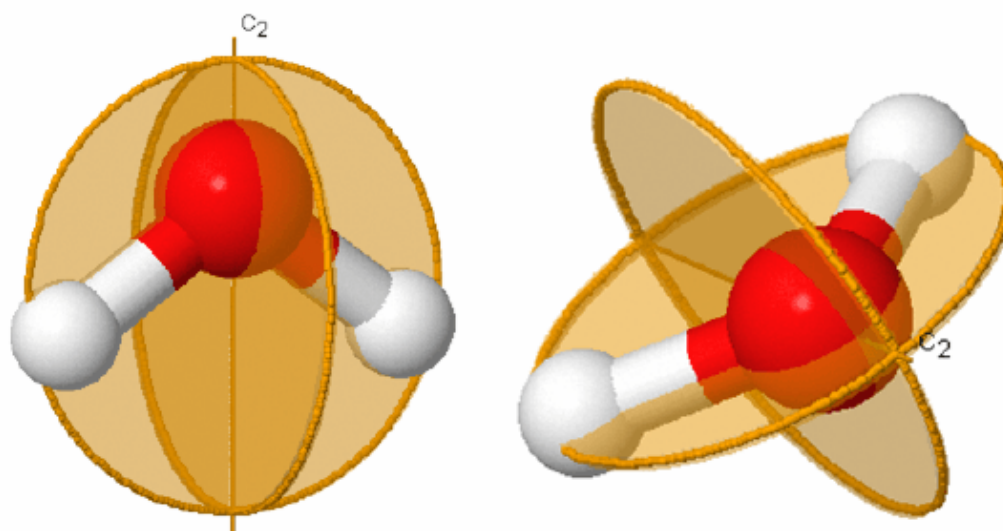


Figure 3.8: Symmetry elements of water viewed from differing perspectives

In the case of water the symmetry elements are E , C_2 (shown), σ_v (xz-plane, perpendicular to the plane of the molecule), and σ_v' (yz-plane, the plane of the molecule). Note that it does not matter whether σ_v represents the xz- or yz-plane. The corresponding symmetry operations for water are $\hat{E}$, C_2 , σ_v , and σ_v' .

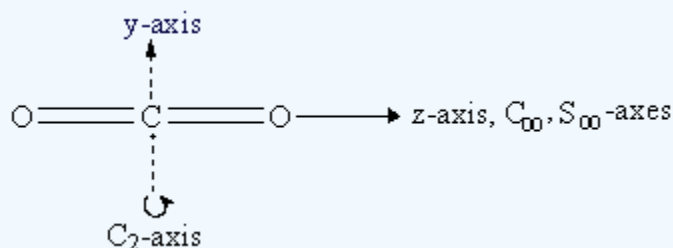


Figure 3.9: Symmetry elements of carbon dioxide

The symmetry elements not shown above are E , σ_v (yz-plane, the plane of the paper), σ_h (xy-plane, perpendicular to the C_2 axis), and i (center of inversion at the coordinate origin). The symmetry operations for carbon dioxide are $\hat{E}$, infinitely many C_2 , C_∞ , infinitely many σ_v , S_∞ , σ_h , and i . A subscript of ∞ means that rotation through any angle about that axis results in a valid symmetry operation. Note that σ_h is identical to S_0 and is often omitted.

Assignment

Reexamine the ball-and-stick models for molecules assigned to you in [Table 3.2](#). Determine all of the symmetry elements and corresponding symmetry operations for each molecule. (Hint: At least one molecule on the list contains the symmetry element σ_h

and one contains an S_3 symmetry element.)

There are a few things to keep in mind while trying to determine the symmetry elements for chemical compounds. The first is that molecules are three-dimensional objects. This means that we can tell the difference between the “front” and “back” or the “top” and “bottom” of planar molecules. For instance, the σ_v reflection of the water molecule across the yz-plane is not the same as the identity operation $\hat{E}$. Second, since atoms of the same kind (or color) are indistinguishable, you may want to number the atoms in the models in order to keep track of the results of the symmetry operations. Finally, when molecules have hexagonal rings with alternating double bonds, all of these bonds---both single and double---are equivalent (e.g. benzene and toluene). It is only the orientation of the atoms themselves that can be “seen” spectroscopically and hence needs to be considered here.

After you have determined the symmetries of the molecules, you can double-check your axis assignments. The z-axis should be the axis of highest C_n symmetry. In H_2O , there is only one C_n axis, C_2 , so it is the z-axis. In CO_2 , there is a C_2 axis and a C_∞ axis, so the C_∞ axis is the z-axis. Check the other molecules to make sure that the z-axis you assigned is the one of highest symmetry. Remember that the axis of highest symmetry may not be unique.

Problem #4: Orders of Symmetry Operations

The order of a symmetry operation is the number of times the operation must be applied to obtain the identity operation, $\hat{E}$. More specifically, the order of a symmetry operation $\hat{A}$ is n , if n is the smallest positive integer such that $\hat{A}^n = \hat{E}$. For instance, for H_2O , the non-identity symmetry operations each have order 2. Note that an inversion always has order 2. The symmetry operation S_4 has order 4 because it must be applied four times in succession to return the molecule to its original orientation when the outside atoms are labeled. (Try it for methane!) Therefore, S_4 generates 4 symmetry operations: S_4 , $S_4^2 = C_2$, S_4^3 , and $S_4^4 = \hat{E}$ of orders 4, 2, 4, and 1, respectively.

Assignment

For each of the molecules assigned to you in Table 3.2, find the order of each symmetry operation. Use the description of order to aid you.

Problem #5: Symmetry Groups

You may have noticed that the set of symmetry operations forms a group under composition of operations, called the symmetry group of the molecule. The symmetry elements of a molecule can be used to determine the group to which the molecule belongs. Using Figure 3.10 and the molecule's symmetry elements, the group can be identified.

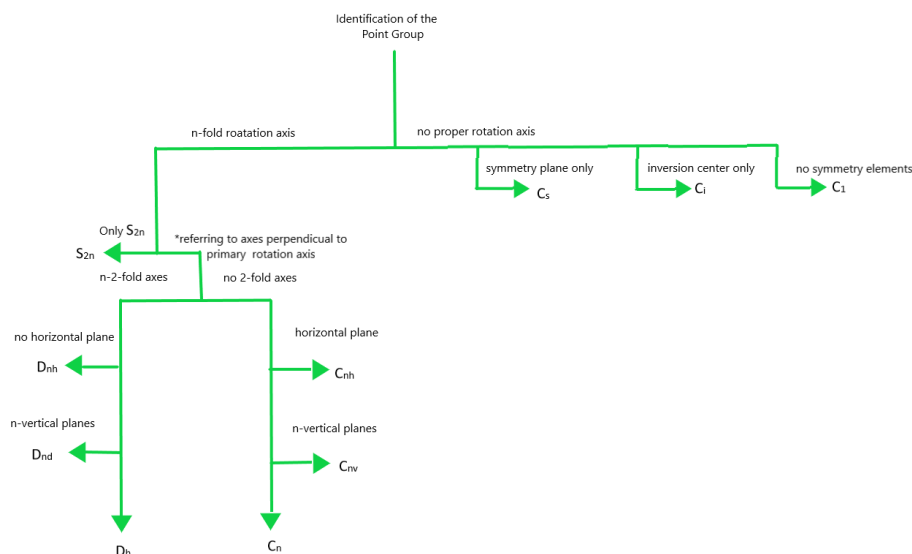


Figure 3.10: Flow Map for identifying the symmetry group to which any molecule belongs.

Assignment

Identify the symmetry group for each of the molecules assigned to you in Table 3.2. The order of a symmetry group is the number of operations which comprise the group. What is the order of each group? Verify by determining the group for your molecules from HyperChem and by examining the character tables in Chapter 12 of McQuarrie and Simon (Chem 110B text) or **Chemical Applications of Group Theory**, by F. A. Cotton. There are also [tutorials](#) available on to help identify the group and practice finding groups of other molecules.

Problem #6: Action of Symmetries on Coordinate Axes

Your overall goal is to identify the molecular origins of the infrared peaks observed in a spectrum of contaminated air. Since a molecule's infrared light absorption depends on how the Cartesian axes transform under the symmetry operations, the next step is to determine what happens to each of the Cartesian axes as the different symmetry operations are performed upon the molecule.

Example 3.5

In Figure 3.11 and Table 3.4, we illustrate this process for water. Note that under the identity operation, $\hat{E}$, none of the axes are inverted or reversed. When the molecule is rotated about the C_2 axis, the orientation of the z-axis remains the same but the x- and y-axes are oriented in the opposite direction; each point (x,y,z) is moved to the point (-x,-y,z). In this case, the rotation is equivalent to multiplying the x and y values by -1. For any operation, -1 indicates that there is a reversal in the orientation of the axis relative to the original orientation, whereas +1 indicates that the orientation remains the same.

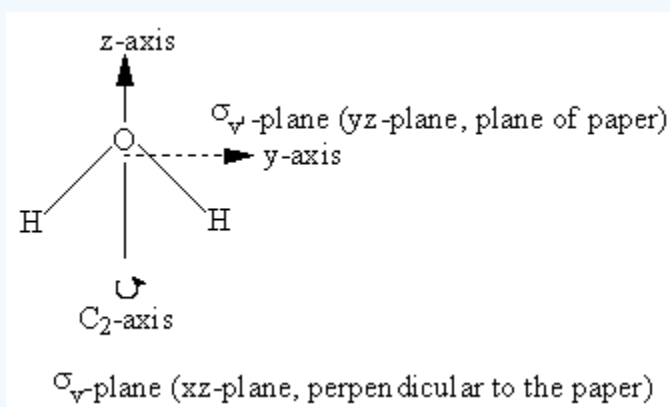


Figure 3.11: The coordinate axes and symmetry elements for water

Table 3.4: How the axes of water transform under the symmetry operations

H ₂ O	$\hat{E}$	C_2	$\sigma_v (xz)$	$\sigma_v' (yz)$
X	1	-1	1	-1
Y	1	-1	-1	1
Z	1	1	1	1

Assignment

Construct similar tables for the **FIRST THREE** molecules assigned to you in Table 3.2. First, check with your TA to make sure you have drawn the Cartesian coordinate axes in the standard way for each of these molecules.

Problem #7: Action of Symmetries on Vibrational Motions

The vibrational motions of the molecule that absorb infrared radiation are the ones that transform under the symmetry operations of the molecule in the same way as do the Cartesian coordinate axes of the molecule. Therefore, we need to determine how the molecular vibrations behave under each of the symmetry operations, so that we can compare them to the transformations of the Cartesian coordinate axes.

Example 3.6

Let us examine the vibrations of water again (Figure 3.12).

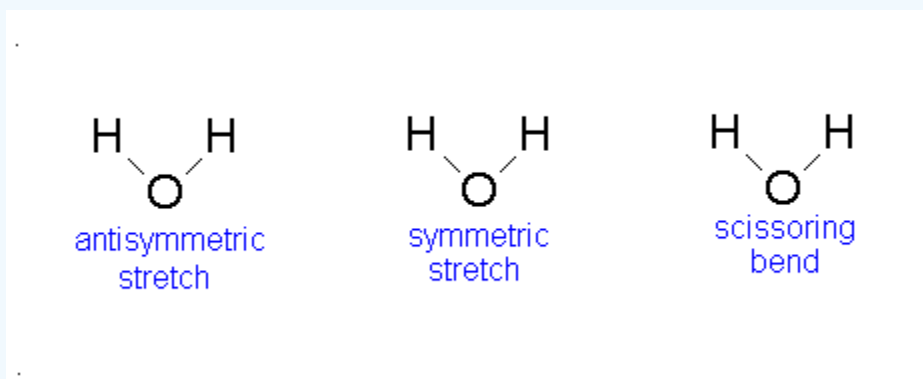


Figure 3.12: Vibrations of water

If we ask how each of the vibrations of water behaves under each of the symmetry operations, we can add more entries to Table 3.5. When we examine what happens to the vibrating molecules as the symmetry operations are performed, we are interested only in whether or not the geometrical orientation of the molecule has changed. For instance, consider the water molecule as it undergoes the asymmetric stretch. Imagine the molecule (or stop the computer program) when it reaches its most extreme position, with one “arm” extended and the other “arm” contracted. Now perform the C_2 operation (rotation by 180°) on this “distorted” molecule. Its orientation after the C_2 operation is different from its orientation before. Note that the configuration has been reversed. The fact that its new position is distinguishable from its original position is represented in Table 3 by -1.

Now let’s examine the water molecule as it undergoes the bend or symmetric stretch. If we perform the C_2 operation (rotation by 180°) on this vibrating molecule, its geometric orientation is unchanged. Its new position is indistinguishable from its original position. The fact that it appears unchanged is represented in Table 3.5 by +1.

Table 3.5: How the axes and vibrations of water transform under the symmetry operations

H ₂ O	$\hat{E}$	C_2	$\sigma_v (xz)$	$\sigma_v' (yz)$
X	1	-1	1	-1
Y	1	-1	-1	1
Z	1	1	1	1
bend	1	1	1	1
symmetric stretch	1	1	1	1
asymmetric stretch	1	-1	-1	1

Assignment

Construct tables as in example 3.6 for the **FIRST THREE** molecules assigned to you in Table 3.2. Examine the motions of the atoms for each different vibration. If you were to imagine the molecule (or stop the computer program) when it reaches its most extreme position, consider how that “version” of the molecular shape would behave under each of the different symmetry

operations. That the molecule is indistinguishable after the symmetry operation is indicated by a 1, while a distinguishable molecule is represented by a -1. Fill in a line in your table for each vibrational motion. Note this procedure works for most, but not all simple molecules. For example, this procedure will not work for your third molecule.

Problem #8: Comparing Symmetry Operations on Axes and Vibrations

When a symmetry operation and an axis transform in the same way, the frequency associated with that symmetry operation will absorb light. By comparing the tables you generated in

Example 3.7

In the case of water, as illustrated in Table 3.5, the bending vibration transforms under the symmetry operations in the same way as does the z-axis. This is also true for the symmetric stretching motion. On the other hand, the asymmetric stretch transforms in the same way as does the y-axis. In this case, all three of the vibrational motions of water would absorb infrared light since each one of them transforms under the symmetry operations as does one of the Cartesian coordinate axes. All of the vibrations also have frequencies that are in the appropriate frequency range. We would expect the infrared spectrum of water to have three peaks corresponding to frequencies of 1840, 3587, and 3652 cm^{-1} .

Assignment

For the **FIRST THREE** molecules assigned to you in Table 3.2, use the tables you constructed in Problem #7 to determine how the vibrational motions transform under the symmetry operations. List the frequency calculated of the ones that transform as do the x-, y-, or z-axes. (You listed the frequencies you need for this problem in Problem #2.) Given that infrared spectrometers operate in the range of about 600 cm^{-1} to 4000 cm^{-1} (wavenumbers), which vibrational frequencies should you observe in the infrared spectra for your molecules? Make a rough sketch of the infrared spectrum you would expect to see for each of your molecules, labeling peaks with their frequencies. Assume that if peaks are separated by less than 25 cm^{-1} , they will not be resolved and will appear as a single peak.

Problem #9: Obtaining Infrared Spectra

You have now reached the experimental portion of this exercise. Before you can measure your contaminated air sample. You will need to measure some common air pollutants. The IR spectra that you measure for these molecules will help you decide which compound is in your air sample.

Assignment

Breathing into the sample compartment of the infrared spectrometer can generate the spectrum of water and carbon dioxide. You will then take spectra of **methyl iodide**, **ethylene**, **acetylene**, **trans-DCE**, and a sample of **contaminated air**, which have already been prepared in infrared cells. Do not be overly concerned if the windows on the cells appear to be hazy; they will work fine. Do you observe the predicted absorptions? If not, try multiplying all of your vibrational frequencies by 0.89 (a factor theoretical chemists recommend to compensate for over calculations). Do they come closer to what you observe now?

Operating the Bruker FTIR Spectrometer (room 3475)

1. Start the **OPUS 6.0 software** on the computer next to the instrument.
2. In the **Measure Menu**, go to the **Advance tab** to set the parameters for the experiment
 - Scans: 16 for sample and 16 for background
 - Resolution: 2.0
 - Signal Gain: 1
 - IR Data Type: Transmittance
 - Go to **Path**: and select the Chem115 directory for data storage
 - Name your sample in the **Filename**: field

3. Close the sample compartment and wait 2-3 minutes for the sample compartment to be purged of air.
4. In the **Measure Menu**, go to the **Basic tab**. Click the “Background Single Channel” button, the instrument will start scanning the background. The progress status will be shown at the bottom of the screen.
5. Open the chamber, and load your sample in. Close the chamber.
6. To acquire your sample scan. Give your sample a description under the **Basic tab**. Run the sample spectrum by clicking on the “**Sample Single Channel**” button. The progress of the scan can be seen at the bottom of the screen.

Operating the FTIR Spectrometer (room 3480)

1. Start the **OPUS 7.2 software** on the computer next to the instrument.
2. In the **Toolbar Menu**, find the **Advance Measurement tab** to set the parameters for the experiment
 - Scans: 16 for sample and 16 for background
 - Resolution: 2.0
 - Signal Gain: auto
 - IR Data Type: Transmittance
 - Go to **Path**: and select the Chem115 directory for data storage
 - Name your sample in the **Filename**: field
3. Close the sample compartment and wait 2-3 minutes for the sample compartment to be purged of air.
4. In the **Measure Menu**, go to the **Basic tab**. Click the “Background Single Channel” button, the instrument will start scanning the background. The progress status will be shown at the bottom of the screen.
5. Open the chamber, and load your sample in. Close the chamber.
6. To acquire your sample scan. Give your sample a description under the **Basic tab**. Run the sample spectrum by clicking on the “**Sample Single Channel**” button. The progress of the scan can be seen at the bottom of the screen.



2017-An Agilent 630 FTIR might be available, ask Paul

Collect a spectrum of water and carbon dioxide:

1. Open the sample compartment.
2. Exhale into the sample compartment.
3. Collect the spectrum by clicking on the “**Sample Single Channel**” button on the **Basic Tab**.

Collect spectra of the four reference samples and your unknown:

1. Open the sample compartment.
2. Put the cell holder with the cell into the sample compartment. Align the holes in the bottom of the holder with the holes in the plate in the bottom of the compartment.
3. Wait 2-3 minutes for the compartment to purge.
4. Name the file.
5. Acquire the spectrum by pressing **Sample Single Channel** button.

To pick peaks: (This procedure may not work depending on the instrument you are using.)

Click on the peak picking icon, or select **Peak Picking** from the **Evaluate** menu.

- The peak picking screen will show up. Choose the **Interactive mode**. Sliding the Threshold square up or down so that all the peaks are below it.
- Each peak above the threshold will be labeled with its frequency and the peak list will be written to a report file that you must save by selecting Save Report from the File pulldown menu.
- You can annotate the plot by selecting the annotator options from the Tools pulldown menu.
- The file path and name are the default title for your plot. You can change this by selecting Title under the Display pulldown menu.
- Once you have picked your peaks and annotated your plot, save the window as before by selecting Save Sample from the File pulldown menu.
- Select Plot from the File pulldown menu. In the Plot menu select the window you wish to print. Check that the size of the plot is correct and then click on the “plot”. This will take 1-2 minutes. Be patient. You can then select another window to print or

click on the “done” button.

Problem #10: Identifying the Pollutant

As a reminder: The sample was taken in a location 20 m away from an open farm field where strawberries were growing. In the field on one side, there were cows grazing; on the other side, there was a natural gas pumping station. Across the street, there was a gas station with an auto repair shop specializing in air conditioner repair and a dry cleaner. This information narrows the list of suspected chemicals to six. Test your air sample and determine the culprit. The spectrometer automatically purges the cell cavity with N_2 , which is transparent in infrared. This removes water and carbon dioxide, which exhibit strong IR absorptions, so you should be able to see the pollutant's spectrum relatively easily.

Assignment

Examine the spectrum from your sample of the unknown, contaminated air. Note that it is a very different spectrum from that of carbon dioxide and water, which is shown in [Figure 3.1](#). Which of the chemicals that you studied is responsible for this spectrum? To which potential polluter described above would you attribute this pollution?

Post-Lab Questions

1. Include all of the information recorded during the assignments.
2. Include all of the FTIR spectra
3. Identify the pollutant and predict the source of the pollutant.
4. Explain the process used to identify the pollutant.

Outside Links

- <http://symmetry.otterbein.edu/index.html>
- [Point group symmetry character tables](#)

References

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4. Skoog, D. A.; Holler, F. J.; Crouch, S. R. *Principles of Instrumental Analysis*, Seventh Edition; Cengage Learning: Boston, MA, 2016.

Contributors and Attributions

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6.4: Molecular Fluorescence

goals

1. Students should have an in-depth understanding of fluorescence after the completion of this lab exercise.
2. Students should be able to optimize the scan parameters to achieve the best emission and excitation spectra.
3. Students should be able to extract key information from the emission and excitation spectra and determine the unknown concentration of a compound.

Introduction

When a molecule absorbs a photon in the ultraviolet or visible (UV VIS) region (180 - 780 nm), an electronic transition occurs within the molecule. This transition involves moving an electron from the singlet ground state to a singlet excited state. After excitation, the molecule will undergo de-excitation to regain its ground state electronic configuration. De-excitation of the molecule can occur in three distinct ways: by collisional deactivation (external conversion), **fluorescence**, or **phosphorescence** (Figure 4.1).

The first relaxation mechanism, **collisional deactivation**, occurs when the excited molecule transfers its excess energy to molecules with which it collides without photon emission. The second mechanism, **fluorescence**, involves the electron returning from the excited singlet state to the ground singlet state accompanied by the emission of a photon of lower energy (longer wavelength) than the absorbed photon; the energy loss is due to vibrational relaxation while in the excited state. When fluorescence is favored, it occurs within about 10^{-8} seconds after absorption. For reference, a picosecond is 10^{-9} seconds. **Phosphorescence**, the third route for de-excitation, occurs when the excited electron enters the lowest triplet state from the excited singlet state by intersystem crossing and subsequently emits a photon in returning from the lowest triplet state to the singlet ground state. These excited singlet-triplet and triplet- ground singlet transitions involve electron reversal (Figure 4.2), which is a low probability occurrence. Thus, the time between absorption and phosphorescence can be from 10^{-2} seconds to several minutes.

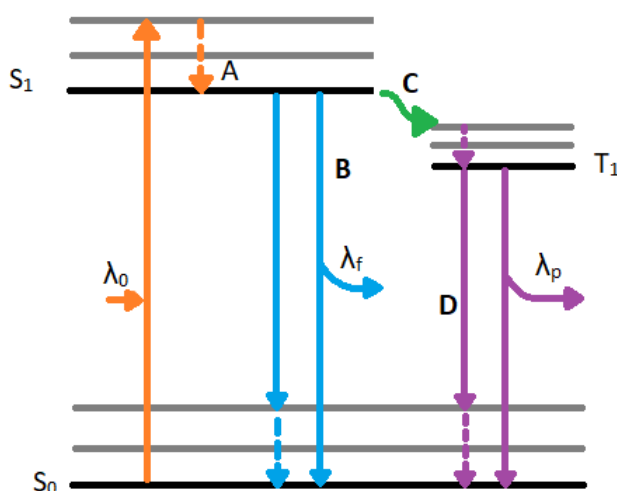


Figure 4.1: Jablonski diagram displaying excitation and de-excitation pathways of a molecule. λ_0 indicates the incident wavelength that is absorbed by the molecule. Once the molecule reached the excited singlet state (S_1) from the ground state singlet (S_0), it can experience vibrational relaxation as seen in A. After relaxing to the lowest energy excited state, a molecule can de-excite by fluorescence as seen in path B. The fluorescence path emits a wavelength (λ_f) which is longer than the absorbed wavelength (λ_0). The alternative, unfavored path moves along path C which involves intersystem crossing. Intersystem

crossing involves a conversion from an excited singlet state (S_1) to an excited triplet state (T_1). After reaching the triplet state and experiencing vibrational relaxing, the molecule will phosphoresce by emitting an even shorter wavelength (λ_p) to reach the ground state singlet (S_0).

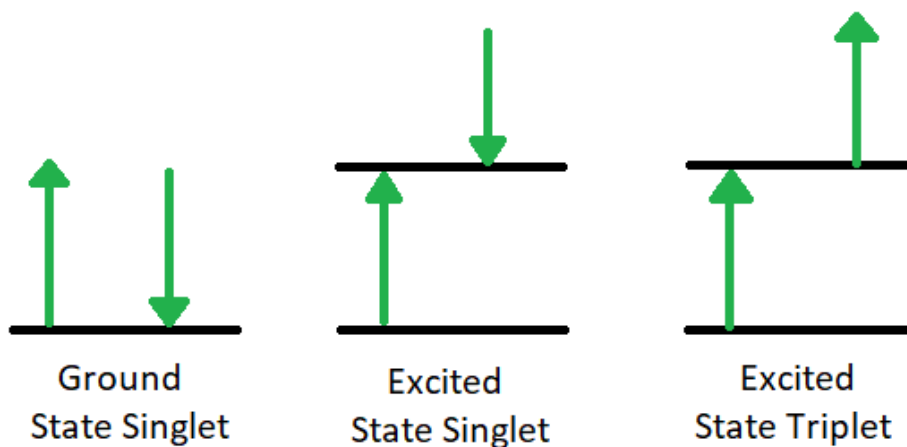


Figure 4.2: Transition from Ground Singlet (A) to Excited Singlet (B) to Excited Triplet (C).

Since our interest involves fluorescence, a typical experimental arrangement is shown in [Figure 4.3](#). This is used in either of two distinct ways. The first, which holds the excitation monochromator M1 fixed, and varies the emission monochromator M2, yields an emission spectrum (the wavelength distribution of light emitted by the excited singlet state). When generating an emission section, the intensity of the transmitted light (I) is measured and compared to the transmitted light's measured wavelength (λ_f). Alternately, M2 can be fixed and M1 varied; this procedure produces an excitation spectrum (a plot of fluorescence intensity as a function of excitation wavelength). The excitation spectrum can be produced by measuring the intensity of the transmitted light (I) and comparing it to the measured wavelength of the incident light (λ_0). Often, the excitation spectrum of a pure compound has exactly the same profile as the absorption spectrum ([Figure 4.1](#)). The principles described by [Figure 4.1](#) can be observed in the excitation and emission spectra. An example of these spectra can be seen in [Figure 4.4](#).

Simple Fluorometer

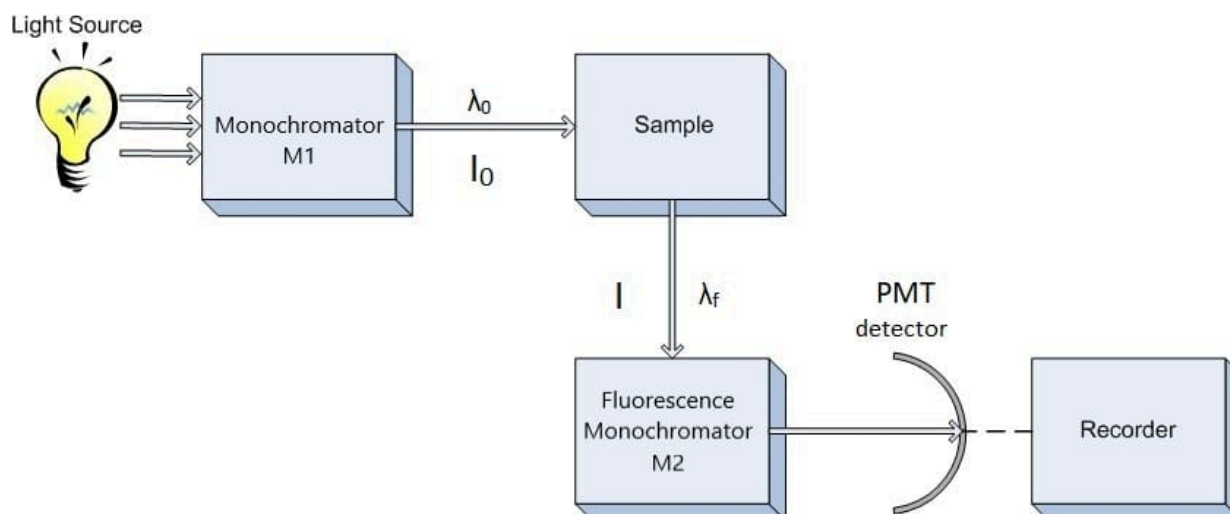


Figure 4.3: Schematic Diagram of Fluorometer. M1 = excitation monochromator, M2 emission monochromator, Photo Multiplier Tube detector.

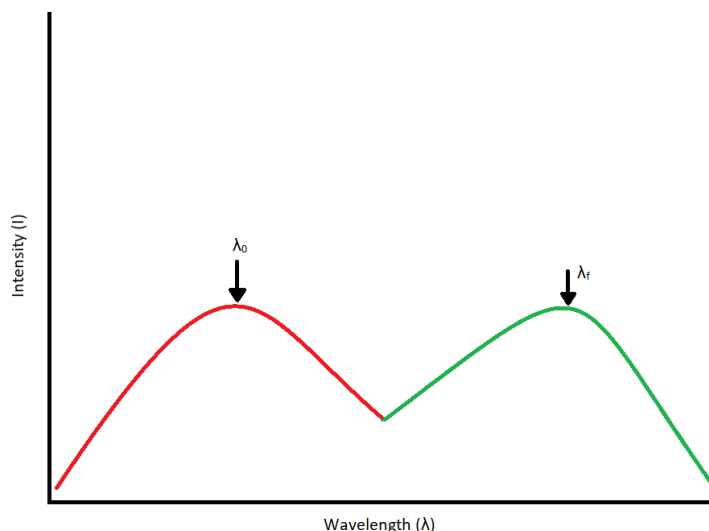


Figure 4.4: Excitation/Absorbance Spectrum (Red) coupled with an emission spectrum (Green). Each spectrum peaks at its respective wavelength. Excitation and emission spectra should overlap and be mirror images of each other.

Coupling the above techniques with a relationship between fluorescence intensity and concentration would be exceptionally useful. Such a relationship can be derived from [Beer's Law](#), which states that the fraction of light intensity transmitted by a sample is

$$\frac{I}{I_o} = 10^{-\epsilon bc} \quad (6.4.1)$$

where,

- I is the transmitted light intensity,
- I_o is the incident intensity,
- ϵ is the molar absorptivity at a given wavelength in units of $L \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$,
- b is the cell path in centimeters, and
- c is the concentration in moles per liter.

The fraction of absorbed light is:

$$1 - \frac{I}{I_o} = 1 - 10^{-\epsilon bc} \quad (6.4.2)$$

from which it follows that the absolute amount of absorbed light is equal to:

$$I_o - I = I_o - I_o (10^{-\epsilon bc}) \quad (6.4.3)$$

The fluorescence intensity, F , is proportional to the amount of light absorbed and fluorescence quantum yield, Φ . Thus,

$$F = k I_o \phi [1 - (10^{-\epsilon bc})] \quad (6.4.4)$$

where k is a proportionality constant. **If dilute solutions are used**, so that less than 2% of the excitation energy is absorbed, the exponential term in Equation 6.4.4 can be approximated by the first two terms in the corresponding [Taylor series](#) expansion

$$e^x \approx 1 + x \quad (6.4.5)$$

Then,

$$F = k I_o \phi \epsilon bc \quad (6.4.6)$$

hence fluorescence intensity is **proportional to concentration, but only at low concentration:**

Instrumentation

In this experiment, two instruments will be used. First, the Agilent HP8453 UV-Vis spectrometer to measure optimal excitation wavelength. You used this instrument in CHE 105, so you may remember how to use it. For the second portion of this experiment, the Varian Eclipse Fluorescence Spectrometer (Figure 4.5) will be used. This instrument is computer-controlled through software running on Windows XP. Detailed operating instructions for the Eclipse will be in the procedure section.



Figure 4.5: UCD's Varian Eclipse Fluorescence Spectrometer

Experimental Procedure

This experiment involves the analysis of [vitamin B₂](#), riboflavin, by measuring its native fluorescence. Riboflavin (Figure 4.6) is a common water-soluble vitamin found in eggs, milk, and other foods, that strongly fluoresces and is very sensitive to light. Its two predominant irradiation decomposition products are lumichrome and lumiflavin, which are themselves highly fluorescent.

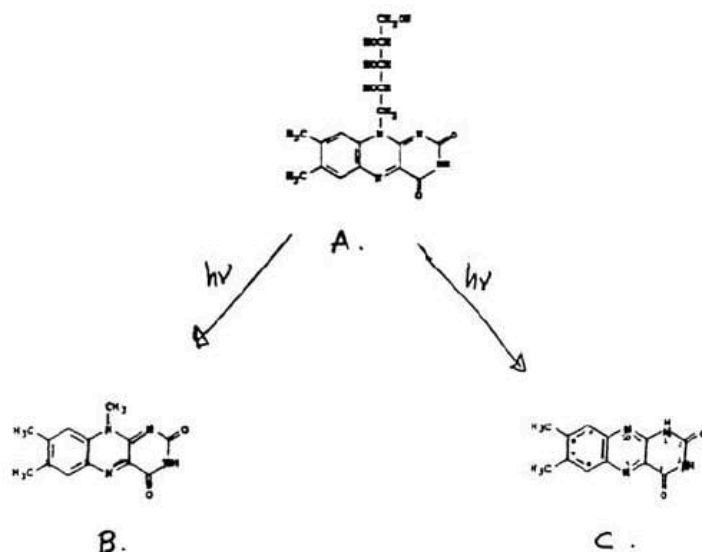


Figure 4.6: Riboflavin (A) and the two most predominant irradiation decomposition products, lumiflavin (B) and lumichrome (C).

The analysis method requires working with riboflavin samples at concentrations well below the part per million (ppm) level. Therefore, it is extremely important that the utmost care is exercised in cleaning and handling all of the glassware and solutions during this experiment.

In the first lab period, the standard and unknown solutions mentioned below should be prepared. Later during this period, you should consult the T.A. about operating the UV VIS spectrophotometer as well as the fluorescence spectrometer. You should also obtain the absorption spectra of riboflavin and perform a practice run on the fluorescence spectrometer.

The second lab period should be used to complete the experiment and Part II of the unknown analysis.

Stock Solutions

1. Obtain the 100 ppm stock standard solution of Riboflavin from the refrigerator or from the T.A.
2. Allow the solution to sit on the counter for a few minutes to equilibrate to room temperature. This is to ensure consistent volume measurements.
3. Deliver 1 mL of the stock solution to a 10 mL volumetric flask and dilute using deionized water. This is your 10 ppm solution.
4. Using a volumetric pipet, deliver 1.00 mL of the 10 ppm solution to a 100 mL volumetric flask, and dilute using deionized water. This is your 100 part per billion (ppb) stock solution.
5. Fill in Table 4.1 with the correct amounts of 100 ppb riboflavin to make the remainder of your standards. Check the table with your TA before you begin the experiment.
6. Make the solutions 2-6 from the table and dilute with deionized water. Make certain that your glassware is clean and rinsed with deionized water.

Table 4.1: Standard Riboflavin Solutions

Solution #	mL of 100 ppb sol.	Dilute to (mL)	Rib. Conc (ppb)
2		50	2
3		50	10
4		50	20
5		50	40
6		50	50

Unknown Solution

The dry milk sample should be reconstituted as indicated on the jar. Record your work (this is a quantitative experiment). The milk samples require special treatment in order to remove interfering fats and proteins, which would otherwise make analysis difficult or impossible. The procedure to accomplish this is outlined below.

1. Find the dry milk sample on the shelves above the weigh station.
2. Reconstitute the dry milk sample by following the directions listed on the sample jar.
3. Take 25 mL of milk sample and add exactly 75 mL of a solution consisting of equal parts 3M HAc and 3M NaCl. Do not use a volumetric flask for this. It is too difficult to stir the solution. Use your 25 mL pipet and a 250 or 400 mL beaker.
4. Stir for 20 minutes.
5. Filter the solution using a Buchner funnel and the vacuum line. Make sure you have a trap between the filtering flask and the house vacuum line.
6. Take a 5 mL aliquot of the filtrate and dilute to 100 mL with deionized water in a volumetric flask. This solution will be used for your analytical measurements.

Instrumental

Part 1: Approximate Excitation Wavelength

The instrument you'll be using in this portion of the experiment is an Agilent HP8453 UV-Vis spectrometer.

The first step in conducting the analysis is to determine the optimum excitation wavelength for riboflavin. When no prior information is available, as in this case, the absorption spectrum can be used to approximate the best excitation wavelength.

Look near the Agilent HP8453 UV-Vis spectrometer for a pack of cuvettes. If you cannot find a pack, ask your T.A. for a new pack. Use the 10 ppm solution to obtain an absorption spectrum of riboflavin from 280 to 600 nm. Use distilled water as your reference solution. If you do not recall how to acquire an absorbance spectrum, check with your TA for instructions. The wavelength of maximum absorbance should be used as an initial excitation wavelength. There may be more than one absorbance peak.

Print the absorbance spectrum

Part 2: Actual Excitation and Emission Maxima

In this part of the experiment, you will determine the optimal instrument operation parameters. Using the approximate excitation wavelengths determined above from the UV-Vis absorbance spectrum, obtain two emission spectra. The emission maximum observed in the two emission spectra will then be used to collect an excitation spectrum to "refine" the choice of excitation wavelength. **You will see that the fluorescence excitation spectrum closely follows the absorbance spectrum.**

You should collect the following spectra:

1. Two emission spectra of the 50 ppb standard solution will be taken. Collect the first spectrum from the first excitation wavelength (~370 to 700 nm). Collect the second spectrum from the second excitation wavelength (~440 to 700 nm). The two approximate excitation wavelengths determined in part 1 should be used to determine the wavelength ranges.
2. Two excitation spectra. One spectrum of the 50 ppb standard and one spectrum of the milk sample you prepared. (280 to ~500nm) Select the scan maximum by taking the larger of the two emission wavelength and subtracting 30 nm as described in step 5 of the "Using the Varian Eclipse Fluorimeter."
3. Print all of the spectra from this section.

Using the Varian Eclipse Fluorimeter

- Ask your TA for the necessary Cuvette for the instrument.

Emission Spectrum

1. Power up the Eclipse with the switch on the front panel, if necessary. The dim light on the front will become green when ready.
2. If the computer is not logged in, log into "Fluorimeter" by clicking on its icon.
3. Double click on the **Scan** icon on the desktop THEN Click on the **Setup** button.
4. Set the excitation wavelength to either of the absorption maxima in the UV-Vis spectrum
5. Set the scan limits. The starting wavelength for the **emission spectrum** should be 30 nm above the excitation wavelength to avoid Rayleigh scattering. The upper wavelength should be 700nm.
6. The scan speed should be set to **Slow** (120 nm/minute) and both slits should be set to 10nm.
7. Click on the **Reports** tab. Set the **Threshold** value to 5. Click the OK button.
8. Click on the green traffic light icon to start the scan.
9. A window will appear where you can name your sample. Put in your desired name and click OK.
10. When the scan is complete, print the spectrum by clicking on the **Print** button.

Excitation Spectrum

1. Obtain **excitation spectrum** by repeating the above steps, start by clicking the **Setup** button. Click on the box that says excitation.
2. Set the emission wavelength to the value you determined above.
3. Record the excitation spectrum from 280nm to about 30nm below the emission wavelength.
4. Collect excitation spectra under the identical conditions for the milk sample you prepared.

Part 3: Standard Curve

At two excitation wavelengths (both ~370 and ~440 nm) you determine the exact wavelengths to use.

1. Double click the **Simple Reads** icon on the desktop.
2. Click on the **Setup** button, and set the emission wavelength and excitation wavelength in the window.
3. Set the excitation and emission slits at 10 nm, and the Average Time at 5 seconds.
4. Click on **Read** to begin data acquisition.
5. Change the excitation wavelength and repeat the procedure.
6. Do this for all your standards, and repeat three times for the unknown sample.
7. Print the data files when you are done.
8. When you are done with the fluorimeter, exit the programs, switch out the instrument, and remember to sign the logbook.
9. Create a standard curve in Excel or Google sheets to determine the concentration of Riboflavin in your unknown solution.

Post-Lab Questions

1. Include all spectra generated during this lab.
2. Include the calibration curve you created in excel.
3. Indicate the calculated concentration of Riboflavin in the milk sample.
4. Describe the relationship between the emission and excitation spectra that you obtained. Are they mirror images?

References

1. Skoog, D. A.; Holler, F. J.; Crouch, S. R. Principles of Instrumental Analysis, Seventh Edition; Cengage Learning: Boston, MA, 2016.

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6.5: Capillary Electrophoresis

Definition

1. Students should develop an understanding of the similarities between electrophoretic methods.
2. Students should develop an understanding of how Electroosmotic Flow and Electrophoretic Mobility effect retention time.
3. Students should use CE data to complement data for gas or liquid chromatography.
4. Students should know how the addition of a component affects partitioning.

Introduction

Electrophoresis is a class of separation techniques in which we separate analytes by their ability to move through a conductive medium in response to an applied electric field. This conductive medium is usually an aqueous buffer. Generally, when the electric field is applied, cations migrate toward the electric field's negatively charged cathode. Anions migrate toward the positively charged anode and neutral species do not experience the electrical field and remain stationary. Ions with larger charge-to-size ratios—which favors ions of larger charge and of smaller size—migrate at a faster rate than ions with smaller charge-to-size ratios. High-Performance Liquid Chromatography (HPLC) or Gas Chromatography (GC) use liquid and gas respectively to force the sample through a column, but electrophoresis utilizes an electric field.

In capillary electrophoresis (CE), the conducting buffer is retained within a capillary tube whose inner diameter is typically 25–75 μm . Samples are introduced into one end of the capillary tube. As the sample migrates through the capillary its components separate and elute from the column at different times. When a component in the sample migrates at a lower rate, the component will be retained and will have a longer retention time. When a sample migrates at a faster rate, the retention time is shorter. Similar to HPLC and GC, the retention times can be used to identify components and determine the amount of component that was in the original sample. In capillary electrophoresis, the movement of ions through the capillary is defined by the ion's electrophoretic mobility. A basic schematic of the instrument can be seen in Figure 6.1.

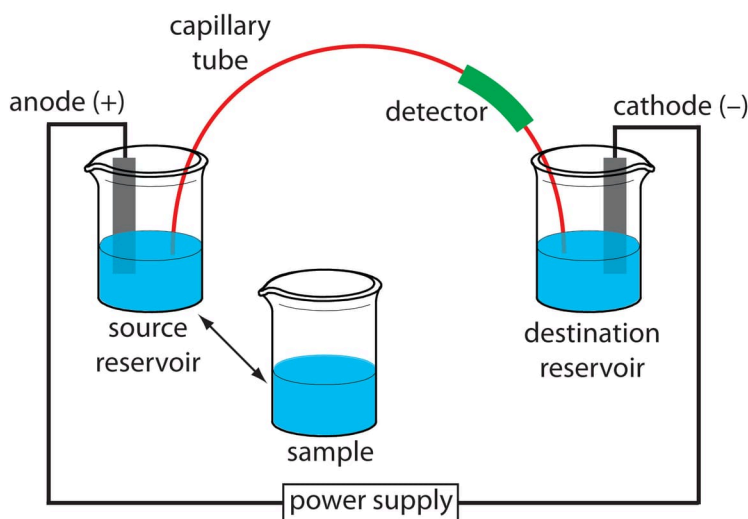


Figure 6.1: Schematic diagram of the basic instrumentation for capillary electrophoresis. The sample and the source reservoir are switched when making injections.

Electrophoretic Mobility

The [electrophoretic mobility](#) (μ_e) describes the ability of a component to migrate. The [electrophoretic mobility](#) (equation 6.1) of

an object in an applied electric field is determined by the charge on the molecule divided by the frictional coefficient (f). [Stokes' Law](#) (Equation 6.2) gives the frictional coefficient of the molecule (f). This value is dependent on size and shape of the molecule as well as the viscosity of the solvent(η).

$$\mu_e = \frac{q}{6\pi\eta r} \quad (6.5.1)$$

$$f = 6\pi\eta r \quad (6.5.2)$$

The velocity of the particle in an applied field is described by equation 6.3. The variable E is the applied electric field. The velocity describes the rate at which the components are moving through the capillary.

$$v = \mu_e E \quad (6.5.3)$$

Gel Electrophoresis

One of the most common forms of electrophoresis that students use is slab gel electrophoresis. In a slab gel, the conducting buffer is retained within a porous gel of agarose or polyacrylamide. Slabs are formed by pouring the gel between two glass plates separated by spacers. Typical thicknesses are 0.25–1 mm. Gel electrophoresis is an important technique in biochemistry where it is frequently used for separating DNA fragments and proteins. Although it is a powerful tool for the qualitative analysis of complex mixtures, it is less useful for quantitative work. Gel electrophoresis is commonly used in biochemistry to separate macromolecules, nucleic acids and proteins. Proteins and nucleic acid fragments are separated by differences in mobility through a sieving gel under the force of an applied electric field. An Example of a gel can be seen in Figure 6.2.

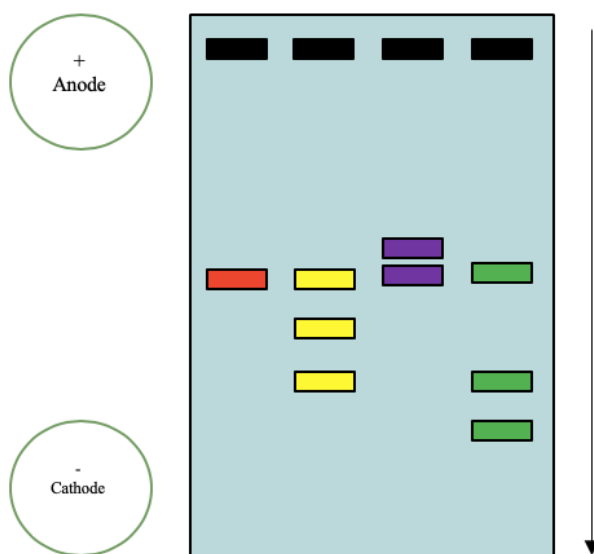


Figure 6.2: Schematic of a standard gel electrophoresis set-up. The travels in the direction of the arrow

Capillary Zone Electrophoresis

The simplest CE method is capillary zone electrophoresis (CZE), a method by which molecules, ions, or particles are separated **solely** by their electrophoretic mobility. This technique is based on the idea that the velocity is proportional to the charge to mass ratio. The capillaries are usually made of silica. In uncoated capillaries at pH greater than 3 the SiOH groups are ionized to SiO⁻ ([Figure 6.3](#)). The deprotonation creates a negative charge along the capillary wall. This leads to a phenomenon called electroosmotic flow (EOF). The EOF is the overall movement of the sample from the anode to the cathode.

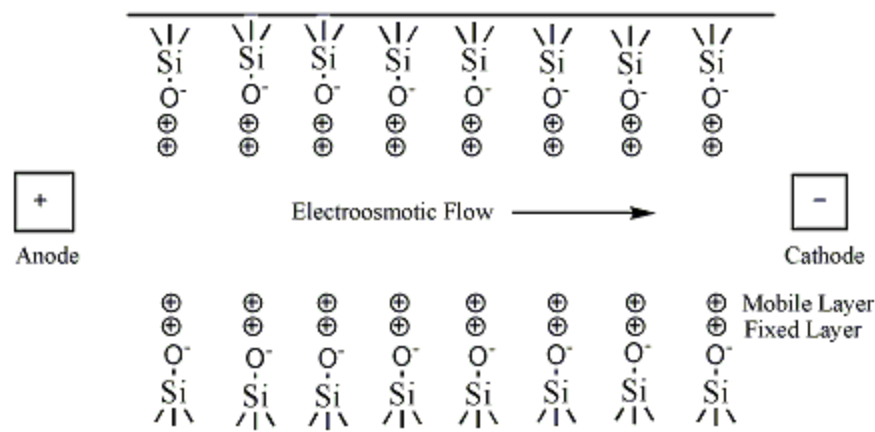


Figure 6.3: SiOH groups being ionized and creating the electroosmotic flow inside of the capillary.

The negative charge on the capillary wall leads to the formation of a double layer of cations along the wall. The inner layer of cations is tightly bound to the capillary wall (cations directly bound to the oxygens of the SiO^- group) and the outer layer is a diffuse layer of cations. A **zeta potential** forms at the boundary between the inner and outer layers of cations. When an electric field is applied, the cations in the diffuse layer move towards the cathode. The cations are more solvated than the anions and pull the bulk solvent towards the cathode. This movement defines the EOF. (Figure 6.4). The relative mobilities of the particles are the same, but now neutral molecules and negative particles are pulled toward the cathode by the EOF.

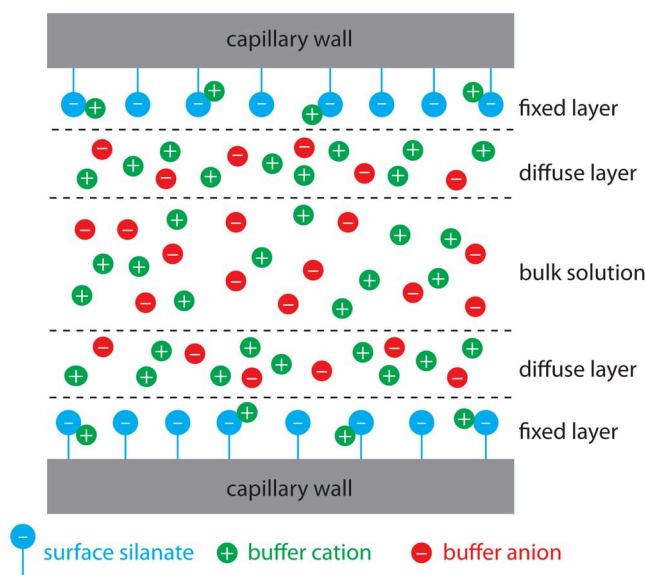


Figure 6.4: Schematic diagram showing the origin of the double layer within a capillary tube. Although the net charge within the capillary is zero, the distribution of charge is not. The walls of the capillary have an excess of negative charge, which decreases across the fixed layer and the diffuse layer, reaching a value of zero in bulk solution.

While the EOF is affecting all of the components in the sample, the cations have a faster velocity because they are attracted to the cathode, anions are unattracted to the cathode, and neutral molecules are neither attracted or unattracted. Neutral molecules will not be separated from one another. Negatively charged particles will be separated because the electrophoretic mobility counters the EOF. A solute's total velocity (v_{tot}) as it moves through the capillary is the sum of its electrophoretic velocity and the electroosmotic flow velocity. This concept is demonstrated in Figure 6.5. Cations elute first in an order corresponding to their electrophoretic mobilities, with small, highly charged cations eluting before larger cations of lower charge. Neutral species elute as a single band with an elution rate equal to the electroosmotic flow velocity. Finally, anions are the last components to elute, with smaller, highly charged anions having the longest elution time.

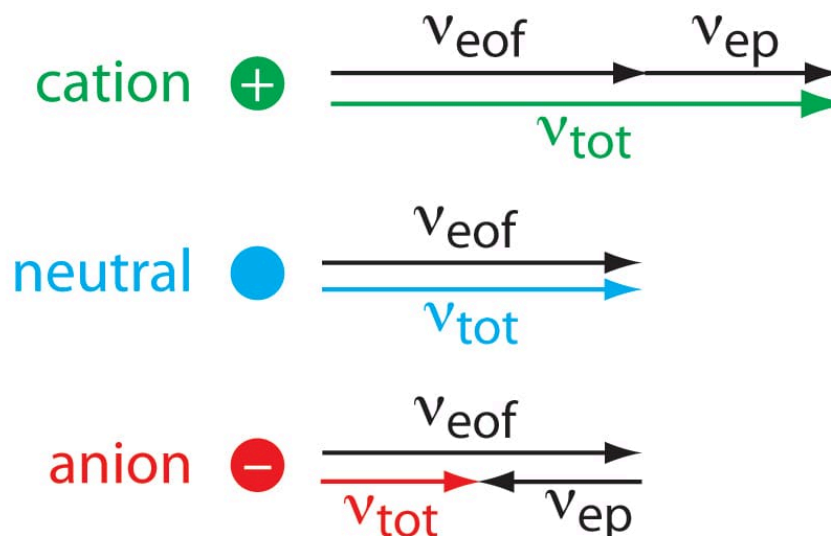


Figure 6.5: Electroosmotic and Electrophoretic flow. A visual explanation for the general elution order in capillary electrophoresis. Each species has the same electroosmotic flow. Cations elute first because they have a positive electrophoretic velocity, v_e . Anions elute last because their negative electrophoretic velocity partially offsets the electroosmotic flow velocity. Neutrals elute with a velocity equal to the electroosmotic flow.

Figure 6.5 can also be described using the following equations:

$$v_{tot} = v_{ep} + v_{eof} \quad (6.5.4)$$

$$(v_{tot})_{cations} > v_{eof} \quad (6.5.5)$$

$$(v_{tot})_{neutrals} = v_{eof} \quad (6.5.6)$$

$$(v_{tot})_{anions} < v_{eof} \quad (6.5.7)$$

The EOF is extremely useful for separating molecules with both positive and negative charges, but the EOF is not necessary. The EOF can be abolished by changing the buffer conditions. Using running buffer at very low pH will abolish the EOF. If low pH is a problem for the stability of the samples, the inside of the capillary can be coated with an uncharged layer. Low concentrations of ionic detergent, below the critical micelle concentration, will also diminish the EOF. There is no separation of molecules with similar charge to mass ratios. It is frequently desirable to improve or alter the separation. Molecules with similar electrophoretic mobilities can be separated by the addition of carrier compounds to the running buffer.

Micellar Electrokinetic Chromatography

The addition of a new species to the running buffer will separate the molecules in the initial sample. This separation can occur based on the molecules' affinities for the added species. For example, uncharged, nonpolar molecules can attract to neutral species, while charged species can attract to added ionic species. There are several different ways to perform this experiment based on the properties of the added species. The addition of cyclodextrins (or micelles) to the running buffer allows the separation of chiral species. This is described as MEKC, or Micellar Electrokinetic Chromatography. This technique can separate compounds (which had similar mobilities in CZE experiments) due to the difference in affinities of the sample molecules for detergent micelles.

Neutral species will partition between the running buffer and the hydrophobic interior of the micelles. The micelles, which are negatively charged, have a retention time greater than the EOF. Thus, as molecules enter the micelles they are slowed down. The stronger an affinity the neutral species has for the micelle, the longer its retention time. The more nonpolar neutral species have the highest affinity for the micelles. Charged particles that have hydrophobic groups will also be retained by interaction with the hydrophobic core of the micelle. Highly positively charged particles will interact with the surface of the micelle and also be retained. These interactions can be seen in Figure 6.6. You can see that the separation of the species in the mixture will be changed by the addition of detergent to the running buffer. You can tailor your separation to exactly suit your needs by

experimenting with different additions to the running buffer.

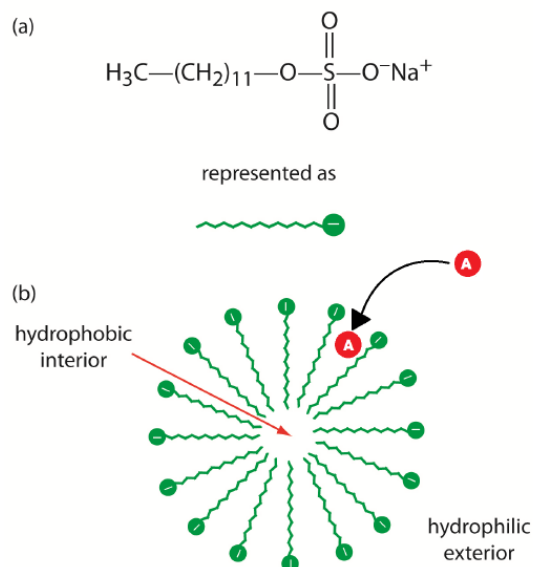


Figure 6.6: Micellar Interactions within the Capillary. (a) Structure of sodium dodecylsulfate and its representation, and (b) cross-section through a micelle showing its hydrophobic interior and its hydrophilic exterior. The cross-section shows the migration of neutral compound “A” into the hydrophobic interior. The v_{ep} of “A” can be retarded due to the partitioning into the micelle.

Instrumentation

In this experiment, you will repeat the analysis you did (or will do) in the [HPLC experiment](#). You will compare two different modes, [Capillary Zone Electrophoresis \(CZE\)](#) and [Micellar Electrokinetic Capillary Chromatography \(MEKC\)](#), to achieve the same separation. Differences in resolution and retention times will be observed and explained. Differences, if any, between the results obtained by HPLC and CE will also be addressed. The Instrument used can be seen in [Figure 6.7](#)

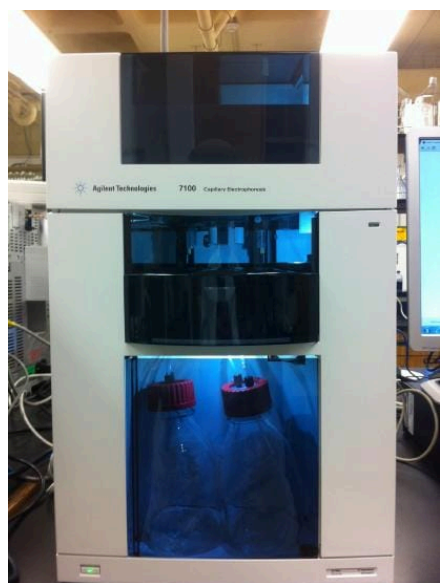


Figure 6.7: Agilent Technologies 7100 Capillary Electrophoresis, UCD Capillary Electrophoresis instrument. Note that multiple vials can be probed sequentially if required. This instrument is located in room 3475.

Experimental Procedure

Part 1: Preparing the Required Solutions

1. The solutions from Lab 2: High-Pressure Liquid Chromatography (HPLC) will be used. If you have already performed that lab, gather your samples. If your group has not performed Lab 2, refer to Part 3: Analysis of Caffeine Beverages in the Procedure section of Lab 2: HPLC.
2. Check the counter with the buffer solutions and identify two buffers that have already been prepared. The buffers are:
 - a. Solution A: 0.05 M borate buffer, pH=9.0.
 - b. Solution B: 0.05M Sodium dodecyl sulfate (SDS), 0.05 M borate buffer, pH=9.0.
3. Filter the stock caffeine solutions using the provided filter and syringe. Add approximately 10 mL into a labeled CE vial. **DO NOT fill the vials more than 75%.**
 - a. The filter and syringe can be reused if the solutions are filtered from low concentration to high concentration.
 - b. The syringes can be found in the plastic drawers near the door of the lab.
 - c. Filter approximately 1 mL and dispose of it into a waste beaker to wash the filter.
 - d. Filter the solution into the appropriate vial. (Figure 6.8) **DO NOT** put tape on the vials; ask your TA for a sharpie to write directly on the vials.
4. Filter the beverage samples into labeled vials using the same technique described in step 3.
5. Fill a labeled CE vial with filtered Solution A buffer and another labeled vial with filtered solution B buffer each about 75% full.



Figure 6.8: Appearance of HPLC/CE vials. The HPLC can also use plastic vials that look quite similar. Image is taken from www.thermofisher.com

****DO NOT dispose of these samples if Lab 2: HPLC has NOT been performed; after completing Lab 2 AND Lab 6, the solutions can be disposed of.****

Part 2: Running a Capillary Zone Electrophoresis Experiment

1. Turn on the instrument (Agilent Technologies 7100 Capillary Electrophoresis Instrument), if it is not already on, by pressing the power button at the bottom left of the front face of the CE. The light in the middle of the button should be green when it's on.
2. Open the *Chemstation* software if it's not already on by double-clicking the "Instrument 2 Online" icon with an image of the CE.

You will start in "Instrument View."

3. The instrument must first be initialized.

- a. Click the "On" button next to the question mark button on the bottom right of the "CE" window.
- b. Click on the power button icon in the top left corner of the "DAD" (diode array detector) window to "make device ready." Wait until the system goes to "Ready." This may take some time if the temperature-controlled zones need to stabilize.

4. Wait until the software goes to "Ready."

- a. In the "CE" window, right-click "Inlet" and unload by selecting "Unload Inlet Lifter."
- b. Do the same for the "Outlet." You should then hear the instrument lowering the vials in positions 1 and 2.
- c. Right-click the sample wheel and select "Get Vial."
- d. "Vial" should be set to "1" - click "Get."
- e. The tray will rotate vial 1 to the front of the instrument.
- f. Open the tray door and remove vials 1 and 2, if they are present.

5. Place the prepared 75% full Solution A vial in space number 1.

6. Fill a second vial 50 % full of Solution A and label this "Buffer A outlet." Place this Vial in space number 2 in the sample wheel.

7. Close the tray door. The tray will rotate back to the operating position.

8. Make sure to use the features on the screen to insert Vial 1 as the inlet and Vial 2 as the outlet.

9. Right click on the regulator pressure icon in the "CE" window and click "Flush" on the menu. Enter a time of "600" seconds and click "OK". The pressure should increase to about 930 mBar and slowly decrease as liquid is pushed from one vial to the other during the flush time. This will wash out any residues from the previous runs and equilibrate the capillary with the running buffer.

10. Make sure that the method is "CAFFEINE.M" and that the sequence is "CAFFEINE.S."

- a. The method and sequence names can be found in the drop-down boxes in the main menu bar.
- b. Check and make sure the CZE method is using 20 kV by selecting "Method" then "Edit Entire Method". Check with your TA what the run time is for the current capillary.
- c. Press "OK" in the first two windows that appear until you get to the "Setup Method" window.
- d. Select the "CE" tab to check the voltage and run time under "Stop Time."
- e. Check that under "Injection," on the right side of the "CE" tab window, there is one row that under "Function" has "Apply Pressure" and under "Parameter" it says "50mbar for 5s (Inlet: Injection Vial Outlet: Outlet Home Vial)." Click "OK".
- f. "OK" through or cancel through the remaining windows.

11. Right click on the tray icon and select "Get Vial."

- a. Set the "Vial" number to 7 and click "Get."
- b. After the tray rotates to the correct position. open the tray door and put all your samples in.
- c. Start at position 4 (leaving position 3 empty) with the 0.01 g/L standard followed by the rest of the standards in increasing concentration, then the three beverage samples. Make sure you keep track of the order in which the samples are run.
- d. Close the tray door.

12. Review the sequence parameters.

- a. On the menu bar, click "Sequence" and select "Sequence Parameters".
- b. Enter an operator name, make sure "Prefix/Counter" is checked, and enter a prefix for your datafile names.
- c. Click "OK."

13. Run the sequence by clicking the "Sequence" Play button above the "DAD Window."

14. After the sequence has started, watch the "Online Plot." You should see a peak for your first standard about 2-3 minutes into the run. The electrical current, which can be monitored in the "CE" window should be 15-16 microamperes.

15. You can print the reports after each sample is run.

- a. When the entire sequence has completed, click "Data Analysis" at the bottom left of the window.
- b. Click on "Chem_115" in the data tree and select your sequence.
- c. Select the data you want and print.
- d. Repeat for all your other data files.
- e. A prompt to save each trial as a PDF will appear after each trial.
- f. Create a file and save the trials to the file.
- g. Print the PDFs.

16. When the sequence is complete, remove the inlet and outlet.

- a. In the "CE" window, right-click "Inlet" and unload by selecting "Unload Inlet Lifter."
- b. Do the same for the "Outlet." You should then hear the instrument lowering the vials in positions 1 and 2.

IMPORTANT

Make sure that you get a peak for each standard. You can always abort the run and restart it if there is a problem.

Part 3: Running a Micellar Electrokinetic Capillary Chromatography Experiment

1. Wait until the software goes to "Ready."

- a. In the "CE" window, right-click "Inlet" and unload by selecting "Unload Inlet Lifter."
- b. Do the same for the "Outlet." You should then hear the instrument lowering the vials in positions 1 and 2.
- c. Right-click the sample wheel and select "Get Vial."
- d. "Vial" should be set to "1" - click "Get."
- e. The tray will rotate vial 1 to the front of the instrument.
- f. Open the tray door and remove vials 1 and 2 from Part 2 of the experiment.
- g. Place the prepared 75% full Solution B vial in space number 1.
- h. Fill a second vial 50 % full of Solution B and label this "Buffer B outlet." Place this Vial in the space number 2 in the

sample wheel.

- i. Close the tray door. The tray will rotate back to the operating position.
- 2.** Make sure to use the features on the screen to insert Vial 1 as the inlet and Vial 2 as the outlet.
- 3.** Right click on the regulator pressure icon in the "CE" window and click "Flush" on the menu. Enter a time of "300" seconds and click "OK."
- 4.** Make sure that the method is "CAFFEINE_MEKC.M" and that the sequence is "CAFFEINE_MEKC.S."
 - a. The method and sequence names can be found in the drop-down boxes in the main menu bar.
 - b. Check and make sure the MEKC method is using 15 kV by selecting "Method" then "Edit Entire Method". Check with your TA what the run time is for the current capillary.
 - c. Press "OK" in the first two windows that appear until you get to the "Setup Method" window.
 - d. Select the "CE" tab to check the voltage and run time under "Stop Time."
- 5.** The samples should already be in the correct position after the CZE experiment.
- 6.** Run the sequence by clicking the "Sequence" Play button above the "DAD Window."
- 7.** You can print the reports after each sample is run.
 - a. When the entire sequence has completed, click "Data Analysis" at the bottom left of the window.
 - b. Click on "Chem_115" in the data tree and select your sequence.
 - c. Select the data you want and print.
 - d. Repeat for all your other data files.
 - e. A prompt to save each trial as a PDF will appear after each trial.
 - f. Create a file and save the trials to the file.
 - g. Print the PDFs.
- 8.** When the sequence is complete, remove the inlet and outlet.
 - a. In the "CE" window, right-click "Inlet" and unload by selecting "Unload Inlet Lifter."
 - b. Do the same for the "Outlet." You should then hear the instrument lowering the vials in positions 1 and 2.
 - c. Right-click the sample wheel and select "Get Vial."
 - d. "Vial" should be set to approximate "4" - click "Get." (This will ensure that you can remove all of the vials from the CE)
 - e. The tray will rotate vial 4 to the front of the instrument.
 - f. Open the tray door and remove all of your vials.
 - g. Close the tray door.

Treatment of Data

- 1.** Before completing the lab, confirm that you have peaks for each of your runs. If you notice any issues with your data, talk with

your TA.

2. Create a calibration curve using the caffeine standard peak height or area versus the concentration. This can be done in *Chemstation* in the lab or at home in excel/google sheets.

- a. To create a calibration curve in *Chemstation*, start by clicking the “data analysis” tab in the bottom left corner of the window. This will open an offline window.
- b. Go to the CHE 115 file and find your data folder and select it.
- c. Double click the first standard run in the sequence window.
- d. Find the “Calibration” tab in the menu bar and select “New Calibration Table.”
- e. A new window “Calibrate: HPLC1” will appear and select “Automatic setup.”
- f. Set the “level” to 1 and put the concentration of the first run in the “Default Amount.” Press “OK.”
- g. Double click the second run and go to the “Calibration” menu bar and click “Add Level.”
- h. Set the “Level” to 2 and enter the second run’s concentration in the “Default Amount.” Press “OK.”
- i. Repeat steps g and h for each of the remaining standard solutions.
- j. The Calibration Table and the Calibration Curve can be viewed in the bottom of the window. Confirm that all the points are there.

3. If you cannot make a calibration curve in *Chemstation* or the curve did not use all of the points, use the data to develop a calibration curve in Excel or Google Sheets

Post-Lab Questions

****Include ALL Data Reports and Calibration Tables****

1. Explain the difference in retention time for the two different experiments.
2. What would you expect to happen to the retention time of the caffeine peak if you decreased the run voltage for the first experiment to 10 kV?
3. Did you get the same answer for the two different CE experiments? Explain.
4. Did you get the same answer as you did for the HPLC experiment? Is this surprising? If the answers are different, suggest some possible explanations. If you have not completed HPLC, you do not need to answer this question.

References

1. Copper, C. L. Capillary Electrophoresis Part I. Theoretical and Experimental Background. *J. Chem. Ed.* **1998**, 75, 343-347. [pdf](#)
2. Copper, C. L.; Whitaker, K. W. Capillary Electrophoresis Part II. Applications. *J. Chem. Ed.* **1998**, 75, 347-351. [pdf](#)
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6.6: Electrospray Mass Spectrometry

Goals

1. Students should gain familiarity with the operation of the instrument.
2. Students should observe the multiple ionization states of a complex molecule.
3. Students should be able to determine the optimal conditions to produce the best mass spectrum.
4. The students should understand how to pick peaks and determine the charge and molecular weight of a complex compound.

In this experiment, you will learn the mechanics of [electrospray mass spectrometry](#) and use this technique to study the effect of different solvents on the three-dimensional structure of proteins.

Introduction

Every mass spectrometer has three essential parts, an ionization source, a [mass analyzer](#), and a detector. There are many ionization techniques. In this class, you will encounter two different ionization methods. In the GC/MS experiment, the molecules of interest were ionized by electron impact. Not only were the molecules ionized they were fragmented into smaller pieces. The pattern of ionized fragments produced is characteristic of the compound and allows one to identify compounds by the mass spectrum produced. This is called a hard ionization technique; in contrast, electrospray ionization is a soft ionization technique. The solution containing the compound of interest is sprayed through a nebulizer to create a fine mist of droplets. The droplets are charged by the large potential difference between the end of the nebulizer needle and the entrance of the capillary (Figure 1). The droplets are dried by the drying gas inside the capillary. As the droplets shrink, the electrostatic repulsion between the charged particles causes some of them to enter the gas phase. The gas-phase ions are drawn through the capillary by the high vacuum in the analyzer. The molecules in solution are ionized but not fragmented. Each molecule in the solution can have a different number of charges attached. This creates a mass spectrum of differently charged particles.

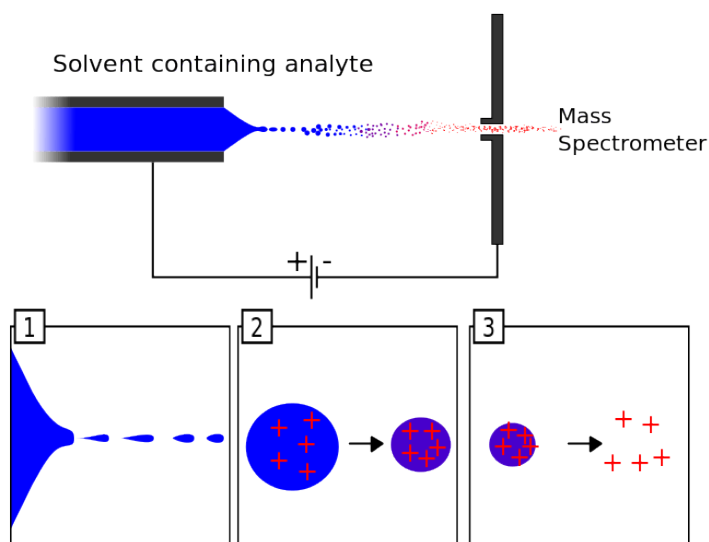


Figure 7.1: Diagram of Electrospray Ionization. (1) Under high voltage, the "Taylor Cone" emits a jet of liquid drops (2) The solvent from the droplets progressively evaporates, leaving them more and more charged (3) When the charge exceeds the Rayleigh limit the droplet explosively dissociates, leaving a stream of charged ions. (CC-SA-BY 3.0; [Evan Mason](#))

In this instrument, the mass analyzer is a modified quadrupole. The quadrupole detector comprises four rods, two positively charged and two negatively charged, maintained by a variable DC potential difference (Figure 2). A variable AC potential also exists between the two rods. The mass of ions that pass through the quadrupole is determined by the strengths of the applied

fields. During a scan, the voltages are ramped up but the ratio of the ac and dc fields is held constant. See section 11B-2 in Skoog for a detailed description. Scans are very quick, on the order of 100 ms. Because of the speed of data acquisition, the quadrupole detector is ideal for the analysis of HPLC output. Once the ions make it through the quadrupole they must be counted by a detector. The detector in your system is a HED (high energy dynode) detector, which is a modified electron multiplier that is more sensitive at higher masses.

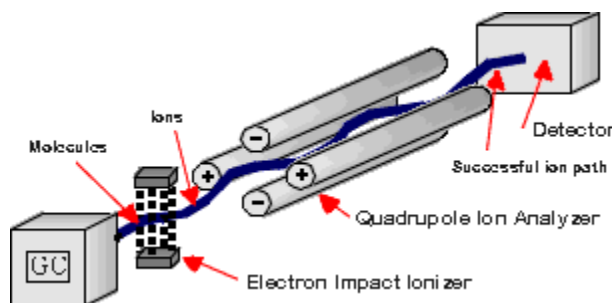


Figure 7.2. Quadrupole Ion selector

The instrument is extremely flexible and can be run in either positive or negative ion selection mode. It can also be run in scan mode where a wide range of masses is detected or it can be used in selected ion mode where only a single ion is detected.

The technique is used primarily for determining the molecular weights of proteins and components of multi-protein complexes. For proteins larger than 3000 daltons, no molecular ion can be observed in this mass spectrometer. A series of ions is produced from each protein molecule. The pattern of peaks is called the charge state envelope. The molecular weight of the protein can be determined based on the distribution of ions in the envelope. The first step is to determine which ions are related. Usually this involves selecting the largest peaks in the mass spectrum. Remember that you are detecting protein molecules to which additional H^+ atoms are attached. The m/z of a particular peak is:

$$\frac{m}{Z} = \frac{M + nH^+}{n} \quad (6.6.1)$$

where M is the mass of the uncharged protein and n is the number of additional protons attached and H^+ is the mass of a single proton. Using several peaks in the charge state envelope you can determine the molecular weight of the protein.

Electrospray ionization is also used to study protein denaturation. When a protein is folded into a compact globular structure many of the ionizable side chains are buried in the core of the protein and are not accessible to solvent. So, they will not be ionized. As the protein unfolds the buried acidic or basic side chains are exposed to solvent and protonated or deprotonated. Thus, the more peaks at high m/z the more unfolded the protein.

Example

When determining the molar mass of a compound, it requires using two adjacent peaks seen in the mass spectrum. Usually, the tallest peak and its neighbor are the best peaks to select. When performing the calculation, the following two equations represent the two adjacent peaks:

$$X = \frac{M + z}{z} \quad (6.6.2)$$

$$Y = \frac{M + z + 1}{z + 1} \quad (6.6.3)$$

The X equation represents the peak with the larger m/z value. This peak is not as ionized as the Y equation which is the peak that has the smaller m/z value. Equations 7.2 and 7.3 can be combined to find the z value or the charge of the molecule (Equation 7.4).

$$z = \frac{Y - 1}{X - Y} \quad (6.6.4)$$

This z value can then be used to solve for the molecule weight. By performing a simple rearrangement of equation 7.2, the molecular weight can be solved for. The rearranged equation can be seen as equation 7.5.

$$M = z(X - 1) \quad (6.6.5)$$

Solution

In the following example, the charge and the molecular weight will be calculated for the following molecule.

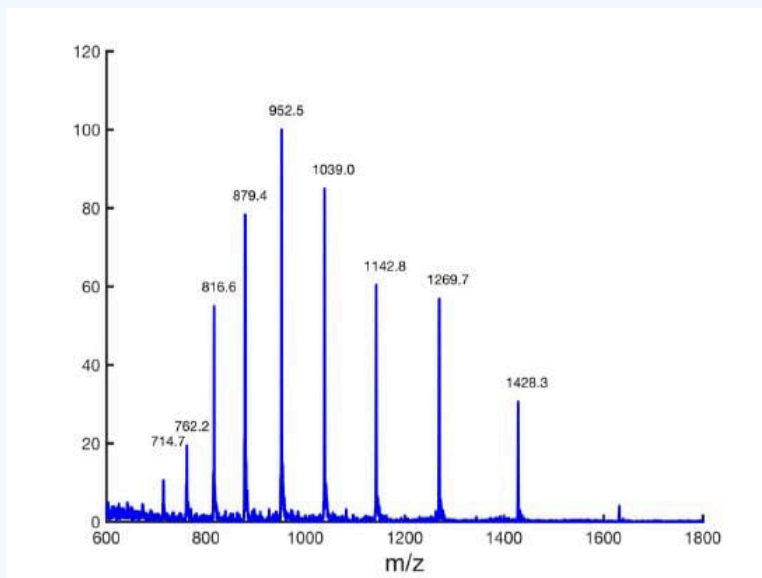


Figure 7.3: Example of a Mass Spectrum.

The first task will be to start by picking two adjacent peaks. The tallest peak is 952.5 m/z, so this will be the Y peak. The X peak will be the adjacent peak with the larger m/z value. In this example, the 1039 m/z peak will be used. These values will be plugged into equation 6.6.4) to determine the charge.

$$z = \frac{952.5 - 1}{1039 - 952.5} \quad (6.6.6)$$

After plugging in these values, this equation results in a z value of +11. This z value can be used to determine the molecular weight by using equation 6.6.5).

$$M = 11(1039 - 1) \quad (6.6.7)$$

After completing this calculation, the molecular weight is calculated to be 11,418 amu. This value is an approximation, but after finding out that the molecule is putidaredoxin which has an actual theoretical molecular weight of 11,419 amu, it shows that this method could approximate the mass very closely.

Principles of protein structure

Proteins are polymers of 20 amino acids. The amino acids can be divided into groups based on their chemical properties. The hydrophobic or non-polar amino acids are leucine, isoleucine, valine, alanine, phenylalanine, methionine, proline and tryptophan; the polar, uncharged residues are glycine, serine, threonine, tyrosine, glutamine, asparagine and cysteine. The most important for mass spectrometry are the charged amino acids, glutamate and aspartate are the acidic side chains, lysine, arginine, and histidine are the basic side chains. The structures for the 20 side chains are shown in Figure 3. Note that the figure contains a few errors: under the positively charged side groups heading, the Asparagine amino acid listed should actually be Arginine (Arg, R) and under the polar, uncharged side groups heading, the Aspartate amino acid listed should actually be Asparagine (Asn, N). Also, Proline should be listed in the nonpolar, aliphatic side groups section as it is hydrophobic.

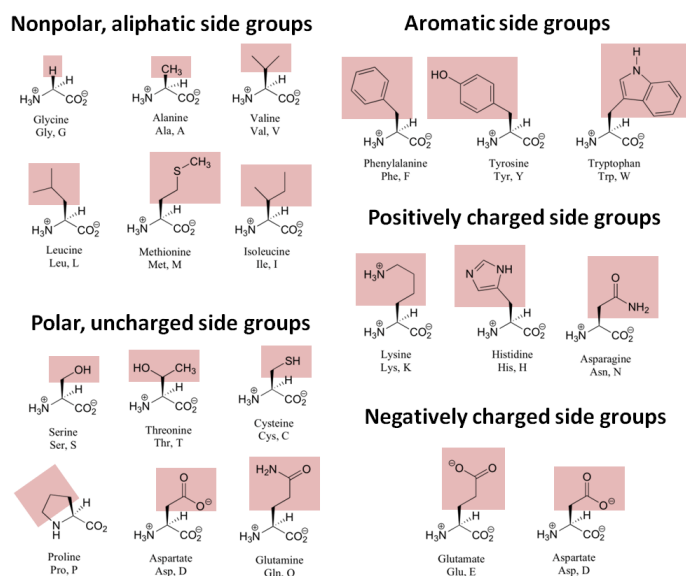


Figure 7.4: The structures of the 20 naturally occurring amino acids.

At pHs above the pK_a for the acidic side chains, those side chains will carry a negative charge. The basic side chains are positively charged at pH less than the pK_a . The pH of the solution in which your protein is prepared will determine how the proteins are studied by mass spectrometry. At low p,H the proteins will carry a net positive charge and at higher pHs, the charge will go through neutral and become positive as the pH increases. The pK_a 's of the amino acids are shown in table 1.

Table 1: pK_a Values for the 20 naturally occurring Amino Acids

Amino Acid	α -carboxylic acid	α -amino	Side chain
Alanine	2.35	9.87	
Arginine	2.01	9.04	12.48
Asparagine	2.02	8.80	
Aspartic Acid	2.10	9.82	3.86
Cysteine	2.05	10.25	8.00
Glutamic Acid	2.10	9.47	4.07
Glutamine	2.17	9.13	
Glycine	2.35	9.78	
Histidine	1.77	9.18	6.10
Isoleucine	2.32	9.76	
Leucine	2.33	9.74	
Lysine	2.18	8.95	10.53
Methionine	2.28	9.21	
Phenylalanine	2.58	9.24	
Proline	2.00	10.60	
Serine	2.21	9.15	
Threonine	2.09	9.10	
Tryptophan	2.38	9.39	
Tyrosine	2.20	9.11	10.07

Amino Acid	α -carboxylic acid	α -amino	Side chain
Valine	2.29	9.72	

The forces that stabilize the three dimensional or tertiary structures of proteins are hydrophobic interactions between the non-polar side chains, hydrogen bonds between carbonyl oxygens and amide nitrogens, electrostatic interactions, usually on the surface of the protein, between charged side chains. Denaturation occurs when the tertiary structure of the protein unfolds (imagine scrunching up a rubber band and then letting go of it.) As the tertiary interactions unfold, so do the secondary structure elements, the alpha helices, and the beta sheets. The pH of the solvent will affect not only the charge of the ionizable side chains it will also destabilize the three-dimensional structure of the protein by disrupting charge-charge and electrostatic interaction that stabilize the structure. Organic solvents also destabilize the structure by interfering with the hydrophobic interactions between nonpolar side chains. Hydrophobic interaction is a term used to describe the phenomenon that nonpolar groups prefer to be in a nonpolar environment. In aqueous solution, the nonpolar side chains will "stick together" to decrease the surface area in contact with the solvent.

Denaturants that are frequently used include, heat, urea, salt, pH and organic solvents. The effect of these denaturants on protein stability give insight into the type of interactions that are more important in stabilizing the tertiary structure.

Experimental Procedure

In this experiment, you will acquire mass spectra of the protein ubiquitin under several different solvent conditions.

- First, you will investigate the effect of pH on protein stability by looking at the mass spectrum at different pH values.
- Make 100 mLs each of the following 5 solutions: Water and acetic acid at pH=7, 2.75, 2.5, 2.1, and 1.8.
- You will be given a 10^{-3} M solution of ubiquitin in water. Prepare 1mL dilutions of 10^{-6} M ubiquitin by diluting the stock ubiquitin in each of the solutions.

HINT: Glacial acetic acid is 100% and the molarity is 17.4. The K_a for acetic acid is 1.76×10^{-5} . Also, remember the [ICE table](#) approach. Use this information to calculate what percentage of acetic acid solutions you need to make. Do this before you come to the lab.

You will also investigate the effect of organic solvents on the stability of ubiquitin.

Make 10 mLs each of the following 4 solutions:

- 20% acetonitrile + 80% pH=2.75 acetic acid and water
- 5% acetonitrile + 95% pH=2.75 acetic acid and water
- 20% methanol + 80% pH=2.75 acetic acid and water
- 40% methanol + 60% pH=2.75 acetic acid and water.

Use the same stock solution of ubiquitin to prepare 1 mL of 10^{-6} M ubiquitin by diluting the ubiquitin in each of the solutions.

Instrumentation

(For a 2013 update go to <http://chemwiki.ucdavis.edu/User:labgod4>)

The instrument you are using is a Varian 325-MS with a Primeline LC Pump (instrument picture below is not the MS-325-TQ).

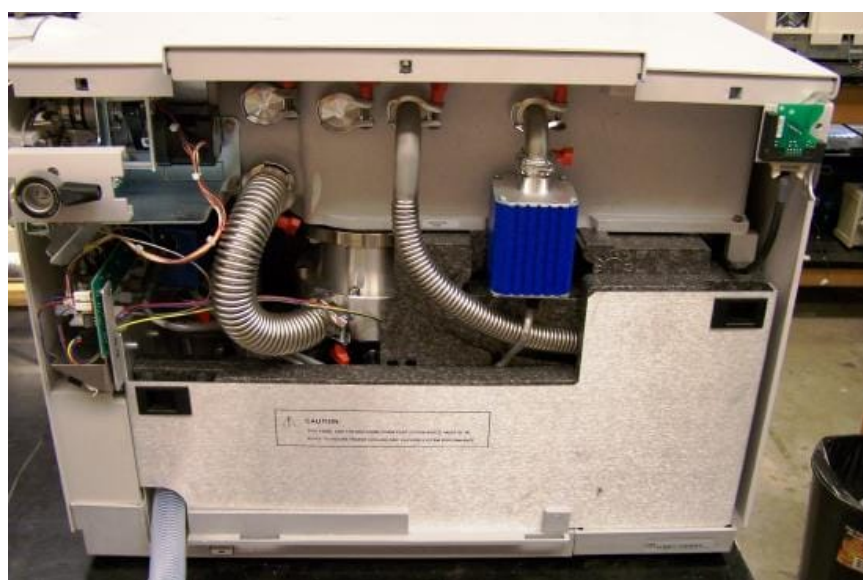
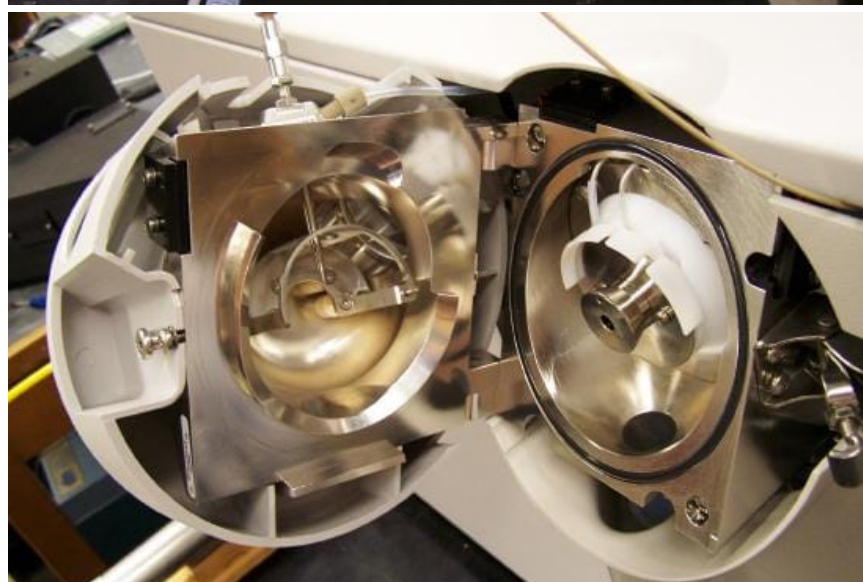


Figure 7.5: Mass Spectrometer in Chem 115. The ESI needle in the middle image.

First, make sure that the solvent reservoirs are sufficiently filled with HPLC grade solvent. Open **System Control**, if it is not already open, by clicking on the top and most left icon (Figure 7.6) on the toolbar at the top of the monitor. This opens up the **Quad View** (Figure 7.7). Make sure you are using view **III** (Figure 7.8) so that the proper quadrants are viewable. Make sure the correct method is loaded. This software should look familiar because it is similar to that used for the HPLC experiment. The desired method for running the ubiquitin samples is '115 50-50 posneg 4 min'. It is a 4-minute method. Changing methods is done by activating the desired method under the file tab of **System Control**.

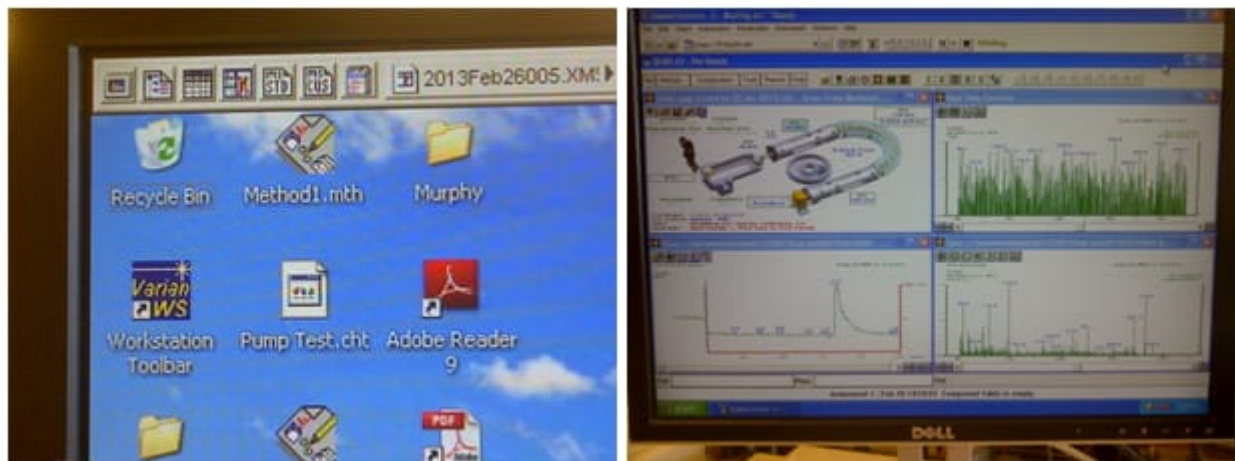


Figure 7.6 **Figure 7.7**

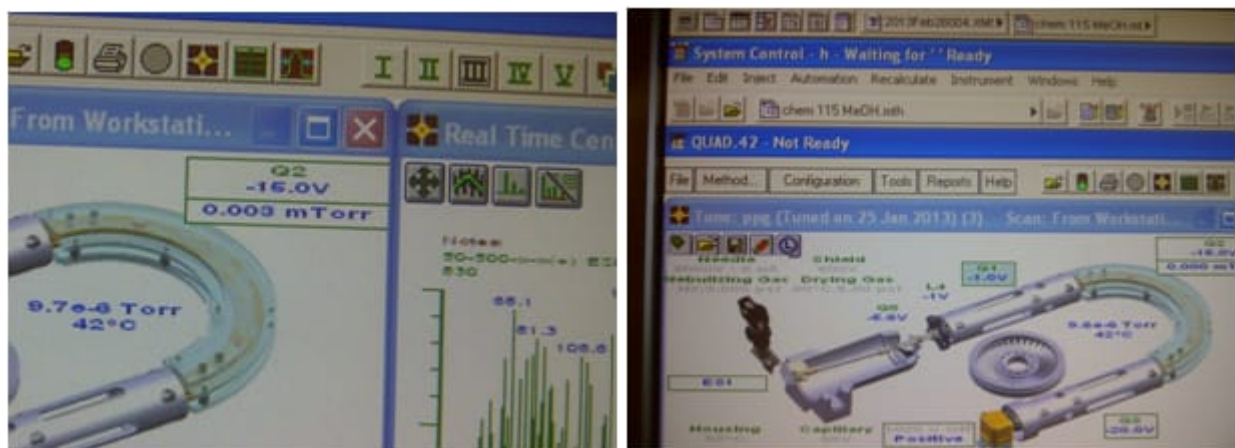


Figure 7.8 **Figure 7.9**

Click **Turn Spray On** in the Quad view (Figure 7.10) to turn on the gases and heaters. Once the temperature reaches 75% of the set value (275-300°C) activate the pumps under the Windows tab under **System Control**, click on 212.24, then "Activate." The spray icon now has a red stream coming from it (Figure 7.11), and the pump lights have turned green.

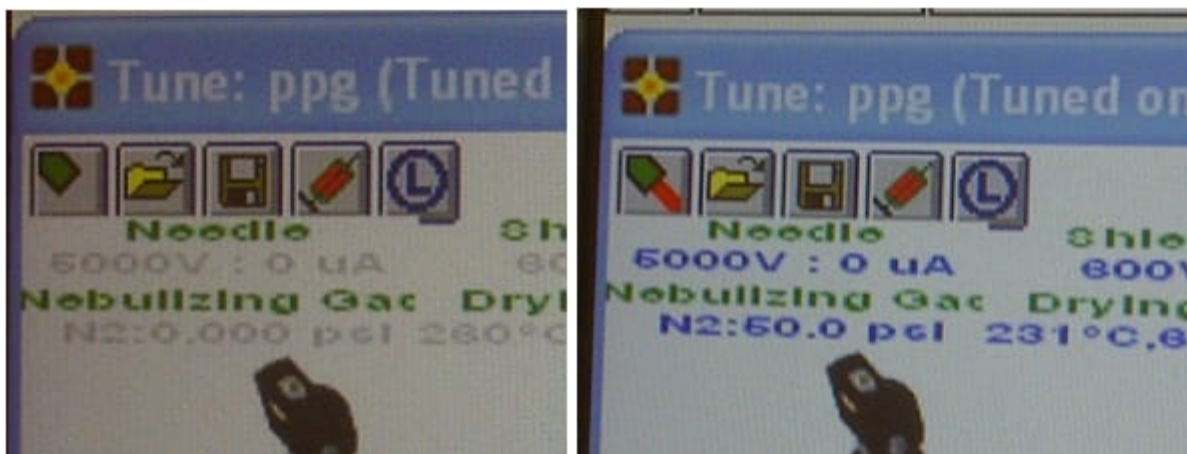


Figure 7.10 Figure 7.11

A few minutes is required for the temperature to stabilize, flush out the sample loop during this time. Add ~10 μL of water into the sample loop in the INJECT position, twice. When you are ready to load your sample, make sure the valve is in the LOAD position. Dilute the first sample (pH 7) 10x to check concentration, and inject around 10. Then, under the Windows tab in **System Control**, click on the Sample List, click Data Files, and change the sample name. Once the API and 325 indicate they are ready (Figure 7.12), it is time to turn on the analyzer. The button turns green when it has been activated (Figure 13).

Now toggle back to the 212.24 LC pump window and click **Start Run** on the pumps. This starts the run and the green analysis timer begins in the area to the right of '325 MS: ready to collect' seen in Figure 7.10. Push the valve button to INJECT your first sample into the LCMS after the run has gone for 1 min. After each run, make sure the instrument is in the "Ready" and "Ready to collect" mode.

To print your chromatograms and mass spectra, click on your file name above **System Control** at the top of the monitor (Figure 7.14) which opens the window that appears in Figure 7.15 and select: View/edit chromatogram. Click on the desired peak and the mass spec data pops up below (Figure 7.16). Use the arrow keys to locate the highest RIC for the peak of interest. Right click on the mouse to label the peak as desired by choosing **New Label for Plot 1** (Figure 7.17). Hover the mouse of the label; when the arrow turns to a 'hand' the label and its indicator can be dragged to their desired locations. Print, using the printer icon, choose active chromatogram and spectrum. Then click on the short stack printer icon to send the data to the printer. Each click prints one copy.

Repeat this for all samples, starting with the flushing of the sample loop.

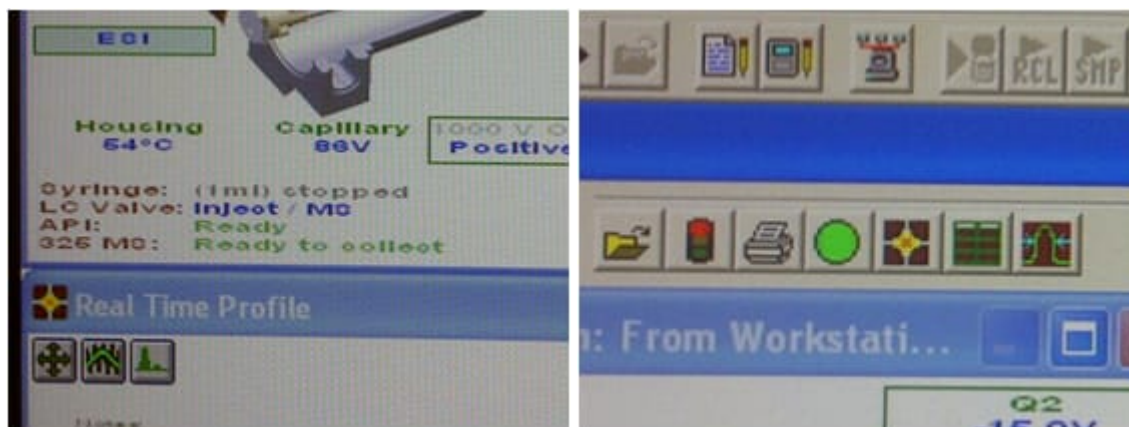


Figure 7.12 Figure 7.13

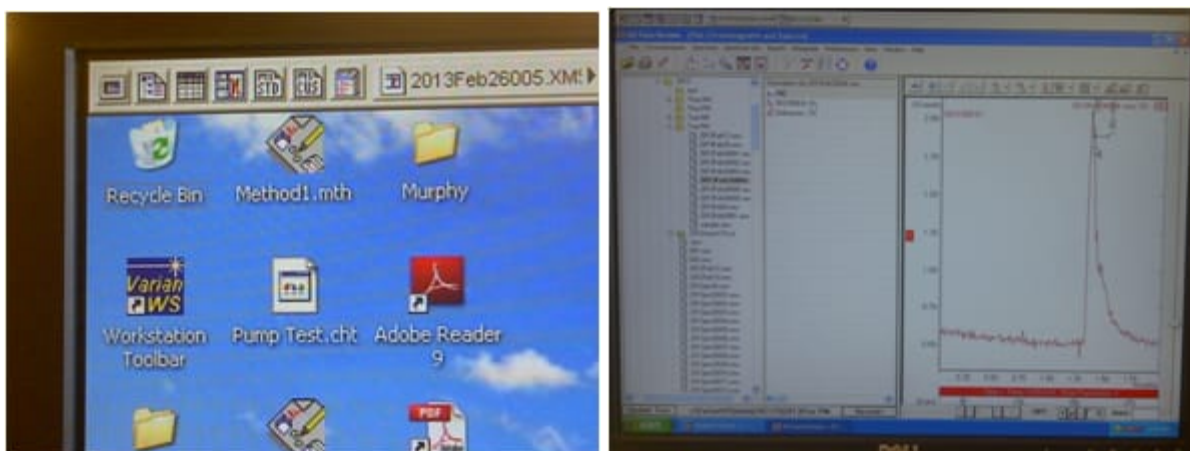


Figure 7.14 Figure 7.15

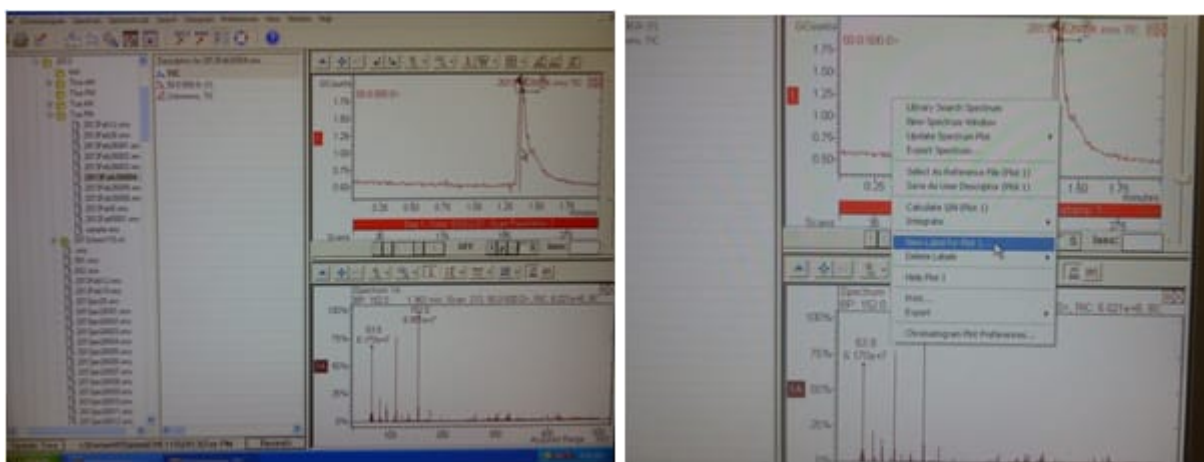


Figure 7.16 Figure 7.17

Shutdown Protocol: Once the analysis is complete, **turn off the spray** by clicking the icon in *Figure 7.11*. The icon now reverts back to how it appears in *Figure 7.10*. Verify that the spray has become a constant drip in the API window. Allow the drip to continue for a few minutes. Toggle back to the **Quad View** to verify that the **Spray is off** and appears as it does in *Figure 7.10* (no red coming out). If it has not been dripping, allow the drip to continue to flush out the needle and chamber for a few minutes. Using the Windows tab in **System Control**, toggle to the 212.24 window and click **Stop Pumps**. It is now safe to close out **System Control** and exit as normal. Make sure to log off to restrict the access to the instrument.

Post-Lab Questions

1. Print the chromatogram and the mass spectra for each "peak" in the chromatogram.
2. Using the pattern of peaks in your mass spectrum of the pH=2.75 sample calculate the molecular weight of ubiquitin. Show all your work.

Include the answers to the following questions in your report:

1. Why is the peak for the pH=7 sample so much smaller than the peaks for all the other conditions?
2. Discuss the differences in the distribution of ions for all the different conditions.
3. What happens to the 3-dimensional structure of the protein as the pH is decreased?
4. What happens as the concentration of the organic solvent is increased? Explain the differences you see with methanol and acetonitrile.

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2. Loo, J. A.; Ogorzalek-Loo, R. R.; Udseth, H. R.; Edmonds, C. G.; Smith, R. D. Solvent Induced Conformational Changes of Polypeptides Probed by Electrospray Ionization Mass Spectrometry. *Rapid Comm. in Mass Spec.* **1991**, *5*, 101.
3. Hofstadter, S. A.; Smith, R. D. Electrospray Ionization Mass Spectrometry. *J. Chem. Ed.* **1996**, *73*, A82.
4. For a 2013 Update go to <http://chemwiki.ucdavis.edu/User:labgod4>

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6.7: Vocabulary

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6.8: GC-MS Procedure and instructions for data analysis

In this experiment, you will extract residual compounds present on paper currency and quantitate the amount of cocaine on each bill using GC-MS.

Materials

- Paper currency: bring your own if possible - the money is not destroyed by the extraction process. We will provide bills if you do not have your own.
- Methanol, analytical grade
- 4 ppm solution of doxepin (Internal Standard)
- Serial dilution of cocaine including internal standard (this will be prepared for you)
- 15 mL centrifuge tube for each dollar bill extraction
- 3 mL syringe and 0.2 µm filter to filter dollar bill extracts
- Agilent CG-MS with Mass Hunter Software
- Two 10 mL beakers; one for syringe wash methanol, one for waste
- 10 µL syringe for injecting samples

Create a standard curve

The standard solutions contain 0.6 ppm doxepin internal standard and cocaine concentrations between 0.1-5 ppm. These solutions are prepared for you. Each analysis takes about 12 minutes.

How to use your time: You should start running the standards using the [operating instructions](#), then prepare your extraction samples while you are waiting between standard injections. After you have created your extraction sample, use the time between injections to compile and analyze your data using the [instructions for data processing in section 5.9](#).

With the software open and the correct method running (see [operating instructions in section 5.8](#)), inject at least four of the standards using **quantitative injection technique**; you can choose which standards to use based on your pre-lab reading.

- Methanol blank**
- 0.1 ppm cocaine** + 0.6 ppm doxepin in methanol
- 0.5 ppm cocaine** + 0.6 ppm doxepin in methanol
- 1.0 ppm cocaine** + 0.6 ppm doxepin in methanol
- 2.5 ppm cocaine** + 0.6 ppm doxepin in methanol
- 5.0 ppm cocaine** + 0.6 ppm doxepin in methanol

During any waiting time while samples are running, begin data analysis using the instructions in Section 5.8.

Ideal milestones for lab progress

Plan to use the entire scheduled time for this experiment. The following is an ideal timeline for progress in this 3-week module. However, real lab work does not always adhere to ideality. Instrument and sample issues are expected, and flexibility is deliberately built into the schedule. You should process the data as it is being collected so that issues are recognised immediately. If you expect a problem with the instrument or samples, stop immediately and notify your instructors.

Suggested timeline (ideal):

- **Week 1:** By the end of the first lab meeting, you have...
 - A complete calibration curve (methanol blank + four standards = 5 injections)

- One of the standards is run in triplicate to gauge the amount of variation between sample runs (2 additional injections)
- your extraction sample is run in triplicate (3 injections)

This is 10 injections at 12 minutes each, or about two hours of time *if you are efficient*. Use time between injections to work on data analysis. Before you leave lab on Day 1, be sure to collect and analyze your data. In other words, create your calibration curve as your data is being collected. If the calibration is going well, you may use any additional time to continue making replicate injections.

- **Week 2:** By the end of the second lab period, you have...
 - *At least* two replicates for each of the calibration points. If time allows, you should inject all of the standards three times total (ie two times on Day 2).
 - *At least* one additional injection of the sample. If time allows, inject twice.
 - Comparison of the data from each day to confirm it is consistent.
- **Week 3:** If things go smoothly on the first two days, this period can be used for writing. However, if you do run into issues or have not met the milestones above, then it is expected that the time in Week 3 is used for data collection.

Ideally, by the end of Day 2 in lab, you would have triplicate data points for each standard solution. However, you can decide when you are satisfied that your standard curve is appropriate to estimate the amount of cocaine present. If not, the third lab meeting should be used for additional data collection. Diagnose any issues with your standard curve using the data analysis methods outlined in Section 5.8.

Instructions for quantitative injection

It is important that the same volume be injected each time if the quantitative precision is to be as good as possible in this experiment. Please inject between 1-2 μL of sample; these instructions are for exactly two μL injections.

1. Wear gloves when handling the syringe.
2. Clean the syringe by drawing up and expelling into waste several full syringe volumes of solvent (the same solvent used in the sample solutions).
3. Empty and dry the syringe by pumping the empty syringe up and down several times.
4. Move the syringe plunger the the 2 μL mark to fill the syringe with a plug of air.
5. Draw up about 2 μL of sample solution by moving the plunger to the 4 μL . This is most easily done by drawing up excess solution and then pushing the plunger back to the goal mark.
6. Dab the tip of the syringe to remove excess sample. Then, pull the syringe plunger back so that you can see the air on both sides of the solution.
7. Measure the volume of solution. This is most easily done by pushing the plug of solution exactly to the zero calibration mark and measuring against the graduated markings.
8. Record the volume of solution to be injected.
9. Inject by pushing the plunger all the way to the zero mark (thus injecting the solution and the air plug). Then determine how much was actually injected.
 - a. Position the needle in the guide of the injection port. Pierce the rubber septum. Smoothly inject {t<1 second}; withdraw the syringe smoothly.
 - b. Draw back the syringe plunger and measure any remaining sample volume. **RECORD this value** and subtract it from the initial total volume. This result is the volume you actually injected. If necessary, use the volume injected to normalize the areas of all peaks for each run.

Preparing the analyte samples

You should prepare your sample for analysis while you are also running the series of standards on the GCMS.

1. Fold each bill accordion style, with about 12 folds. Insert the folded bill far into a 15 mL centrifuge tube.
2. Use a digital pipette to add exactly 5.00 mL of methanol into the tube with the folded bill.
3. Invert the tube for at least 10 minutes.
4. Using tweezers, carefully remove the bill from the tube. Try to leave as much of the methanol in the tube as possible.
5. In a 2 mL volumetric flask, transfer 1.000 mL of the extraction solution, then add enough internal standard solution (4 ppm

doxepin in methanol) to reach a final concentration of 0.6 ppm doxepin. Fill to the calibration mark with MS-grade methanol. (Note: this step dilutes your extraction solution and you should account for this in any calculations).

- Immediately seal the vial containing the extraction solution and put parafilm around the cap's seal. Then store this sample in case you need it again in the future.
- 6. Use a 3 mL syringe with a 0.2 μ m filter to filter the contents of the volumetric flask into a clean glass vial that can be sealed to prevent evaporation. Treat this solution as precious - do not throw it away.

Treatment of Data

The general criteria for writing and submitting lab reports are found in the [orientation module](#). Specific additional expectations for this module are given below.

1. Include the following results in the lab report: (assume general expectations apply, e.g. label plots and figures with titles, axes titles, sample calculations, etc)
 - I. Standards
 1. A good chromatogram of each standard sample (peaks labeled with r.t. and identity of each compound of interest). Ideally, this would be one figure with chromatograms stacked on the same X axis. Don't give us several pages of individual chromatograms, please!
 2. A mass spectrum of cocaine and doxepin (with labeled peaks).
 3. Results of the library search of one standard solution.
 - II. Calibration
 1. Calibration plot of C_A (cocaine concentration) vs $\frac{S_A}{S_{IS}}$ including a linear fit with y-intercept, slope, and errors.
 2. Derived relative response factor (K) and its associated error.
 - III. Quantitation
 1. How much cocaine is detected on each bill analyzed in units of $\frac{\mu g}{mL}$ (ppm) and $\frac{\mu g}{bill}$
 2. Include concise error analysis.
 - IV. Identification of other compounds on the bill
 1. What compounds, if any, were detected in analyte solutions that were not present in the standard solutions? Provide the results of the library search.
 2. Provide the Mass Spectra of notable compounds detected.
2. In your discussion, be sure to address points from the pre-lab assignment, and..
 - I. Discuss the relationship between boiling points, molecular structure, and retention time of the compounds.
 - II. Explain why doxepin appears as two peaks, and suggest how we might increase separation cocaine from doxepin.
 - III. Discuss the fragmentation pattern you observed and how it supports assignment of the compound. Address how molecular structure yield the observed mass ions.
 - IV. Discuss and interpret the results of the library search on your dollar bill

References

1. McLafferty, F. *Interpretation of Mass Spectra*; 3rd ed.; University Science Books: Mill Valley, CA, 1980.
2. Silverstein, R. M.; Bassler, G. C.; Morrill, T. C. *Spectrometric Interpretation of Organic Compounds*; 4th ed.; Wiley: New York, NY, 1981.
3. Skoog, D. A.; Holler, F. J.; Nieman, T. A. *Principles of Instrumental Analysis*, Fifth Edition; Harcourt Brace: Philadelphia, 1998; 591-621.
4. Heimbuck, C.A.; Bower, N. Teaching Experimental Design Using a GC-MS Analysis of Cocaine on Money: A Cross-Disciplinary Laboratory. 79, 10, 2002.

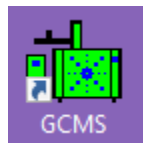
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6.9: Instructions for Manual Operation of the Agilent GCMS

Data is collected using Mass Hunter software, which is available on the GCMS computers using the "GCMS" shortcut on the computer desktop.

Please note that the Mass Hunter GCMS software is used only for data acquisition. Data software is done using a different software package (see next section).



Agilent 7820A/5975 GCMS Operation

1. Check to ensure that there is sufficient pressure of He mobile phase in the carrier gas tank.
2. Locate the Mass Hunter **GCMS software icon** located in the upper left corner of the computer desktop. Double click to open.
3. Allow a few minutes for the software to initialize. The green bars at the top left of the MassHunter window indicate when the instrument is ready.
4. Load the desired method using the Method tab. If the desired method is not listed in the quick access menu, you can find all methods in D:\MassHunter\GCMS\1\methods.
 - Choose your method and load, loading takes a few minutes.
 - The green action bar will confirm when the method loading is complete.
 - Have your TA check the method to make sure students did not save changes to it accidentally.
 - The loaded method is displayed in small font the across the top of the MassHunter window. For example, the method "Standby.M" is loaded as seen in the figure below
5. When the method has been loaded and the instrument is ready, you can prepare to inject your sample. Press the green arrow button to "Run" a method.

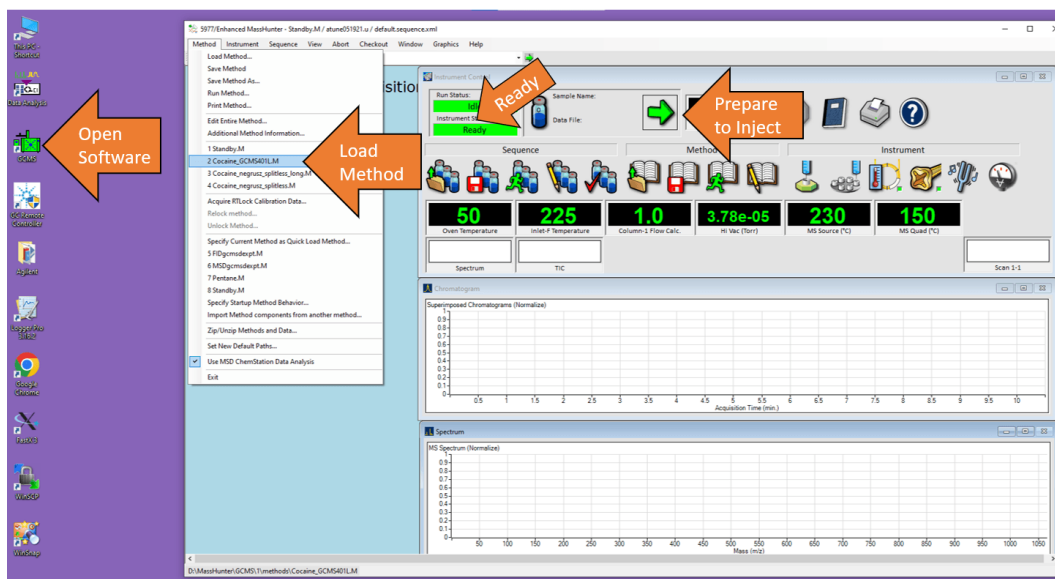


Figure 6.9.1: Infographic for how to open the GCMS MassHunter software, run a method, and prepare the instrument to inject a sample. (CC-BY-SA; Kathryn Haas for Duke Chem.)

6. After you press the "Run Method" icon (the green arrow), the Start Run window will open. You should choose the directory where you will save and retrieve your data files, enter the a file name, and sample information.
 - The data folder for your course is in the C: Drive, specifically C:\GCMS\1\data\CHEM401....
 - Create a new folder with your name inside your course folder if one does not exist.
 - Name your file making sure to retain the ".D" suffix. The recommended file naming format is using the date YYYYMMDD and underscores such as "20220902_standard1.D". Add the other information: user, sample name, info.

- Record the directory, file name, and the identity of the sample in your notebook so that when you need to access the data later, you know where to find it and which file holds the data from which sample.

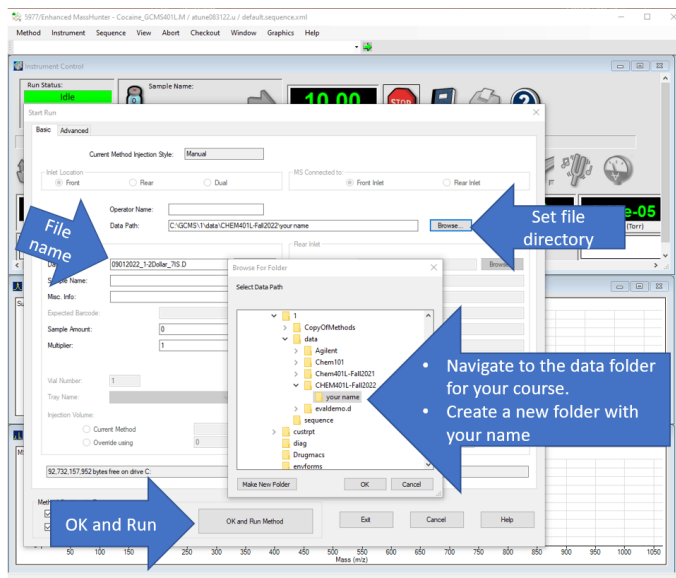


Figure 6.9.2: Infographic to show how to create a new folder to store your GCMS data. (CC-BY-SA; Kathryn Haas for Duke Chem.)

- When your file directory and file name are entered correctly, press the "OK and RUN METHOD" button. The GC/MS will prepare for your sample injection.
- Wait until the "GC Acquisition" window opens and prompts you with **"Waiting for Remote Start"** (see Figure 6.9.3, left). The inject your sample into the front inlet and immediately press the Start button on the front of the instrument.

Warning

DO NOT override anything! You will get two messages asking if you want to override a default setting. Keep your hands off the keyboard and mouse! **NEVER OVERRIDE THE SOLVENT DELAY**, doing so will drastically shorten the lifetime of the filaments inside the Mass Spec, potentially render the instrument inoperable, and likely result in increased chemistry laboratory fees.

- First message: "Waiting for remote start". Do not manually override. Instead, inject your sample and immediately press the start run button on the Instrument itself.
- Second Message: "Override Solvent Delay". **Press NO!**

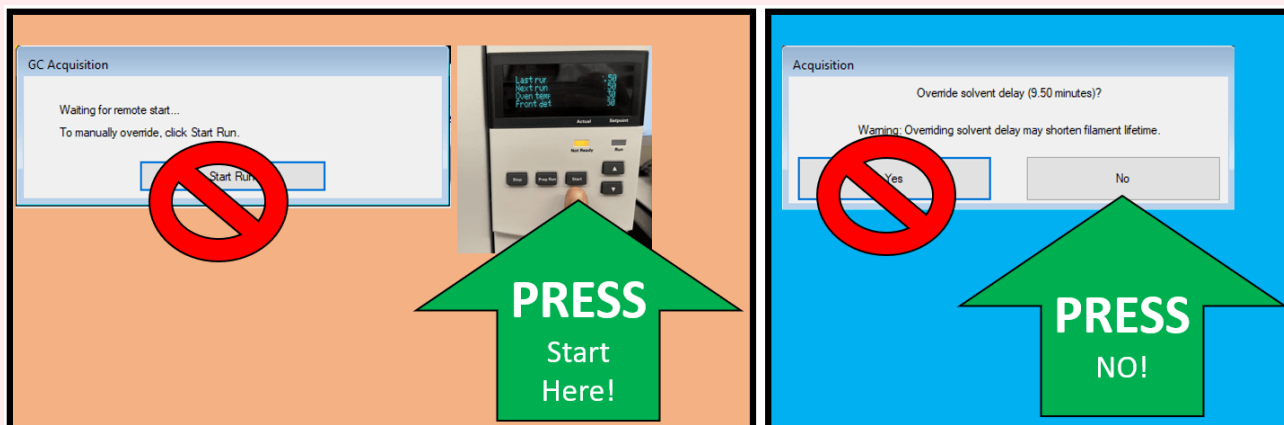


Figure 6.9.3: How to initiate a run after injection of sample in the inlet. Use the button at the front of the instrument to Start the run. Do NOT override the solvent delay. (CC-BY-SA; Kathryn Haas for Duke Chem.)

- Set a timer for 11 minutes** - each run takes exactly 11 minutes. As your run progresses and the 9.5-minute solvent delay has passed, you should see peaks appear in the chromatograph windows. As soon as the run has finished, there is a 2-minute "equilibration time" before the next sample can be injected. During the equilibration time, the oven temperatures must be

adjusted (about 1 min) and there is a additional 1 min delay before the next sample can be injected.

10. As soon as the 11-minute run has finished, repeat the steps above and inject your next sample. During the 2-minute equilibration time after the run, press the green arrow button and prepare the software for the next injection (repeat steps 5-7 above).

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6.10: CAFFEINE SERIAL DILUTION AND ANALYSIS USING (UV-VIS) SPECTROSCOPY

Learning Objectives

After completion of the UV-vis module you will be able to:

- Use analytical techniques to create a serial dilution starting from pure, solid material. (Use an analytical balance, volumetric glassware, and pipettes)
- Operate an Agilent Cary double beam UV-vis spectrometer and collect UV-visible absorption data.
- Use a UV-visible spectrometer to identify the wavelength of maximum absorption of a light-absorbing analyte, and select the appropriate wavelength for quantitative analysis.
- Create a series of standard solutions using serial dilution, generate a calibration curve, and validate the calibration using a validation standard (control) solution of the analyte.
- Determine if UV-vis is an appropriate technique to quantitate a single analyte in a mixture.

6.10.1: Pre-lab assignment

6.10.2: TA Notes

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6.10.1: Pre-lab assignment

Please read this entire module on ultraviolet-visible (UV-Vis) absorption spectroscopy. This module should take less than one lab period. It is part of a two-part series with the theme of caffeine analysis in beverages. First, in this module, you will create a serial dilution of caffeine and characterize the solutions using UV-vis. **Store your solutions in sealed vials for use in the next module.** You have three objectives:

1. Create a series of caffeine solutions for use in this and the next module.
2. Determine the wavelength of maximum absorbance for each solution.
3. Validate the serial dilution using a control.

Written Pre-lab Assignment

Complete the following in your lab notebook and be prepared to discuss these points with your TA.

1. In writing, please explain the advantages to using multi-point calibration compared to single-point calibration.
2. Describe how you should choose appropriate wavelengths in the following scenarios:
 - a. to quantitate a single component in solution.
 - b. to quantitate two or more components in a solution.
3. Prepare a procedure for creation of the caffeine standard solutions described in this module.
4. Prepare a table in your notebook to record absorbance data.
5. Some of your caffeine standards have absorbances exceeding 1 absorbance unit, meaning they are too concentrated to be measured reliably in their current form using a standard cuvette. Suggest a strategy to overcome this limitation so that you can still construct a calibration curve that includes all your standard solutions. *Hint: Consider the components of Beer's Law.*

6.10.2: TA Notes

This page is directed toward laboratory teaching assistants, but is deliberately left open for students to read.

TA Notes

The goal is that after completion of this module students will be able to:

- Use analytical techniques to create a serial dilution starting from pure, solid material. (Use an analytical balance, volumetric glassware, and pipettes)
- Use a full-spectrum UV-vis to measure the absorbance of a series of solutions.
- Determine the best wavelength to monitor the absorbance of an analyte.
- Validate a series of standard solutions using a control.

Pre-Semester Preparations

Do the following during the TA training and orientation week(s)

1. Check that the materials listed below are available and sufficient for all students to use them. If they are running low, please create more or inform the instructor.
 - **Caffeine, 99%:** There should be at least ~25 g of caffeine powder available. If not, please inform the lab director.
 - **Volumetric Glassware:** Be sure that there is sufficient glassware for four students to complete the experiment simultaneously. Please inspect the glassware for chips or obvious contamination. Clean or dispose of glassware as needed and inform the instructor if more glassware should be added to the lab space.
2. Complete instrument training with Dr. Haas.
3. Perform the entire experiment as if you were a student.
4. Write and submit a report as if you were a student (this and the HPLC part of the experiment).
5. Practice grading your and your partner's report using the grading rubric.

Pre-Lab Preparations

When you are on TA duty, arrive at least 20-30 minutes early to prepare and ready the following materials for students.

- Check to be sure that caffeine powder is in its place (notify the instructor if we should order more).
- Check to be sure there are cuvettes available for students to use. They should be **quartz** cuvettes, not glass.
- Remind students to sign into lab
- Check that the student's pre-lab is complete
 - Check their proposed calibration points and ensure that the control solution is approximately in the middle of their range.
- Tell students to gather their glassware as soon as they arrive and get set up on a bench space so that they can make solutions.
- They should clean their glassware prior to use.
- The Cary 100 UV-vis instruments (two of them) should be turned on at the beginning of lab period. Let the students be involved in this.

Helping your students

During the laboratory session, you are expected to be present and available for the entire 4.5 hours. Your job is to help students to complete the experiment safely and successfully, while upholding expectations and academic standards for the students.

In this particular laboratory session, please provide explicit instruction on the following:

- **Weighing solid material:** They can begin by weighing their caffeine when their glassware is clean. Please show students how to use the balances correctly and remind them that there is error associated with the measurement. (refer to P-chem materials...we assume that it is in the last digit of the reported number.) Show them how to quantitatively transfer their material into a volumetric flask, rinsing the weighing container into the flask with water.
- **For using volumetric flasks:** Remind them to use good technique. We can assume students know how to use these, but.....It takes a while to dissolve the solid material. **DON'T LET THEM MOVE ON TOO QUICKLY!** With powder in flask, fill it about 1/2 to 3/4 with solvent, swirl and sonicate as necessary to dissolve the solid before filling to the line.

Help students to analyze data immediately upon collection. They should validate their standard curve using a control solution. The control solution is provided, and it should be within the range of their calibration curve. If the control is outside their calibration range, a new control should be created. The students should treat the control as a sample in their calibration template, then compare their derived concentration to the known concentration. If the values are the same, then the curve is validated. If not, the students should re-create the standards using correct analytical technique. The students should proceed to the HPLC module only after the calibration is validated.

Encourage students to proceed to the next module and begin creating methods for the HPLC if they have validated their curve and there is additional time in the period.

Shut down/ clean up

15 minutes prior to the end of the period, students should stop lab work and clean up. They are to leave the room in clean condition prior to the end of the period (period ends at 17:55).

- Remind students when it is time to clean up.
- Remind students to save their samples!
- Turn off the UV-vis
- Remind students that all glassware should be returned to the storage locations.
- Any samples that are stored should be labeled clearly with the student's name and section.
- Remind students to sign out with the time they leave and get your signature.
- Check the TA checklist for additional items to be completed.

6.11: DETERMINATION OF COCAINE ON BILLS USING GAS CHROMATOGRAPHY, MASS SPECTROMETRY (GC-MS)

Goals

After completing this module, students will be able to:

1. Explain the principles of gas-liquid partitioning.
2. Predict relative retention on a GC column based on molecular structure.
3. Program a GCMS method and operate the instrument to collect useful data.
4. Interpret fragmentation patterns of Electron Ionization Mass Spectra.
5. Use the mass spectra data library to identify the components in a mixture.
6. Determine the response factor of an analyte.
7. Quantitate an analyte using an internal standard.

6.11.1: GC-MS Pre-Lab Assignment

6.11.2: GC-MS Procedure and instructions for data analysis

6.11.3: TA Notes

6.11.4: Archive of GCMS procedure

6.11.4.1: Instructions for Data Processing using MSD ChemStation Data Analysis Software

6.11.4.2: Instructions for Operating the Agilent 7820A/5975 GCMS (Manual)

6.11.4.3: Archived GC-MS Pre-Lab Assignment (Fall 2024)

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6.11.1: GC-MS Pre-Lab Assignment

What is in your pocket? If you have paper currency in your pocket, it is likely that you are carrying trace amounts of illicit drugs, including cocaine. Several studies have shown that about 80% of U.S. dollar bills are [contaminated with cocaine](#). In this experiment, you will use Gas Chromatography-Mass Spectrometry (GC-MS) to perform forensic analysis on money, and quantitate the amount of cocaine on bills.

Pre-Lab Assignment

1. Read the entire contents of this module.
2. Use Web of Science or another literature database to find the article below and read it. You will use a GC-MS method based largely on this paper. Be on the lookout for the ions they focused on in single ion monitoring (SIM) mode for cocaine and the doxepin internal standard. You will also use doxepin as an internal standard: Negrusz, A.; Perry, J. L.; Moore, C. M. *J. Forensic Sci.* **1998**, 43 (3), 626.
3. Use Web of Science or another literature database to research cocaine and other substances that might be found on money. Make a prediction about what you might look for on your bills during this experiment.
4. Answer the pre-lab questions below.
5. Please bring at least one piece of paper currency with you for analysis (at least one per student). The money will not be destroyed during analysis. (Foreign Bills are welcome!)

*If you do not have paper currency, please contact your instructors to borrow some at least 24 hours in advance.

Pre-Lab Questions:

1. Draw the structures of cocaine and doxepin and give their molecular masses.
2. Use an internet search to find the Mass Spectrum of cocaine and doxepin. What is(are) the most abundant ion(s) in each case?
3. Predict at least one other compound that might be present in your dollar extracts. Find the masses of each of your predicted compounds and determine likely fragments that will appear in their MS. You can find mass spectra for many substances here: <https://webbook.nist.gov/chemistry/>
4. What is the goal of this experiment? In other words, what data will be collected in this experiment?
5. Using scientific literature, what results have been produced by similar experiments in the past? How do these experiments differ from the one we are performing?
6. What is the purpose of this experiment? In other words, why do we care about the data being collected? What importance is there to this data in the real world?

Sources:

https://en.wikipedia.org/wiki/Contaminated_currency

6.11.2: GC-MS Procedure and instructions for data analysis

In this experiment, you will extract residual compounds present on paper currency and quantitate the amount of cocaine on each bill using GC-MS.

Materials

- Paper currency: One bill for each student. Bring your own if possible - the money is not destroyed by the extraction process. *We can provide bills if you do not have your own, but please let us know >24 hours in advance.
- Methanol, analytical grade
- 4 ppm solution of doxepin (Internal Standard)
- Serial dilution of cocaine including internal standard (this will be prepared for you)
- 15 mL glass tube for each dollar bill extraction
- volumetric glassware and pipettes
- 3 mL plastic syringe and 0.2 μm filter to filter dollar bill extracts
- Agilent CG-MS with Mass Hunter Software
- Two 10 mL beakers; one for syringe wash methanol, one for waste
- 10 μL syringe for injecting samples

Create a standard curve

The standard solutions contain 0.6 ppm doxepin internal standard and cocaine concentrations between 0.1-5 ppm. These solutions are prepared for you. Each analysis takes about 12 minutes, and each injection can be performed automatically using an automated liquid sampler (ALS).

How to use your time: We have two instruments that may be shared by several students in a lab period. One person should work on one instrument at a time. During the lab period, two students may begin using the software on the instrument computer (one on each instrument), while remaining students begin sample preparation. After the first set of students is finished setting up one calibration series in the sequence on the computer, you will swap places.

Each student should do the following during the first lab period:

- Prepare at least one extract sample for analysis.
- Inspect the instrument method that will be used for analysis.
- Enter calibration standards and sample information into a sequence file.

As a group, you should:

- After all students have had a turn to enter their sample information and inspect the method, start the analysis (Run the sequence file).
- Set up the sequence to run each calibration standard in triplicate.
- Set up the sequence to run each sample in triplicate.

You should stay in the lab the entire first period to ensure your sequence continues to run as expected. Do not throw away any samples yet! You can begin setting up your data analysis method during this period even if the samples are not injected yet.

During the second period, begin quantitative data analysis using the instructions. If necessary, repeat the calibration.

During the third period, continue analysis and writing of your report in the classroom with instructors on hand to help as needed.

With the software open and the correct method running (see [operating instructions in section 5.8](#)), set up a sequence to inject a methanol blank and at least four of the standards. You may use all of the standards if you wish, or choose which standards to use based on your pre-lab reading. Each student should set up the sequence for at least one calibration series of their choice.

- A. **Methanol blank**
- B. **0.1 ppm cocaine** + 0.6 ppm doxepin in methanol
- C. **0.5 ppm cocaine** + 0.6 ppm doxepin in methanol
- D. **1.0 ppm cocaine** + 0.6 ppm doxepin in methanol
- E. **2.5 ppm cocaine** + 0.6 ppm doxepin in methanol
- F. **5.0 ppm cocaine** + 0.6 ppm doxepin in methanol
- G. **10.0 ppm cocaine** + 0.6 ppm doxepin in methanol
- H. **15.0 ppm cocaine** + 0.6 ppm doxepin in methanol
- I. **20.0 ppm cocaine** + 0.6 ppm doxepin in methanol

During any waiting time while samples are running, begin creating the batch and method for data analysis using the instructions in Section 5.8.

Ideal milestones for lab progress

Plan to use the entire scheduled time for this experiment. The following is an ideal timeline for progress in this 3-week module. However, real lab work does not always adhere to ideality. Instrument and sample issues are expected, and flexibility is deliberately built into the schedule. You should process the data as it is being collected so that issues are recognized immediately. If you expect a problem with the instrument or samples, stop immediately and notify your instructors.

Suggested timeline (ideal):

- **Week 1:** By the end of the first lab meeting, you have...
 - A complete calibration curve (methanol blank + four standards = 5 injections)
 - Injection of all student samples
 - Replicate runs are programmed into the sequence and are running (check the Helium before you leave!)
 - You have begun to create data analysis methods.
- **Week 2:** By the end of the second lab period, you have...
 - *Repeated any injections as necessary.*
 - *A complete report for quantitative analysis.*
 - A complete list of compounds found in your samples (compared to the NIST library).
 - If applicable, a comparison of the data collected on different days to confirm it is consistent.
- **Week 3:** If things go smoothly in the first two sessions, this period can be used for writing. However, if you do run into issues or have not met the milestones above, then it is expected that the time in Week 3 is used for data collection.

Ideally, by the end of Day 2 in lab, you would have triplicate data points for each standard solution. However, you can decide when you are satisfied that your standard curve is appropriate to estimate the amount of cocaine present. If not, the third lab meeting should be used for additional data collection. Diagnose any issues with your standard curve using the data analysis methods outlined in Section 5.8.

Preparing the analyte samples

You should prepare your sample for analysis while you are also running the series of standards on the GCMS.

1. Fold each bill accordion style, with about 12 folds. Insert the folded bill far into a 15 mL centrifuge tube.
2. Use a digital pipette to add exactly 5.00 mL of methanol into the tube with the folded bill.
3. Invert the tube for at least 10 minutes.
4. Using tweezers, carefully remove the bill from the tube. Try to leave as much of the methanol in the tube as possible.
5. In a 2 mL volumetric flask, transfer 1.000 mL of the extraction solution, then add enough internal standard solution (4 ppm doxepin in methanol) to reach a final concentration of 0.6 ppm doxepin. Fill to the calibration mark with MS-grade methanol. (Note: this step dilutes your extraction solution and you should account for this in any calculations).
 - Immediately seal the vial containing the extraction solution and put parafilm around the cap's seal. Then store this

sample in case you need it again in the future.

6. Use a 3 mL syringe with a 0.2 μm filter to filter the contents of the volumetric flask into a clean glass vial that can be sealed to prevent evaporation. Treat this solution as precious - do not throw it away.
 - Label two autosampler vials with your initials and section number
 - Remove the plunger from the syringe.
 - Assemble the filter onto the front of the syringe.
 - Hold the filter tip inside of a labeled autosampler vial.
 - Pour the contents of the volumetric flask into the back of the syringe with one hand while holding the filter and autosampler vial with the other hand.
 - Replace the Plunger and gently press the plunger to force filtered sample into the vial. Change to the second vial after 1 mL has flowed into the first vial.
 - Cap both vials with a cap and septum.
 - Place one sample into each autosampler.

Treatment of Data

The general criteria for writing and submitting lab reports are found in the [orientation module](#). Specific additional expectations for this module are given below.

1. Include the following results in the lab report: (assume general expectations apply, e.g. label plots and figures with titles, axes titles, sample calculations, etc)
 - I. Standards
 1. A good chromatogram of each standard sample (peaks labeled with r.t. and identity of each compound of interest). Ideally, this would be one figure with chromatograms stacked on the same X axis. Don't give us several pages of individual chromatograms, please!
 2. A mass spectrum of cocaine and doxepin (with labeled peaks).
 - II. Calibration
 1. Calibration plot of C_A (cocaine concentration) vs $\frac{S_A}{S_{IS}}$ including the most appropriate fit with y-intercept, slope (or other fit parameters), and errors.
 - Note that the Quantitative Analysis software does not provide residuals analysis, nor does it provide uncertainty in the parameters of the fit, nor the uncertainty in the derived concentrations of your samples. You can use another fitting program to derive these things and properly analyze the calibration and derived concentrations, and the associated uncertainty.
 - Note that response is not always linear. A robust way to make the choice between linear and quadratic fits of your calibration data is to do a residuals analysis. (Recall that this was part of your CHEM401 lectures during the first few weeks of the semester!) Again, the computer software does not help you with this and so it is something you would do in a different data analysis program (eg Excel or Matlab).
 2. Give the equation of the calibration line with associated error and specify the response factor (K).
 - III. Quantitation
 1. How much cocaine is detected on each bill analyzed in units of $\frac{\mu\text{g}}{\text{mL}}$ (ppm) and $\frac{\mu\text{g}}{\text{bill}}$
 2. Include concise error analysis.
 - IV. Identification of other compounds on the bill
 1. What compounds, if any, were detected in analyte solutions that were not present in the standard solutions? Provide the results of the library search of dollar bill extraction (with blank subtraction; otherwise also show the library search on the blank).
 2. Provide the Mass Spectra of notable compounds detected.
2. In your discussion, be sure to address points from the pre-lab assignment, and..
 - I. Discuss the relationship between boiling points, molecular structure, and retention time of the compounds.
 - II. Explain why doxepin appears as two peaks, and suggest how we might increase separation cocaine from doxepin.
 - III. Discuss the fragmentation pattern you observed and how it supports assignment of the compound. Address how molecular structure yield the observed mass ions.
 - IV. Discuss and interpret the results of the library search on your dollar bill

References

1. McLafferty, F. *Interpretation of Mass Spectra*; 3rd ed.; University Science Books: Mill Valley, CA, 1980.
2. Silverstein, R. M.; Bassler, G. C.; Morrill, T. C. *Spectrometric Interpretation of Organic Compounds*; 4th ed.; Wiley: New York,

NY, 1981.

3. Skoog, D. A.; Holler, F. J.; Nieman, T. A. *Principles of Instrumental Analysis*, Fifth Edition; Harcourt Brace: Philadelphia, 1998; 591-621.
4. Heimbuck, C.A.; Bower, N. Teaching Experimental Design Using a GC-MS Analysis of Cocaine on Money: A Cross-Disciplinary Laboratory. 79, 10, 2002.

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- [Lab 5: Gas Chromatography/Mass Spectrometry \(GC/MS\)](#) by W. R. Fawcett, John Berg, P. B. Kelley, Carlito B. Lebrilla, Gang-yu Liu, Delmar Larsen, Paul Hrvatin, David Goodin, and Brooke McMahon is licensed [CC BY-NC-SA 4.0](#).

6.11.3: TA Notes

This page is directed toward laboratory teaching assistants, but is deliberately left open for students to read.

TA Notes

After completing this module your students will be able to...

- Explain the principles of gas-liquid partitioning.
- Predict relative retention on a GC column based on molecular structure.
- Program a GCMS method and operate the instrument to collect useful data.
- Interpret fragmentation patterns of Electron Ionization Mass Spectra.
- Use the mass spectra data library to identify the components in a mixture.
- Determine the response factor of an analyte.

Quantitate an analyte using an internal standard.

Pre-Semester Preparations

Do the following during the TA training and orientation week(s)

1. Check that the materials listed below are available and sufficient for all students to use them. If they are running low, please create more or inform the instructor.
 - **4 ppm doxepin in methanol.** This solution is stored in the refrigerator. Please keep the stock flask cold and take out a small amount for use during the lab. Please seal the flask with parafilm after each use and return it to the refrigerator.
 - **Methanol, mass spectrometry grade.** The MS grade methanol is stored in a flammable cabinet near the windows, and poured into a working container for students to use. Avoid contamination!
 - **Calibration standards:** Calibration standards contain 0.6 ppm doxepin and various concentrations of cocaine. Instructions for their preparation are below. Usually, these are prepared once at the beginning of the semester and used by all students during the semester. The standards should last the entire semester if treated carefully. If using the autosampler vials, the septa should be changed after each series of injections to prevent evaporation of the methanol and the standards becoming unusable.

Preparation of calibration standards

Cocaine standard solution is purchased as 1 mL of a 1.0 mg/mL solution. This is the same as 1000 ppm.

To make a series of calibration standards, 5 mL each: First create 5 mL of a 100 ppm solution of cocaine in 10 mL of methanol. This requires a 10x dilution of the standard. So use 0.5 mL of standard and dilute to 5 mL.

$$\frac{0.5 \text{ mL} \times 1000 \text{ ppm cocaine}}{5 \text{ mL}} = 100 \text{ ppm cocaine working solution}$$

You will need the 100 ppm cocaine working solution and a 4 ppm doxepin solution. Create the standards using the table below. Add cocaine and doxepin to a 5 mL volumetric flask, then dilute to the volumetric mark with MS-grade methanol. The final concentration of doxepin in each solution is 0.6 ppm.

Concentration of Cocaine	Vol. of 100 ppm cocaine solution	Vol. of 4 ppm doxepin (mL)

20 ppm cocaine	1000 μL	750 μL
15 ppm cocaine	750 μL	750 μL
10 ppm cocaine	500 μL	750 μL
7.5 ppm cocaine	375 μL	750 μL
5 ppm cocaine	250 μL	750 μL
2.5 ppm cocaine	125 μL	750 μL
1 ppm cocaine	50 μL	750 μL
0.5 ppm cocaine	25 μL	750 μL
0.1 ppm cocaine	5 μL	750 μL

- During the TA training week(s) you should run all standards in triplicate on both GCMS instruments.
 - Take note of the variation that you see in the triplicate data points on each instrument and discuss this with the instructor to confirm the instruments are performing as expected. Determine if the response varies across different days.
 - Confirm that the response to the solutions is as expected, and to identify any potential issues with the calibration standards.
 - Identify the concentration range where the response is linear and nonlinear on each instrument.
- Complete instrument training with Dr. Haas.
- Perform the entire experiment as if you were a student (except, you should inject all available standards and measure concentrations for at least three bill extracts).
- Write and submit a report as if you were a student.
- Practice grading your and your partner's report using the grading rubric.

Pre-Lab Preparations

When you are on TA duty, arrive at least 20-30 minutes early to prepare and ready the following materials for students.

- Get some doxepin solution out of the refrigerator and put it in a sealed vial for students to use. Do this at least 20 minutes prior to the start of lab time so that the solution can reach room temperature prior to use.
- Refill the MS-grade methanol working container. Avoid contamination!

- Check that all standards are present and that there is sufficient volume of each.
- Check that the He gas is sufficient. If it is getting low, please inform the instructor and/or change the tank.
- Remind students to sign into lab
- Check that the student's pre-lab is complete
- Determine whether students have brought their own currency for analysis, and if not, make arrangements for them to use lab currency.
- Tell students to gather their glassware as soon as they arrive and get set up on a bench space so that they can make solutions.
- They should clean their glassware (if they are wise!) prior to use.

Helping your students

During the laboratory session, you are expected to be present and available for the entire 4.5 hours. Your job is to help students to complete the experiment safely and successfully, while upholding expectations and academic standards for the students.

For Manual Injections:

If you are not using the automatic liquid sampler (ALS), you should begin by demonstrating how to inject samples on the GCMS. Start immediately by demonstrating an injection with a methanol blank. Then discuss the pre-lab assignment while the methanol blank is running.

Keep students on task during the lab period. Students should set a timer for 12 minutes, and prepare to run the next sample as the instrument equilibrates between runs. During the 12-minute down time, students should prepare their extract sample, then work on creating their data analysis method, then analyze each sample and adding the data to their calibration template while the next sample is running. By the end of the first period, students should have completed one injection of each standard so they have a complete calibration curve (or at least a first try), they should also complete replicate injections of their sample, and replicate injections of at least one calibration point. If all is well, continue replicates for the remaining time. Otherwise, if something is wrong with the instrument or with the standards, discuss the situation with the instructor and plan to re-start during the next period if necessary.

Encourage students to use *at least* two full lab periods (assuming a 3-period module) to perform lab work. Ideally, data is actively processed during collection and students are able to finalize data collection and begin writing their reports during the third week of lab.

For Automatic Injections:

If you are using the automatic liquid sampler (ALS) accessory to perform automatic injections, begin by introducing students to the GCMS and show them the instrument method options in the software. Point out the oven settings, the ALS settings, and let them explore the other options in the GC method control window. Then show them the options in the MS method control options. Be sure not to overwrite the method by accident. Then, show students the sequence table and explain to them how to build a sequence. Ask each student to build a sequence table on the two available instruments. They should program the sequence to inject the blank and a series of standards. If any students are waiting during this time, ask them to start preparing their samples. After the first students have build their sequence, then tell students to switch - any students who did not get a chance to build the sequence should now add their samples and another series of standards to the sequence, while other students should prepare their samples. Before the students leave, they should inject all samples and standards in triplicate. Students can share triplicate calibration data, but they should each have a unique sample or samples to analyze.

Shut down/ clean up

15 minutes prior to the end of the period, students should stop lab work and clean up. They are to leave the room in clean condition prior to the end of the period (17:55).

- Remind students when it is time to clean up.
- Turn the instruments into standby by opening the standby method
- Remind students that all glassware should be returned to the storage locations.
- Any samples that are stored should be labeled clearly with the student's name and section.

- Remind students to sign out with the time they leave and get your signature.
- Check the TA checklist for additional items to be completed.

6.11.4: ARCHIVE OF GCMS PROCEDURE



-   Topic hierarchy  

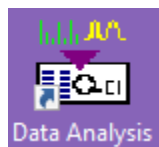
6.11.4.1: Instructions for Data Processing using MSD ChemStation Data Analysis Software

6.11.4.2: Instructions for Operating the Agilent 7820A/5975 GCMS (Manual)

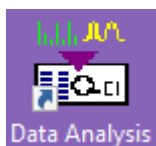
6.11.4.3: Archived GC-MS Pre-Lab Assignment (Fall 2024)

6.11.4.1: Instructions for Data Processing using MSD ChemStation Data Analysis Software

Data analysis is accomplished using a different software than was used for data collection. The software that is used for data analysis is the MSD Chem Station Data Analysis software, and it is available on the GCMS computers using the "Data Analysis" shortcut on the desktop.



Open and view your data file(s) using the Data Analysis software



1. If not already open, open the **Data Analysis** program
2. Find your data file in the file navigator at the left side of the window - double click on the file name to load the data. **If you do not see the file you want in the lefthand file navigator, go to **File--> Load Data File**.
 - The default view shows the chromatograms. TIC means "total ion chromatogram". The TIC of the scan and of the single ion monitoring (SIM) modes will be shown.
 - You can zoom in on a peak or region by clicking and dragging the cursor over the region you want to enlarge. Un-zoom by double clicking on the spectrum window.
 - To view the Mass Spectrum (MS), double right-click on the peak of interest or right click and drag to see the MS of a region. The MS should appear as a new window at the bottom of the screen. Notice the peak's retention time is displayed at the top of the mass spectrum sub window. The abundance is observed as the y-axis of both the chromatogram and mass spectrum.
 - Different regions of the peak can give varying information; therefore, it is important to survey places within the same peak.
 - Abundances should be in the 1×10^5 - 1×10^6 range. Abundances of 1×10^7 and greater are too large and indicate a possible over-injection, too concentrated of a sample, and/or wrong method. Extremely large abundances will shorten the filament lifetime. Conversely, abundances less than 1×10^5 are also not desired and indicate injector problems, diluted sample, and/or the wrong method. Report abundances that are too high or too low to your TA immediately.

Qualitative Data Processing

1. **Search for compounds:** Do this for one of your standards and for each of your analyte samples. Go to **Spectrum--> Library Search Report**. Choose the screen as a destination and choose the genie.e integration parameter file. Press OK, and in moments the report should appear on the screen. You can copy/paste the report or save it as a text file.
 - **NOTE:** The default library, "DEMO" is quite limited. To discover more compounds present in your samples, you may switch to the more comprehensive NIST library. To do this, navigate to **Spectrum --> Select Library**.
 - Replace the library path in "Search Order 1" by selecting **Browse...** on row 1 and selecting "NIST.L"
 - Hit "OK" and perform the Library Search Report as described above.
2. **Extract Ion Chromatograms (EIC):** Go to **Chromatograms --> Extract Ion Chromatograms**. Choose at least one of the masses expected for cocaine (303,182,82 m/z), and one for doxepin (58 amu). Then click on the peaks in the chromatogram to view the MS and determine the retention time for each compound. Record the extracted masses that you use and the retention time of each compound in your notebook - you will need it for the next step.

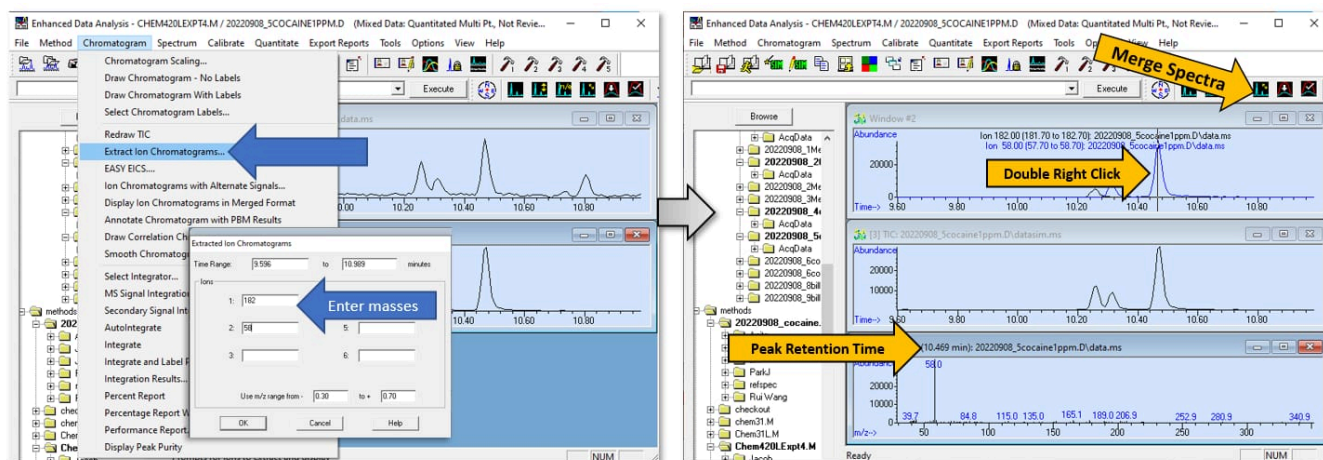


Figure 6.11.4.1.1: Infographic showing how to create an extracted ion chromatogram (EIC) from the total ion chromatogram (TIC). (CC-BY-SA; Kathryn Haas for Duke Chem.)

Quantitative Processing

To generate a calibration curve, you will plot C_A (cocaine concentration) vs $\frac{S_A}{S_{IS}}$, which is the ratio of the peak area of the cocaine analyte (S_A) divided by the peak area of the doxepin internal standard (S_{IS}). Those peak areas may be calculated by the following method:

1. **Make a compounds list for Quantitation:** Now you will create a quantitation method for cocaine and for doxepin.
 - a. Go to **Method --> Load Method...** and select Default.M. Then press OK.
 - b. Go to **Method --> Save Method...** and create a name for your method with the format YYYYMMDD_cocaine_YOURINITIALS.M. Then press OK. This will create a copy of the Default.M method that you can edit according to your data analysis needs.
 - c. Go to **Calibrate --> Edit Compounds...** The Edit Compounds window will appear.
 - At the left of the Edit Compounds window, right-click on the "Compound Database" line and choose **Insert Compound Below Current Compound**. Repeat three times so that there are four compounds in the list.
 - Edit the compound name to indicate the compound, quant signal, and target mass (e.g. "doxepin_SCAN_58" and "doxepin_SIM_58").
 - Enter the compound name, the retention time of the compound, the type of signal for quantitation and the target mass. In the type of signal, use both Scan Ion and SIM Ion for each compound.

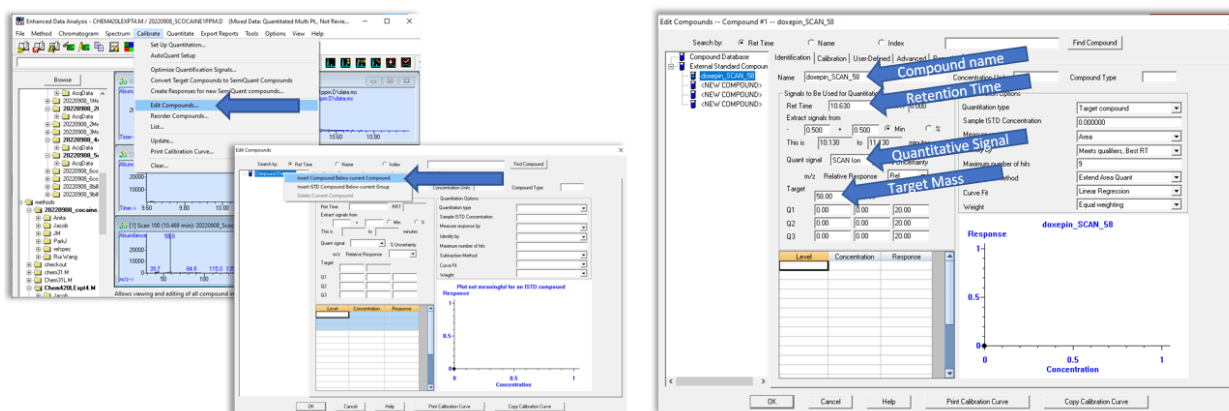


Figure 6.11.4.1.2: Infographic showing the steps for creating a method for quantitative analysis. (CC-BY-SA; Kathryn Haas for Duke Chem.)

- d. After you have created the compound list, press OK.
 - e. Save the method using **Method-->Save Method...**
2. **Run the quantitation: Quantitate--> Calculate.**
 - a. A report will be generated and will appear at the bottom of the window. Scroll down to view the quantitation results.
 3. **Check and edit the peak integration as necessary.** Before assuming that the results are correct, view the integration and

adjust if necessary.

- Go to **View-->QEdit QUANT Results**. Click "start" on the list of compounds to view the integration of each peak.
- If necessary, adjust the integration by choosing the compound in the "Quick Qedit" window. Then right click and drag across the bottom of the peak to be integrated.
- If the quantitation shows a nonsense result, or there is no integration because the peak was not found, it may be because the target peak was very small or absent. Choose the analyte that appeared as a strange result in the "Quick Qedit" window. In the chromatogram window, right-click and drag over the baseline where the peak should be. A red line will appear over where you dragged to indicate where the software will integrate the peak.
- Press **Exit** in the "Quick Qedit" window when you are finished, and choose "Yes" to the prompt that asks you if you'd like to save the changes to the quantitation.
- If you changed the integrations, go to **Quantitate--> Generate Report** to generate an updated report using the adjusted integration.

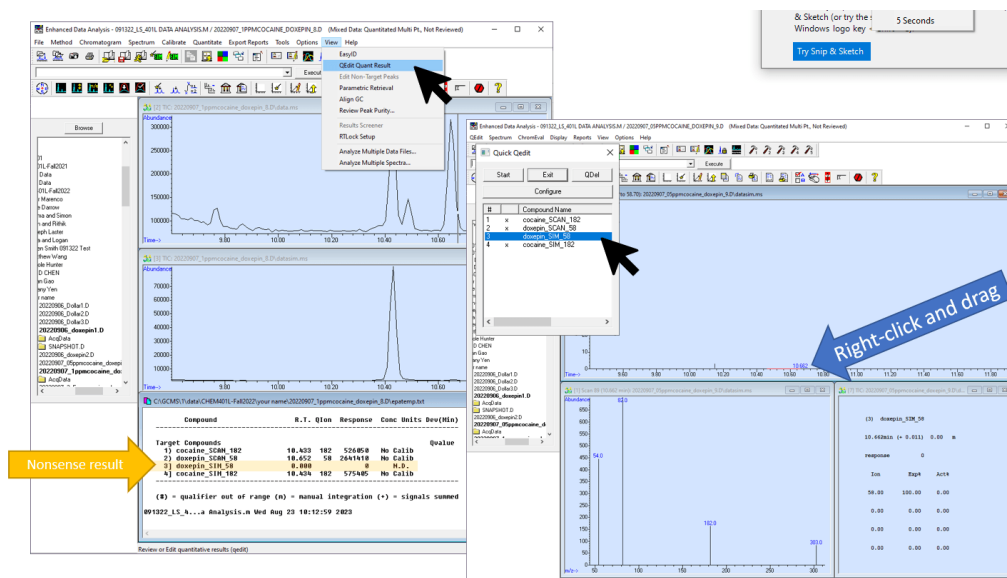


Figure 6.11.4.1.3: How to manually change the quantitation in the case that peaks are not integrated correctly or there is no peak and the result is nonsense. (CC-BY-SA; Kathryn Haas for Duke Chem.)

- Copy the Results into a spreadsheet or text file.** Probably the easiest way to do this is using Excel. Copy the text block exactly as shown below. Then copy/paste into excel. Use the Text Import Wizard to convert the text into spreadsheet format. Then you can copy/paste the relevant cells into another spreadsheet where you will organize and process your data.

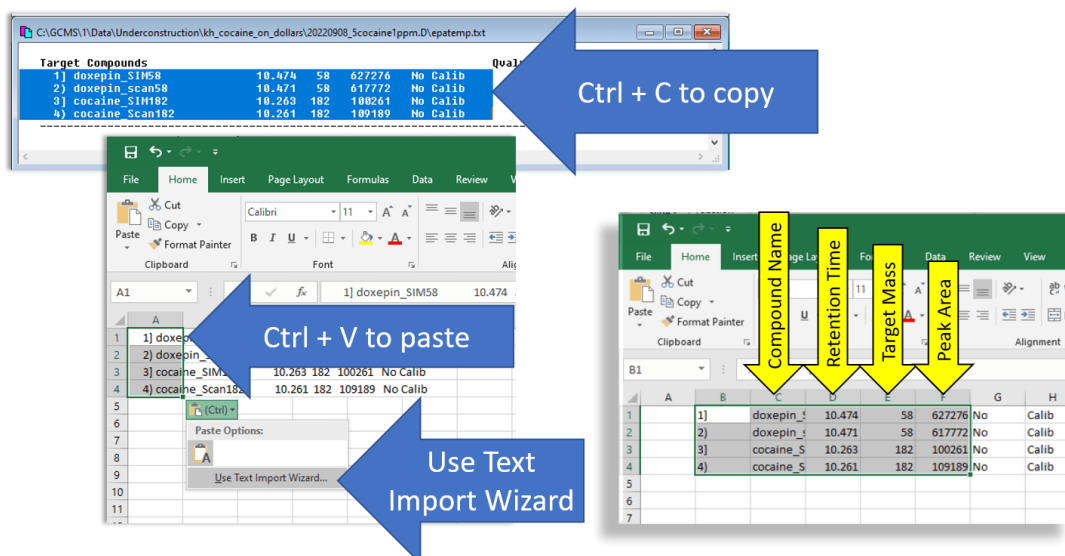


Figure 6.11.4.1.4: Infographic showing the steps for copying and pasting the quantitative data into Microsoft Excel. (CC-BY-SA; Kathryn Haas for Duke Chem.)

- Now you can repeat the quantitation for the next file. Simply open the next file for quantitation and choose **Quantitate--**

>**Calculate** and repeat the process outlined in steps 2-3 above.

How to Create figures for reports

Creating nice figures from the Data Analysis program is a bit harder than it should be. Here are some instructions for creating "ok" figures. If you figure out a way to export data and create figures in a better way, please let us know!

General tips

- Double click with both mouse buttons to annotate. The annotation doesn't show up on the screen (ugh!) but it will be there when you copy/paste.
- Capturing data: One option is to copy the spectrum using the "copy" button in the toolbar and paste it into a powerpoint or word file.  The other option is to copy the screen using the Snipping Tool software. 
- From the powerpoint, you can annotate and arrange spectra easily, then screen shot to capture your work (shift + windows + S)

Arranging chromatograms in the Data Analysis Program

1. To create a figure with overlaid chromatograms, like the one shown below in Figure 6.11.4.1.5, start by opening a "fresh" copy of the Data Analysis program.

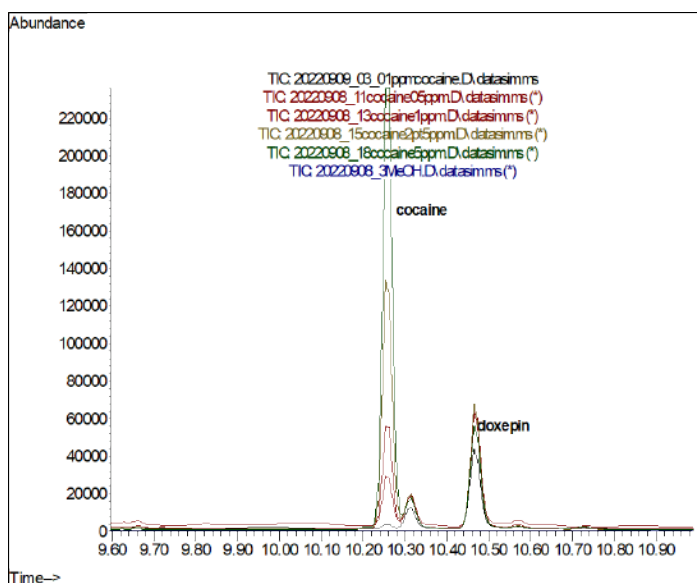


Figure 6.11.4.1.5: An example set of overlaid (aka merged) TIC chromatograms with analytes annotated. (CC-BY-SA; Kathryn Haas for Duke Chem.)

2. Go to the folder where your data is stored and open one of the files. Then choose **View-->Analyze multiple data files...** and choose a representative data file for each chromatogram you want to view.

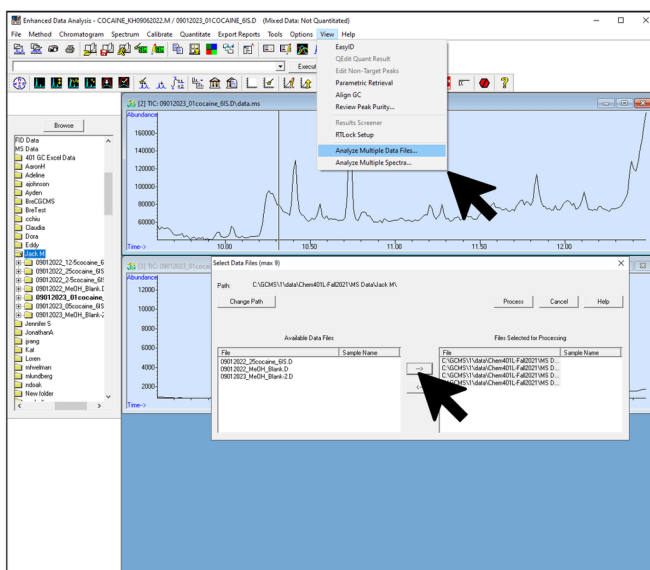


Figure 6.11.4.1.6: Infographic that shows how to choose files for an overlaid set of chromatograms. (CC-BY-SA; Kathryn Haas for Duke Chem.)

- Choose whether you want to view the TIC or a specific mass. You will need to repeat this for each file, and be sure all are the same!
 - Enter "0" (zero) for the TIC
 - Enter a specific m/z value for the SIM chromatogram.
 - Leave all other lines as "-1" (negative 1) to indicate they are unused.

Ex: Import only the TIC
(enter "0" in the first line, all other lines are "-1")

Enter m/z values (1-1050 amu; TIC=0)

M/Z #1	0
M/Z #2	-1
M/Z #3	-1
M/Z #4	-1
M/Z #5	-1
M/Z #6	-1

OK Cancel

Ex: Import the TIC and the SIM for m/z = 82
(enter "0" in the first, "82" in the second, all other "-1")

Enter m/z values (1-1050 amu; TIC=0)

M/Z #1	0
M/Z #2	82
M/Z #3	-1
M/Z #4	-1
M/Z #5	-1
M/Z #6	-1

OK Cancel

Figure 6.11.4.1.7: How to choose the appropriate chromatograms to view multiple data files at once. 0 = TIC, -1 = nothing. (CC-BY-SA; Kathryn Haas for Duke Chem.)

- Go to **Chromatogram--> Overlay Chromatograms** to overlay them. Choose only one "type" of chromatogram for each file (for example, select only "M/Z: 0" for each file to overlay the TIC's for each file. The figure below shows an "overlay" of TIC from four files. Note that this is actually a "stacked" view though the software seems to call this an "overlay".

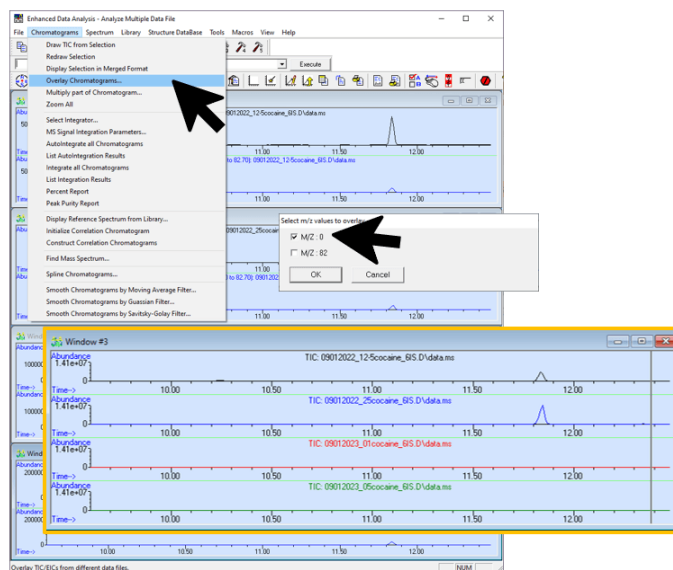


Figure 6.11.4.1.8: How combine the appropriate chromatograms into one window. (CC-BY-SA; Kathryn Haas for Duke Chem.)

- With the multiple spectra window (the stacked view) highlighted, go to **Chromatogram--> Display Selected in Merge Format** to "merge" the stacked spectra. Now the view is what most people refer to as an "overlay".

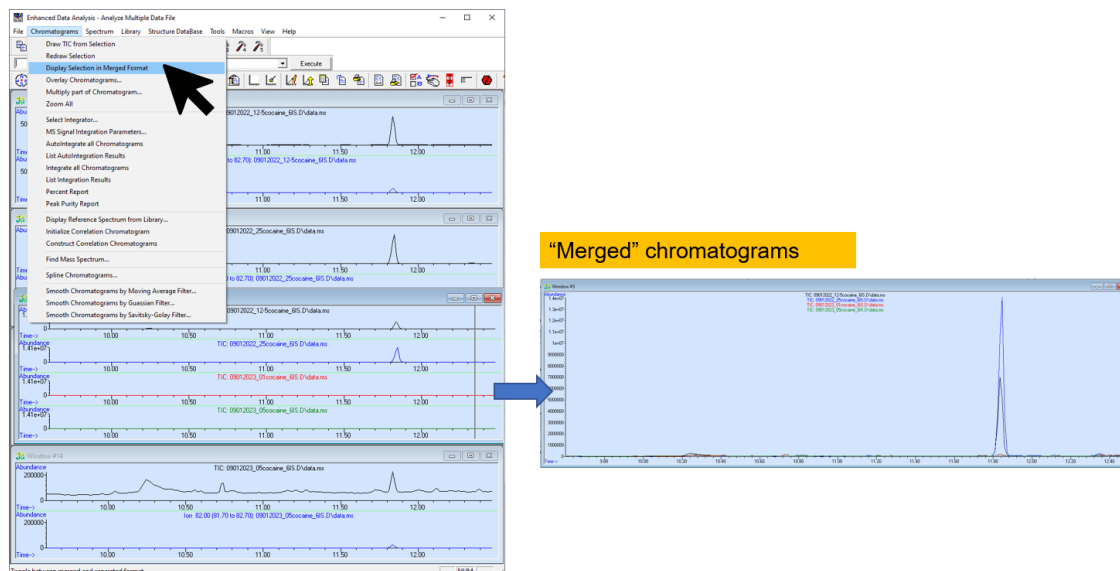


Figure 6.11.4.1.9: How to merge (overlay) the chromatograms. (CC-BY-SA; Kathryn Haas for Duke Chem.)

- To annotate, double-click near the location where you would like to add an annotation. Specify the justification of the text. Then click "Add". To remove the annotation, double click on it and select "Delete".

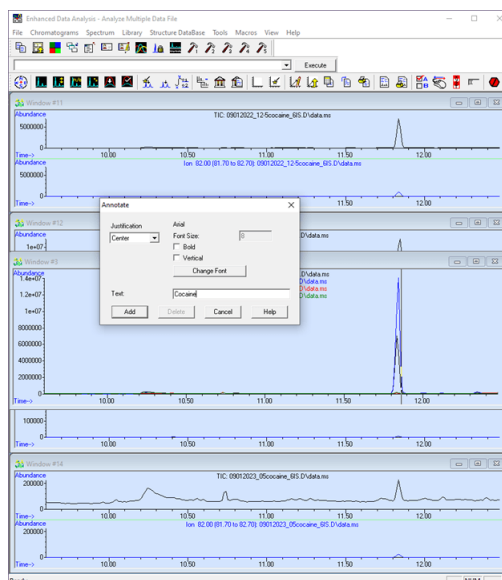


Figure 6.11.4.1.10: Double click on the chromatogram to add annotations. (CC-BY-SA; Kathryn Haas for Duke Chem.)

7. Choose the "Edit Colors" tool  from the top menu and specify colors of background or other aspects of the plot.
8. Use the Snip tool to capture your chromatograms and then paste the chromatograms in your report or other Word document.

Using a plotting program to generate better chromatograms

As seen in the above figure, the jpg chromatograms generated by Data Analysis are difficult to label and interpret. An alternate qualitative data analysis approach is to export individual chromatograms as CSV files which may be analyzed in Excel to produce cleaner plots.

1. Open your data file of interest in Data Analysis
2. Go to **File --> Export Data To CSV File...** and select **Current File - Select Destination**
3. Select **Chromatogram in R0**
4. Navigate to **Desktop --> 401L Fall YYYY** and create a folder with your name to store your data analysis files, if you haven't already done so. Rename and save the CSV file here.
5. Open this CSV file using a program that can plot data (MatLab, Excel, Origin, etc.)
 - You will notice that Data Analysis has exported your chromatogram as a series of points where Column A is retention time (min) and Column B is abundance.
 - This file contains both data from the TIC (marked by a cell containing "TIC: [sample name]\data.ms") and the SIM (found below the TIC data, marked by "TIC: [sample name]\datasim.ms")
 - To plot one of these chromatograms, highlight over all of the data points for either the TIC or SIM and navigate to **Insert --> Scatter --> Scatter with Straight Lines**
6. Repeat this process for all other samples and overlay the data into a single plot to produce a merged chromatogram similar to the one in the figure above.

This page titled [6.11.4.1: Instructions for Data Processing using MSD ChemStation Data Analysis Software](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by Kathryn Haas.

- [Lab 5: Gas Chromatography/Mass Spectrometry \(GC/MS\)](#) by W. R. Fawcett, John Berg, P. B. Kelley, Carlito B. Lebrilla, Gang-yu Liu, Delmar Larsen, Paul Hrvatin, David Goodin, and Brooke McMahon is licensed [CC BY-NC-SA 4.0](#).

6.11.4.2: Instructions for Operating the Agilent 7820A/5975 GCMS (Manual)

Data is collected using Mass Hunter software, which is available on the GCMS computers using the "GCMS" shortcut on the computer desktop.

Please note that the Mass Hunter GCMS software is used only for data acquisition. Data software is done using a different software package (see next section).



Agilent 7820A/5975 GCMS Operation

1. Check to ensure that there is sufficient pressure of He mobile phase in the carrier gas tank.
2. Locate the Mass Hunter **GCMS software icon** located in the upper left corner of the computer desktop. Double click to open.
3. Allow a few minutes for the software to initialize. The green bars at the top left of the MassHunter window indicate when the instrument is ready.
4. Load the desired method using the Method tab. If the desired method is not listed in the quick access menu, you can find all methods in D:\MassHunter\GCMS\1\methods.
 - Choose your method and load, loading takes a few minutes.
 - The green action bar will confirm when the method loading is complete.
 - Have your TA check the method to make sure students did not save changes to it accidentally.
 - The loaded method is displayed in small font at the across the top of the MassHunter window. For example, the method "Standby.M" is loaded as seen in the figure below
5. When the method has been loaded and the instrument is ready, you can prepare to inject your sample. Press the green arrow button to "Run" a method.

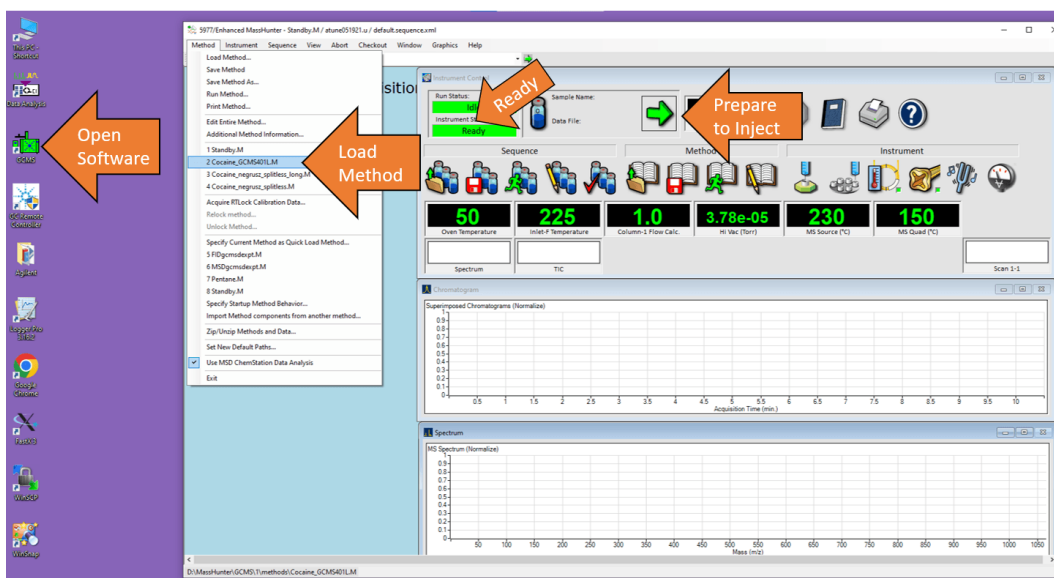


Figure 6.11.4.2.1: Infographic for how to open the GCMS MassHunter software, run a method, and prepare the instrument to inject a sample. (CC-BY-SA; Kathryn Haas for Duke Chem.)

6. After you press the "Run Method" icon (the green arrow), the Start Run window will open. You should choose the directory where you will save and retrieve your data files, enter the a file name, and sample information.
 - The data folder for your course is in the D: Drive, specifically D:\MassHunter\GCMS\1\data\CHEM401L...
 - Create a new folder inside the folder for your course if one does not exist. A good format for the file name is

"D:\MassHunter\GCMS\1\data\CHEM401L-Fall2024"

- Name your file making sure to retain the ".D" suffix. The recommended file naming format is using the date YYYYMMDD and underscores such as "20220902_standard1.D". Add the other information: user, sample name, info.
- Record the directory, file name, and the identity of the sample in your notebook so that when you need to access the data later, you know where to find it and which file holds the data from which sample.

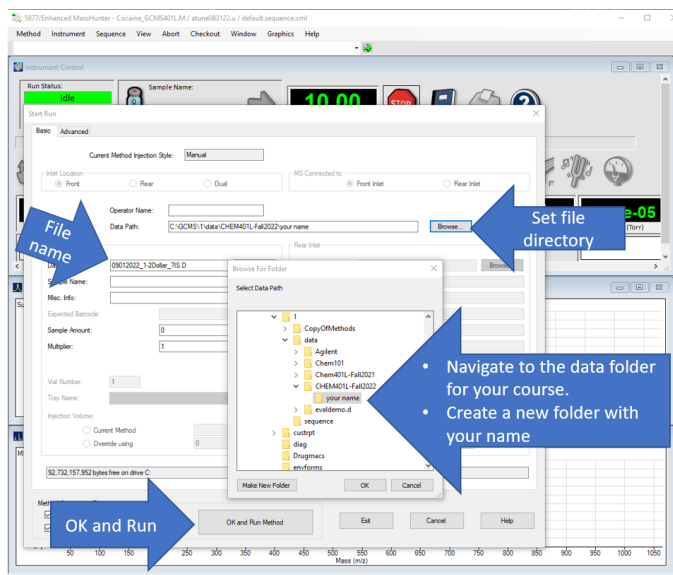


Figure 6.11.4.2.2: Infographic to show how to create a new folder to store your GCMS data. (CC-BY-SA; Kathryn Haas for Duke Chem.)

- When your file directory and file name are entered correctly, press the "OK and RUN METHOD" button. The GC/MS will prepare for your sample injection.
- Wait until the "GC Acquisition" window opens and prompts you with "**Waiting for Remote Start**" (see Figure 6.11.4.2.3, left). The inject your sample into the front inlet and immediately press the Start button on the front of the instrument.

Warning

DO NOT override anything! You will get two messages asking if you want to override a default setting. Keep your hands off the keyboard and mouse! **NEVER OVERRIDE THE SOLVENT DELAY**, doing so will drastically shorten the lifetime of the filaments inside the Mass Spec, potentially render the instrument inoperable, and likely result in increased chemistry laboratory fees.

- First message: "Waiting for remote start". Do not manually override. Instead, inject your sample and immediately press the start run button on the Instrument itself.
- Second Message: "Override Solvent Delay". **Press NO!**

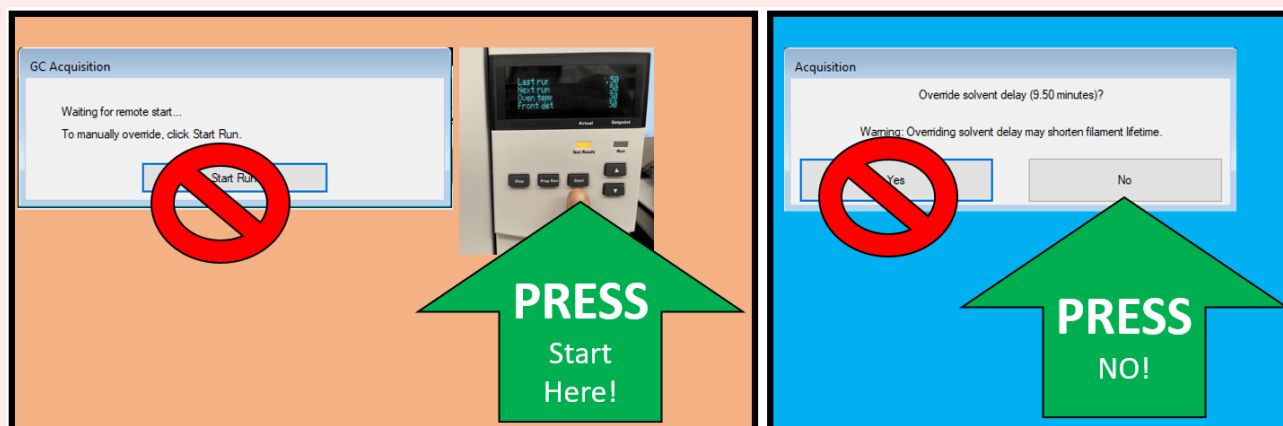


Figure 6.11.4.2.3: How to initiate a run after injection of sample in the inlet. Use the button at the front of the instrument to Start the run. Do NOT override the solvent delay. (CC-BY-SA; Kathryn Haas for Duke Chem.)

9. **Set a timer for 11 minutes** - each run takes exactly 11 minutes. As your run progresses and the 9.5-minute solvent delay has passed, you should see peaks appear in the chromatograph windows. As soon as the run has finished, there is a 2-minute "equilibration time" before the next sample can be injected. During the equilibration time, the oven temperatures must be adjusted (about 1 min) and there is a additional 1 min delay before the next sample can be injected.
10. As soon as the 11-minute run has finished, repeat the steps above and inject your next sample. During the 2-minute equilibration time after the run, press the green arrow button and prepare the software for the next injection (repeat steps 5-7 above).

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6.11.4.3: Archived GC-MS Pre-Lab Assignment (Fall 2024)

What is in your pocket? If you have paper currency in your pocket, it is likely that you are carrying trace amounts of illicit drugs, including cocaine. Several studies have shown that about 80% of U.S. dollar bills are [contaminated with cocaine](#). In this experiment, you will use Gas Chromatography-Mass Spectrometry (GC-MS) to perform forensic analysis on money, and quantitate the amount of cocaine on bills.

Pre-Lab Assignment

1. Read the entire contents of this module.
 2. Use Web of Science or another literature database to find the article below and read it. You will use a GC-MS method based largely on this paper. Be on the lookout for the ions they focused on in single ion monitoring (SIM) mode for cocaine and the doxepin internal standard. You will also use doxepin as an internal standard:
 - Negrusz, A.; Perry, J. L.; Moore, C. M. *J. Forensic Sci.* **1998**, 43 (3), 626.
 3. Use Web of Science or another literature database to research cocaine and other substances that might be found on money. Make a prediction about what you might look for on your bills during this experiment.
 4. Answer the pre-lab questions below.
 5. Please bring at least one piece of paper currency with you for analysis (at least one per student). The money will not be destroyed during analysis. (Foreign Bills are welcome!)
- *If you do not have paper currency, please contact your instructors to borrow some at least 24 hours in advance.

Pre-Lab Questions:

1. Draw the structures of cocaine and doxepin and give their molecular masses.
2. Use an internet search to find the Mass Spectrum of cocaine and doxepin. What is(are) the most abundant ion(s) in each case?
3. Predict at least one other compound that might be present in your dollar extracts. Find the masses of each of your predicted compounds and determine likely fragments that will appear in their MS. You can find mass spectra for many substances here: <https://webbook.nist.gov/chemistry/>
4. Describe the effect of a gradient temperature program compared to isothermal gas chromatography.
5. List the settings for the GC and MS found in the Negrusz paper.

Sources:

https://en.wikipedia.org/wiki/Contaminated_currency

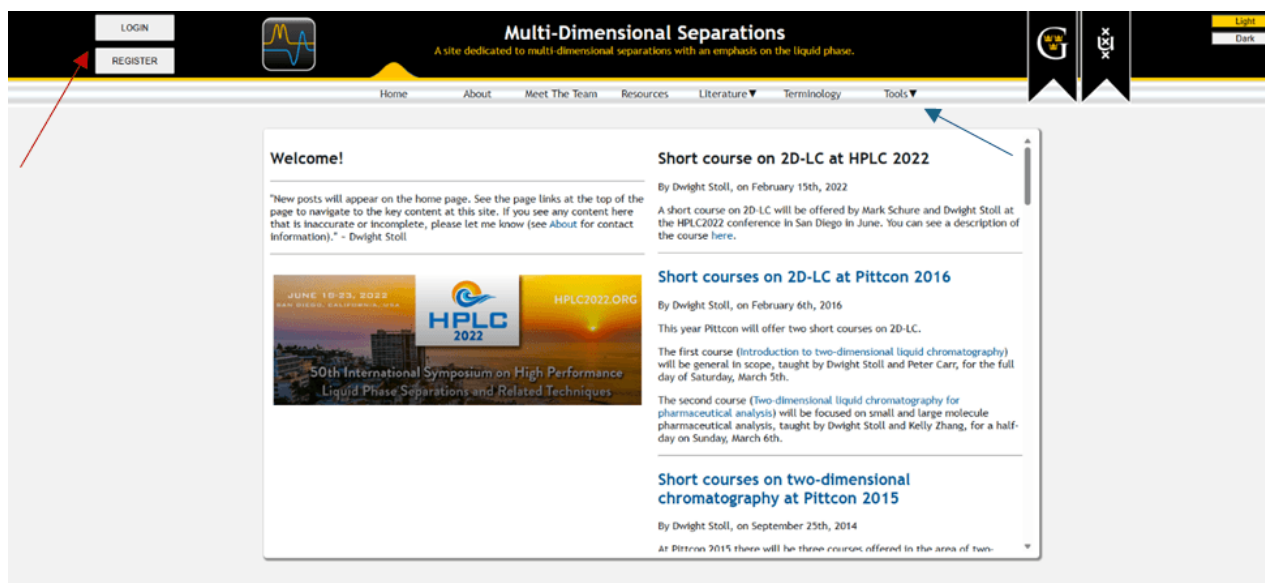
6.13: HPLC and UV-vis Prelab Assignment

Please read this entire module on ultraviolet-visible (UV-Vis) absorption spectroscopy. This module should take less than one lab period. It is part of a two-part series with the theme of caffeine analysis in beverages. First, in this module, you will create a serial dilution of caffeine and characterize the solutions using UV-vis. **Store your solutions in sealed vials for use in the next module.** You have three objectives:

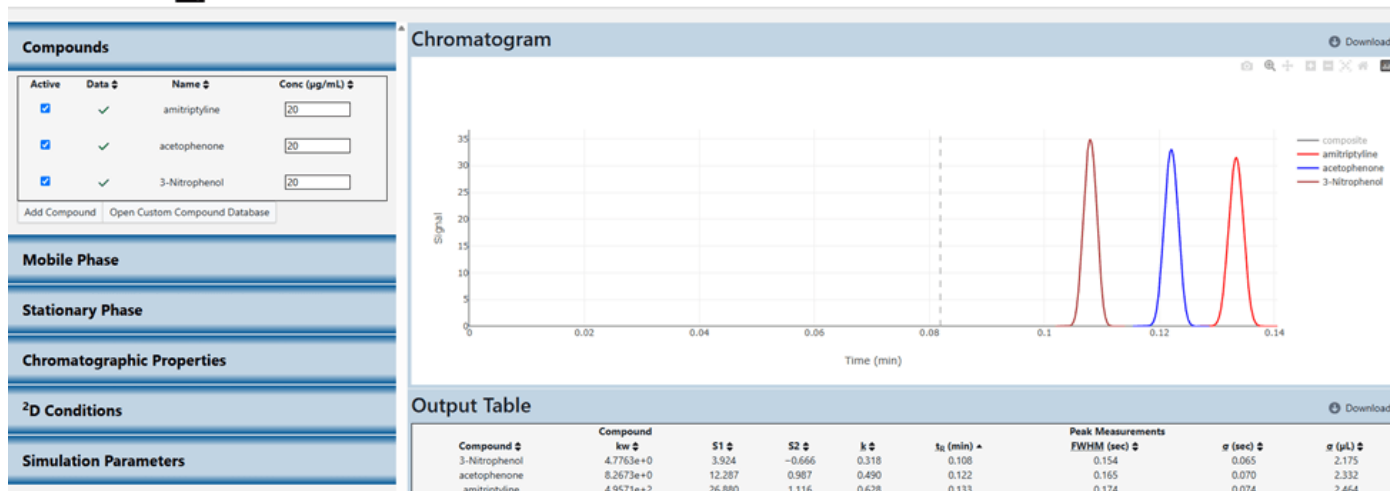
1. Create a series of caffeine solutions for use in this and the next module.
2. Determine the wavelength of maximum absorbance for each solution.
3. Validate the serial dilution using a control.

HPLC Simulation Pre-lab Assignment

1. (red arrow) Open the [Multi-Dimensional Separations Website](#) (do not use Safari). Click “login” on the top left. Username: jcm166, password: Duke401L
2. (blue arrow) Select tools, then MultiSim LC [BETA]

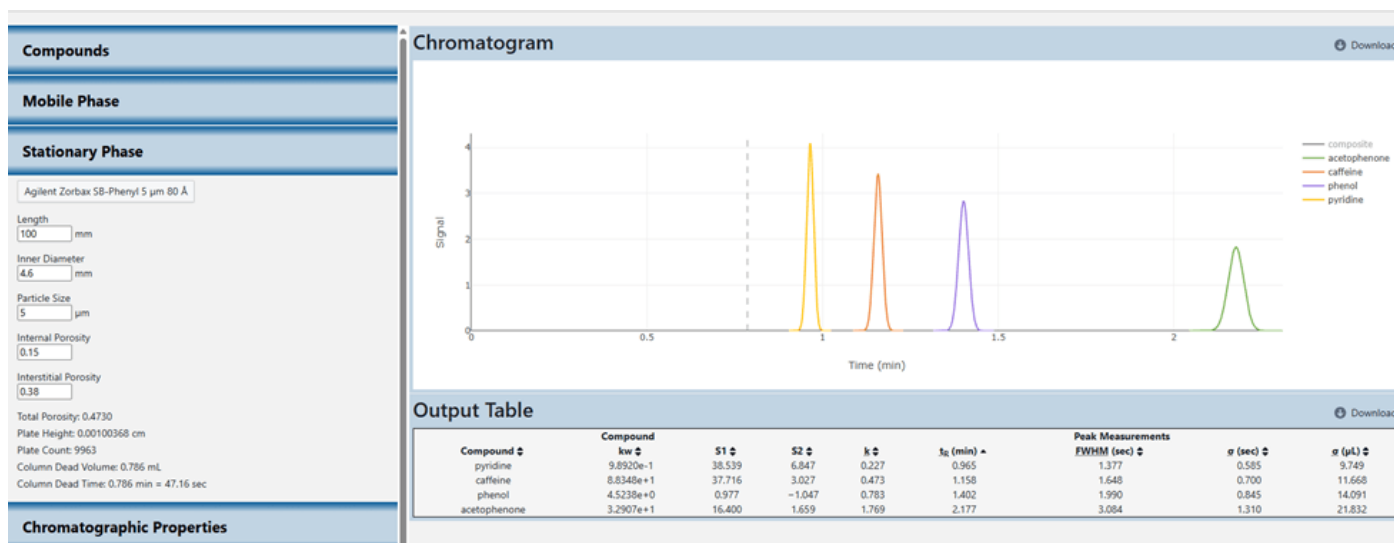


3. Under compounds, click “add compound.” Uncheck the default compounds and select caffeine, acetophenone, phenol, and pyridine



4. Use the Mobile Phase tab to select the appropriate mobile phase conditions. Select ACN as your mobile phase B. Changing "Mobile Phi" changes the percent of mobile phase B in the mobile phase mixture.

5. Under the stationary phase tab, you can change the column parameters. For our applications, we will be using the Agilent Zorbax SB C18 column, with 100mm x 4.6mm dimensions with 5µm pore size. The other parameters can be ignored for now



6. Finally, under the Chromatographic Properties tab, set the flow rate to 1mL/min and keep the rest of the parameters as they are.

Feel free to play around with these parameters to see how changing the parameters affects the retention time and resolution of peaks!

Pre-lab Assignment

1. Look up the structures of the four compounds. Based on their structures, why do these compounds elute in the order that they do?

2. Play around with the mobile phase composition by changing the value under "Mobile Phi". This changes the percent acetonitrile in the theoretical mobile phase composition. Why does increasing the amount of acetonitrile in the mobile phase decrease the retention time of these compounds? Does the change in retention time of compounds scale linearly with acetonitrile composition?

3. You may notice that the retention time of the four compounds do not change at the same rate relative to one another when the mobile phase composition is adjusted. Why do you think this is?
4. Under the Mobile Phase tab, you can change the simulation to a gradient method. This gradually increases the percent of mobile phase B from Φ Initial to Φ Final over a certain amount of time (Gradient Time). What effects on the peak shape/ elution time when you run the simulation with a mobile phase gradient?
5. (optional) If you wish to measure [caffeine] in your favorite beverage, bring some to lab.

Complete the following in your lab notebook and be prepared to discuss these points with your TA.

1. This experiment involves the creation of a multi-point calibration curve. What are the advantages to using multi-point calibration compared to single-point calibration?
2. When making your HPLC methods in this experiment, you will be tasked with selecting a wavelength for the detector. Assuming you only care about quantitating caffeine, how should the best wavelength(s) be chosen? If you wanted to quantitate another compound using a two-component solution, how would you determine the best wavelength(s) to use?
3. Prepare a procedure for creation of the caffeine standard solutions described in this module.
4. Prepare a table in your notebook to record absorbance data.
5. Some of your caffeine standards have absorbances exceeding 1 absorbance unit, meaning they are too concentrated to be measured reliably in their current form using a standard cuvette. Suggest a strategy to overcome this limitation so that you can still construct a calibration curve that includes all your standard solutions. *Hint: Consider the components of Beer's Law.*

6.14: Pre-Lab Assignment

Please read this entire module on ultraviolet-visible (UV-Vis) absorption spectroscopy. This module should take less than one lab period. It is part of a two-part series with the theme of caffeine analysis in beverages. First, in this module, you will create a serial dilution of caffeine and characterize the solutions using UV-vis. **Store your solutions in sealed vials for use in the next module.** You have three objectives:

1. Create a series of caffeine solutions for use in this and the next module.
2. Determine the wavelength of maximum absorbance for each solution.
3. Validate the serial dilution using a control.

Written Pre-lab Assignment

Complete the following in your lab notebook and be prepared to discuss these points with your TA.

1. In writing, please explain the advantages to using multi-point calibration compared to single-point calibration.
2. Describe how you should choose appropriate wavelengths in the following scenarios:
 - a. to quantitate a single component in solution.
 - b. to quantitate two or more components in a solution.
3. Prepare a procedure for creation of the caffeine standard solutions described in this module.
4. Prepare a table in your notebook to record absorbance data.
5. Some of your caffeine standards have absorbances exceeding 1 absorbance unit, meaning they are too concentrated to be measured reliably in their current form using a standard cuvette. Suggest a strategy to overcome this limitation so that you can still construct a calibration curve that includes all your standard solutions. *Hint: Consider the components of Beer's Law.*

During the in-lab portion of this module, you will use different mobile phase conditions to separate mixtures of compounds using reverse-phase HPLC. Please prepare for the in-lab meeting by reading the entire contents of this Module and completing the tasks described below:

Pre-lab assignment: learn the theory that explains separation of compounds using reverse phase chromatography

Investigate parabens: In Part 1, you will separate a mixture of parabens. Parabens are a class of chemicals widely used as preservatives in the cosmetic and pharmaceutical industries. Take the time to learn about parabens and look up general paraben structures to develop an understanding of their chemical structure and polarity. The paraben mixture that you will work with in lab has already been prepared for you and contains **4-hydroxy benzoic acid**, **methyl-4-hydroxy benzoate**, **ethyl-4-hydroxy benzoate**, and **propyl-4-hydroxy benzoate**. Draw these structures and order them according to their relative polarity.

Simulated separation: Go to go to the [Multi-Dimensional Separations website \(click here\): http://www.multidlc.org/hplcsim/hplcsim.html](http://www.multidlc.org/hplcsim/hplcsim.html). You will use this website to simulate chromatographic separation of a mixture of parabens. Follow the steps in the box below:

Follow these steps to simulate the HPLC separation of paraben compounds:

1. Open the [Multi-Dimensional Separations Website](http://www.multidlc.org/hplcsim/hplcsim.html) (do not use Safari). Take note of three regions of the screen: (1) **The controls are on the left** (highlighted with a red box and arrow in the figure below). With the controls, you can add or remove compounds, and change the solvent conditions and gradient parameters. (2) A simulated chromatogram is shown at the right. The simulation uses the [van Deemter equation \(discussed previously\)](#) along with physical properties of the analytes to predict their elution time and peak width in a standard C18 reverse-phase HPLC column. (3) The list of compounds and their respective parameters are listed at the bottom of the screen.

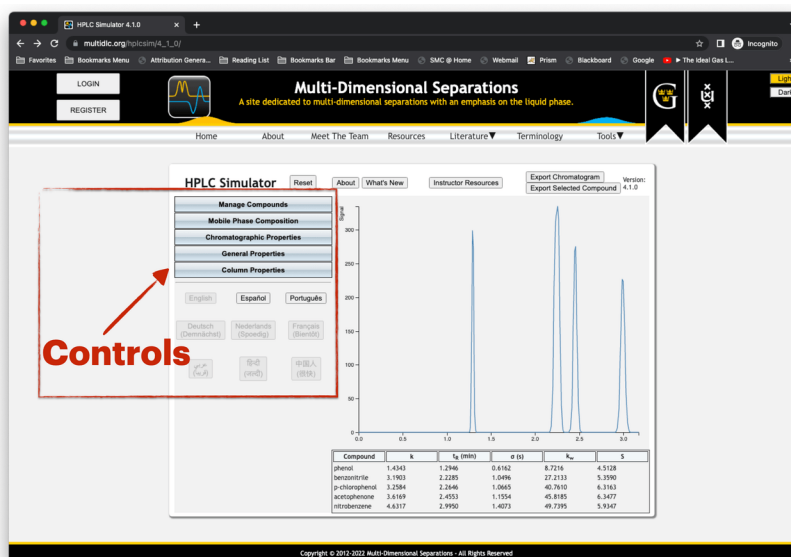


Figure 6.14.1: The opening page of the HPLC Simulator. The controls panel is highlighted with a red box and arrow.

- Go to **Manage Compounds** tab of the control panel (top). Select the available paraben compounds (three of them will be in the paraben mixture you will work with in lab) and deselect the compounds that were pre-selected by default. Note that each paraben compound has multiple possible names - their common names are used in the control panel, but their IUPAC names are given in this manual.
- Notice that you can **locate each compound** in the chromatogram. Examine the chromatogram at the right, and the compounds list table listed under the chromatogram. The retention time (t_R) is given for each compound. The y axis of the chromatogram corresponds to t_R . Click on each of the compounds in the list (below the chromatogram). The selected compound will appear orange in the Simulated Chromatogram window.
- Go to **Column Properties** tab of the control panel (bottom). Notice there is only one option for the column (Agilent SB-C18 column), but there are options to change the dimensions of the column (length, inner diameter, porosity, and particle size). Match the dimensions of the columns in our labs at Duke (column details are in the section on [HPLC components at Duke](#)). Take note of the calculated void volume and void time (t_0).
- Go to **Column Properties** tab of the control panel. Change the temperature to 20°C or something close to room temperature. You can change the temperature by sliding the blue dot to the left and right - when you do this, the chromatogram will change accordingly. Notice the effect that temperature has on the retention time of all analytes.
- Go to **Mobile Phase Composition**.
 - Change solvent B to Methanol and select **Isocratic Elution Mode**. Change the percent of solvent B to match the five isocratic conditions that you will use in Part 1 of the in-lab portion. Observe how the peak resolution, peak shape, and retention times change with different solvent conditions.
 - Select **Gradient Elution Mode**. The default is a linear gradient from 5% to 95% solvent B over 5 minutes. Play around with the length of time of the gradient, and then try some different gradient conditions and observe how the peak resolution and peak shapes change.

Pre-lab questions:

- Draw the structures of the compounds that will be used in part 1 of the in-lab portion, as well as any addition parabens that were used in the simulation. Compare your predicted elution order to the simulated elution order. Explain why the compounds elute in this order.
- One of the components in our real mixture, 4-hydroxy benzoic acid, is not available in the simulated list of compounds. Where do you expect this compound to elute relative to the other parabens? Please consider that this molecule is unique compared to the other parabens due to its carboxylic acid functional group, and explain whether that factor should increase or decrease its retention time.
- Describe the effect of a gradient elution on resolution and peak shape, compared to an isocratic elution.
- Make a list of materials needed to conduct calibration of the HPLC and measurement of the [caffeine] in beverage samples.
- What wavelength(s) will you use to monitor caffeine as it elutes from the HPLC?
- (optional) If you wish to measure [caffeine] in your favorite beverage, bring some to lab.

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