

Active Learning Worksheets for Analytical Chemistry

Faculty Resources and Answer Keys

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Open Educational Resource

Designed for use with *Analytical Chemistry 2.1* by David Harvey

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Active Learning Worksheets for Analytical Chemistry Front Matter

This collection includes active-learning worksheets, answer keys, and instructor-facing faculty resources for a one-semester analytical chemistry course. The roadmap below summarizes the worksheets by unit, estimated completion time, recommended sequencing, technology needs, and associated sections from Harvey's Analytical Chemistry 2.1. The worksheets are designed to be adaptable; instructors may use the full sequence, select individual activities, or reorder materials to match their own course structure.

Analytical Foundations

Worksheet	Time (mins)	Use/ Sequencing	Tech Needed	Analytical Chemistry 2.1 Sections
1. Applying the Analytical Problem-Solving Framework	45-60	Can be used independently; introduces core ideas	Internet	1.2: The Analytical Perspective . “ Rapid coronavirus tests: a guide for the perplexed ” (Nature, Feb 2021)
2. Evaluating Glassware Accuracy and Precision	75	Sequential foundation	None	3.4 Selecting an Analytical Method 4.2 Characterizing Experiment Errors
3. Correcting Mass and Propagating Uncertainty	90-120	Builds on #2	None	16.9 Correcting Mass for the Buoyancy of Air 4.3 Propagation of Uncertainty
4. Designing a Representative Sampling Strategy	60	Can be used independently; introduces core ideas	None	7.1: The Importance of Sampling 7.2: Designing a Sampling Plan 7.3: Implementing the Sampling Plan
5. Analyzing and Interpreting Experimental Error	60-135	Best after #2-4; synthesis activity	Excel	4.2 Characterizing Experiment Errors 4.4 The Distribution of Measurements and Results 4.6 Statistical Methods for Normal Distributions
6. Solution Preparation and Error Considerations	75-90	Builds on #2-3	None	2.2: Concentration 2.5: Preparing Solutions

Equilibrium Chemistry I: Solubility, Acid-Base, and Buffers

Worksheet	Time (mins)	Use/ Sequencing	Tech Needed	Analytical Chemistry 2.1 Sections
7. Solubility and Back Titration Problem Solving	50-65	Standalone with equilibrium prep	None	6.4 Equilibrium Constants for Chemical Reactions 6.7 Solving Equilibrium Problems 9.2: Acid-Base Titrations
8. Fundamentals of Acid-Base Reactions	45-60	Acid-base foundation	None	6.4 Equilibrium Constants for Chemical Reactions 6.7: Solving Equilibrium Problems
9. Designing and Evaluating Buffer Systems	60-90	Builds on #8	None	6.8: Buffer Solutions
10. Titration Curve Calculations for Monoprotic Acids	75-90	Builds on #8-9	None	6.4 Equilibrium Constants for Chemical Reactions 6.7: Solving Equilibrium Problems 6.8: Buffer Solutions 9.2: Acid-Base Titrations

Equilibrium Chemistry II: Polyprotic Systems and Activity

Worksheet	Time (mins)	Use/ Sequencing	Tech Needed	Analytical Chemistry 2.1 Sections
11. Polyprotic Acid and Base Reactions and pH Calculations	60-90	Builds on #8-10	None	6.4 Equilibrium Constants for Chemical Reactions 6.7: Solving Equilibrium Problems 6.8: Buffer Solutions
12. Plotting and Interpreting Polyprotic Titration Curves	60-75	Synthesis of #10-11	Excel	6.4 Equilibrium Constants for Chemical Reactions 6.7: Solving Equilibrium Problems 9.2: Acid-Base Titrations 16.11: Acid Dissociation Constants
13. Identifying Principal Species by pH	45-60	Builds on #9-11	None	6.6: Ladder Diagrams 6.8: Buffer Solutions
14. Titration Curve Calculations and Analysis for Diprotic Systems	90-115	Builds on #10-13	None	6.4 Equilibrium Constants for Chemical Reactions 6.7: Solving Equilibrium Problems 6.8: Buffer Solutions 9.2: Acid-Base Titrations
15. Charge and Mass Balance, Ionic Strength, and Activity	50-75	Can stand alone after equilibrium review	None	6.7: Solving Equilibrium Problems 6.9: Activity Effects

Spectroscopic Methods

Worksheet	Time (mins)	Use/ Sequencing	Tech Needed	Analytical Chemistry 2.1 Sections
16. Concepts and Calculations Associated with Light	60-75	Spectroscopy foundation	None	10.1: Overview of Spectroscopy 10.2: Spectroscopy Based on Absorption 10.5: Emission Spectroscopy 10.6: Photoluminescent Spectroscopy
17. Spectrophotometer Components and Comparisons	60-75	Recommended after #16	None	10.1: Overview of Spectroscopy 10.2: Spectroscopy Based on Absorption 10.3: UV/Vis and IR Spectroscopy
18. Beer's Law, Mixtures, and Deviations	45-60	Builds on #16-17	None	10.1: Overview of Spectroscopy 10.2: Spectroscopy Based on Absorption 10.3: UV/Vis and IR Spectroscopy
19. Atomic Spectroscopy Part 1: Fundamentals and Flame AA Instrumentation	60-70	Instrument-specific and modular	None	4.7: Detection Limits 10.1: Overview of Spectroscopy 10.2: Spectroscopy Based on Absorption 10.3: UV/Vis and IR Spectroscopy 10.4: Atomic Absorption Spectroscopy
20. Atomic Spectroscopy Part 2: Interferences, Calibration, and Sensitivity	60-90	Builds on #19	None	5.2: Calibrating the Signal 5.3: Determining the Sensitivity 5.4: Linear Regression and Calibration Curves 10.4: Atomic Absorption Spectroscopy 10.5: Emission Spectroscopy 10.7: Atomic Emission Spectroscopy
21. Uncertainty Calculations in Standard Curves	50-75	Builds on calibration basics and #20	Excel	5.4: Linear Regression and Calibration Curves

Electrochemical Methods

Worksheet	Time (mins)	Use/ Sequencing	Tech Needed	Analytical Chemistry 2.1 Sections
22. Electrochemistry Basics	60-80	Electrochemistry foundation	None	Review General Chemistry notes from <i>Chemistry 2e</i> from OpenStax 17.1 Review of Redox Chemistry 17.2 Galvanic Cells 17.3 Electrode and Cell Potentials 17.4 Potential, Free Energy, and Equilibrium
23. Exploring Ion-Selective Electrodes	55-70	Builds on #22	None	11.2: Potentiometric Methods

Chromatographic Methods and Method Selection

Worksheet	Time (mins)	Use/ Sequencing	Tech Needed	Analytical Chemistry 2.1 Sections
24. Chromatography Basics	50-65	Chromatography foundation	None	12.1: Overview of Analytical Separations 12.2: General Theory of Column Chromatography
25. Comparison of GC and HPLC	50-65	Builds on #24	None	12.4: Gas Chromatography 12.5: High-Performance Liquid Chromatography
26. HPLC Simulations	75-90	Recommended after #24-25	Internet	12.3: Optimizing Chromatographic Separations 12.5: High-Performance Liquid Chromatography
27. Selecting an Instrument	55-70	Best near end of course; synthesis activity	None	Review prior units; see Sections 11.2-11.4 as needed 11.2: Potentiometric Methods 11.3: Coulometric Methods 11.4: Voltammetric and Amperometric Methods

About This Collection

These worksheets were developed to support a flipped, activity-based approach to teaching CHEM 310: Chemical Analysis, a 300-level analytical chemistry course at North Central College in Naperville, Illinois. Since Fall 2016, I have used and refined these materials over ten semesters, continually improving the in-class learning experience through student feedback, performance data, and classroom observation.

Why These Materials Exist

Research supports the effectiveness of active learning in improving conceptual understanding, retention, and student performance in STEM disciplines, including analytical chemistry. However, when I first flipped my course, I quickly discovered that the greatest challenge wasn't creating videos or assigning textbook readings. It was writing high-quality in-class activities that used time meaningfully. This set of worksheets grew out of that need.

Over the years, the materials have evolved through multiple course offerings and been tested in classes of 8-20 students. They are organized to support students in building both their conceptual understanding and problem-solving fluency in real analytical chemistry contexts.

Developing Analytical Intuition and Experimental Design

Beyond mastering quantitative calculations and instrumental theory, a primary objective of this collection is to put students in the "shoes of the instructor" as the experimental designer. Throughout the curriculum, students are frequently asked not just to perform a measurement, but to justify the choice of method based on real-world constraints.

- Precision vs. Speed: Students evaluate how glassware selection and multi-step vs. single-step dilutions impact both the time required for a protocol and the final propagated uncertainty (Worksheets 2, 3, 6).
- Cost and Instrument Availability: Activities require students to compare instrument specifications to determine which is appropriate for trace analysis versus routine teaching lab use based on budget and sensitivity needs (Worksheets 17, 20, 23).
- Waste Management: Students weigh the accuracy of a method against its environmental impact, specifically calculating the volume of toxic waste generated by different preparation strategies (Worksheet 6).
- Methodological Decision-Making: Capstone scenarios require students to match specific techniques (e.g., atomic spectroscopy vs. ion-selective electrodes) to diverse analytical problems, justifying their selection based on detection limits, selectivity, and portability (Worksheets 23, 27).

By focusing on these trade-offs, the curriculum aims to transform students from those who can follow procedures into analysts who can design and troubleshoot an analytical process.

How These Worksheets Are Designed to Be Used

Most worksheets are designed to take 1–2 class periods (50–100 minutes) and are aligned with key sections of *Analytical Chemistry 2.1* by David Harvey. This alignment allows the collection to serve as a "plug-and-play" resource for instructors already using the Harvey text as their primary OER. While these worksheets are presented in a linear order that mirrors the textbook, they are designed to be modular and flexible. Instructors should feel empowered to reorder units to match their specific syllabus or laboratory schedule.

Ideally, students work in groups of 3–4 to check each other's math, reasoning, and interpretation. As formative activities, these worksheets are most effective when the instructor circulates throughout the room to check for understanding and provide "just-in-time" guidance. Pre-identified "Facilitated Discussion Pauses" (detailed in the Faculty Resources for each answer key) pinpoint specific moments where the instructor should stop group work for a brief, 5-minute whole-class debrief on a threshold concept or complex calculation.

While optimized for a "flipped" classroom, these materials are flexible enough to be adapted as in-class collaborative activities to replace traditional lectures, as pre-lab assignments to prepare students for specific instrumental techniques or statistical methods, or as supplemental practice for high-stakes topics like error propagation or polyprotic titration calculations.

Pacing and Flexible Implementation

The estimated completion times for each worksheet are intended as planning guides rather than strict limits. In my own course, student groups often work through these activities at different speeds during class. When students finish early and have already completed the preparation for the next topic, they may begin the next worksheet. If the class is not ready to move ahead, early finishers can use the remaining time for related online homework, lab-data analysis, or follow-up calculations. At times, students may be asked to complete a portion of a worksheet outside of class if they do not finish during the class period. When this occurs, I typically review key points in the following class and provide answer guidance through the learning management system so students can check their understanding.

For longer worksheets, the faculty resources identify natural stopping points that can be used to divide the activity across class periods, assign part of the worksheet as follow-up work, or help instructors decide which sections to emphasize during class. These stopping points are based on conceptual transitions within the worksheet rather than a fixed time cutoff, since class periods, student preparation, and course formats vary across institutions.

Outcomes and Student Impact

Student performance on standardized assessments was higher in the flipped, worksheet-driven course offerings than in earlier traditional lecture offerings. In my traditional lecture sections (2014–2015), students averaged in the 59th percentile with a median at the 60th percentile on the American Chemical Society Analytical Chemistry standardized final exam ($n = 33$). In the flipped model (2016–2026), that average rose to the 66th percentile with a 73rd percentile median score ($n = 122$). Beyond the exam data, I have found that this structure changes the way students use class time. They talk through calculations, compare reasoning with peers, catch mistakes before they become habits, and ask more specific questions because they are actively working with the material. This format gives students more time to practice analytical thinking with support, rather than encountering the hardest parts only when they are studying alone.

What's Included in This OER Collection

This OER collection is available in two companion versions: a student worksheet compilation and an instructor compilation containing faculty resources and suggested answers.

- Worksheets for key analytical chemistry concepts: sampling, statistics, error propagation, titration, acid–base chemistry, spectroscopy, chromatography, and electrochemistry
- Instructor-facing answer keys
- Estimated time guides
- Reading alignment with Analytical Chemistry 2.1 by Harvey
- Modular and chapter-based organization for flexible course integration
- Supporting spreadsheet templates, simulator links, and other supplemental materials for selected activities, when applicable

Using the Answer Keys

The instructor compilation includes faculty resources, pacing notes, discussion prompts, and suggested answers intended for instructor use. The suggested answers are intended to support instructors in facilitating discussion and evaluating student work. In some cases, the answers include additional explanation, examples, or context beyond what students would be expected to generate independently. These expanded responses are included as instructor support and should not necessarily be interpreted as the minimum level of detail required for a correct student response. Instructors should use their discretion in determining appropriate expectations based on course emphasis, assigned readings, and classroom discussion.

Access, Licensing, and Attribution

This collection is shared openly through Illinois OER Commons. All materials are editable and downloadable and are intended for adaptation and reuse. Instructors are encouraged to adapt, remix, and reorder these worksheets to fit their own course structure, laboratory sequence, institutional context, and student needs.

Portions of these materials, including selected figures, tables, excerpts, and adapted questions, are reproduced or adapted from David Harvey's *Analytical Chemistry 2.1*, which is also licensed under CC

BY-NC-SA 4.0. Individual figures, tables, excerpts, and adapted activities include source attribution and indicate when modifications were made. Instructors who revise these materials should retain existing source attributions and add appropriate attribution for any additional content they incorporate.

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This collection includes both student-facing worksheets and instructor-facing answer keys. The answer-key files include faculty resources, pacing notes, discussion prompts, and suggested answers intended for instructor use. Instructors should remove or withhold instructor-facing notes before distributing materials to students.

Acknowledgments

The development of this worksheet collection would not have been possible without the insight, encouragement, and contributions of many members of the North Central College community. I extend deep thanks to the students in CHM 210/CHEM 310 over the past ten years, whose feedback, persistence, and thoughtful engagement helped shape and improve these materials semester after semester. I am grateful to my colleagues in the Chemistry Department for supporting a flipped-classroom model and for offering valuable conversations on pedagogy, sequencing, and assessment. I also thank the Oesterle Library faculty and staff, especially Thomas Jencks and Michelle Boule Smith, for leading the OER grant team and for their support with formatting and dissemination. Many thanks as well to Noah Cooperider, Student Disability Services Coordinator at North Central College, for reviewing these materials and providing guidance on accessibility and flexibility standards. I am also grateful to the reviewers and editors of the Analytical Sciences Digital Library (ASDL) for their thoughtful feedback and suggestions, which helped strengthen the clarity and usefulness of these materials.

Finally, this project is made possible in part by state funds from the Illinois State Library made possible by Illinois General Assembly HB5535 as administered by the Consortium of Academic and Research Libraries in Illinois. Their support of open, inclusive, and adaptable instructional resources has been instrumental in bringing this collection to life.

Use of Generative AI Tools

Generative AI tools were used as part of the revision process for this collection. ChatGPT by OpenAI and NotebookLM by Google were used to support high-level review, improve organization and consistency, refine language and formatting, brainstorm examples, organize modular content, and draft or revise portions of the instructor-facing faculty resources and front matter.

The student-facing worksheets, answer keys, and faculty resources were reviewed, revised, and edited by the author, who remains responsible for the accuracy of the chemistry, the alignment with course goals, the sequencing of activities, and the final pedagogical decisions. AI-generated suggestions were accepted, revised, rejected, or rewritten based on the author's disciplinary expertise and classroom experience. Faculty adopting or adapting these materials should follow their institution's policies and guidelines regarding the use, attribution, and disclosure of generative AI.

Teaching Analytical Chemistry with Purpose

This OER resource is built on the belief that students learn analytical chemistry best when they practice it: interpreting data, troubleshooting procedures, comparing methods, and applying logic to solve real problems. My goal is for these worksheets to make that kind of thinking easier to teach, easier to practice, and easier for students to see as part of analytical chemistry. If you adopt or adapt these materials, I welcome collaboration and feedback.

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Active Learning Worksheets for Analytical Chemistry

#1 Analytical Foundations: Applying the Analytical Problem-Solving Framework

Faculty Resources

(Delete before sharing with students):

- **Analytical Context and Framing:** I use this worksheet on the first day of class to introduce the analytical problem-solving framework shown in Figure 1 and to show students why analytical chemistry matters. The COVID testing context works well because most students remember that testing was a real problem that had to be solved quickly, and many pre-health students can immediately see the connection to clinical decision-making. I would avoid treating this as a “history of COVID” lesson or as a paper students need to master in detail. The point is that, in 2020, the analytical problem was not simply to “make a test.” It was to balance speed, cost, reliability, accessibility, and public-health need.
- **Key Concepts and Common Challenges:** Students often confuse sensitivity, which relates to detecting true positives, with specificity, which relates to avoiding false positives. They may also struggle with the idea that an “imperfect” rapid test can still be analytically useful if the problem definition prioritizes speed, cost, and accessibility over absolute detection limits. I use this worksheet to preview ideas that will come up throughout the semester, including method selection, validation, precision, accuracy, sensitivity, specificity, detection limits, and trade-offs in analytical design.
- **Facilitated Discussion Pauses:** This works best as a group discussion activity. Students can work through the questions in small groups, but I like to reserve the last 10–15 minutes of class for whole-class discussion. Pause after Question 4, which focuses on validation. Ask the class: “If RT-PCR is the gold standard, why would rapid tests still be useful?” This helps students articulate the trade-offs that are central to analytical method development. Once Question 7 is wrapped up, have a broader class discussion about the design decisions students generated.
- **Calculation Support:** While this activity is mostly conceptual, help students connect it to quantitative thinking by asking them to define what “low viral load” means in terms of concentration and detection limits. If they have not heard terms like detection limit yet, mention that these are ideas they will learn more formally later in the semester. The goal is not for students to complete detailed calculations here, but to start seeing analytical chemistry as a quantitative decision-making process.
- **Synthesis and Extension Questions:** For Question 7, push students to make a design decision: “As the designer, would you rather your test have a 5% false-negative rate or a 5% false-positive rate? Why?” This is a good place to emphasize that there may not be one universally correct answer; the better answer depends on the purpose of the test, the consequences of each type of error, and the setting in which the method will be used.
- **Pacing Note:** This worksheet typically fits within one class period if students complete the reading before class, even with some course introduction or syllabus material. If time is limited, Questions 1–6 can be completed during class and Question 7 can be assigned as a follow-up reflection or used to start the next class. I do not usually treat the article itself as testable content; I use it as a vehicle for introducing how students will be expected to think in analytical chemistry.

Active Learning Worksheets for Analytical Chemistry

#1 Analytical Foundations: Applying the Analytical Problem-Solving Framework

Answers

Before You Begin

- Read [1.2: The Analytical Perspective](#).
- Read “[Rapid coronavirus tests: a guide for the perplexed](#)” (Nature, Feb 2021)¹

What You Should Already Know

- General familiarity with how scientists design experiments
- Introductory understanding of experimental error, validation, and iteration

What You’ll Learn

- Define and describe the key steps in the analytical problem-solving process
- Identify how these steps are used in the context of rapid COVID-19 test development
- Evaluate scientific work using performance metrics (e.g., accuracy, sensitivity)

What You’ll Practice

- Analyze how real-world analytical problems are framed and solved
- Describe the role of controls, validation, and reproducibility in method development
- Connect scientific literature to the analytical framework

Estimated Completion Time: 45-60 minutes

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Active Learning Worksheets for Analytical Chemistry, Version 1.0, 2026

¹ Guglielmi, G. Rapid Coronavirus Tests: A Guide for the Perplexed. *Nature* **2021**, 590 (7845), 202–205.
<https://doi.org/10.1038/d41586-021-00332-4>.

The Analytical Approach

Many analytical chemists describe this perspective as an analytical approach to solving problems.

Although there likely are as many descriptions of the analytical approach as there are analytical chemists, it is convenient to define it as the five-step process shown in Figure 1.2.1. Introductory text in this section is adapted from David Harvey, *Analytical Chemistry 2.1*, Section 1.2, licensed under CC BY-NC-SA 4.0.

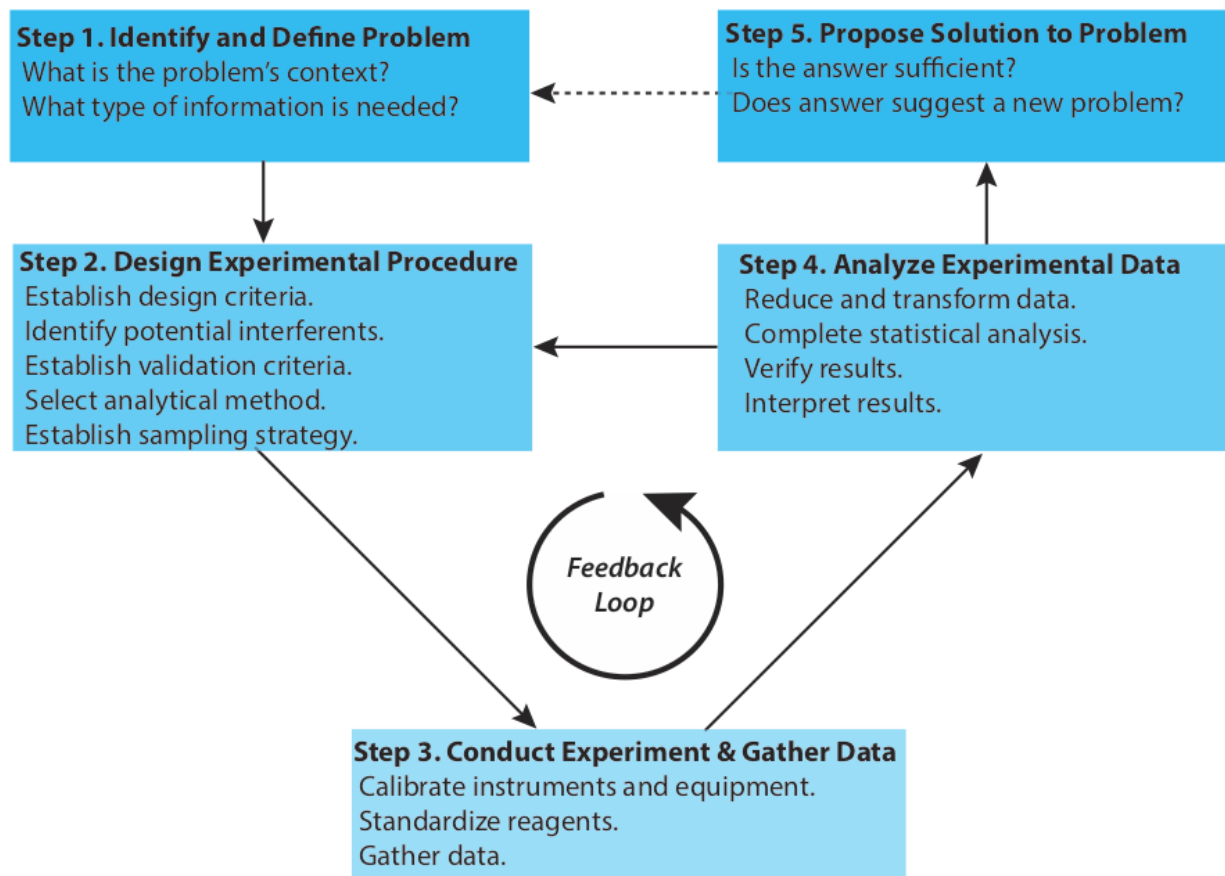


Figure 1. Flow diagram showing one view of the analytical approach to solving problems. Adapted from Figure 1.2.1 in David Harvey, *Analytical Chemistry 2.1*, licensed under CC BY-NC-SA 4.0. Harvey's figure was modified after Atkinson, G. F. *J. Chem. Educ.* **1982**, 59, 201–202.

This analytical approach can be used to solve problems that arise in everyday life. For example, when the pandemic spread rapidly in 2020, it became imperative to determine who was infected with COVID-19. Read “[Rapid coronavirus tests: a guide for the perplexed](#)” (Nature, Feb 2021). As you read the article, pay particular attention to how it emulates the analytical approach and consider the following questions, which were adapted from David Harvey, *Analytical Chemistry* 2.1, Section 1.2, licensed under CC BY-NC-SA 4.0.

Questions

1. What is the analytical problem, and why is it significant from both a societal and a scientific perspective?

The analytical problem involves developing rapid, accurate, and accessible tests to detect COVID-19 infections. The societal significance is the essential need for accurate diagnostics to control the

pandemic, guide isolation measures, and allocate medical resources effectively. The scientific significance is that it pushed advancements in diagnostic technology, emphasizing the need for sensitivity, specificity, and scalability in analytical methods.

2. What factors (e.g., accuracy, precision, sensitivity, specificity) were most important in designing the experiments? How do these relate to the key performance indicators of an analytical method?

Accuracy and precision were important to ensure consistent and correct results to gain public and healthcare trust. Sensitivity and specificity were vital for detecting true positives while minimizing false positives/negatives. These factors align with validation metrics in analytical chemistry, ensuring that the method is both reliable and practical.

3. What experimental controls and standards did the authors use, and how did they address potential sources of error? How might these strategies influence the reproducibility of results?

The authors likely used known positive and negative samples to calibrate and validate their tests. They may have included controls to verify the absence of cross-reactivity with other coronaviruses. These likely optimized reagents and strict procedural guidelines to minimize variability and systematic errors. By standardizing methods and reporting protocols, they ensured the method could be replicated across different labs and settings.

4. How did the authors validate their method? Consider both internal validation (e.g., replicates) and external validation (e.g., comparison with gold-standard methods).

Multiple replicates under controlled conditions confirmed the test's reliability within the same experimental framework. Then, comparisons with RT-PCR, the gold standard for COVID-19 detection, allowed them to assess the sensitivity and specificity of rapid tests in real-world conditions.

5. Did the authors iterate through steps of method development (e.g., problem definition, data collection, analysis)? Identify specific examples of iteration in the article.

Yes, the development of rapid tests followed an iterative process. First, the problem was identified: the need for rapid and accurate COVID-19 detection methods. Then, initial experiments on antigen and molecular tests were conducted to evaluate performance. These tests were then refined based on observed challenges, such as false negatives due to low viral loads. For example, adjustments to sample preparation and assay design were likely based on initial feedback and testing outcomes.

6. Was the analytical problem resolved effectively? Evaluate the solution based on the balance of scientific rigor, practicality, and impact.

The problem was resolved to a significant degree; rapid tests became a cornerstone of the pandemic response. The methods were validated against established benchmarks, demonstrating reliability. The tests were inexpensive, fast, and easy to use, making them accessible globally. They played a key role in identifying and isolating cases, though challenges like false negatives persisted.

7. If you were to design a follow-up study to improve upon this work, what would you focus on, and why?

There are many possible follow-up studies. For instance, one could work to improve sensitivity for low viral loads to ensure earlier detection to prevent spread. Another option would be to design tests that are equally effective in resource-limited settings. A third option could be to develop tests capable of detecting multiple pathogens simultaneously. Such studies would build on the existing methods, addressing their limitations and broadening their applications.

Active Learning Worksheets for Analytical Chemistry

#2 Analytical Foundations: Evaluating Glassware Accuracy and Precision

Faculty Resources

(Delete before sharing with students):

- **Analytical Context and Framing:** Bring a 100 mL volumetric flask and a 25 mL pipet to the front of the room. Ask: “If I use this pipet four times to fill the flask, is it just as accurate as using the flask once?” This usually gets students to notice that repeated use of smaller glassware is not automatically equivalent to one larger-volume measurement. Having students physically see the glassware, rather than only looking at pictures, helps make the accuracy and precision comparisons feel more concrete.
- **Key Concepts and Common Challenges:** The primary hurdle is distinguishing absolute tolerance, such as ± 0.03 mL, from percent relative tolerance. Students often assume smaller glassware is better because the absolute tolerance is smaller, without realizing the percent relative tolerance may be significantly higher. Question 7 is an important place to reinforce that absolute and relative tolerance answer different questions. This idea comes back later when students compare the data in Tables 2 and 3 and evaluate accuracy and precision from actual measurements.
- **Facilitated Discussion Pauses:** After students rank the data in Tables 2 and 3, pause to compare Alex’s and Denise’s data. This is a useful time to distinguish between random error, such as Alex’s scatter, and systematic error, such as Denise’s consistent “blow-out” error. Also remind students that some rankings may include ties. For example, if two students have equivalent accuracy, students do not need to force a ranking between them.
- **Calculation Support:** For Table 1, consider splitting the calculations among group members so students can compare patterns without spending too much class time on repeated calculations. Remind students that percent relative standard deviation is the standardized way to compare the spread or precision of different volume measurements regardless of the total volume. For the later data-analysis questions, encourage students to connect each calculation back to the meaning of accuracy, precision, systematic error, or random error rather than treating the calculations as isolated steps.
- **Synthesis and Extension Questions:** I use the final questions to push students beyond simply ranking the glassware or choosing the option with the smallest tolerance. By this point in the worksheet, students have compared absolute and relative tolerance, interpreted real measurement data, and distinguished accuracy from precision. The synthesis step is asking them to use all of that information to make a practical analytical decision. In lab, the most accurate glassware is not automatically the best choice. Sometimes the sample is limited, the reagent is expensive, the waste stream matters, or the glassware choice makes the procedure harder than it needs to be. I want students to start seeing accuracy as one part of a larger analytical decision, not the only factor that matters.
- **Pacing Note:** If this worksheet needs to be divided across class periods, a natural stopping point is after Question 9. Up to that point, students focus on glassware labels, tolerances, and expected accuracy and precision. The remaining questions shift to interpreting experimental data and comparing real student performance. If time is limited, the data-analysis section can be assigned as follow-up work, but students benefit from at least some class discussion of Tables 2 and 3 because that is where the accuracy/precision distinction becomes more practical.

Answer Keys for Active Learning Worksheets for Analytical Chemistry

#2 Analytical Foundations: Evaluating Glassware Accuracy and Precision

Before You Begin

- Read [3.4 Selecting an Analytical Method](#)
- Read [4.2 Characterizing Experiment Errors](#)

What You Should Already Know

- Basic understanding of significant figures and measurement uncertainty.
- Conceptual familiarity with moles, solutions, and concentration (especially for volumetric work).
- Introduction to error types (systematic vs. random).
- Experience using volumetric glassware (pipets, flasks, graduated cylinders) even if not in-depth.

What You'll Learn

- Define and distinguish between accuracy, precision, systematic error, and random error.
- Interpret volumetric glassware labels and explain their significance in quantitative analysis.
- Use manufacturer-provided tolerances to determine the expected measurement range for glassware.
- Calculate percent error, standard deviation, and relative standard deviation from experimental data.
- Compare the precision and accuracy of different types and sizes of volumetric glassware.
- Evaluate real-world student data to identify sources and types of experimental error.

What You'll Practice

- Analyze experimental data to identify patterns in accuracy and precision.
- Apply quantitative reasoning to calculate and interpret error metrics.
- Draw conclusions about the reliability of different measurement techniques based on statistical evidence.
- Explain how procedural differences can lead to systematic or random errors.
- Rank experimental performance based on well-justified quantitative criteria.

Estimated Completion Time: 75 minutes

Author: Rebecca L. Sanders

Active Learning Worksheets for Analytical Chemistry, Version 1.0, 2026

Labels on Volumetric Glassware

1. Obtain from your instructor a 100 mL Class A volumetric flask. Identify what each of the following labels represents:

- A: **Class A Glassware**
- TC: **To contain the nominal volume**
- 20 °C: **Calibrated to hold the specified volume at 20 °C**
- 100 mL: **Nominal volume**
- ± 0.08 mL: **Tolerance**
- $\Phi 13$ **Standard taper stopper size 13**

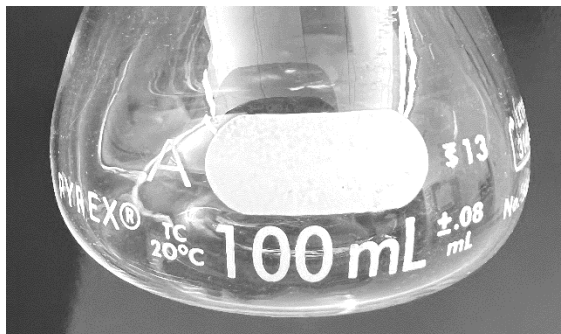


Figure 1. Image of a 100 mL volumetric flask.

2. Obtain from your instructor a 15 mL Class A volumetric pipet. Identify what each of the following labels represents:
 - 15 mL: **Nominal volume**
 - TD: **To deliver**
 - 20 °C: **Calibrated to hold the specified volume at 20 °C**
 - ± 0.03 mL: **Tolerance**

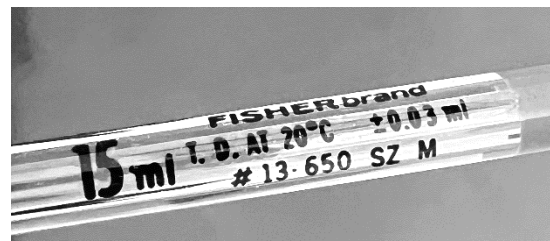


Figure 2. Image of a 15 mL volumetric pipet.

1. The manufacturers of analytical instruments and equipment, such as glassware and balances, usually provide a statement of the item's maximum **measurement error**, or **tolerance**. For example, a 10-mL volumetric pipet has a tolerance of ± 0.02 mL, which means the pipet delivers an actual volume within the range 9.98–10.02 mL at a temperature of 20 °C. Although we express this tolerance as a range, the error is determinate; that is, the pipet's expected volume is a fixed value within this stated range. Text adapted from David Harvey, *Analytical Chemistry 2.1*, Section 4.2, licensed under CC BY-NC-SA 4.0.

On the following page, Table 1 shows the measurement errors associated with Class A volumetric flasks and volumetric pipets. Obtain any necessary glassware from your instructor to fill in the missing information in the table. Then, calculate percent relative tolerance for any glassware that is needed and fill out the information in table 1.

Table 1. Measurement errors for Class A volumetric glassware. Adapted from Table 4.2.1 in David Harvey, *Analytical Chemistry 2.1*, licensed under CC BY-NC-SA 4.0.

Volumetric Flasks			Volumetric Pipets		
Capacity (mL)	Tolerance (mL)	Percent Relative Tolerance (%)	Capacity (mL)	Tolerance (mL)	Percent Relative Tolerance (%)
5	±0.02	0.4	1	±0.006	0.6
10	±0.02	0.2	2	±0.006	0.3
25	±0.03	0.12	5	±0.01	0.2
50	±0.05	0.1	10	±0.02	0.2
100	±0.08	0.08	15	±0.03	0.2
250	±0.12	0.05	25	±0.03	0.12
500	±0.20	0.04	50	±0.05	0.1
1000	±0.30	0.03	100	±0.08	0.08

3. For lab, you will use a 25 mL volumetric flask. What is the tolerance of the 25 mL volumetric flask? What is the expected volume range (in mL) that should be contained by the volumetric flask?

Tolerance = ± 0.03 mL; Range = 24.97-25.03 mL

4. For lab, you will use a 25 mL volumetric pipet. What is the tolerance of the 25 mL volumetric pipet? What is the expected volume range (in mL) that should be delivered by the volumetric pipet?

Tolerance = ± 0.03 mL; Range = 24.97-25.03 mL

Accuracy describes how closely the result of an experiment agrees with the “true” or expected result. Adapted from David Harvey, *Analytical Chemistry 2.1*, Section 4.2, licensed under CC BY-NC-SA 4.0. We can express accuracy as an absolute error, e

$$e = \text{obtained result} - \text{expected result}$$

or as a percentage error, % e

$$\%e = \frac{|\text{obtained result} - \text{expected result}|}{\text{expected result}} \times 100$$

Precision measures the variability of the individual results of a sample that is measured multiple times. The closer the agreement between individual analyses, the more precise the results. We use standard deviations to determine the spread in a data set and percent relative standard deviation to compare standard deviations from one data set to another data set.

$$\% \text{relative standard deviation} = \frac{\text{standard deviation}}{\text{average}} \times 100$$

5. Based on the tolerance of the 25 mL volumetric flask and pipet listed in table 1, which would you expect to be more accurate? More precise?

They have the same tolerance so they should both, in theory, be equally accurate and precise.

6. Consider the tolerance of the 15 and 25 mL volumetric pipets listed in table 1. Which would be more accurate? Why?

While both have tolerance values of ± 0.03 mL, their percent relative tolerance differs. The 15 mL and 25 mL flasks have a percent relative tolerance values of 0.2% and 0.12%, respectively. Therefore, the 25 mL volumetric pipet should be more accurate because it has the smaller percent relative tolerance.

7. If you wanted to make a solution at a known concentration as accurately as possible, would you choose to make the solution using glassware that holds larger volumes or smaller volumes? Why?

You would choose a larger piece of glassware because it generally has a lower percent relative tolerance.

8. Describe three reasons why you might not choose to measure a volume using an approach that is most accurate approach.

There are numerous reasons why one might choose to measure a volume using a less accurate approach. You might not want to generate as much waste, you might be working with a costly material or have a limited supply of a chemical that you can't measure in large quantity. You may not even need to measure a volume as accurately as possible.

Data Collected Using Volumetric Glassware

Consider the accuracy and precision of four students using 25 mL Class A volumetric flasks and pipets to measure 25 mL of deionized water in triplicate. Their results are listed in the tables below.

Table 2. Individual and average volumes contained by a 25 mL Class A Volumetric Flask.

Student	Trial 1	Trial 2	Trial 3	Average Volume (mL)	% Error	Standard Deviation (mL)	% Relative Standard Deviation
Alex	25.02	25.36	25.08	25.15	0.61	0.18	0.7
Billy	25.06	25.09	25.12	25.09	0.36	0.03	0.12
Charlie	24.98	25.17	24.87	25.01	0.03	0.15	0.6
Denise	24.89	24.91	24.93	24.91	0.36	0.02	0.08

Table 3. Individual and average volumes delivered by a 25 mL Class A Volumetric Pipet.

Student	Trial 1	Trial 2	Trial 3	Average Volume (mL)	% Error	Standard Deviation (mL)	% Relative Standard Deviation
Alex	24.76	24.79	24.64	24.73	1.1	0.08	0.3
Billy	24.79	25.11	25.17	25.02	0.09	0.20	0.8
Charlie	25.15	25.11	25.04	25.10	0.40	0.06	0.2
Denise	25.50	25.48	25.35	25.44	1.8	0.08	0.3

9. Based on tables 2 and 3,

- a. Rank the four students from most accurate to least accurate when using the 25 mL volumetric flask. What values did you use to determine your ranking?

Based on percent error, Charlie > Billy = Denise > Alex

- b. Rank the four students from most precise to least precise when using the 25 mL volumetric flask. What values did you use to determine your ranking?

Based on percent relative standard deviation, Denise > Billy > Charlie > Alex

- c. Rank the four students from most accurate to least accurate when using the 25 mL volumetric pipet. What values did you use to determine your ranking?

Based on percent error, Billy > Charlie > Alex > Denise

- d. Rank the four students from most precise to least precise when using the 25 mL volumetric pipet. What values did you use to determine your ranking?

Based on percent relative standard deviation, Charlie > Alex = Denise > Billy

10. Did the accuracy of a student using a particular piece of glassware predict how precise the student was with that glassware? Why or why not?

No, accuracy and precision are different from one another. While Alex was the least precise and accurate with the 25 mL volumetric flask compared to the other three students, this does not link accuracy with precision. You can have students that are both accurate and precise. Students that are precise but not accurate. Students that are accurate but not precise. Students that are neither precise nor accurate. Any combination is possible.

11. Using tables 1 and 2, did the students' average volumes fall within the expected tolerance of the 25 mL volumetric flasks? Explain.

Only Charlie's average volume for the volumetric flask falls within the listed tolerance of the flask.

12. Using tables 1 and 3, did the students' measurements fall within the expected tolerance of the 25 mL volumetric pipets? Explain.

Only Billy's average volume for the volumetric pipet falls within the listed tolerance of the pipet.

13. When using the volumetric pipet, Denise was blowing out the last drop of water using her pipet bulb, yielding volumes that were higher than expected. Is this an example of systematic or random error? Why?

This is a systematic (determinate) error causing the measured volume to be higher than expected. This error can be identified and corrected for by having Denise practice and correcting her technique with the pipet.

14. If any average volumes from the student data fell outside of the expected tolerance of the glassware, list at least three reasons why that may have occurred. For each error, list if it was a systematic or random error.

There are numerous possible systematic and random errors that could cause a student to have average volumes that fall outside the expected tolerance range of the glassware. Some examples:

- 1. Glassware that is not clean, causing water to stick inside the volumetric pipet (could be random or systematic)***
- 2. Students reading the meniscus incorrectly in a random fashion (random error) or consistently reading it the wrong way (systematic error)***
- 3. Students could be too hasty with draining the pipets and not let all the solution that should drain out be collected. This could be systematic if they did the same mistake consistently or random if their handling of the glassware was unpredictable between trials.***
- 4. Students fail to account for a temperature difference in the room (systematic error)***
- 5. Students don't notice they have air bubbles in their pipet during some measurements (random error)***
- 6. Students are using a pipet with a chipped tip that doesn't hold the nominal volume anymore (systematic error)***

16. Summarize three key things you learned from this activity.

Any number of answers are appropriate here, but hopefully students are highlighting differences in glassware sizes as well as the difference between accuracy and precision. Then, they can hopefully start to identify the range of errors that occur when using glassware in the lab.

Active Learning Worksheets for Analytical Chemistry

#3 Analytical Foundations: Correcting Mass and Propagating Uncertainty

Faculty Resources

(Delete before sharing with students):

- **Analytical Context and Framing:** I use this worksheet to show students that uncertainty does not disappear just because the calculation gets more complicated. The buoyancy correction is a useful reminder that even routine mass measurements have assumptions built in, and the propagation problems help students track how uncertainty from balances, glassware, dilutions, and calculations affects the final result.
- **Key Concepts and Common Challenges:** Propagating uncertainty through logarithms, especially for pH and pOH, is usually difficult for students. Emphasize that the uncertainty in concentration and the uncertainty in pH are related, but they do not follow the same rules as simple multiplication or division.
- **Facilitated Discussion Pauses:** Pause after comparing Method A and Method B. Many students initially assume that the one-step method must be better because it has fewer steps. They often overlook what it means to measure a very small mass such as 10.2 mg and how much uncertainty that introduces.
- **Calculation Support:** Make sure students identify all sources of uncertainty in Method A: the balance, the first volumetric flask, the pipet, and the second volumetric flask. Students often calculate only one part of the uncertainty and then underestimate the total uncertainty in the final concentration.
- **Synthesis and Extension Questions:** Ask students: "If your lab only had a top-loading balance (± 0.1 g), how would that change your entire experimental design for making this specific solution?" This helps them connect uncertainty propagation to real experimental design decisions.
- **Pacing Note:** This worksheet builds directly on Worksheet 2's glassware/tolerance work and works well as a two-part activity. A natural stopping point is after Question 5. Up to that point, students practice buoyancy corrections, uncertainty in pH or pOH, and basic propagation of uncertainty. The remaining questions shift to solution preparation and comparison of experimental strategies, where students apply uncertainty propagation in a more realistic analytical context.

Answer Keys for Active Learning Worksheets for Analytical Chemistry

#3 Analytical Foundations: Correcting Mass and Propagating Uncertainty

Before You Begin

- Read about buoyancy: [16.9 Correcting Mass for the Buoyancy of Air](#)
- Read about propagation of error: [4.3 Propagation of Uncertainty](#)

What You Should Already Know

- Molarity, dilution, and stoichiometry
- Logarithmic relationships in acid/base chemistry (pH, pOH).
- Significant figures and precision.
- Familiarity with volumetric glassware and tolerance values

What You'll Learn

- Explain the effect of buoyancy on mass measurements and apply correction formulas
- Calculate absolute and relative uncertainties for derived quantities (e.g., $[H^+]$, $[OH^-]$, molarity)
- Propagate uncertainty using multiplication, division, subtraction rules
- Evaluate alternative experimental strategies based on uncertainty in final concentration
- Interpret how uncertainty accumulates through sequential measurements

What You'll Practice

- Apply quantitative reasoning in uncertainty propagation
- Compare methods for solution preparation based on propagated uncertainty
- Assess the contribution of instruments and techniques to total uncertainty
- Communicate error and uncertainty using scientific notation and conventions

Estimated Completion Time: 90-120 minutes

Author: Rebecca L. Sanders, *Active Learning Worksheets for Analytical Chemistry*, Version 1.0, 2026

Useful Equations:

- $W_v = W_a * \left[1 + \left(\frac{1}{D_o} - \frac{1}{D_w}\right) * 0.0012\right]$ where W_v is the object's true weight in vacuo, W_a is the object's true weight in air, D_o is the density of the object, and D_w is the density of the brass weights (assume 8.40 g/mL).
- $[H^+] = 10^{-pH}$
- $pH = -\log[H^+]$
- $[OH^-] = 10^{-pOH}$
- $pOH = -\log[OH^-]$
- $uncertainty\ in\ [H^+] = 2.303[H^+](uncertainty\ in\ pH)$
- $uncertainty\ in\ pH = \frac{uncertainty\ in\ [H^+]}{2.303[H^+]}$
- $uncertainty\ in\ [OH^-] = 2.303[OH^-](uncertainty\ in\ pOH)$
- $uncertainty\ in\ pOH = \frac{uncertainty\ in\ [OH^-]}{2.303[OH^-]}$
- $\% relative\ error_{total} = \sqrt{(\%error_1)^2 + (\%error_2)^2 + \dots}$
- $absolute\ error_{total} = \sqrt{error_1^2 + error_2^2 + \dots}$

Buoyancy Calculation

1. If a 20.00 mL volumetric pipet delivered 19.9741 g of water at 21.0 °C, what is the true mass of the water? What is its true volume? The density of water at 21.0 °C is 0.9979955 g/mL.

$$W_v = W_a * \left[1 + \left(\frac{1}{D_o} - \frac{1}{D_w} \right) * 0.0012 \right]$$

$$W_v = 19.9741 * \left[1 + \left(\frac{1}{0.9979955} - \frac{1}{8.40} \right) * 0.0012 \right]$$

$$\text{True mass: } W_v = 19.9953 \text{ g}$$

$$\text{True volume: } 19.9953 \text{ g} * \frac{1 \text{ mL}}{0.9979955 \text{ g}} = 20.0354 \text{ mL}$$

Errors in pH Measurements

2. The pH of a solution is 6.05 ± 0.03 . Find $[H^+]$ and its absolute and percent relative uncertainties.

$$[H^+] = 10^{-pH} = 10^{-6.05} = 8.913 * 10^{-7} \text{ M}$$

$$\text{Uncertainty in } [H^+] = 2.303 * 8.913 * 10^{-7} \text{ M} * 0.03$$

$$\text{Uncertainty in } [H^+] = 6 * 10^{-8} \text{ M}$$

$$[H^+] = 10^{-pH} = 10^{-6.05} = 8.9 * 10^{-7} \pm 0.6 * 10^{-8} \text{ M } (\pm 7\%)$$

3. A solution of KOH was standardized to have a concentration of $0.1012 \pm 0.0009 \text{ M}$. What is the pOH of the solution and its absolute uncertainty?

$$pOH = -\log[OH^-] = -\log(0.1012) = 0.9948$$

$$\text{Uncertainty in } [OH^-] = 2.303 * [OH^-] * (\text{uncertainty in } pOH)$$

$$0.0009 = 2.303 * 0.1012 * (\text{uncertainty in } pOH)$$

$$\text{uncertainty in } pOH = 0.004$$

$$pOH = 0.995 \pm 0.004$$

Propagation of Error Calculations

Find the absolute uncertainty and percent relative uncertainty for each answer.

4. $[14.12 (\pm 0.07) \div 3.98 (\pm 0.02)] \times 6.6821 (\pm 0.0008)$

$$\frac{14.12}{3.98} * 6.6821 = 23.7063 \text{ (round at the end once uncertainty determined)}$$

$$\text{relative uncertainty} = \sqrt{\left(\frac{0.07}{14.12}\right)^2 + \left(\frac{0.02}{3.98}\right)^2 + \left(\frac{0.0008}{6.6821}\right)^2}$$

$$\text{relative uncertainty} = \sqrt{(0.0049575)^2 + \left(\frac{0.02}{3.98}\right)^2 + \left(\frac{0.0008}{6.6821}\right)^2}$$

$$\text{relative uncertainty} = \sqrt{0.000024577 + 0.0000252519 + 0.000000014} = 0.007$$

$$\% \text{ relative uncertainty} = 0.7\%$$

$$\text{Absolute uncertainty: } 23.71 \pm 0.17$$

5. $[2.36 (\pm 0.10) \times 7.55 (\pm 0.04)] - 0.17 (\pm 0.05)$

$$2.36 * 7.55 - 0.17 = 17.648 \text{ (round at the end when uncertainty determined)}$$

$$\text{relative uncertainty} = \sqrt{\left(\frac{0.10}{2.36}\right)^2 + \left(\frac{0.04}{7.55}\right)^2} = \sqrt{0.001795 + 0.000028} = 0.0427$$
$$= 4.3\% \text{ relative uncertainty}$$

$$\text{absolute uncertainty} = 0.043 * (2.36 * 7.55) = 0.76$$

$$\text{absolute uncertainty} = \sqrt{(0.76)^2 + (0.05)^2} = 0.76 = 0.8$$

$$\% \text{ relative uncertainty} = \frac{0.76}{17.648} * 100 = 4\%$$

$$\text{answer: } 17.6 \pm 0.8 (\pm 4\%)$$

Propagation of Error When Making Solutions

You need to make a solution of 0.00111 M CdCl_2 as accurately and precisely as possible. To make the solution, you could make it in one of two ways.

Possible Method A: Two-step

6. Calculate the formula mass of CdCl_2 to the correct number of significant figures. The atomic mass of Cd is 112.41 ± 0.01 g/mol and the atomic mass of Cl is 35.45 ± 0.01 g/mol.

$$112.41 + 2 * (35.45) = 183.31 \text{ g/mol}$$

7. What is the absolute uncertainty in the formula mass of CdCl_2 ?

$$\text{absolute uncertainty} = \sqrt{(0.01)^2 + (0.01)^2 + (0.01)^2} = 0.017 = 0.02 \text{ g/mol}$$

8. A solution was made by dissolving 0.1018 ± 0.0002 grams of CdCl_2 in a 50.00 ± 0.05 mL volumetric flask with distilled water. What is the molarity? What are the absolute and percent relative uncertainties of the solution's molarity?

$$M = \frac{0.1018 \text{ g}}{0.05000 \text{ L}} \times \frac{1 \text{ mol}}{183.31 \text{ g}} = 0.011107 \text{ M} \text{ (rounds to 4 significant figures, 0.01111M, but for now, we will keep an additional digit and round as needed at the end.)}$$

$$\% \text{ relative uncertainty} = \sqrt{\left(\frac{0.05}{50.00} \times 100\right)^2 + \left(\frac{0.02}{183.31} \times 100\right)^2 + \left(\frac{0.0002}{0.1018} \times 100\right)^2} = \sqrt{0.01 + 0.00012 + 0.0386} = 0.22\% \text{ relative uncertainty}$$

$$\text{Absolute uncertainty} = 0.011107 \text{ M} * 0.0022 = 0.00002 \text{ M}$$

$$\text{Molarity: } 0.01111 \pm 0.00002 \text{ M}$$

9. You need a 1:10 dilution of the solution from #8 to make your solution. Using a 5.00 ± 0.01 mL volumetric pipet, you dilute the solution from #8 into a new 50.00 ± 0.05 mL volumetric flask. What is the molarity of the diluted solution? What are the absolute and percent relative uncertainties of the solution's molarity?

$$M_1V_1 = M_2V_2$$

$$(0.01111\text{ M})(5.00\text{ mL}) = M_2(50.00\text{ mL})$$

$$M_2 = 0.001111\text{ M}$$

$$\begin{aligned} \% \text{ relative uncertainty} &= \sqrt{\left(\frac{0.01}{5.00} \times 100\right)^2 + \left(\frac{0.05}{50.00} \times 100\right)^2 + \left(\frac{0.00002}{0.01111} \times 100\right)^2} \\ &= \sqrt{0.04 + 0.01 + 0.0484} = 0.3\% \text{ relative uncertainty} \end{aligned}$$

$$\text{Absolute uncertainty} = 0.001111\text{ M} \times 0.003 = 0.000003\text{ M}$$

$$\text{Molarity: } 0.001111 \pm 0.000003\text{ M}$$

Possible Method B: One-step

10. You could have made the solution in one step by using one tenth of the original mass of CdCl_2 . This solution was made by dissolving 0.0102 ± 0.0002 grams of CdCl_2 in a 50.00 ± 0.05 mL volumetric flask with distilled water. The resulting solution would have yielded a solution with 0.00111 ± 0.00002 M CdCl_2 . Calculate the percent relative standard deviation for this approach. Which method (A or B) results in a final solution concentration with less uncertainty? Why? Be sure to consider the uncertainty in each individual measurement and how it contributes to the overall uncertainty.

$$\text{Percent relative uncertainty} = \frac{0.00002}{0.00111} \times 100 = 2\% \text{ relative error}$$

Method A yields a final concentration with a lower percent relative error. While Method A has more steps, Method B relies on measuring a very small mass (only 10.2 mg!), which is difficult to measure reliably. The stepwise dilution from Method A will produce a lower percent relative error if executed properly because measuring at least four significant figures on an analytical balance (100.0 mg) will give less error than measuring a smaller mass.

Active Learning Worksheets for Analytical Chemistry

#4 Analytical Foundations: Designing a Representative Sampling Strategy

Faculty Resources

(Delete before sharing with students):

- **Analytical Context and Framing:** I use this worksheet as a fun way to show students how a simple question can become an analytical chemistry problem. I usually do not tell students this activity is coming until class starts. I pose the question: “Is a Double Stuf Oreo truly double stuffed? And how could we answer that analytically?” At first, it sounds like a simple cookie question, but as students look at previous attempts and begin designing their own study, they see how many choices are involved in defining the population, collecting samples, preparing the sample, measuring the crème, and interpreting the results. This helps students see that sampling design is part of analytical chemistry, not just a preliminary step before the “real” measurement.
- **Key Concepts and Common Challenges:** Defining the population of interest is harder than students expect. They often say “all Oreos,” but they need to narrow that to something measurable, such as “regular and Double Stuf Oreos purchased in the U.S. during the current year” or “packages purchased from local stores this semester.” Students also need to distinguish between the larger population they care about and the actual samples they can reasonably collect and measure. This is a useful place to ask practical questions: Where would you buy the cookies? How many packages would you buy? Would you sample from multiple stores or lots? How would you randomize which cookies are measured?
- **Facilitated Discussion Pauses:** After evaluating the Truth in Advertising study, pause to discuss the limitations of using a kitchen scale that reads only to the nearest gram. This is a good opportunity to connect sampling limitations with measurement limitations: even a larger sample does not fully solve the problem if the measurement tool is not appropriate. If you are willing to bring Oreos to class, this is also a good time to let students physically examine the sample. They may notice that the cookies are crumbly, that the crème may not separate cleanly, and that the product may not behave exactly the way they remember from childhood.
- **Calculation Support:** I usually let students brainstorm a little before steering them toward a single “best” approach. They come up with surprisingly creative ideas, from freezing the cookies to scraping the crème to weighing whole cookies and wafers separately. The point is not to make the Oreo method perfect; the point is to show that sample preparation is a source of analytical judgment and possible bias.
- **Synthesis and Extension Questions:** Ask students: “If you had a fixed budget, how would you divide your resources between buying more packages, measuring more cookies, and improving the measurement technique?” I want students to see sampling, measurement quality, cost, and feasibility as connected decisions. In real analytical work, they often have to weigh their options when resources are limited. This worksheet can also be used as a planning period for an actual lab activity, where students design a feasible sampling and measurement plan based on class size, available time, and available equipment.
- **Pacing Note:** This worksheet is likely to fit within one class period if students stay focused during group discussion. If time is limited, Questions 1–4 can be used to evaluate the previous Oreo studies during class, and the sampling-plan questions can be completed as follow-up work or used to begin the next class. This activity can be extended into a lab or adapted to a different real-world sample, but the key goal is the same: students should practice turning a familiar question into a clearly defined analytical problem with a defensible sampling plan.

Answer Keys for Active Learning Worksheets for Analytical Chemistry

#4 Analytical Foundations: Designing a Representative Sampling Strategy

Before You Begin

- Read [7.1: The Importance of Sampling](#)
- Read [7.2: Designing a Sampling Plan](#)
- Read [7.3: Implementing the Sampling Plan](#)

What You Should Already Know

- Differences between population and sample
- Basics of random and representative sampling
- Sources of sampling bias
- How to calculate mean and standard deviation

What You'll Learn

- Design a representative sampling plan for Oreo cookies
- Determine sample size and sampling locations to minimize bias
- Identify variables to control during sample preparation and measurement

What You'll Practice

- Collaboratively plan a sampling and measurement strategy
- Evaluate the impact of sample source, preparation, and handling
- Justify sampling decisions based on analytical chemistry principles

Estimated Completion Time: 60 minutes

Author: Rebecca L. Sanders

Active Learning Worksheets for Analytical Chemistry, Version 1.0, 2026

Backstory: The Double Stuf Oreo Debate

In 2013, a high school math teacher named Dan Anderson made headlines when his students tested whether Double Stuf Oreos® really had double the crème filling of regular Oreos®. They discovered that the Double Stuf cookies only had about 1.86 times the crème.² The story spread quickly, with coverage from CNN, NPR, and others, sparking a swirl of public curiosity and snack-food skepticism.³

In Dan Anderson’s original study, he weighed ten full cookies of each type to find the average mass for one cookie was 11.57 grams for regular Oreos and 14.67 grams for Double Stuf Oreos. Then he weighed five wafers to find an average wafer mass was 3.98 grams. He used the average cookie mass and subtracted the average mass of two wafers to find the average mass of Oreo and Double Stuf Oreo crème. The scale used read to the nearest hundredth of a gram.

1. What are the strengths of Dan Anderson’s sampling method?

Some examples include:

- *Weighing 10 cookies gives a reasonable sample size for a high school experiment.*
- *Using a balance readable to 0.01 g improves precision.*
- *Separating crème mass via subtraction (cookie – 2 * wafer) is clever and accessible.*
- *Inexpensive and reproducible setup.*

2. What are some limitations or possible sources of error in Anderson’s approach?

Some examples include:

- *Only 10 cookies sampled—may not represent full production variation.*
- *Assumes uniformity in wafer mass across cookies (both regular and Double Stuf), which may not hold.*
- *Separation not physically measured and assumes all crème is intact and wafers are equal.*
- *Potential bias from a single package or store.*

² Anderson, D. Oreo: Original vs. Double vs. Mega. Recursive Process. <http://blog.recursiveprocess.com/2013/03/03/oreo-original-vs-double-vs-mega/> (accessed July 2, 2025).

³ Shoichet, C. E. Teacher and Students Say Double Stuf Oreos Are Not Double Stuf. CNN. <https://www.cnn.com/2013/08/21/us/oreo-high-school-experiment/> (accessed July 2, 2025).

In response, *Truth in Advertising* conducted their own investigation in 2022 and found that while Double Stuf Oreos may not have exactly double the crème, they do consistently contain about twice as much crème as regular Oreos, supporting Nabisco's claim.⁴ However, Nabisco still provides no published standard defining what "double" means on the packaging.

In the Truth in Advertising study, they relied on a kitchen scale that only measured to the nearest gram. The crème of a pack of each variety of cookies was measured with average crème masses of 3.1 and 6.2 grams for regular and Double Stuf Oreos, respectively.

3. What are the strengths of the Truth in Advertising approach?

Some examples include:

- *Uses the entire pack provides better sampling across a batch.*
- *Simple method for measuring total crème: direct and efficient.*
- *Realistic method for consumer-grade investigation.*

4. What are limitations or drawbacks of the Truth in Advertising study?

Some examples include:

- *Scale only reads to nearest gram.*
- *No per-cookie variability assessed.*
- *No clear info about number of cookies tested.*
- *Only one package of each type tested.*
- *Not statistically rigorous for hypothesis testing.*

Develop a Sampling Plan

As analytical chemists, you now have the tools to weigh in. Can we design a more robust sampling plan to finally settle the debate? Your team will plan how to collect data, choose how many cookies to sample (and from where), and define how to analyze your results to determine: Does a Double Stuf Oreo® from this year actually contain double the crème?

Note: These answers are suggestions. Modify based on your class's needs.

5. What is your population of interest?

All Double Stuf and regular Oreos sold in your country in the current year.

⁴ Truth in Advertising. *Double Stuf Oreos*. <https://truthinadvertising.org/articles/double-stuf-oreos/> (accessed July 2, 2025)

6. How many total cookies will you sample?

At least 30 of each type (enough for comparison and statistical tests).

7. How many packages will you sample from?

Minimum 3 per type (reduces batch/store bias).

8. How many cookies will you sample per package?

10 cookies per package (select randomly or use every nth cookie).

9. Where will you get the Oreos®?

Walmart, Target, Amazon, and local stores for diversity.

Handling and Preparation

10. How will you handle and store cookies to ensure consistency?

Keep sealed until day of experiment. Avoid humidity or heat. Store at room temp.

11. What measurements will you make?

Mass of full cookie, mass of separated wafers, mass of crème (by subtraction and direct can be used to spot any errors in measurements or data recording).

12. What tools will you use for measurements?

Analytical balance (± 0.0001 g), spatula, tweezers, labeled weigh boats or paper.

Experimental Design Considerations

13. What variables must be controlled to ensure accuracy?

- *Same person separating crème (or consistent method).*
- *Same analytical balance and weigh procedure.*
- *Use same batch of weigh paper or containers.*
- *Avoid mid-experiment interruptions or shifts in environment.*

14. What qualitative observations might be helpful to record?

Crème distribution (uniform? double-sided?), crème color, cookie breakage, residue on wafers, ease of crème separation from wafers.

15. Will you blind your samples or randomize order? Why?

Yes. Randomize the order of cookie measurements. Optionally blind cookie type (especially if comparing by weight alone).

16. How will you record your data?

Shared Google Sheet or shared Excel file. Use ID codes for each cookie. Include mass, observations, and time stamp.

Data Analysis Planning

17. How will you calculate and compare average crème mass?

- *Average crème mass and standard deviation for each Oreo type*
- *Ratio of Double Stuf to regular*

18. What statistical tests might help determine if differences are significant?

- *t-test (independent, two-sample) to compare mean crème mass*
- *Q-test or Grubbs test to reject outliers*
- *95% confidence intervals*

Reflection

19. What's one thing you'll need to be especially careful about during sampling or measuring?

- *Ensuring clean separation of crème without residue*
- *Preventing cross-contamination or scale drift*
- *Being consistent with tare process and labeling*

Optional Extension

20. Would your answer change based on year, location, or package size? How could you design a broader study?

- *Compare crème content across:*
 - *Years (longitudinal sampling)*
 - *Packaging types (family-size, mini, seasonal)*
 - *Regions (Midwest vs. East Coast)*
- *Partner with other schools or classes to expand data pool.*

Active Learning Worksheets for Analytical Chemistry

#5 Analytical Foundations: Analyzing and Interpreting Experimental Error

Faculty Resources

(Delete before sharing with students):

- **Analytical Context and Framing:** Connect this worksheet directly to the previous activities: “In Worksheet #2, we thought about accuracy, precision, and measurement error. In Worksheet #4, we designed an Oreo sampling plan. Now we are looking at what happens when real data are collected.” I use this worksheet to show students that even careful measurements have variability and that statistical tools help us decide what the data can and cannot support. The Oreo data are intentionally realistic rather than perfectly clean, which gives students practice working with the kind of spread and ambiguity they may see in actual lab data.
- **Key Concepts and Common Challenges:** Students often need help distinguishing a documented blunder from a statistical outlier. A blunder can only be corrected when there is evidence, such as a notebook entry or clear documentation. A suspicious value without documentation should be evaluated statistically rather than removed simply because it looks wrong. Students should also recognize that differences between data sets can come from individual technique, shared procedural choices, sample variability, or limitations of the measurement process.
- **Facilitated Discussion Pauses:** After students compare the instructor data with the student data, pause to discuss what kinds of errors or variation could explain the differences. Ask: “Is this difference telling us something about the cookies, the sampling process, the measurement technique, or the person collecting the data?” After the F-test comparison between the instructor and Student 1, ask: “Why do we check the variances before choosing which t-test to use?” This reinforces that comparison of means depends on whether the two data sets have similar precision.
- **Calculation Support:** Students typically need support when they reach the formal statistical tests, especially the F-test and t-test calculations. For Questions 18 and 19, it can help to write the final numerical answers on the board so students can check their progress. If their answers do not match, you can then help them locate where the calculation went wrong rather than having every group wait for individual confirmation. I still like students to do some of these calculations by hand because it helps them understand what Excel is doing later and appreciate why spreadsheet tools are useful when the setup is correct.
- **Synthesis and Extension Questions:** Focus on Student 3’s high value. Ask: “If you suspect this value is a typo but do not have documentation, what is the most defensible way to handle it?” This is where I usually talk about the difference between correcting a documented error and deciding what to do with an undocumented questionable value. I also use this as a place to reinforce the importance of written data collection and detailed observations in lab notebooks. This worksheet prepares students to analyze and interpret their own data later, rather than just calculating statistics from a provided example.
- **Pacing Note:** This worksheet synthesizes error, sampling, and data interpretation from Worksheets #2–4. It can vary substantially in length depending on whether students complete the statistical tests and Excel analysis during class. A natural stopping point is after Question 15. Up to that point, students focus on identifying sources of error, distinguishing systematic error, random error, and blunders, and interpreting experimental data qualitatively. The remaining questions shift to formal statistical analysis, including confidence intervals, outlier tests, F-tests, t-tests, and optional Excel-based comparisons. The Excel section can be treated as optional; it is most valuable if students will later analyze their own lab data using Excel. If time is limited, the instructor can provide some calculated values and focus class time on interpretation.

Answer Keys for Active Learning Worksheets for Analytical Chemistry

#5 Analytical Foundations: Analyzing and Interpreting Experimental Error

Before You Begin

- Read [4.2 Characterizing Experiment Errors](#)
- Read [4.4 The Distribution of Measurements and Results](#)
- Read [4.6 Statistical Methods for Normal Distributions](#)
- Reference critical values from these tables: [16.4: Critical Values for t-Test](#), [16.5: Critical Values for F-Test](#), [16.6: Critical Values for Dixon's Q-Test](#), and [16.7: Critical Values for Grubb's Test](#)

What You Should Already Know

- How to calculate mean and standard deviation
- Definitions of systematic error, random error, and blunders
- Basic familiarity with confidence intervals
- The concept of a population mean vs. sample mean

What You'll Learn

- Identify, classify, and interpret sources of error in data collection
- Calculate and interpret standard deviation and confidence intervals
- Apply statistical logic (t-test, Q-test, etc.) to evaluate data sets
- Determine whether differences in data sets are statistically significant

What You'll Practice

- Analyze real experimental data with appropriate skepticism
- Make data-driven decisions about data quality and inclusion
- Collaborate to classify types of error and determine potential correction strategies
- Communicate statistical findings clearly and accurately

Estimated Completion Time: 60-135 minutes

Author: Rebecca L. Sanders

Active Learning Worksheets for Analytical Chemistry, Version 1.0, 2026

Useful Equations

- $\mu = \bar{X}_A \pm \frac{ts_A}{\sqrt{n_A}}$
- $F_{\text{exp}} = \frac{s_A^2}{s_B^2}$ where $S_A > S_B$ so that $F_{\text{exp}} > 1$.
- $t_{\text{exp}} = \frac{|\bar{X}_A - \bar{X}_B|}{\sqrt{\frac{s_A^2}{n_A} + \frac{s_B^2}{n_B}}}$ Degrees of freedom: $\nu = \frac{\left(\frac{s_A^2}{n_A} + \frac{s_B^2}{n_B}\right)^2}{\left(\frac{s_A^2}{n_A}\right)^2 + \left(\frac{s_B^2}{n_B}\right)^2} - 2$
- $t_{\text{exp}} = \frac{|\bar{X}_A - \bar{X}_B|}{s_{\text{pool}}} * \sqrt{\frac{n_A n_B}{n_A + n_B}}$
where $s_{\text{pool}} = \sqrt{\frac{(n_A - 1)s_A^2 + (n_B - 1)s_B^2}{n_A + n_B - 2}}$ Degrees of freedom = $n_A + n_B - 2$.
- $G_{\text{exp}} = \frac{|X_{\text{out}} - \bar{X}|}{s}$
- $Q_{\text{exp}} = \frac{\text{gap}}{\text{range}}$

Errors in Data Collection

Several students and an instructor sampled Oreo cookies to determine the average mass of cream inside an Oreo cookie. Each dataset was obtained by weighing the cream from individual cookies after carefully separating it from the wafers. The purpose of this activity is to explore how data collection techniques and errors influence results and to practice identifying and categorizing these errors. Then, you will explore how to statistically analyze the data that was collected.

While identifying the cream mass may seem simple at first, there are many places where errors could occur. With your group, discuss each of the errors below. Describe how they may impact the **mean** and **standard deviation** of a dataset. Classify each error as **systematic**, **random**, or a **blunder** and suggest whether it could be corrected. A blunder is a human mistake or error that results in an incorrect measurement or data recording.

1. **Wafer crumbs are left in the cream mass** due to incomplete separation.

Impact: Increases the mass, leading to a systematic overestimation of the cream mass.

Error Type: Systematic.

Correction: Yes, by improving separation technique.

2. **The masses were collected using an analytical balance with the top glass door left open.**

Impact: Causes random fluctuations in mass measurements.

Error Type: Random.

Correction: Yes, by ensuring the balance door is closed during measurements.

3. **Environmental vibrations causing the balance to fluctuate** slightly during measurements.

Impact: Introduces random errors and increased variability in the data.

Error Type: Random.

Correction: Yes, by stabilizing the balance environment.

4. **When a student enters a cream mass into a spreadsheet, they switch two numbers, recording 3.9055 g instead of the measured mass of 3.0955 g.**

Impact: Large error in a single data point, drastically affecting mean and standard deviation.

Error Type: Blunder.

Correction: Yes, by verifying and correcting the entry.

5. All cookies were selected cookies from a single package that may not represent the larger population.

Impact: Systematic bias if the package is not representative of the population.

Error Type: Systematic.

Correction: No, unless sampling from multiple packages.

6. For the first cream measurement, a student tared the balance, then recorded the mass of the cream and the weigh paper it was placed on as just the cream mass.

Impact: Introduces a systematic error in the first measurement.

Error Type: Blunder.

Correction: Yes, by re-measuring the affected sample.

Now that you have evaluated a variety of possible errors in data collection, let's explore some data collected by one instructor and four students. The data was collected as follows:

- **Sampling:** Nine cookies were randomly selected by each experimenter from a larger population of several Oreo packages to represent the average cream mass for regular Oreos.
- **Preparation:** The cream was carefully separated from the wafers using a spatula, attempting to remove all the cream without contamination.
- **Measurement:** The cream was weighed on an analytical balance tared with a piece of weigh paper, and the masses were recorded.

Table 1. Measured cream masses (in grams) for regular Oreos across nine trials conducted by the instructor and four students. The table includes calculated mean and standard deviation values for each dataset. Data was modified from student data collected in CHEM 310 in Fall 2023.

Trial	Instructor	Student 1	Student 2	Student 3	Student 4
Trial 1	3.1239	3.2294	2.4386	2.6371	2.7599
Trial 2	2.8006	2.8632	2.6885	2.8363	2.8345
Trial 3	2.9186	3.0003	2.4315	3.5606	3.1014
Trial 4	2.9051	3.2717	3.3358	4.3764	3.3360
Trial 5	3.0650	3.2944	2.6176	3.2903	3.1120
Trial 6	2.8268	3.1742	2.9423	3.1744	2.0281
Trial 7	3.1425	3.2014	2.8790	2.7902	2.9948
Trial 8	3.2187	3.4146	2.6721	2.7701	2.7695
Trial 9	3.0857	2.9160	2.1463	2.7098	2.9734
Mean (g)	3.0097	3.1517	2.6835	3.1272	2.8788
Standard Deviation (g)	0.1498	0.1853	0.3441	0.5609	0.3686

Evaluating the Instructor's Data

7. Review the instructor's data. The instructor's cream masses were $3.01 \pm 0.15 \text{ g}$. What does the standard deviation represent?

It represents the variability or spread of the cream mass measurements around the mean.

8. Are there any obvious sources of error? What might explain the variability in the standard deviation?

Possible environmental factors, balance fluctuations, or inconsistent cream separation. Variability could also stem from small sample size.

9. How does the instructor's mean and standard deviation compare to the other datasets? What do those numbers imply?

Mean similar, in the middle of others. The standard deviation (0.15 g) is lower, suggesting more consistent measurements.

10. We don't know the actual mass of cream in regular Oreos, but we could calculate a 95% confidence interval.

- a) Based on the instructor's data, calculate the 95% confidence interval of Oreo cream mass.

$$\mu = \bar{X}_A \pm \frac{ts_A}{\sqrt{n_A}}$$
$$\mu = 3.0097 \pm \frac{2.306 * 0.1498}{\sqrt{9}}$$

$$\mu = 3.01 \pm 0.12 \text{ g}$$

- b) What does a 95% confidence interval represent? What does the other 5% represent?

A 95% confidence interval represents the range where μ is likely to fall with 95% confidence. The remaining 5% accounts for sampling error.

Evaluating the Students' Data

11. Review the data collected by student 1. Are there any obvious sources of error? What might explain the variability in the standard deviation? Compared to the instructor?

Comparison: *Higher mean (3.1517 g) and standard deviation (0.1853 g).*

Possible Errors: *Over-measurement or environmental factors.*

12. Review the data collected by student 2. How does their mean and standard deviation compare to the instructor's data? Are there any obvious sources of error?

Comparison: *Lower mean (2.6835 g) and higher variability (0.3441 g).*

Possible Errors: *Contamination or mismeasurement.*

13. Review the data collected by student 3. How does their mean and standard deviation compare to the instructor's data? Are there any obvious sources of error?

Comparison: *Similar mean (3.1272 g) but significantly higher standard deviation (0.5609 g).*

Possible Errors: *Data entry error or variability in separation technique.*

14. Review the data collected by student 4. How does their mean and standard deviation compare to the instructor's data? Are there any obvious sources of error?

Comparison: *Lower mean (2.8788 g) and higher variability (0.3686 g).*

Correction: *Typo correction to 3.0281 g could improve results.*

15. Upon reviewing student 4's notebook pages, the instructor finds that 2.0281 g entry was a typo that was supposed to be 3.0281 g. Based on student 4's documentation, the instructor can fix this blunder. The instructor predicts a similar data entry error occurred with student 3's 4.3764 g data point given that no other data point is 4 grams or more. However, student 3 only collected data digitally. What should the instructor do?

In the absence of paper trail, a test for an outlier is the only way to exclude the 4.3764 g data point.

Statistical Analysis to Remove Data Points and Compare Data Sets

16. Calculate if any data points can be excluded from student 2's data set.

The data point furthest from the mean is 3.3358 g.

$$G_{exp} = \frac{|3.3358 - 2.6835|}{0.3441} = 1.896$$

G_{exp} (1.896) < G_{table} (2.110) so all data points should be kept.

17. Calculate if any data points can be excluded from student 3's data set.

The data point furthest from the mean is 4.3764 g.

$$G_{exp} = \frac{|4.3764 - 3.1272|}{0.5609} = 2.227$$

G_{exp} (2.227) > G_{table} (2.110) so the 4.3764 g should be rejected.

From the remaining 8 data points, the new mean is 2.9711 g and the new standard deviation is 0.3299 g. Another outlier test from these 8 data points concluded all 8 data points can be kept.

18. Are the mean masses for the instructor and student 1 significantly different at 95% confidence? *Hint: Use an F-test first to determine which t-test should be used.*

$$F_{exp} = \frac{s_A^2}{s_B^2} = \frac{0.1853^2}{0.1498^2} = 1.53 < F_{table} = 3.438$$

Use the t-test where there is not a significant difference in standard deviations (assume equal variance).

$$t_{exp} = \frac{|\bar{X}_A - \bar{X}_B|}{s_{pool}} * \sqrt{\frac{n_A n_B}{n_A + n_B}} = \frac{|3.1517 - 3.0097|}{0.1685} * \sqrt{\frac{9*9}{9+9}} = 1.79$$

$$\text{where } s_{pool} = \sqrt{\frac{(n_A-1)s_A^2 + (n_B-1)s_B^2}{n_A + n_B - 2}} = \sqrt{\frac{(9-1)*(0.1853)^2 + (9-1)*(0.1498)^2}{9+9-2}} = \sqrt{\frac{0.2747+0.1795}{16}} = 0.1685$$

Degrees of freedom: $n_A + n_B - 2 = 9 + 9 - 2 = 16$

$$t_{exp} = 1.79 < t_{table} = 2.120$$

There is not a significant difference in means at the 95% confidence levels.

19. Are the mean masses for the instructor and student 2 significantly different at 95% confidence?

$$F_{exp} = \frac{s_A^2}{s_B^2} = \frac{0.3441^2}{0.1498^2} = 5.28 > F_{table} = 3.438$$

Use the t-test where there is a significant difference in standard deviations (assume unequal variance).

$$t_{exp} = \frac{|\bar{X}_A - \bar{X}_B|}{\sqrt{\frac{s_A^2}{n_A} + \frac{s_B^2}{n_B}}} = \frac{|2.6835 - 3.0097|}{\sqrt{\frac{0.3441^2}{9} + \frac{0.1498^2}{9}}} = 2.61 < t_{table} = 2.20$$

$$\text{Degrees of freedom: } \nu = \frac{\left(\frac{s_A^2}{n_A} + \frac{s_B^2}{n_B}\right)^2}{\frac{\left(\frac{s_A^2}{n_A}\right)^2}{n_A+1} + \frac{\left(\frac{s_B^2}{n_B}\right)^2}{n_B+1}} - 2 = \frac{\left(\frac{0.3441^2}{9} + \frac{0.1498^2}{9}\right)^2}{\frac{\left(\frac{0.3441^2}{9}\right)^2}{9+1} + \frac{\left(\frac{0.1498^2}{9}\right)^2}{9+1}} - 2 = 11$$

There is a significant difference in means at the 95% confidence levels.

Optional: Using Microsoft Excel to Compare Data Sets

Because t-test calculations by hand can be cumbersome, it is useful to know the same calculations can be done in Microsoft Excel.

- Open Microsoft Excel. All raw data for individual data points needs to be entered in Excel. For example, below are two data sets with five trials each.

	A	B	C
1	Example Data		
2	Trial	Set 1	Set 2
3	1	2.5	2.6
4	2	2.6	2.7
5	3	2.5	2.7
6	4	2.7	2.8
7	5	2.6	3.0
8	Average	2.580	2.760
9	Stdev	0.084	0.152

- Make sure the Data Analysis package is installed to conduct statistical tests. This can be done by opening File → Options → Add-ins. Make sure the Analysis ToolPak is enabled.
- Under the Data tab, select “Data Analysis”. A menu will open with several analysis tools. Select “F-test Two-Sample for Variances”. Enter both data sets, making sure the data set with the larger standard deviation is put in variable range 1. An example is shown below:

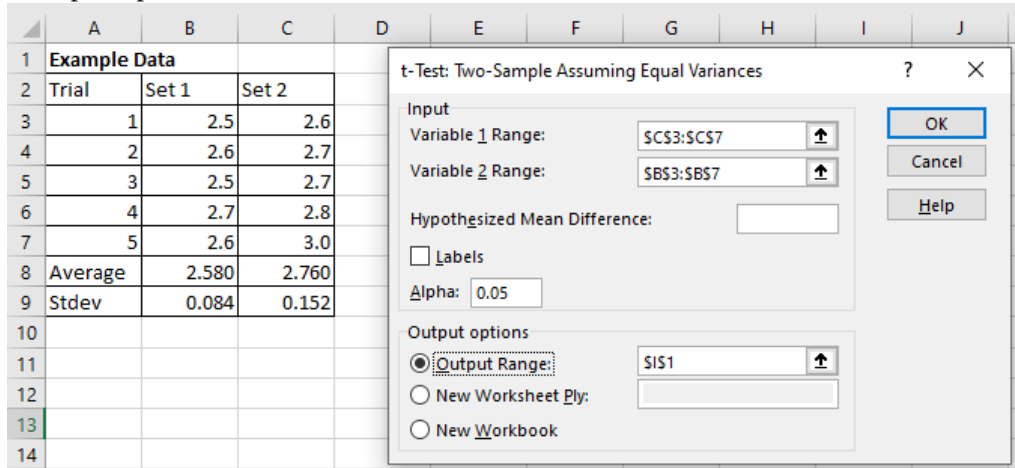
The screenshot shows the 'F-Test Two-Sample for Variances' dialog box. The 'Input' section has 'Variable 1 Range' set to '\$C\$3:\$C\$7' and 'Variable 2 Range' set to '\$B\$3:\$B\$7'. The 'Alpha' is set to '0.05'. The 'Output options' section has 'Output Range' selected with the range '\$E\$1'.

- The output will give an F value, which is F_{exp} and an F Critical value, which is F_{table} . In the example below $F_{exp} = 3.285714$, which is less than $F_{table} = 6.388233$. Since $F_{exp} < F_{table}$, the standard deviations are not significantly different at a 95% confidence level (Alpha: 0.05).

	A	B	C	D	E	F	G
1	Example Data				F-Test Two-Sample for Variances		
2	Trial	Set 1	Set 2				
3	1	2.5	2.6				
4	2	2.6	2.7				
5	3	2.5	2.7				
6	4	2.7	2.8				
7	5	2.6	3.0				
8	Average	2.580	2.760				
9	Stdev	0.084	0.152				
10							

		Variable 1	Variable 2
Mean		2.76	2.58
Variance		0.023	0.007
Observations		5	5
df		4	4
F		3.285714	
P(F<=f) one-tail		0.137926	
F Critical one-tail		6.388233	

- Since $F_{exp} < F_{table}$, the standard deviations are not significantly different. Use the "t-test: Two sample assuming equal variance" under Data Analysis to compare the means. If the standard deviations are significantly different, use the "t-test: Two sample assuming unequal variance". Use the data set with the larger mean for variable range 1. An alpha (or p value) of 0.05 represents a 95% confidence level. This can be changed if a different confidence level is desired. For example, if the t-test was conducted at a 90% confidence level, change alpha to be 0.10. An example input for the t-test is shown below.



- The output will give a t Stat value, which is t_{exp} , and a t Critical two-tail value, which is t_{table} . It can be helpful to highlight the important values from the F-test and t-test output tables. In this example, $t_{exp} = 2.32379$, which is greater than $t_{table} = 2.306004$. In this example, the means of set 1 and set 2 are significantly different at the 95% confidence level.

	A	B	C	D	E	F	G	H	I	J	K
1	Example Data				F-Test Two-Sample for Variances				t-Test: Two-Sample Assuming Equal Variances		
2	Trial	Set 1	Set 2								
3	1	2.5	2.6		Variable 1 Variable 2				Variable 1 Variable 2		
4	2	2.6	2.7		Mean	2.76	2.58		Mean	2.76	2.58
5	3	2.5	2.7		Variance	0.023	0.007		Variance	0.023	0.007
6	4	2.7	2.8		Observations	5	5		Observations	5	5
7	5	2.6	3.0		df	4	4		Pooled Variance	0.015	
8	Average	2.580	2.760		F	3.285714			Hypothesized Mean Difference	0	
9	Stdev	0.084	0.152		P(F<=f) one-tail	0.137926			df	8	
10					F Critical two-tail	6.388233			t Stat	2.32379	
11									P(T<=t) one-tail	0.024315	
12									t Critical one-tail	1.859548	
13									P(T<=t) two-tail	0.048631	
14									t Critical two-tail	2.306004	

20. Using Microsoft Excel, determine if the mean masses for the instructor and student 1 significantly different at a 95% confidence level? Compare your answer calculated in Excel to the one you calculated by hand in question 18.

F-Test Two-Sample for Variances

	Variable 1	Variable 2
Mean	3.1517	3.009656
Variance	0.0343	0.022451
Observations	9	9
df	8	8
F	1.5289	
P(F<=f) one-tail	0.281	
F Critical one-tail	3.4381	

$F_{exp} = 1.5289 < F_{table} = 3.4381$ so there is not a significance difference in standard deviations at the 95% confidence level. Use t-Test for two-sample assuming equal variance.

t-Test: Two-Sample Assuming Equal Variances

	Variable 1	Variable 2
Mean	3.151689	3.009656
Variance	0.034325	0.022451
Observations	9	9
Pooled Variance	0.028388	
Hypothesized Mean Difference	0	
df	16	
t Stat	1.788249	
P(T<=t) one-tail	0.046343	
t Critical one-tail	1.745884	
P(T<=t) two-tail	0.092687	
t Critical two-tail	2.119905	

$t_{exp} = 1.7882 < t_{table} = 2.1199$ so there is not a significant difference means at 95% confidence.

21. Using Microsoft Excel, determine if the mean masses for the instructor and student 2 significantly different at a 95% confidence level? Compare your answer calculated in Excel to the one you calculated by hand in question 19.

F-Test Two-Sample for Variances

	Variable 1	Variable 2
Mean	2.683522	3.009656
Variance	0.118407	0.022451
Observations	9	9
df	8	8
F	5.274007	
P(F<=f) one-tail	0.015043	
F Critical one-tail	3.438101	

$F_{exp} = 5.274 > F_{table} = 3.438$ so there is a significance difference in standard deviations at the 95% confidence level. Use t-Test for two-sample assuming unequal variance.

t-Test: Two-Sample Assuming Unequal Variances

	Variable 1	Variable 2
Mean	2.683522	3.009656
Variance	0.118407	0.022451
Observations	9	9
Hypothesized Mean Difference	0	
df	11	
t Stat	-2.60691	
P(T<=t) one-tail	0.012196	
t Critical one-tail	1.795885	
P(T<=t) two-tail	0.024392	
t Critical two-tail	2.200985	

Any results from t_{stat} should be treated as absolute values. $t_{exp} = 2.607 > t_{table} = 2.201$ so there is a significant difference means at 95% confidence. Note: When calculations are done by hand, round difference may give 10 degrees of freedom instead of 11.

Active Learning Worksheets for Analytical Chemistry

#6 Analytical Foundations: Solution Preparation and Error Considerations

Faculty Resources

(Delete before sharing with students):

- **Analytical Context and Framing:** I use the scenario of lead in drinking water because there are lead service lines on portions of our campus, which is located in a historic district. This makes the solution-preparation calculations feel more realistic and less procedural. I frame this worksheet as a move from thinking about single measurements to thinking about how several measurements combine when preparing stock solutions and standards. Remind students that preparing standards is not just a routine setup step. If the standard is wrong, every sample result built from that standard is questionable, and that could affect how someone interprets a possible health risk.
- **Key Concepts and Common Challenges:** Students may struggle when converting between mg/mL, ppm, and molarity. Watch especially for factors of 1000 and for confusion about when 1 ppm can be treated as 1 mg/L for dilute aqueous solutions. Students also need to recognize that the largest contribution to error depends on the size of the measurement and the tool being used. A small absolute uncertainty can become important when the measured volume or mass is small.
- **Facilitated Discussion Pauses:** After students calculate the percent relative error for Method 1, pause and have the class compare the sources of error. The micropipette will often be a major contributor. Ask: “Why might we still use a micropipette even if it contributes more relative error than the volumetric glassware?” This is a good place to reinforce that analytical decisions often involve balancing accuracy, available equipment, waste, convenience, and practical constraints.
- **Calculation Support:** Check students’ reasoning around “AAA,” or “Always Add Acid.” Make sure they understand that diluting concentrated nitric acid is exothermic and that adding acid to water is a safer way to dissipate heat. The propagated-error calculations for preparing standards can take a while, so students may need help staying organized. Encourage them to identify each measurement step, the tool used, and the relative contribution of that step to the final uncertainty.
- **Synthesis and Extension Questions:** Ask students: “If Method 2 is more accurate but generates substantially more lead-containing waste, which method would you choose? How would you justify your choice as an analytical chemist trying to balance accuracy, safety, waste, and practicality?” Question 12 may require substantial support because students often do not know where to start when asked to design a set of standards. It can help to give them an initial constraint, such as: “What if every standard were prepared in a 50 mL volumetric flask?” From there, students can choose reasonable target concentrations or delivery volumes and adjust the plan based on the glassware available. The standards do not need to be perfectly evenly spaced as long as they cover the needed concentration range and can be prepared accurately with available tools.
- **Pacing Note:** This activity builds on Worksheets #2–3 for glassware, uncertainty, and solution preparation. The calculations can take longer than students expect, especially when they propagate error through multiple solution-preparation steps or design a set of standards. If this worksheet needs to be divided across class periods, a natural stopping point is after Question 7. Up to that point, students focus on preparing and evaluating a stock solution. The remaining questions shift to preparing a set of standards and comparing methods based on accuracy, waste, safety, and practicality. Instructors could also assign the method-comparison or standard-design questions as follow-up work if class time is limited.

Answer Keys for Active Learning Worksheets for Analytical Chemistry

#6 Analytical Foundations: Solution Preparation and Error Considerations

Before You Begin

- Read about concentration: [2.2: Concentration](#)
- Read about preparing solutions: [2.5: Preparing Solutions](#)
- Read about propagation of error: [4.3: Propagation of Uncertainty](#)

What You Should Already Know

- Molarity, ppm, and mg/mL unit conversions
- Glassware tolerances and accuracy
- How to use volumetric pipets and flasks
- Understanding why acids are used to stabilize certain ions
- Safety protocols for handling concentrated acids

What You'll Learn

- Convert between concentration units: ppm, molarity, and mg/mL
- Select appropriate glassware to minimize relative error
- Prepare stock and standard solutions using multiple methods
- Propagate error through multi-step dilution procedures
- Compare methods by speed, accuracy, waste, and practicality

What You'll Practice

- Interpret procedural trade-offs when designing a solution protocol
- Identify sources of error and strategies for mitigation
- Apply green chemistry principles to lab-scale planning
- Justify method selection based on analytical context

Estimated Completion Time: 75-90 minutes

Author: Rebecca L. Sanders

Active Learning Worksheets for Analytical Chemistry, Version 1.0, 2026

Useful Equations

- $1 \text{ ppm} = 1 \text{ mg/L}$ for dilute, aqueous solutions
- $C_1V_1 = C_2V_2$
- $\% \text{ relative error}_{\text{total}} = \sqrt{(\% \text{error}_1)^2 + (\% \text{error}_2)^2 + \dots}$
- $\text{absolute error}_{\text{total}} = \sqrt{\text{error}_1^2 + \text{error}_2^2 + \dots}$

In this unit, you have explored how different pieces of glassware can be used for different purposes. You have learned that tolerance generally increases with glassware size, but percent relative tolerance decreases with glassware size. You've done calculations showing how error is propagated when making solutions and you have learned to select glassware, when appropriate, to limit percent relative error. In this worksheet, we will try these concepts together as you think about applying these skills to make solutions and sets of standards.

Preparing a Stock Solution

Hannah wants to measure the concentration of lead leaching from aging lead service lines and solder in a historic district's water system using atomic spectroscopy. Aside from collecting and preparing samples, she will need to prepare standards from a stock solution. Hannah wants to purchase a 500 mL stock solution of lead (207.2 g/mol) standard solution that is 1 mg/mL Pb^{2+} in 2% nitric acid (HNO_3) from ThermoFisher⁵. However, the shipping time will take too long so she decides to make the stock solution instead.

1. The stock solution should be 1 mg/mL Pb^{2+} . What concentration is that in units of ppm? What concentration is that in units of molarity? *Hint: 1 ppm = 1 mg/L in dilute, aqueous solutions.*

A solution that is 1 mg/mL = 1000 mg/L or 1000 ppm.

$$\frac{1000 \text{ mg } Pb^{2+}}{L} * \frac{1 \text{ g } Pb^{2+}}{1000 \text{ mg } Pb^{2+}} * \frac{1 \text{ mol } Pb^{2+}}{207.2 \text{ g } Pb^{2+}} = 0.004826 \text{ M } Pb^{2+}$$

2. Why is the unit of ppm likely used instead of molarity?

The unit of ppm is used because when working with dilute concentrations, it is often easier to discuss numbers that are larger whole numbers rather than small numbers.

3. She will have to use primary standard grade $Pb(NO_3)_2$ (m.w. 331.2 g/mol) to make the stock solution. What mass of $Pb(NO_3)_2$ is needed to make a 500 mL solution?

$$0.5000 \text{ L} * \frac{1000 \text{ mg } Pb^{2+}}{L} * \frac{1 \text{ g } Pb^{2+}}{1000 \text{ mg } Pb^{2+}} * \frac{331.2 \text{ g } Pb(NO_3)_2}{207.2 \text{ g } Pb^{2+}} = 0.7992 \text{ g } Pb(NO_3)_2$$

⁵Thermo Fisher Scientific. Lead standard solution, for AAS, 1 mg/ml Pb in 2-5% HNO_3 . <https://www.thermofisher.com/order/catalog/product/196075000> (accessed July 1, 2025).

4. The stock solution should contain 2-5% HNO_3 . In Hannah's lab, she has access to commercially available concentrated HNO_3 , which is 68 wt.% HNO_3 . How much HNO_3 will she need to use? Why is HNO_3 included in the stock solution?

This measurement doesn't need to be very accurate because a range of concentrations is acceptable. In this case, you could calculate having 3% HNO_3 , which would require ~ 22 mL of concentrated HNO_3 . The acid is included to ensure that the Pb^{2+} doesn't precipitate out of solution.

5. What tool should be used to measure the volume of HNO_3 ? Her labmate reminds her of the acronym "AAA", which means "always add acid." What does that mean and why is it important?

The volume can be measured with a repipet dispenser or with a graduated cylinder. When concentrated acid is added to water, it is a very exothermic reaction. To safely control the exothermic reaction, it is better to add acid to water already in the volumetric flask to disperse out that exothermic reaction.

6. Describe the steps needed to make the stock solution. Make sure to include the order and what tools must be used.

Measure solid, quantitatively transfer to the flask, make sure to rinse the weigh boat with DI water, fill up volumetric flask at least half full, swirl to dissolve solid, carefully add HNO_3 , fill to the line with DI water, invert many times.

7. After preparing the solution, the final concentration is 1000.0 ± 0.4 ppm. The original stock solution⁶ Hannah wanted to order had a concentration of 997 to 1003 $\mu\text{g/mL}$ of Pb^{2+} in 2 to 5% HNO_3 . Was she able to make a solution that is accurate as one that she could have purchased?

Yes, her absolute error is smaller than the one listed for the commercially available stock solution.

⁶Thermo Fisher Scientific. Lead standard solution, for AAS, 1 mg/ml Pb in 2-5% HNO_3 . <https://www.thermofisher.com/order/catalog/product/196075000> (accessed July 1, 2025).

Preparing a Set of Standards

Now Hannah needs to make a set of five standards ranging from 0.1-1.0 ppm Pb^{2+} . She needs a minimum of 50 mL per standard. She is debating between two different methods. In her lab, she has access to 10, 25, 50, 100, 200, 250, and 500 mL Class A volumetric flasks and 1, 2, 3, 4, 5, 10, 15, 20, and 25 mL volumetric pipets. She also has access to micropipettes measuring 2-20, 20-200, and 100-1000 μL .

Method 1. Single Step Preparation of Standards

One option for Hannah is to take the 1000.0 ppm stock solution and use micropipettes and 50 mL volumetric flasks to make her standards. Below highlights five standards that can be prepared by micropipetting volumes of the 1000.0 ± 0.4 ppm stock solution into different 50 mL volumetric flasks.

Table 1. Possible tools used to make five Pb^{2+} standards from 0.1-1.0 ppm as well as the percent relative error in each standard.

Calibration Standard Pb^{2+} Concentration (ppm)	Volume of Pb^{2+} to Deliver with Micropipette (μL)	Volumetric Flask (mL)	% Relative Error
1.0	50 ± 0.4	50 ± 0.05	0.8
0.8	40 ± 0.4	50 ± 0.05	1.0
0.5	25 ± 0.4	50 ± 0.05	1.5
0.2	10 ± 0.05	50 ± 0.05	0.5
0.1	5 ± 0.05	50 ± 0.05	

8. Which micropipette should be used for each measurement?

The 20-200 μL micropipette should be used for the 50, 40, and 25 μL measurements and the 2-20 μL micropipette should be used for the 5 and 10 μL measurements.

9. In the lab, Hannah realizes that there are no clean 50 mL volumetric flasks. She cleans several, but then is concerned that they won't dry quickly. Does she need to wait for them to dry before she can use them? Why or why not?

The volumetric flasks can be wet as long as they are wet with the solvent used to fill the volumetric flasks, in this case, DI water.

10. The percent relative error of the resulting solution concentration has been calculated for four of the five standards.

- a. Calculate the missing percent relative error.

$$\%e = \sqrt{\left(\frac{0.05}{5} \times 100\right)^2 + \left(\frac{0.05}{50} \times 100\right)^2 + \left(\frac{0.4}{1000.0} \times 100\right)^2} = 1.0\%$$

- b. Which measurement is contributing most to the percent relative error (error in concentration, error in micropipette volume, or error in volumetric flask volume)?

The micropipette volume measurement has the greatest percent relative error (1%), causing the most uncertainty in the final solution concentration.

Method 2: Multi-step Preparation of Standards

Hannah will use more than one step to prepare her standards. First, Hannah will make a 1:100 dilution of her stock solution, making a 10.00 ppm Pb^{2+} solution. Then, she will use volumetric pipets and volumetric flasks to make the standards. She decides to keep the same five standard solution concentrations as she selected for Method 1.

11. Propose how Hannah can make her 1:100 dilution of her stock solution.

There are several options here. One option would be to take 5.0 mL of the 1000.0 ± 0.4 ppm solution and dilute it into 500.0 mL. Based on the glassware available 1.0 or 2.0 mL into 100.0 mL or 200.0 mL, respectively, would also be options.

12. Now propose what combination of volumetric pipets and volumetric flasks can be used to prepare the following five standards. Be mindful of the size of the glassware she has access to when selecting each pipet and flask that will be used.

One option is filled out in the table below. Note that students will have to make a few choices to either make more than 50 mL of solution to achieve a desired concentration (e.g. 0.1 ppm) or they may have to change the concentration of one or more calibration standards to make things work with the glassware that is available.

Table 2. Possible volumes and tools used to make five Pb^{2+} standards from 0.1-1.0 ppm as well as the percent relative error in each standard.

Calibration Standard Pb^{2+} Concentration (ppm)	Volume of 1:100 Pb^{2+} to Deliver with Volumetric Pipets (mL)	Volumetric Flask (mL)	Percent Relative Error (%)
1.0	5 ± 0.01	50 ± 0.05	0.3
0.8	4 ± 0.01	50 ± 0.05	0.3
~~0.5~~ 0.4	2 ± 0.01	50 ± 0.05	0.5
0.2	1 ± 0.006	50 ± 0.05	0.6
0.1	1 ± 0.006	100 ± 0.08	0.6

13. Assign each group member one or two standards to calculate percent relative error using glassware tolerance data, then compile your results in the table.

Using the 5 mL into 500 mL dilution, the resulting solution is 10.00 ± 0.02 ppm. Then, using the information in the table, the percent relative errors have been calculated for each solution.

14. One of the volumetric pipets that Hannah needs to use appears to have a film dried on it. Can she still use it? Explain.

The volumetric pipet should be washed and then rinsed with the solution to be pipetted. It needs to be cleaned, but doesn't have to be dry before being used.

Comparing Method 1 and Method 2

Hannah must decide which method she will use to prepare her standards. Several factors can be considered when deciding between method 1 and method 2.

15. Which method would be faster? Why?

Method 1 has fewer steps, so it would be faster.

16. Which method would be more accurate? Why?

Method 2 produces solutions that have, on average, less percent relative error, making those solutions more accurate. However, there are more steps, so it is possible that measurement mistakes with more steps could result in solutions that are not as accurately prepared.

17. Which method would require the least amount of cleanup?

Method 1 has less glassware and fewer steps, requiring less cleanup.

18. Which method would generate the least amount of waste?

Method 1 has fewer steps and uses less total solution, so it would generate less waste.

19. Which method would you select overall? Why?

Method 1 seems better except for being less accurate. Depending on how accurately you need to prepare your standards would likely dictate which method would be selected.

20. Pb^{2+} is a toxic metal ion that requires proper disposal. After completing the experiment, Hannah realized she only needed about 10 mL of each standard. Consider the method you selected.

a. What total volume of waste was generated?

Method 1: At least $500\text{ mL} + 50\text{ mL} * 5 = 750\text{ mL}$

Method 2: At least $500\text{ mL} + 500\text{ mL} + 50\text{ mL} * 4 + 100\text{ mL} = 1300\text{ mL}$

b. Are there ways to minimize waste generation for that method?

An obvious choice would be to make less of the original stock solution because very little of it was needed to prepare the standards. For example, 0.1599 g of $Pb(NO_3)_2$ could have been dissolved in 50 mL to reduce the amount of starting material used and waste generated.

Another option would have been to make a more dilute stock solution. For example, 0.1599 g of $Pb(NO_3)_2$ could have been dissolved in 500 mL, yielding a 100.0 ppm stock solution. Then, the volume measurements for the micropipette would have been larger, reducing errors with micropipettes (50-500 μL).

Smaller volumes of the standards (maybe 25 mL) could have been made as well.

Active Learning Worksheets for Analytical Chemistry

#7 Equilibrium Chemistry: Solubility and Back Titration Problem Solving

Faculty Resources

(Delete before sharing with students):

- **Analytical Context and Framing:** I start by pointing out that titrations are not always quick and straightforward. Many titrations students have seen before involve reactions that happen quickly and give clear endpoints, but real samples do not always behave that nicely. Sometimes the analyte dissolves slowly, has limited solubility, or does not give a convenient direct titration. This worksheet connects solubility calculations, K_{sp} , and back titrations to show how analytical chemists design around those limitations and still get useful information from a sample.
- **Key Concepts and Common Challenges:** Students may need review of solubility equilibrium, concentration conversions, balanced reactions, and RICE tables. The back-titration section often causes confusion because students want to set moles of NaOH equal to moles of magnesium carbonate. Emphasize that the NaOH tells them how much excess acid remained, not how much carbonate was originally present. Students need to subtract the excess acid from the acid initially added before connecting the reaction back to the original sample.
- **Facilitated Discussion Pauses:** After the lead phosphate calculation, pause to connect the chemistry to water quality. Discuss why phosphate can be added to municipal water supplies and how the common ion effect can help keep dissolved lead concentrations low. This is a good place to remind students that solubility equilibria are not just abstract K_{sp} problems; they can affect real decisions about water treatment, contamination, and public health.
- **Calculation Support:** Students may struggle with several layers of the calculations, including unit conversions, reaction stoichiometry, RICE-table setup, and algebra. Encourage them to write the chemical reaction first, identify what quantity is known, and only then choose the calculation pathway. In the back-titration problem, have students label the moles of acid initially added, the moles of acid left over, and the moles of acid that reacted with the sample. This helps them see why the subtraction step is the key to the calculation.
- **Synthesis and Extension Questions:** Ask students: “If magnesium carbonate dissolves slowly, why not just wait longer and do a direct titration? What makes a back titration more practical for a commercial or quality-control lab?” This helps students see that indirect analysis is not just a mathematical trick. It can be a practical solution when dissolution, reaction rate, endpoint detection, or sample behavior makes direct measurement difficult.
- **Pacing Note:** This worksheet may fit within one class period if students are comfortable with Gen Chem equilibrium and titration stoichiometry, but it can take much longer if they need review of K_{sp} , RICE tables, unit conversions, or algebra. If time is limited, focus class time on the solubility setup and back-titration logic, then assign remaining equilibrium or calculation practice as follow-up work. With the successive-approximation piece removed, the worksheet should feel more focused and less likely to derail before students reach the main analytical point.

Answer Keys for Active Learning Worksheets for Analytical Chemistry

#7 Equilibrium Chemistry: Solubility and Back Titration Problem Solving

Before You Begin

- Read about precipitation and solubility: [6.4 Equilibrium Constants for Chemical Reactions](#)
- Read about the common ion effect and the method of successive approximations: [6.7 Solving Equilibrium Problems](#)
- Read about back titrations: [9.2: Acid–Base Titrations](#)

What You Should Already Know

- General stoichiometry and mole conversions
- Writing balanced chemical equations
- Acid-base neutralization reactions
- Understanding of pH and concentration units (mol/L , ppm, ppb)

What You'll Learn

- Write K_{sp} expressions and solve for molar solubility
- Interpret solubility in real-world systems (antacids, water quality)
- Perform stoichiometric calculations for back titrations
- Convert between ppm/ppb and molarity in environmental chemistry contexts

What You'll Practice

- Use equilibrium concepts to predict solubility outcomes
- Apply titration data to indirectly quantify analyte mass
- Justify assumptions and approximations in complex chemical systems
- Communicate multi-step calculations using standard conventions

Estimated Completion Time: 50-65 minutes

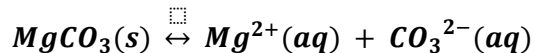
Author: Rebecca L. Sanders

Active Learning Worksheets for Analytical Chemistry, Version 1.0, 2026

Solubility Reactions and Calculations

1. $MgCO_3$ is one ingredient used in antacids. While it is insoluble in water, it becomes soluble under acidic conditions.

a. Write the K_{sp} reaction and expression for $MgCO_3$.



$$K_{sp} = [Mg^{2+}][CO_3^{2-}]$$

b. Find the concentrations of Mg^{2+} and CO_3^{2-} in a saturated solution of $MgCO_3$. The K_{sp} of magnesium carbonate is 3.5×10^{-8} .



$$K_{sp} = [Mg^{2+}][CO_3^{2-}]$$

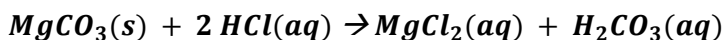
$$3.5 \times 10^{-8} = x^2$$

$$x = 1.9 \times 10^{-4} M = [Mg^{2+}] = [CO_3^{2-}]$$

Back Titrations

You could perform a titration to determine the amount of $MgCO_3$ in an antacid tablet. However, you could not do this titration directly because $MgCO_3$ is not soluble in water. Instead, a back titration must be performed, which is necessary when a **direct titration is not possible**. For example, with solids such as $MgCO_3$ or $CaCO_3$, the endpoint may be difficult to detect because the solid dissolves slowly. Another example involves volatile substances such as ammonia and iodine, where inaccuracy arises due to loss of the substance during titration. In back titration, a **known excess of one reagent A** is allowed to react with an **unknown amount of B**. The **amount of unreacted A** is then determined by **titration** with a **reagent C of known concentration**. From the titration results, the **amount of unreacted A** and the **amount of B** can be found by simple stoichiometric calculations.

2. A 1.0131 g tablet containing $MgCO_3(s)$ was dissolved in 100.0 mL of 0.2015 M $HCl(aq)$. The excess $HCl(aq)$ was back titrated with 15.68 mL of 0.1025 M $NaOH(aq)$.
- a. Write the balanced reaction of $MgCO_3(s)$ with $HCl(aq)$.



- b. How many total moles of HCl were added?

$$0.2015 M HCl * 0.1000 L = 0.02015 \text{ moles } HCl$$

- c. How many moles of HCl reacted with the carbonate? (Hint: Using the calculated total moles of HCl added, subtract the moles of $NaOH$ used to neutralize the excess HCl . The difference is the amount of HCl that reacted with $MgCO_3$.)

$$0.1025 M NaOH * 0.01568 L = 0.0016072 \text{ moles } NaOH \text{ reacted with excess } HCl$$

$$0.02015 \text{ moles} - 0.0016072 \text{ moles} = 0.01854 \text{ moles } HCl \text{ reacted with } MgCO_3$$

- d. How many grams of $MgCO_3$ (m.w. 84.3139 g/mol) reacted with the HCl ?

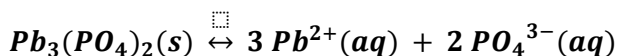
$$0.01854 \text{ moles } HCl \times \frac{1 \text{ mole } MgCO_3}{2 \text{ moles } HCl} \times \frac{84.3139 \text{ g } MgCO_3}{1 \text{ mole } MgCO_3} = 0.7817 \text{ g } MgCO_3$$

- e. What percent of the tablet was made up of $MgCO_3$?

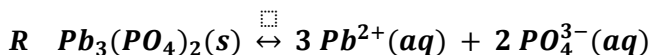
$$\frac{0.7817 \text{ g}}{1.0131 \text{ g}} \times 100 = 77.16\% MgCO_3$$

K_{sp} Calculations

3. $Pb_3(PO_4)_2$ is highly insoluble. Write the K_{sp} reaction and expression for $Pb_3(PO_4)_2$.



4. Find the concentrations of Pb^{2+} and PO_4^{3-} in a saturated solution of $Pb_3(PO_4)_2$. The K_{sp} of lead (II) phosphate is 3.0×10^{-44} .



<i>I</i>	0	0
<i>C</i>	+3x	+2x
<i>E</i>	3x	2x

$$K_{sp} = [Pb^{2+}]^3 [PO_4^{3-}]^2$$

$$3.0 \times 10^{-44} = (3x)^3 (2x)^2$$

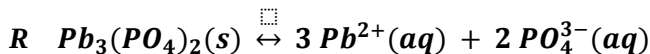
$$x = 7.7 \times 10^{-10} M$$

$$[Pb^{2+}] = 3x = 2.3 \times 10^{-9} M$$

$$[PO_4^{3-}] = 2x = 1.5 \times 10^{-9} M$$

5. Phosphate is added to municipal water to reduce the solubility of Pb^{2+} (207.2 g/mol) that may have been released from lead pipes. If a municipal water supply contains 5.0 ppm PO_4^{3-} (m.w. 94.97 g/mol), what is the maximum possible concentration of Pb^{2+} (in ppb) in the water supply? (Hints: Assume 1 ppm = 1 mg/L and 1 ppb = 1 µg/L.)

$$\frac{5.0 \text{ mg } PO_4^{3-}}{L} * \frac{1 \text{ g}}{1000 \text{ mg}} * \frac{1 \text{ mol}}{94.97 \text{ g}} = 5.3 \times 10^{-5} M PO_4^{3-}$$



<i>I</i>	-	0	$5.3 \times 10^{-5} M$
<i>C</i>	-	+3x	+2x
<i>E</i>	-	3x	$5.3 \times 10^{-5} + 2x$

$$K_{sp} = [Pb^{2+}]^3 [PO_4^{3-}]^2$$

$$3.0 \times 10^{-44} = (3x)^3 (5.3 \times 10^{-5} + 2x)^2$$

Assume 2x is small so that $x = 0$

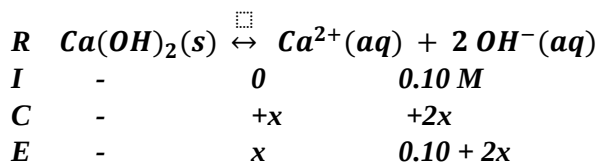
$$3.0 \times 10^{-44} = 27x^3 (5.3 \times 10^{-5})^2$$

$$x = 7.4 \times 10^{-13} M$$

$$[Pb^{2+}] = 3x = 2.2 \times 10^{-12} M * \frac{207.2 \text{ g } Pb}{1 \text{ mol } Pb} * \frac{10^6 \text{ µg}}{1 \text{ g } Pb} = 4.6 \times 10^{-4} \text{ ppb } Pb^{2+}$$

6. $\text{Ca}(\text{OH})_2$ can be used to remove impurities and suspended particles from water. What is the concentration of Ca^{2+} at equilibrium when $\text{Ca}(\text{OH})_2$ is dissolved in a solution with an initial pH of 13.00? The K_{sp} of calcium hydroxide is 6.5×10^{-6} . (Hint: Use the pH to determine the initial concentration of OH^- .)

$$[\text{OH}^-] = 10^{-1.00} = 0.10 \text{ M}$$



$$K_{sp} = [\text{Ca}^{2+}][\text{OH}^-]^2$$

$$6.5 \times 10^{-6} = (x)(0.10 + 2x)^2$$

Assume $2x$ is small so that $x = 0$

$$6.5 \times 10^{-6} = (x)(0.10)^2$$

$$6.5 \times 10^{-6} = 0.01x$$

$$x = 6.5 \times 10^{-4} \text{ M}$$

$$\text{Check assumption: } \frac{2 \times 6.5 \times 10^{-4}}{0.10} \times 100 = 1.3\% < 5\%$$

$$[\text{Ca}^{2+}] = x = 6.5 \times 10^{-4} \text{ M}$$

Active Learning Worksheets for Analytical Chemistry

#8 Equilibrium Chemistry: Fundamentals of Acid–Base Reactions

Faculty Resources

(Delete before sharing with students):

- **Analytical Context and Framing:** I start by separating the everyday meaning of strong/weak from the chemical meaning. In chemistry, strong and weak do not mean concentrated or dangerous; they describe the extent of dissociation. I present this worksheet as a review of ideas students likely saw in general chemistry, but I use it to make sure everyone has the acid–base foundation they need before moving into buffers, titration curves, and polyprotic systems.
- **Key Concepts and Common Challenges:** Amphiprotic species can be difficult for students because they want to classify a species as only an acid or only a base. For species such as HSO_4^- , ask students to write one reaction where it donates a proton and one reaction where it accepts a proton.
- **Facilitated Discussion Pauses:** Pause after the acid-strength ranking question. Ask: “If chloroacetic acid has a larger K_a than acetic acid, what does that suggest about how the chlorine atom affects the conjugate base?” This helps students connect numerical K_a values to molecular structure and stability.
- **Calculation Support:** Use the weak-acid calculations as a just-in-time reminder about the 5% rule. If the approximation works in one problem but fails in the next, stop and point out why successive approximations are sometimes necessary rather than optional.
- **Synthesis and Extension Questions:** Ask students: “You have 0.010 M HCl and 0.010 M HF. Which solution has the lower pH, and why does that matter if you are calculating how much neutralizing agent is needed for a spill?”
- **Pacing Note:** This worksheet is designed primarily as a review, but students may be coming to it with very different timelines and levels of mastery since general chemistry. Some may have seen this material recently, while others may not have used it in several years. If time is limited, focus class discussion on acid/base classification, conjugate pairs, and the first weak-acid calculation. The remaining calculation practice can be assigned as follow-up work.

Answer Keys for Active Learning Worksheets for Analytical Chemistry

#8 Equilibrium Chemistry: Fundamentals of Acid–Base Reactions

Before You Begin

- Read about acid-base reactions: [6.4 Equilibrium Constants for Chemical Reactions](#)
- Read about the method of successive approximations: [6.7: Solving Equilibrium Problems](#)

What You Should Already Know

- General chemical equations and molarity
- pH, pOH, and the autoionization of water
- Definitions of acids, bases, and conjugates
- Equilibrium expressions and ICE tables

What You'll Learn

- Identify strong and weak acids and bases from formulas
- Write dissociation and hydrolysis reactions
- Identify conjugate acid–base pairs
- Use K_a to calculate equilibrium concentrations and pH
- Compare acid strength based on K_a or pK_a

What You'll Practice

- Translate word prompts into chemical equations
- Apply equilibrium concepts to real systems
- Evaluate strength of acids/bases using numerical data
- Communicate reasoning clearly in multi-step calculations

Estimated Completion Time: 45-60 minutes

Author: Rebecca L. Sanders

Active Learning Worksheets for Analytical Chemistry, Version 1.0, 2026

Useful Equations

- $pH = -\log[H^+]$
- $pH + pOH = 14.00$
- $[H^+] * [OH^-] = K_a * K_b = K_w = 1.0 * 10^{-14}$
- $pK_a = -\log(K_a)$
- $pK_a + pK_b = pK_w = 14.00$

Strong Acids and Bases

1. List the strong acids and bases.

Strong acids:

- Hydrochloric acid (HCl)
- Hydrobromic acid (HBr)
- Hydroiodic acid (HI)
- Nitric acid (HNO_3)
- Perchloric acid (HClO_4)
- Sulfuric acid (H_2SO_4)

Strong bases:

- Lithium hydroxide (LiOH)
- Sodium hydroxide (NaOH)
- Potassium hydroxide (KOH)
- Rubidium hydroxide (RbOH)
- Cesium hydroxide (CsOH)
- Calcium hydroxide ($\text{Ca}(\text{OH})_2$)
- Strontium hydroxide ($\text{Sr}(\text{OH})_2$)
- Barium hydroxide ($\text{Ba}(\text{OH})_2$)

2. What is the K_a for HClO_4 ?

HClO_4 is a strong acid so the K_a is greater than 1.

3. Find the pH and $[\text{H}^+]$ of a solution containing 0.010 M HClO_4 .

HClO_4 is a strong acid, so $[\text{H}^+] = 0.010 \text{ M}$.

$$\text{pH} = -\log[\text{H}^+] = -\log(0.010 \text{ M}) = 2.00$$

4. Barium hydroxide is typically classified as a strong base. However, it is not completely soluble with a $K_{sp} = 3 \times 10^{-4}$. What is the pH of 0.035 M $\text{Ba}(\text{OH})_2$, which is below the saturation limit for barium hydroxide?

Because 0.035 M is below the saturation limit for barium hydroxide, this concentration can exist in solution. Therefore, $\text{Ba}(\text{OH})_2$ acts as a strong base as this concentration, so for every one mole of $\text{Ba}(\text{OH})_2$, two moles of $\text{OH}^-(\text{aq})$ are produced in solution.

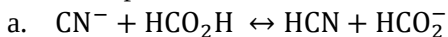
$$[\text{OH}^-] = 2 \times 0.035 \text{ M} = 0.070 \text{ M}$$

$$\text{pOH} = -\log[\text{OH}^-] = -\log(0.070 \text{ M}) = 1.15$$

$$\text{pH} = 14.00 - \text{pOH} = 12.85$$

Brønsted–Lowry Conjugate Acid-Base Pairs

5. Label the Brønsted–Lowry acid and base on each side of the reaction and identify each conjugate acid/base pair.

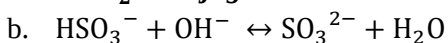


CN^- : Base

HCO_2H : Acid

HCN : Conjugate acid

HCO_2^- : Conjugate base



Acid Base CB CA

OH^- : Base

HSO_3^- : Acid

H_2O : Conjugate acid
 SO_3^{2-} : Conjugate base

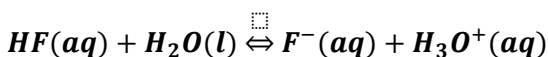
Weak Acids and Bases

6. HSO_4^- is amphiprotic and can act as both an acid and a base. Write its conjugate acid and conjugate base.

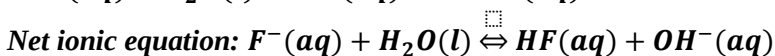
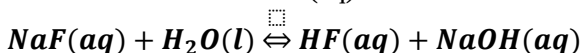
Conjugate acid: H_2SO_4

Conjugate base: SO_4^{2-}

7. Write the reaction of $HF(aq)$ and water.



8. Write the reaction of $NaF(aq)$ and water.

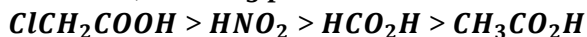


9. Below lists K_a values for several acids.

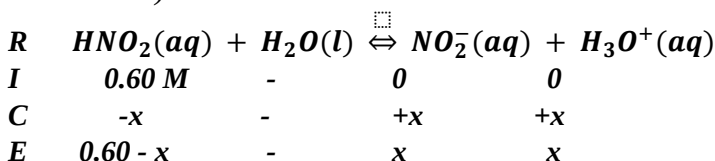
CH_3CO_2H	1.75×10^{-5}
$ClCH_2COOH$	1.36×10^{-3}
HCO_2H	1.80×10^{-4}
HNO_2	7.1×10^{-4}

- a. Rank these acids from strongest to weakest based on K_a values. Briefly explain your ranking.

The bigger the K_a (or the smaller the pK_a), the stronger the acid because more of the acid dissociates, releasing protons into the solution.



- b. What are the equilibrium concentrations of $[H^+]$, $[OH^-]$, $[HNO_2]$, and $[NO_2^-]$ in a 0.60 M solution of HNO_2 ? What is the pH of the solution? (Hint: You can write the anion, NO_2^- , as just A^- in your reaction if you prefer. This is especially helpful for complicated or unknown anion of a weak acid.)



$$K_a = \frac{[NO_2^-][H_3O^+]}{[HNO_2]}$$

$$7.1 \times 10^{-4} = \frac{[x][x]}{[0.60-x]} \text{ assume } x \text{ in the denominator is small}$$

$$7.1 \times 10^{-4} = \frac{x^2}{0.60}$$

$$x = 2.1 \times 10^{-2} M = [H_3O^+] = [NO_2^-]$$

$$\frac{0.021}{0.60} \times 100 = 3.5\%$$

Good assumption because $< 5\%$

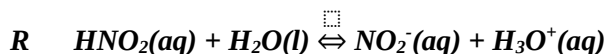
$$pH = -\log(0.021) = 1.69$$

$$[HNO_2] = 0.60 - 0.021 = 0.58 \text{ M}$$

$$pOH = 14.00 - 1.69 = 12.31$$

$$[OH^-] = 10^{-12.31} = 4.9 \times 10^{-13} \text{ M}$$

- c. What are the equilibrium concentrations of $[H^+]$, $[OH^-]$, $[HNO_2]$, and $[NO_2^-]$ in a 0.060 M solution of HNO_2 ? What is the pH of the solution?



$$I \quad 0.060 \text{ M} \quad - \quad 0 \quad 0$$

$$C \quad -x \quad - \quad +x \quad +x$$

$$E \quad 0.060 - x \quad - \quad x \quad x$$

$$K_a = \frac{[NO_2^-][H_3O^+]}{[HNO_2]}$$

$$7.1 \times 10^{-4} = \frac{[x][x]}{[0.060-x]} \quad \text{assume } x \text{ in the denominator is small}$$

$$7.1 \times 10^{-4} = \frac{x^2}{0.060}$$

$$x_1 = 6.5 \times 10^{-3} \text{ M} \quad \text{Bad assumption because } < 5\% \frac{0.0065}{0.060} \times 100 = 11\%$$

Using successive approximations

$$7.1 \times 10^{-4} = \frac{[x][x]}{[0.060-0.0065]} \quad \text{Plug in } x_1 \text{ in the denominator}$$

$$7.1 \times 10^{-4} = \frac{x^2}{0.0535}$$

$$x_2 = 6.2 \times 10^{-3} \text{ M}$$

$$7.1 \times 10^{-4} = \frac{[x][x]}{[0.060-0.0062]} \quad \text{Plug in } x_2 \text{ in the denominator}$$

$$7.1 \times 10^{-4} = \frac{x^2}{0.0538}$$

$$x_3 = 6.2 \times 10^{-3} \text{ M}$$

$$pH = -\log(0.0062) = 2.21$$

$$[HNO_2] = 0.060 - 0.0062 = 0.054 \text{ M}$$

$$pOH = 14.00 - 2.21 = 11.79$$

$$[OH^-] = 10^{-11.79} = 1.6 \times 10^{-12} \text{ M}$$

Active Learning Worksheets for Analytical Chemistry

#9 Equilibrium Chemistry: Designing and Evaluating Buffer Systems

Faculty Resources

(Delete before sharing with students):

- **Analytical Context and Framing:** Use the biological buffer scenario to emphasize that buffer design is not just about hitting the right pH. Tell students: “You may have several possible buffer systems, but one might be toxic, one might be expensive, and one might become less useful under certain storage conditions. A buffer can be chemically reasonable and still be a poor practical choice.”
- **Key Concepts and Common Challenges:** Formal concentration is often the sticking point. Students may calculate $[HA]$ or $[A^-]$ correctly but forget that the formal concentration is the total concentration of all forms in the acid–base pair. Encourage them to set up the problem as two equations: one from Henderson–Hasselbalch and one from mass balance.
- **Facilitated Discussion Pauses:** Pause at the 2-aminophenol distribution plot. Have students identify the approximate pK_a values by finding where the relevant species have equal concentrations. This visual approach often helps students understand why $pH = pK_a$ when the conjugate acid and base forms are present in equal amounts.
- **Calculation Support:** When students calculate the volume of glacial acetic acid needed, remind them to first determine the required concentration of acetic acid from the buffer calculation and then use a dilution calculation to convert that concentration into a measurable volume.
- **Synthesis and Extension Questions:** Ask students: “If you choose a buffer system where the target pH is near the edge of the useful buffer range, what could go wrong when you actually prepare or use the solution?” This helps students see why buffer choice is not just about finding a pK_a somewhere nearby; the buffer needs to resist pH change under realistic conditions.
- **Pacing Note:** This activity builds on Worksheet #8, but can stand alone if students know the basics of acid/base chemistry. The timing depends heavily on students’ prior comfort with buffer chemistry. It can move quickly if students remember Henderson–Hasselbalch and conjugate acid–base pairs, but it may take longer if they need to reconstruct the buffer logic from the beginning. If this worksheet needs to be divided across class periods, a natural stopping point is after Question 4. Up to that point, students focus on buffer preparation, formal concentration, Henderson–Hasselbalch calculations, and interpreting a simple acid/conjugate-base distribution plot. The remaining questions extend this reasoning to polyprotic systems, speciation plots, and buffer selection in more realistic contexts.

Answer Keys for Active Learning Worksheets for Analytical Chemistry

#9 Equilibrium Chemistry: Designing and Evaluating Buffer Systems

Before You Begin

- Read about buffers: [6.8: Buffer Solutions](#)

What You Should Already Know

- Using pK_a and Henderson-Hasselbalch equation
- Writing and balancing acid–base reactions
- Understanding strong vs. weak electrolytes
- RICE tables and equilibrium reasoning

What You'll Learn

- Design buffer systems using weak acid/base pairs and pK_a values
- Perform quantitative buffer calculations (pH, composition, concentration)
- Interpret and apply speciation curves for polyprotic acids
- Evaluate buffer strategies using acid/base equilibrium concepts

What You'll Practice

- Use mathematical models (e.g., Henderson–Hasselbalch) to solve real lab problems
- Sketch and interpret pH-dependent concentration plots
- Communicate acid-base reasoning using clear, quantitative language
- Justify buffer choices in experimental and environmental contexts

Estimated Completion Time: 60-90 minutes

Author: Rebecca L. Sanders

Active Learning Worksheets for Analytical Chemistry, Version 1.0, 2026

Useful Equations

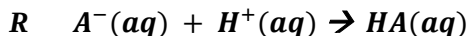
- $pH = -\log[H^+]$
- $pH + pOH = 14.00$
- $[H^+] * [OH^-] = K_a * K_b = K_w = 1.0 * 10^{-14}$
- $pKa = -\log(K_a)$
- $pK_a + pK_b = pK_w = 14.00$
- $pH = pK_a + \log(base/acid)$

Making Buffers

1. In the laboratory, you mix 4.5 g of solid sodium acetate (82.03 g/mol) with 500.0 mL of 0.100 M HCl. What is the pH of the solution? The pK_a of acetic acid is 4.76.

$$4.5 \text{ g sodium acetate} * \frac{1 \text{ mol}}{82.03 \text{ g}} = 0.055 \text{ mol sodium acetate or } A^-$$

$$500.0 \text{ mL } H^+ * \frac{1 \text{ L}}{1000 \text{ mL}} * \frac{0.100 \text{ mol HCl}}{1 \text{ L}} = 0.0500 \text{ mol}$$



$$I \quad 0.055 \quad 0.0500 \quad 0$$

$$C \quad -0.0500 \quad -0.0500 \quad +0.0500$$

$$E \quad 0.005 \quad 0 \quad 0.0500$$

$$pH = pK_a + \log\left(\frac{A^-}{HA}\right)$$

$$pH = 4.76 + \log\left(\frac{0.005}{0.0500}\right)$$

$$pH = 3.76$$

2. Suppose you want to make a buffer with a formal concentration of 0.28 M and a pH of 4.94. Calculate how much acetic acid and sodium acetate you would use to make 1 L of this buffer. Glacial acetic acid is 17.5 M. (Hint: the formal concentration is the sum of $[HA]$ and $[A^-]$ in solution.)

Use two equations and two unknowns.

$$1. \quad pH = pK_a + \log\left(\frac{A^-}{HA}\right)$$

$$2. \quad [HA] + [A^-] = 0.28 \text{ M}$$

$$pH = pK_a + \log\left(\frac{A^-}{HA}\right)$$

$$4.94 = 4.76 + \log\left(\frac{A^-}{HA}\right)$$

$$0.18 = \log\left(\frac{A^-}{HA}\right)$$

$$10^{0.18} = \frac{A^-}{HA}$$

$$1.51 = \frac{A^-}{HA}$$

$$1.51[HA] = [A^-] \text{ substitute into equation 2}$$

$$[HA] + [A^-] = 0.28 \text{ M}$$

$$[HA] + 1.51[HA] = 0.28 \text{ M}$$

$$2.51[HA] = 0.28 \text{ M}$$

$$[HA] = 0.11 \text{ M}$$

$$[A^-] = 0.17 \text{ M}$$

$$[HA]: M_1V_1 = M_2V_2$$

$$(0.11 \text{ M})(1 \text{ L}) = (17.5 \text{ M})V_2$$

$$V_2 = 0.0063 \text{ L} = 6.3 \text{ mL glacial acetic acid}$$

$$[A^-] = 1 \text{ L} * \frac{0.17 \text{ mol } A^-}{1 \text{ L}} * \frac{82.03 \text{ g}}{1 \text{ mol}} = 14 \text{ g sodium acetate}$$

Understanding Species Distributions in Buffers

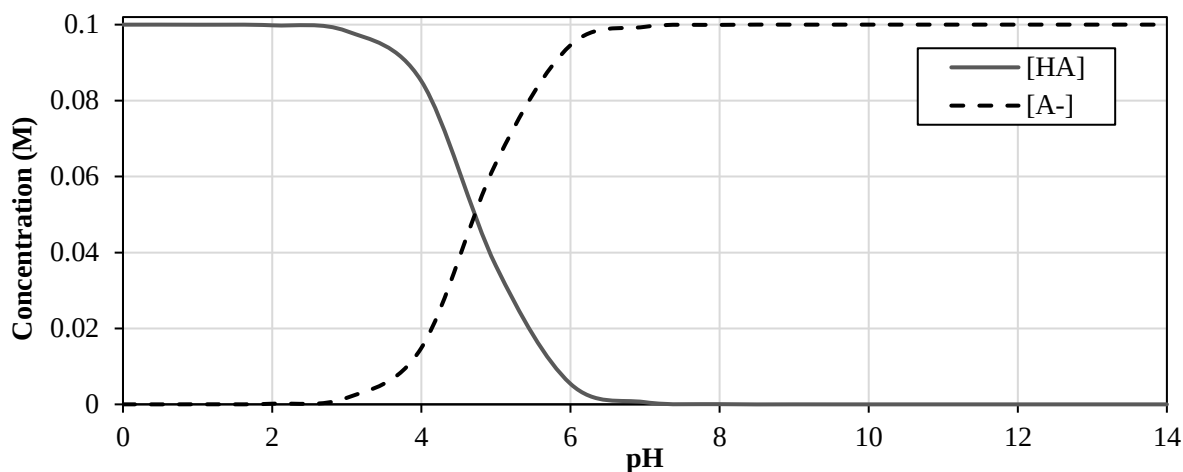


Figure 1. Distribution plot as a function of pH for a 0.10 M acetic acid-acetate buffer. The acetic acid concentration is plotted as a straight line and the acetate concentration is plotted as a dashed line.

3. The concentration of acetic acid and acetate in a buffer containing 0.10 M total concentration can be plotted as a function of pH.

- a. Where is the pK_a on this graph? What are the concentrations of acetic acid (HA) and acetate (A^-) at the pK_a ?

Where the lines cross, $pH = pK_a \approx 4.7$

- b. When $pH = pK_a - 1$, what are the concentrations of acetic acid (HA) and acetate (A^-) at the pK_a ?

$[HA] \approx 0.09 \text{ M}$ and $[A^-] \approx 0.01 \text{ M}$

- c. When $pH = pK_a + 1$, what are the concentrations of acetic acid (HA) and acetate (A^-) at the pK_a ?

$[HA] \approx 0.01 \text{ M}$ and $[A^-] \approx 0.09 \text{ M}$

- d. Is acetic acid/acetate a suitable buffer system at pH 6.00? Why or why not?

No, the buffer should have a pH within 1 pH unit of the pK_a , so acetic acid and acetate could make a buffer between 3.76 to 5.76.

4. Ammonium (NH_4^+) has a pK_a of 9.244.

- a. Name three ways you could make a buffer of ammonium (NH_4^+) and ammonia (NH_3).

- 1. Mix an ammonium containing salt (like NH_4NO_3 or NH_4Cl) with ammonia**
- 2. Mix an ammonium containing salt (like NH_4NO_3 or NH_4Cl) with a strong base like $NaOH$**
- 3. Mix ammonia with a strong base like HCl or HNO_3**

- b. Sketch $[NH_4^+]$ and $[NH_3]$ for a buffer with a formal concentration of 0.050 M.

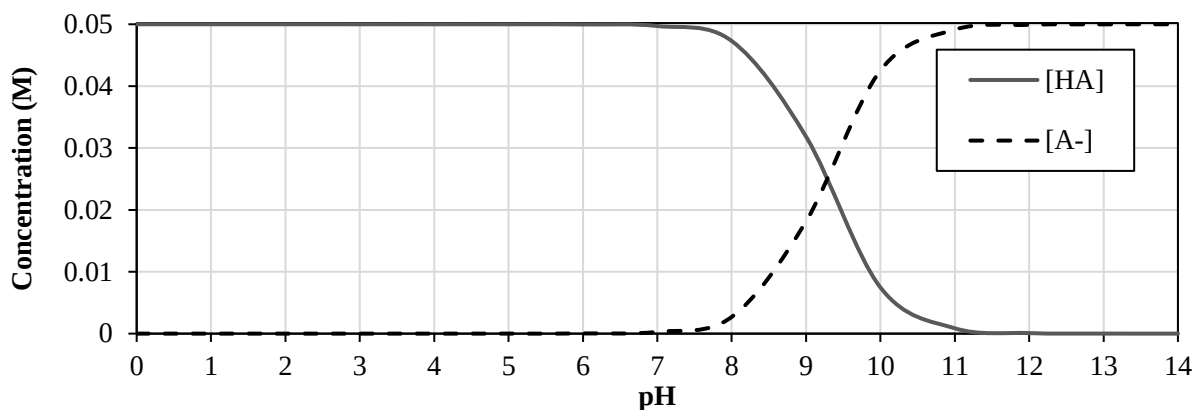


Figure 2. Empty graph to plot the distribution of $[NH_4^+]$ and $[NH_3]$.

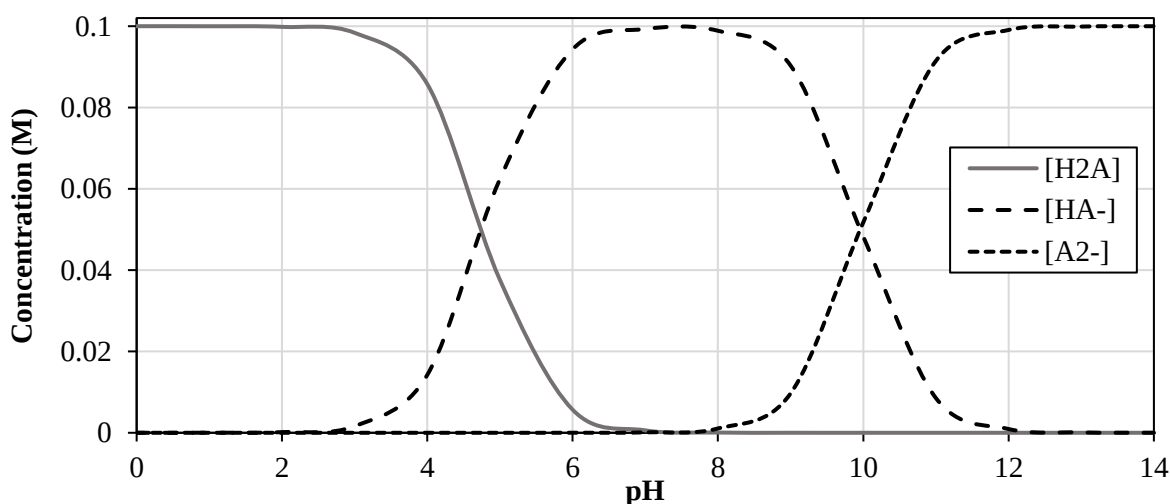


Figure 3. Distribution plot as a function of pH for 0.10 M 2-aminophenol, a diprotic acid. The acidic form (H_2A) concentration is plotted as a straight line, intermediate form (HA^-) concentration is plotted as a long-dashed line, and the basic form (A^{2-}) concentration is plotted as a short-dashed line.

5. These plots can be used to visualize the concentration of various species in solution using polyprotic acids and bases. For example, 2-aminophenol is a diprotic acid (H_2A). The acidic form is labeled H_2A , the intermediate form with one proton lost is labeled HA^- , and the basic form with both protons lost is labeled as A^{2-} .
 - a. Use the plot to determine the approximate pK_a values of 2-aminophenol where $[H_2A] = [HA^-]$ and $[HA^-] = [A^{2-}]$. pK_{a1} represents the first proton lost from H_2A and pK_{a2} will represents the second proton lost from H_2A .

$pK_{a1} \approx 4.8$ and $pK_{a2} \approx 10.0$ (actual values are 4.78 and 9.97)

- b. What pH ranges can 2-aminophenol be used to make a buffer? Why? What conjugate acid-base pair is present in each buffer?

Buffers could be made in the pH ranges of 3.8-5.8 or 9.0-11.0.

- c. A classmate suggests to make a buffer at pH 9.30 by mixing H_2A and HA^- . Another classmate suggests mixing HA^- with $NaOH$. Of the two strategies, which would you choose and why?

H_2A and HA^- can only be made to make a buffer in the pH ranges of 3.6-5.6, so the first option would not work. The second option could work because the pH of the intermediate form, HA^- , can be brought up to pH 9.30.

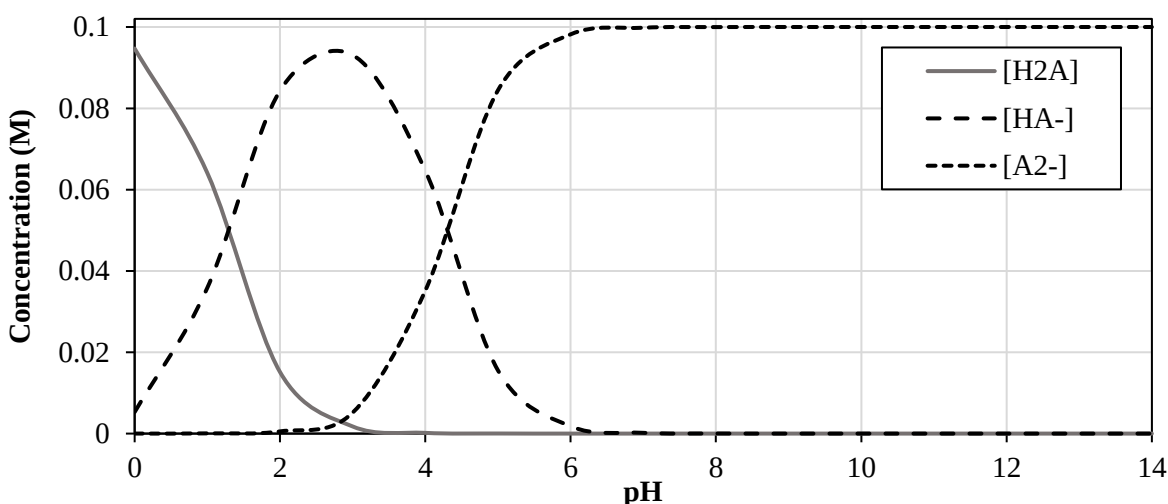


Figure 4. Distribution plot as a function of pH for 0.10 M oxalic acid, a diprotic acid. The acidic form (H_2A) concentration is plotted as a straight line, intermediate form (HA^-) concentration is plotted as a long-dashed line, and the basic form (A^{2-}) concentration is plotted as a short-dashed line.

6. The figure above depicts the speciation of 0.10 M oxalic acid as a function of pH. Oxalic acid has $pK_{a1} = 1.252$ and $pK_{a2} = 4.266$.

- a. On the figure, the concentration of $[H_2A]$ never reaches 0.10 M. Why?

pK_{a1} is too low for the concentration of $[H_2A]$ to reach 0.10 M by $pH = 0$.

- b. On the figure, the concentration of $[HA^-]$ never reaches 0.10 M. Why?

pK_{a1} and pK_{a2} are too close together for $[HA^-]$ to reach 0.10 M. Because pK_{a1} and pK_{a2} are close (1.252 and 4.266), HA^- is always being formed and consumed over the same pH range.

7. Sketch the concentrations of $[H_3PO_4]$, $[H_2PO_4^-]$, $[HPO_4^{2-}]$, and $[PO_4^{3-}]$ across pH if total concentration of all phosphate containing species is 0.10 M and $pK_{a1} = 2.148$, $pK_{a2} = 7.199$, and $pK_{a3} = 12.35$.

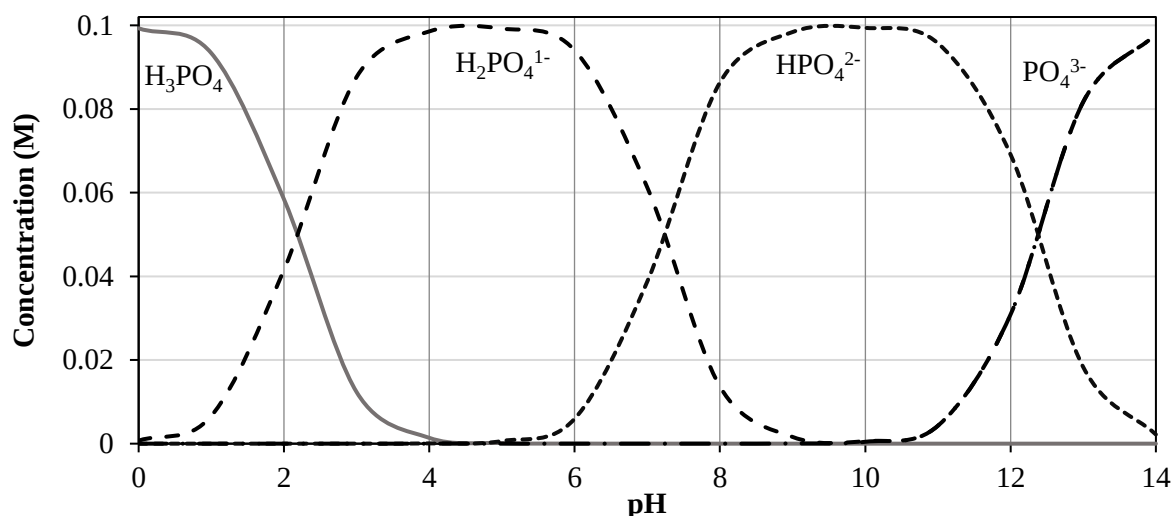


Figure 5. Empty graph to plot the distribution of $[NH_4^+]$ and $[NH_3]$.

8. You have three possible conjugate pairs that could work for pH 7.4, which is needed for a biological study. One is toxic, one is expensive, and one has low solubility at 4 °C. Which do you choose for your study? List two questions you would ask your instructor before ordering the chemicals.

The expensive buffer is likely the best choice because it is the only one that guarantees the experiment will actually work. While we always try to be mindful of costs, a "cheap" experiment that fails is a total waste of time and resources. The toxic buffer could kill the cell line or inhibit the enzyme we are trying to study or introduce chemical interferences that mess up our signal and make our data unreliable. Plus, as we saw in Worksheet #6, generating toxic waste creates long-term disposal problems and environmental risks. The low solubility buffer is likely an issue because most biological work happens on ice or in a cold room to keep samples stable. If the buffer has low solubility at 4 °C, it will precipitate. As we learned in Worksheet #7, once the concentration of the dissolved species changes due to precipitation, the equilibrium is destroyed, and the pH will shift, likely ruining the biological sample.

There are many questions students could ask their instructors, but the questions will likely focus on chemical compatibility, budget, and protocols. For example, students could ask:

- "Does our specific biological system have a known sensitivity to this particular buffer that might cause an interference?"*
- "Since the only chemically viable buffer for a cold-room study is significantly more expensive, do we have the budget for this reagent?"*
- "Can the protocol be changed to run the experiment at room temperature?"*

Active Learning Worksheets for Analytical Chemistry

#10 Equilibrium Chemistry: Titration Curve Calculations for Monoprotic Acids

Faculty Resources

(Delete before sharing with students):

- **Analytical Context and Framing:** I like to present titration calculations as predictable once students understand what is present in solution. A titration curve shows the pH changing across the experiment, but students can calculate the pH at any point if they can identify the major species and the chemistry controlling that region of the curve. I emphasize that the most important step is recognizing what is present at each point or region; once students understand the conceptual chemistry, the math becomes much easier. This worksheet focuses on monoprotic titrations because students need this foundation before moving into the polyprotic titration calculations that come later in the unit.
- **Key Concepts and Common Challenges:** Students often forget to account for dilution at the equivalence point. Emphasize that after titrant is added, the relevant concentration must be calculated using the new total volume, not the initial volume alone.
- **Facilitated Discussion Pauses:** Pause after the half-equivalence calculation. Ask: “Why is the pH equal to the pK_a here? What happened to the log term in the Henderson–Hasselbalch equation?”
- **Calculation Support:** In the weak base/strong acid section with Tris, remind students that the curve starts at a basic pH and moves downward. For the initial pH, they should use the base behavior of Tris. In the buffer region, they can use the pK_a of the conjugate acid form.
- **Synthesis and Extension Questions:** Ask students: “If you were titrating a very weak acid with $pK_a = 9$, would the pH jump at the equivalence point be larger or smaller than the jump for aspirin with $pK_a = 3.5$? Why would this matter when selecting an indicator?”
- **Pacing Note:** This activity builds on acid/base and buffer logic from Worksheets 8–9. This worksheet divides naturally between the two sets of titration calculations. A natural stopping point is after Question 1, which focuses on the weak acid/strong base titration. The second part begins with the weak base/strong acid titration and allows students to apply similar reasoning to a curve that starts at high pH and moves downward.

Answer Keys for Active Learning Worksheets for Analytical Chemistry

#10 Equilibrium Chemistry: Titration Curve Calculations for Monoprotic Acids

Before You Begin

- Read about acid-base reactions: [6.4 Equilibrium Constants for Chemical Reactions](#)
- Read about the method of successive approximations: [6.7: Solving Equilibrium Problems](#)
- Read about buffers: [6.8: Buffer Solutions](#)
- Read about acid-base titrations: [9.2: Acid–Base Titrations](#)

What You Should Already Know

- Stoichiometry and limiting reagents
- Henderson-Hasselbalch equation
- pH , pK_a , and K_w relationships
- Interpreting titration curves

What You'll Learn

- Determine pH at various points during a titration
- Identify buffer regions and equivalence points
- Use moles and volumes to determine remaining acid/base concentrations
- Apply Henderson–Hasselbalch to buffer solutions

What You'll Practice

- Translate titration steps into quantitative models
- Use appropriate acid/base theory at each titration stage
- Explain pH changes with clear reasoning and chemical evidence

Estimated Completion Time: 75-90 minutes

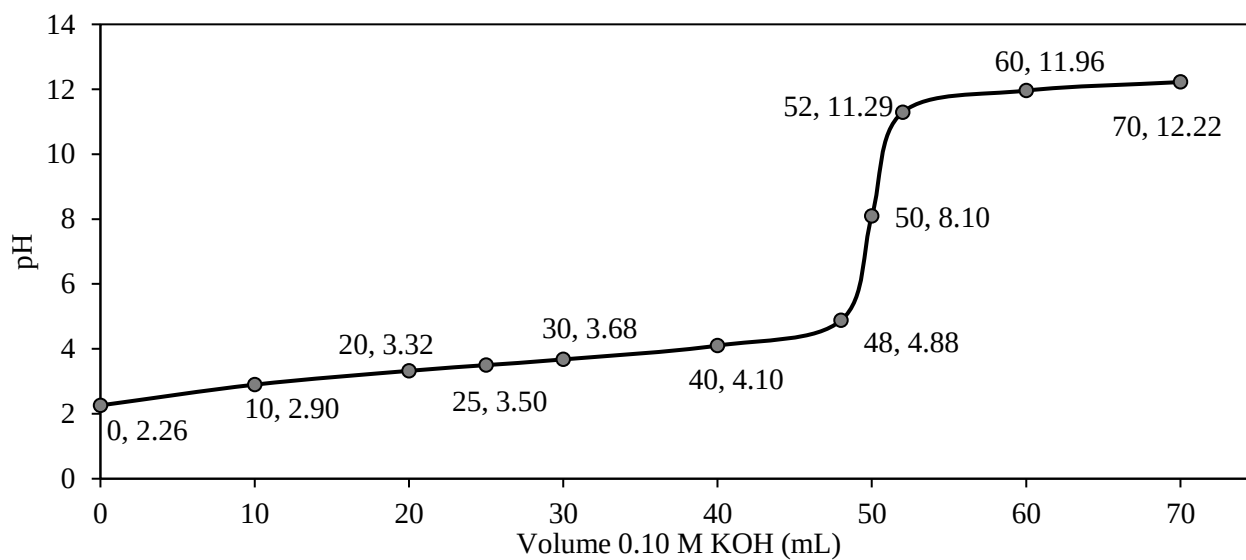
Author: Rebecca L. Sanders

Active Learning Worksheets for Analytical Chemistry, Version 1.0, 2026

Useful Equations

- $M_A V_A = M_B V_B$
- $pH = -\log[H^+]$
- $pH + pOH = 14.00$
- $[H^+] * [OH^-] = K_a * K_b = K_w = 1.0 * 10^{-14}$
- $pK_a = -\log(K_a)$
- $pK_a + pK_b = pK_w = 14.00$
- $pH = pK_a + \log(base/acid)$

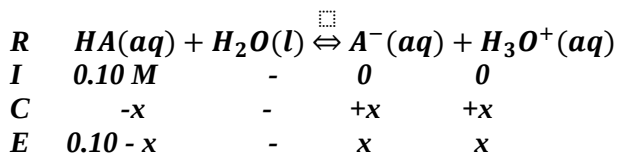
Weak Acid/Strong Base Titration Calculation



- Aspirin (acetylsalicylic acid) acts as a weak monoprotic acid. A 50.0 mL solution of 0.10 M aspirin was titrated with 0.10 M KOH. Verify the pH values on the graph by calculating the pH after 0.0, 25.0, 40.0, 50.0, and 70.0 mL of KOH was added to the aspirin solution. The pK_a of aspirin is 3.50.
 - Equivalence volume

$$\begin{aligned}
 M_A V_A &= M_B V_B \\
 (0.10\text{ M})(50.0\text{ mL}) &= (0.10\text{ M})V_B \\
 V_B &= 50.0\text{ mL} = V_e
 \end{aligned}$$

- 0.0 mL KOH



$$K_a = 10^{-3.50} = 3.16 \times 10^{-4} = \frac{x^2}{0.10 - x} \text{ assume } x \text{ in the denominator is small}$$

$$3.16 \times 10^{-4} = \frac{x^2}{0.10}$$

$$x_1 = 5.6 \times 10^{-3}\text{ M} = [H_3O^+]$$

$$\text{Bad assumption } \left(\frac{0.0056}{0.10} \times 100 = 5.6\% > 5\% \right)$$

Use x_1 as the x value in the denominator to calculate x_2

$$3.16 \times 10^{-4} = \frac{x^2}{0.10 - 0.0056}$$

$$x_2 = 5.5 \times 10^{-3}\text{ M} = [H_3O^+]$$

Use x_2 as the x value in the denominator to calculate x_3

$$3.16 \times 10^{-4} = \frac{x^2}{0.10 - 0.0055}$$

$$x_3 = 5.5 \times 10^{-3}\text{ M} = [H_3O^+]$$

$$pH = -\log(5.5 \times 10^{-3}\text{ M}) = 2.26$$

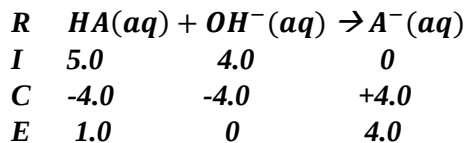
- c. 25.0 mL KOH

$$\text{At } \frac{1}{2} V_e, \text{pH} = \text{p}K_a = 3.50$$

- d. 40.0 mL KOH

$$40.0 \text{ mL KOH} * 0.10 \text{ M KOH} = 4.0 \text{ mmol KOH} = 4.0 \text{ mmol OH}^-$$

$$50.0 \text{ mL} * 0.10 \text{ M HA} = 5.0 \text{ mmol HA}$$



$$\text{pH} = \text{p}K_a + \log\left(\frac{\text{A}^-}{\text{HA}}\right)$$

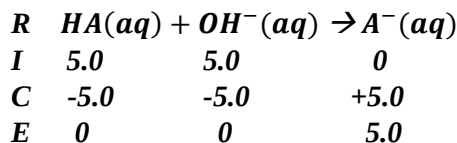
$$\text{pH} = 3.50 + \log\left(\frac{4.0}{1.0}\right)$$

$$\text{pH} = 4.10$$

- e. 50.0 mL KOH

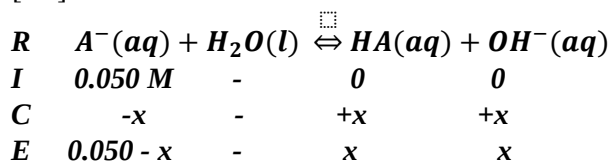
$$50.0 \text{ mL KOH} * 0.10 \text{ M KOH} = 5.0 \text{ mmol KOH} = 5.0 \text{ mmol OH}^-$$

$$50.0 \text{ mL} * 0.10 \text{ M HA} = 5.0 \text{ mmol HA}$$



All of the HA has been converted to A^- . Now, a RICE diagram for the basic form (A^-) in water is written after finding the diluted concentration of A^- .

$$[\text{A}^-] = 5.0 \text{ mmol A}^- / 100.0 \text{ mL} = 0.050 \text{ M}$$



$$K_b = 10^{-10.50} = 3.16 * 10^{-11} = \frac{x^2}{0.050 - x} \quad \text{assume the x in the denominator is small}$$

$$3.16 * 10^{-11} = \frac{x^2}{0.050}$$

$$x = 1.3 * 10^{-6} \text{ M} = [\text{OH}^-] \quad \text{Good assumption} \left(\frac{0.0000013}{0.050} * 100 = 0.0025\% < 5\% \right)$$

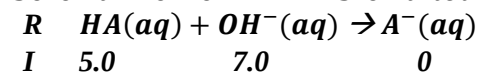
$$\text{pOH} = -\log(1.3 * 10^{-6} \text{ M}) = 5.90$$

$$\text{pH} = 14.00 - 5.90 = 8.10$$

- f. 70.0 mL KOH

$$70.0 \text{ mL KOH} * 0.10 \text{ M KOH} = 7.0 \text{ mmol KOH} = 7.0 \text{ mmol OH}^-$$

$$50.0 \text{ mL} * 0.10 \text{ M HA} = 5.0 \text{ mmol HA}$$



<i>C</i>	-5.0	-5.0	+5.0
<i>E</i>	0	2.0	5.0

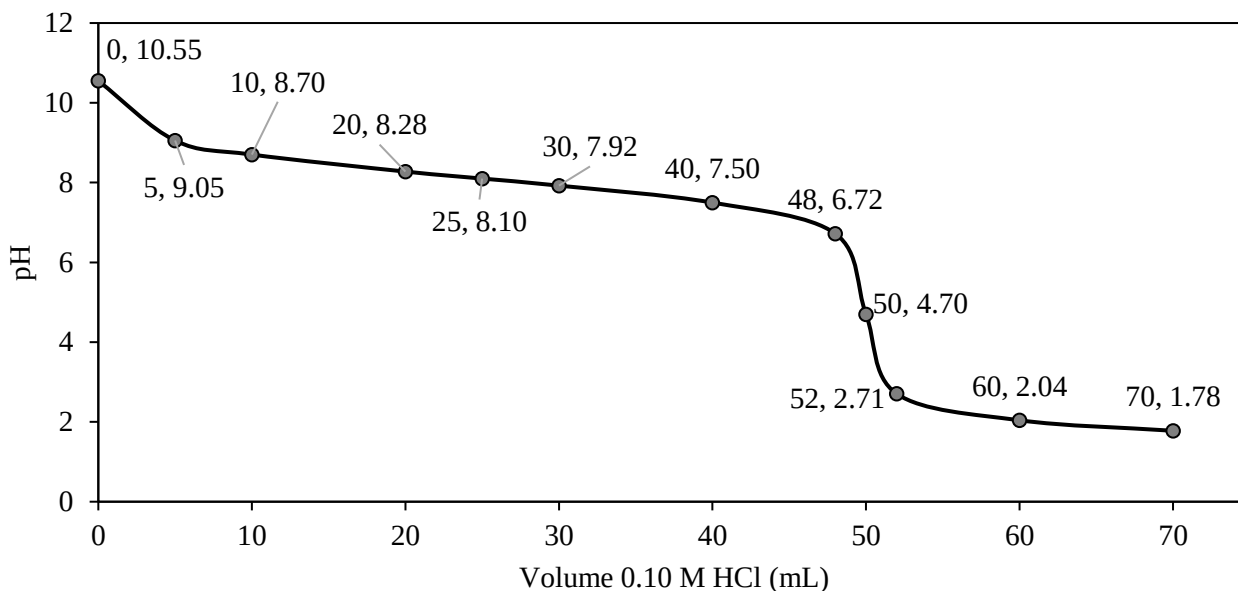
At this point, there is excess OH^- in solution, which dictates the pH of the solution.

$$[\text{OH}^-] = \frac{2.0 \text{ mmol}}{120.0 \text{ mL}} = 0.017 \text{ M}$$

$$\text{pOH} = -\log(0.017 \text{ M}) = 1.78$$

$$\text{pH} = 14.00 - 1.78 = 12.22$$

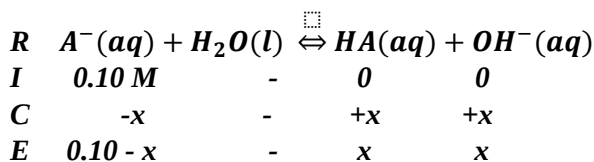
Weak Base/Strong Acid Titration Calculation



2. Tris (tris(hydroxymethyl)aminomethane) is a weak base that accepts protons from HCl. Use the base form as $[\text{A}^-]$ and the conjugate acid as $[\text{HA}]$. A 50.0 mL solution of 0.10 M Tris base was titrated with 0.10 M HCl. Verify the pH values on the graph by calculating the pH after 0.0, 20.0, 25.0, 50.0, and 60.0 mL of HCl was added to the Tris base solution. The acidic form of Tris has $\text{p}K_a$ of 8.10.
- a. Equivalence volume

$$\begin{aligned} M_A V_A &= M_B V_B \\ (0.10 \text{ M}) V_A &= (0.10 \text{ M})(50.0 \text{ mL}) \\ V_A &= 50.0 \text{ mL} = V_e \end{aligned}$$

- b. 0.0 mL HCl



$$K_b = 10^{-5.90} = 1.26 \times 10^{-6} = \frac{x^2}{0.10 - x}$$

assume the x in the denominator is small

$$1.26 \times 10^{-6} = \frac{x^2}{0.10 - x}$$

$$x = 3.5 \times 10^{-4} \text{ M} = [\text{OH}^-] \quad \text{Good assumption} \left(\frac{0.00011}{0.10} \times 100 = 0.22\% < 5\% \right)$$

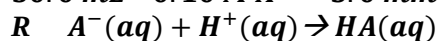
$$pOH = -\log(3.5 \times 10^{-4} M) = 3.45$$

$$pH = 14.00 - 3.45 = 10.55$$

c. 20.0 mL HCl

$$20.0 \text{ mL HCl} \times 0.10 \text{ M HCl} = 2.0 \text{ mmol HCl} = 2.0 \text{ mmol H}_3\text{O}^+$$

$$50.0 \text{ mL} \times 0.10 \text{ M A}^- = 5.0 \text{ mmol A}^-$$



$$I \quad 5.0 \quad 2.0 \quad 0$$

$$C \quad -2.0 \quad -2.0 \quad +2.0$$

$$E \quad 3.0 \quad 0 \quad 2.0$$

$$pH = 8.10 + \log\left(\frac{A^-}{HA}\right)$$

$$pH = 8.10 + \log\left(\frac{3.0}{2.0}\right) = 8.28$$

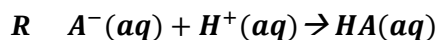
d. 25.0 mL HCl

$$\text{At } \frac{1}{2}V_e, pH = pK_a = 8.10$$

e. 50.0 mL HCl

$$50.0 \text{ mL HCl} \times 0.10 \text{ M HCl} = 5.0 \text{ mmol HCl} = 5.0 \text{ mmol H}_3\text{O}^+$$

$$50.0 \text{ mL} \times 0.10 \text{ M A}^- = 5.0 \text{ mmol A}^-$$



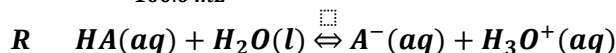
$$I \quad 5.0 \quad 5.0 \quad 0$$

$$C \quad -5.0 \quad -5.0 \quad +5.0$$

$$E \quad 0 \quad 0 \quad 5.0$$

All of the A^- has been converted to HA . Now, a RICE diagram for the acidic form (HA) in water is written after finding the diluted concentration of HA .

$$[HA] = \frac{5.0 \text{ mmol HA}}{100.0 \text{ mL}} = 0.050 \text{ M}$$



$$I \quad 0.050 \text{ M} \quad - \quad 0 \quad 0$$

$$C \quad -x \quad - \quad +x \quad +x$$

$$E \quad 0.050 - x \quad - \quad x \quad x$$

$$K_a = 10^{-8.10} = 7.94 \times 10^{-9} = \frac{x^2}{0.050 - x} \quad \text{assume the } x \text{ in the denominator is small}$$

$$7.94 \times 10^{-9} = \frac{x^2}{0.050 - x}$$

$$x = 2.0 \times 10^{-5} \text{ M} = [H_3O^+]$$

$$\text{Good assumption } \left(\frac{0.000020}{0.050} \times 100 = 0.040\% < 5\% \right)$$

$$pH = -\log(2.0 \times 10^{-5} \text{ M}) = 4.70$$

60.0 mL HCl

$$60.0 \text{ mL HCl} \times 0.10 \text{ M HCl} = 6.0 \text{ mmol HCl} = 6.0 \text{ mmol H}_3\text{O}^+$$

$$50.0 \text{ mL} * 0.10 \text{ M } A^- = 5.0 \text{ mmol } A^-$$

<i>R</i>	$A^-(aq) + H^+(aq) \rightarrow HA(aq)$		
<i>I</i>	5.0	6.0	0
<i>C</i>	-5.0	-5.0	+5.0
<i>E</i>	0	1.0	5.0

At this point, there is excess H^+ in solution, which dictates the pH of the solution.

$$[H^+] = \frac{1.0 \text{ mmol}}{110.0 \text{ mL}} = 0.0091 \text{ M}$$

$$pH = -\log(9.1 * 10^{-3} \text{ M}) = 2.04$$

Active Learning Worksheets for Analytical Chemistry

#11 Equilibrium Chemistry: Polyprotic Acid and Base Reactions and pH Calculations

Faculty Resources

(Delete before sharing with students):

- **Analytical Context and Framing:** I use this worksheet as another step toward the larger goal of being able to predict the pH at any point along a polyprotic acid–base titration curve. Before students can do that well, they need to understand which conjugate acid–base pair is present, how the K_a and K_b values are numbered, and which equilibrium controls the pH in a given situation. I usually emphasize that K_{a1} refers to the first proton lost, while K_{b1} refers to the first proton gained. Students should not try to memorize shortcuts like “ K_{a1} goes with K_{b2} ” because the relationship depends on how many acidic protons are in the system.
- **Key Concepts and Common Challenges:** This is one of the places where students are tempted to memorize shortcuts, and the shortcuts often break down. Students often mix up the relationship between K_a and K_b values in polyprotic systems, especially when switching between the acidic form, intermediate forms, and fully deprotonated basic form. I try to get them to trace the acid–base relationship instead: What species is acting as the acid? What species is acting as the base? Which proton-transfer step are we actually using? That reasoning is slower at first, but it is much more reliable than memorizing which K_a or K_b goes with which species. Question 5 is a common place where students slow down because they have to connect the species, the direction of proton transfer, and the correct equilibrium constant all at once.
- **Facilitated Discussion Pauses:** Pause after question 5 or the sulfurous acid intermediate-form calculation. Ask: “Which proton-transfer step are we actually using here, and which K_a or K_b value belongs with that step?” This helps students move away from memorized pairings and toward tracing the acid–base relationship between the specific species in front of them.
- **Calculation Support:** For the sulfurous acid problem, help students set up the logic stepwise. I often encourage students to temporarily write the system using generic forms such as H_2A , HA^- , and A^{2-} rather than focusing first on the full chemical formulas. This can reduce the cognitive load of tracking formulas while they are still trying to decide which species is acting as the acid or base. Students may also need to see that these problems are not one large equilibrium table, but a sequence of related equilibrium calculations.
- **Synthesis and Extension Questions:** Ask students: “Suppose you are preparing a pH 8.80 solution for an experiment and phosphate is the only buffer system available in the lab. What would you tell someone who asks why this is not a good choice?” I want students to use the pK_a values and available species to justify the decision, rather than simply saying that the numbers “do not work.”
- **Pacing Note:** This activity builds on Worksheets #8-10. If this worksheet needs to be divided across class periods, a natural stopping point is after Question 5. Up to that point, students focus on K_a/K_b relationships for polyprotic systems and choosing appropriate conjugate acid–base pairs for buffers. The remaining questions shift to calculating the pH of the acidic, intermediate, and basic forms of sulfurous acid.

Answer Keys for Active Learning Worksheets for Analytical Chemistry

#11 Equilibrium Chemistry: Polyprotic Acid and Base Reactions and pH Calculations

Before You Begin

- Read about acid-base reactions: [6.4 Equilibrium Constants for Chemical Reactions](#)
- Read about the method of successive approximations: [6.7: Solving Equilibrium Problems](#)
- Read about buffers: [6.8: Buffer Solutions](#)

What You Should Already Know

- Acid/base equilibria and K_a/K_b concepts
- Understanding of conjugate acid–base pairs
- Henderson-Hasselbalch equation
- Stoichiometry and ICE tables

What You'll Learn

- Write equilibrium reactions and expressions for polyprotic systems
- Perform conversions between K_a and K_b conversions and pK_a and pK_b conversions
- Identify appropriate buffer systems for target pH ranges
- Calculate species concentrations at equilibrium for diprotic/triprotic acids

What You'll Practice

- Translate chemical data into appropriate equations and models
- Apply equilibrium problem-solving strategies
- Evaluate buffer construction strategies under constraints
- Communicate speciation outcomes with precision

Estimated Completion Time: 60-90 minutes

Author: Rebecca L. Sanders

Active Learning Worksheets for Analytical Chemistry, Version 1.0, 2026

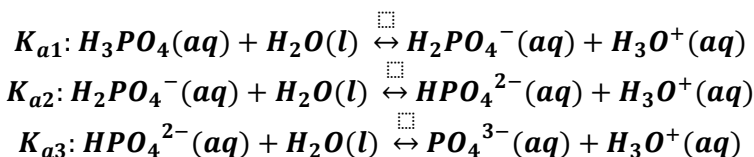
Useful Equations

- $pH = -\log[H^+]$
- $pH + pOH = 14.00$
- $[H^+] * [OH^-] = K_a * K_b = K_w = 1.0 * 10^{-14}$
- $pK_a = -\log(K_a)$
- $pK_a + pK_b = pK_w = 14.00$
- $pH = pK_a + \log(base/acid)$

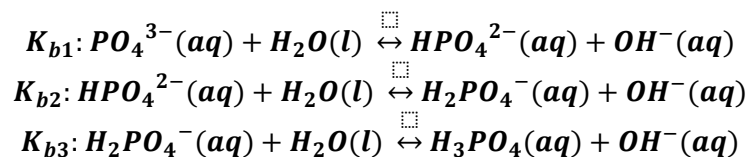
Acid and Base Dissociation Constants for Polyprotic Acids and Bases

Consider the triprotic acid, H_3PO_4 . H_3PO_4 has $pK_{a1} = 2.15$, $pK_{a2} = 7.20$, and $pK_{a3} = 12.38$.

1. Write the K_{a1} , K_{a2} , and K_{a3} reactions for H_3PO_4 .



2. Write the K_{b1} , K_{b2} , and K_{b3} reactions for Na_3PO_4 . (Use PO_4^{3-} to write net ionic equations.)



3. What is pK_{b1} ?

Use $pK_{a3} = 12.38$

$$pK_{b1} = 14.00 - pK_{a3} = 1.62$$

4. What is K_{b3} ?

Use $pK_{a1} = 2.15$

$$pK_{b3} = 14.00 - pK_{a1} = 11.85$$

$$K_{b3} = 10^{-11.85} = 1.4 \times 10^{-12}$$

Making Buffers With Polyprotic Acids and Bases

5. You need to make some buffers. In the lab, you have access to HCl , H_3PO_4 , and Na_2HPO_4 . For each target pH, suggest if you can make it with the chemicals available. If you can, suggest two ways it could be made. If it cannot be made, explain why it cannot be made.
- a. You want to make a buffer with a pH of 6.80.

A buffer could be made by mixing the appropriate amounts of H_3PO_4 and Na_2HPO_4 or by mixing the appropriate amounts of HCl and Na_2HPO_4 .

- b. You want to make a buffer with a pH of 8.80.

A buffer cannot be made because a pH of 8.80 is not within 1 pH unit of any of the pK_a s of H_3PO_4 .

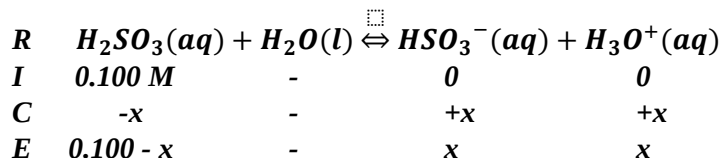
- c. You want to make a buffer with a pH of 11.80.

While pK_{a3} is close enough to the desired pH, you would need $NaOH$ or Na_3PO_4 to make a buffer at that pH.

Calculating the pH of Polyprotic Acids and Bases

Sulfurous acid (H_2SO_3) is a diprotic acid with $pK_{a1} = 1.91$ and $pK_{a2} = 7.18$.

6. What is the pH of 0.100 M of the acidic form of sulfurous acid? What are the concentrations of the acidic form, intermediate form, and basic form in this solution at equilibrium?



$$K_a = 10^{-1.91} = 1.23 \times 10^{-2} = \frac{x^2}{0.100 - x} \quad \text{assume } x \text{ in the denominator is small}$$

$$1.23 \times 10^{-2} = \frac{x^2}{0.100}$$

$$x_1 = 3.5 \times 10^{-2} M = [H_3O^+]$$

Bad assumption ($\frac{0.035}{0.100} * 100 = 35\% > 5\%$)

Use x_1 as the x value in the denominator

$$1.23 \times 10^{-2} = \frac{x^2}{0.100 - 0.035}$$

$$x_2 = 2.8 \times 10^{-2} M = [H_3O^+]$$

Use x_2 as the x value in the denominator

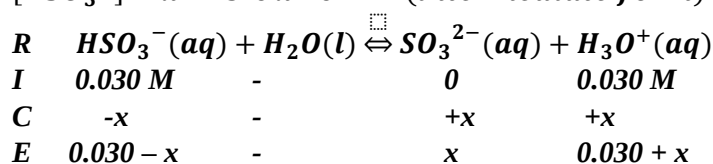
$$1.23 \times 10^{-2} = \frac{x^2}{0.100 - 0.028}$$

$$x_2 = 3.0 \times 10^{-2} M = [H_3O^+]$$

$$pH = -\log(3.0 \times 10^{-2} M) = 1.52$$

$$[H_2SO_3] = 0.100 - 0.030 = 0.070 M \text{ (acidic form)}$$

$$[HSO_3^-] = x = 3.0 \times 10^{-2} M \text{ (intermediate form)}$$



$$K_a = 10^{-7.18} = 6.6 \times 10^{-8} = \frac{(x)(0.030 + x)}{(0.030 - x)}$$

You can assume both the x in the numerator in $(0.030 + x)$ and the x in the denominator $(0.030 - x)$ are small.

$$6.6 \times 10^{-8} = \frac{(x)(0.030)}{(0.030)}$$

Then, the 0.030 in the numerator and denominator cancel out, leaving

$$x = 6.6 \times 10^{-8}$$

Good assumption ($\frac{6.6 \times 10^{-8}}{0.030} * 100 = 2.2 \times 10^{-4}\% < 5\%$)

$$x = 6.6 \times 10^{-8} M = [SO_3^{2-}]$$

7. What is the pH of 0.100 M of the intermediate form of sulfurous acid?

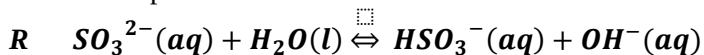
The pH of the intermediate form (HSO_3^-) is equal to the average of pK_{a1} and pK_{a2} .

$$pH = \frac{1}{2} (pK_{a1} + pK_{a2})$$

$$pH = \frac{1}{2} (1.91 + 7.18)$$

$$pH = 4.54$$

8. What is the pH of 0.100 M of the basic form of sulfurous acid?



$$I \quad 0.100 \text{ M} \quad - \quad 0 \quad 0$$

$$C \quad -x \quad - \quad +x \quad +x$$

$$E \quad 0.100 - x \quad - \quad x \quad x$$

$$pK_{a2} = 7.18$$

$$pK_{b1} = 14.00 - 7.18 = 6.82$$

$$K_{b1} = 10^{-6.82} = 1.5 \times 10^{-7} = \frac{x^2}{0.100 - x} \quad \text{assume } x \text{ in the denominator is small}$$

$$1.5 \times 10^{-7} = \frac{x^2}{0.100}$$

$$x = 1.2 \times 10^{-4} \text{ M} = [\text{OH}^-]$$

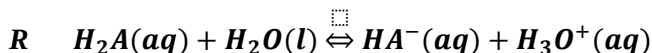
$$\text{Good assumption } \left(\frac{0.00012}{0.100} \times 100 = 0.12\% < 5\% \right)$$

$$pOH = -\log(1.2 \times 10^{-4} \text{ M}) = 3.91$$

$$pH = 14.00 - 3.91 = 10.09$$

8-hydroxyquinoline is a diprotic acid with $pK_{a1} = 4.9$ and $pK_{a2} = 9.81$.

9. What is the pH of 0.050 M of the acidic form of 8-hydroxyquinoline?



$$I \quad 0.050 \text{ M} \quad - \quad 0 \quad 0$$

$$C \quad -x \quad - \quad +x \quad +x$$

$$E \quad 0.050 - x \quad - \quad x \quad x$$

$$K_a = 10^{-4.9} = 1.3 \times 10^{-5} = \frac{x^2}{0.050 - x} \quad \text{assume } x \text{ in the denominator is small}$$

$$1.3 \times 10^{-5} = \frac{x^2}{0.050}$$

$$x = 8 \times 10^{-4} \text{ M} = [\text{H}_3\text{O}^+] \quad \text{Good assumption } \left(\frac{0.0008}{0.050} \times 100 = 2\% < 5\% \right)$$

$$pH = -\log(8 \times 10^{-4} \text{ M}) = 3.1$$

10. What is the pH of 0.050 M of the intermediate form of 8-hydroxyquinoline?

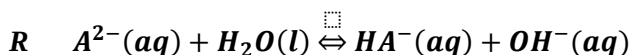
The pH of the intermediate form is equal to the average of pK_{a1} and pK_{a2} .

$$pH = \frac{1}{2} (pK_{a1} + pK_{a2})$$

$$pH = \frac{1}{2} (4.9 + 9.81)$$

$$pH = 7.4$$

11. What is the pH of 0.050 M of the basic form of 8-hydroxyquinoline? What are the concentrations of the basic form, intermediate form, and acidic forms in this solution at equilibrium?



$$I \quad 0.050 \text{ M} \quad - \quad 0 \quad 0$$

$$C \quad -x \quad - \quad +x \quad +x$$

$$E \quad 0.050 - x \quad - \quad x \quad x$$

$$pK_{a2} = 9.81$$

$$pK_{b1} = 14.00 - 9.81 = 4.19$$

$$K_{b1} = 10^{-4.19} = 6.5 \times 10^{-5} = \frac{x^2}{0.050 - x} \quad \text{assume } x \text{ in the denominator is small}$$

$$6.5 \times 10^{-5} = \frac{x^2}{0.050}$$

$$x = 1.8 \times 10^{-3} \text{ M} = [OH^-]$$

Good assumption (.0018/.050 x 100 = 3.6% < 5%)

$$pOH = -\log(1.8 \times 10^{-3} \text{ M}) = 2.75$$

$$pH = 14.00 - 2.75 = 11.25$$

$$[A^{2-}] = 0.050 - 0.0018 = 0.048 \text{ M}$$

$$[HA^-] = x = 1.8 \times 10^{-3} \text{ M}$$

$$[H_2A] = K_{b2} = 10^{-9.1} = 8 \times 10^{-10} \text{ M} \quad (\text{use } pK_{a1} \text{ to find } pK_{b2})$$

Active Learning Worksheets for Analytical Chemistry

#12 Equilibrium Chemistry: Plotting and Interpreting Polyprotic Titration Curves

Faculty Resources

(Delete before sharing with students):

- **Analytical Context and Framing:** I use this worksheet to introduce students to the kind of diprotic acid titration curve analysis they will need for lab. The goal is not just to read a curve, but to use a well-collected titration curve to estimate both pK_a values and the molar mass of an unknown diprotic acid. I emphasize that these pieces of evidence may not all point perfectly to the same unknown, so students need to think about which evidence is most reliable and what kinds of experimental error could affect each value.
- **Key Concepts and Common Challenges:** Students may expect every diprotic acid titration curve to show two obvious buffer regions or two clearly separated equivalence points. Emphasize that when the pK_a values are close together, the buffer regions can overlap and appear as one broad region rather than two distinct plateaus. Students also need to recognize that the curve can provide multiple pieces of evidence, including approximate pK_a values, equivalence-point volumes, and molar mass.
- **Facilitated Discussion Pauses:** Pause after students identify the endpoint from the titration curve and derivative plots. Ask: “What evidence from the graph is most convincing for the endpoint, and what evidence is more approximate?” This is a good place to discuss why the zero crossing in the second derivative plot is usually more defensible than simply eyeballing the steepest part of the original titration curve.
- **Calculation Support:** The most common calculation errors occur when students move from endpoint volume to molar mass. Remind students to use the amount of acid actually titrated, not necessarily the total mass originally dissolved. In the second example, the student dissolves 0.750 g of unknown in 250.0 mL but titrates only a 50.00 mL aliquot, so only 0.150 g of acid is present in the titration. Students also often identify the second equivalence-point volume first, but they should use the first equivalence-point volume to calculate molar mass because one mole of base has reacted per mole of diprotic acid at the first equivalence point.
- **Synthesis and Extension Questions:** Ask students: “If the molar mass points toward one possible unknown but the pK_a values point toward another, which evidence do you trust more? What experimental errors could affect each type of evidence?” I like this question because it feels more like real data analysis than a textbook matching exercise. The molar mass, pK_a values, and curve shape may not all point perfectly to the same unknown, and that is a useful discomfort for students. I want them to practice saying which evidence they trust most and why, instead of assuming that one imperfect number settles the whole question.
- **Pacing Note:** This activity is a synthesis of the titration and speciation work from worksheets #10-11. It can vary in length depending on whether students create the Excel plots themselves or interpret prepared figures. If the worksheet needs to be divided across class periods, a natural stopping point is after students interpret the titration curve and derivative plots to identify the endpoint. The remaining questions can then focus on calculating molar mass, estimating pK_a values, evaluating evidence, and identifying the unknown acid. The final page is mostly instructional support for students who need to understand how first- and second-derivative plots are generated; it does not need to be treated as a required calculation section unless students are making the plots themselves.

Answer Keys for Active Learning Worksheets for Analytical Chemistry

#12 Equilibrium Chemistry: Plotting and Interpreting Polyprotic Titration Curves

Before You Begin

- Read about acid-base reactions: [6.4 Equilibrium Constants for Chemical Reactions](#)
- Read about the method of successive approximations: [6.7: Solving Equilibrium Problems](#)
- Read about acid-base titrations: [9.2: Acid–Base Titrations](#)
- Acid dissociation constants: [16.11: Acid Dissociation Constants](#)

What You Should Already Know

- Concepts of weak acid/base equilibria and pK_a
- Understanding of diprotic acid behavior during titration
- Reading and interpreting titration curves
- Familiarity with first and second derivative plots in titration analysis

What You'll Learn

- Analyze titration curves of diprotic acids to identify equivalence and buffer regions
- Use first and second derivatives to more precisely identify titration endpoints
- Estimate pK_a values and molar mass from experimental titration data
- Match experimental data to likely unknowns using a table of reference acids

What You'll Practice

- Construct and interpret Excel-based titration and derivative plots
- Use critical reasoning to evaluate graphical data
- Practice identifying dominant species and pK_a relationships in polyprotic systems

Estimated Completion Time: 60-75 minutes

Author: Rebecca L. Sanders

Active Learning Worksheets for Analytical Chemistry, Version 1.0, 2026

Interpreting a Diprotic Titration Curve

In lab, students were asked to track the pH during the titrations of unknown diprotic acids with a strong base, NaOH. The goal is for them to plot the titration curves and interpret those curves to identify their assigned unknown diprotic acid. In Table 1, a list of possible unknown diprotic acids is shown.

Table 1. Possible unknown diprotic acids

Name	Formula	Molar Mass (g/mol)	pK_{a1}	pK_{a2}
Oxalic acid	$C_2H_2O_4$	90.03	1.252	4.266
Malonic acid	$C_3H_4O_4$	104.06	2.847	5.696
Maleic acid	$C_4H_4O_4$	116.07	1.910	6.332
Succinic acid	$C_4H_6O_4$	118.09	4.207	5.636
Malic acid	$C_4H_6O_5$	134.09	3.459	5.097
D-tartaric acid	$C_4H_6O_6$	150.09	3.036	4.366

Example 1

Jamie conducted a titration of 0.1940 g of an unknown diprotic acid dissolved in distilled water with 0.09801 M NaOH. Then Jamie plotted the pH of the titration as a function of the volume of 0.09801 M NaOH added (Figure 1).

1. Describe the general features of the titration curve. Circle any buffer region(s) and roughly identify the endpoint volume from Figure 1.

It appears to have a buffer region from ~2 – 35 mL. The rough endpoint volume is 37 mL.

2. In a diprotic titration curve, you might expect two distinct buffer regions to be observed. In this example, the titration curve looks to only contain one buffer region even though it is one of the diprotic acids in Table 1. Why?

It is likely that the two pK_a values are close enough together that two distinct buffer regions are not observed.

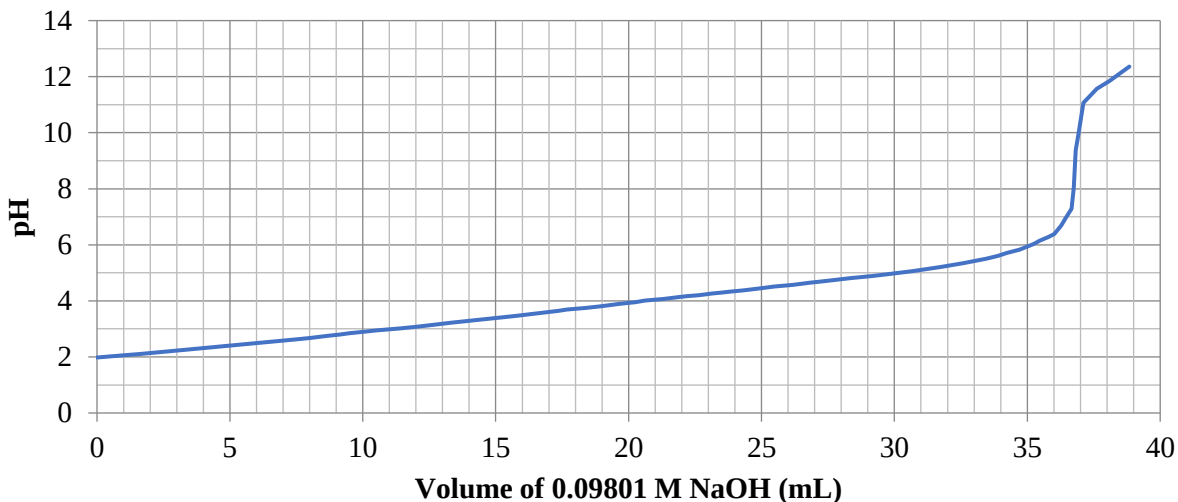


Figure 1. Titration curve of Jamie's unknown diprotic acid with 0.09801 M NaOH.

Analyzing Derivatives to Find Endpoints

The rough endpoint that you identified in question 1 represents the second endpoint of the unknown diprotic acid because all of the unknown acids have two pK_a values in the acidic pH region. While Figure 1 can be used to roughly identify the second endpoint of the titration, first and second derivatives of the titration curve can be calculated and plotted to more accurately identify the endpoint of the titration curve. Jamie prepared some additional figures to determine the second endpoint. They plotted the first derivative as a function of average volume of titrant added (Figure 2), the second derivative as a function of average of average volume of titrant added (Figure 3), and a zoom in on the x-axis of Figure 3 to see where the second derivative shows a local maximum or minimum, indicating the point of steepest slope (i.e., the inflection point) on the original titration curve (Figure 4). Instructions for how to make these graphs are included at the end of this worksheet.

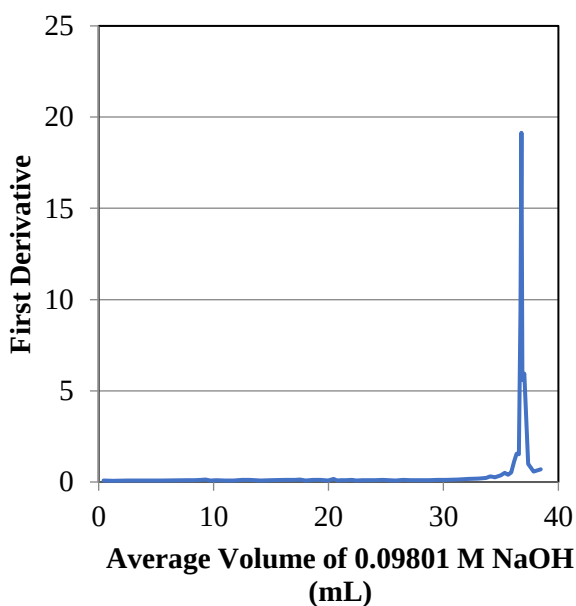


Figure 2. First derivative of the titration curve of an unknown diprotic acid with 0.09801 M NaOH.

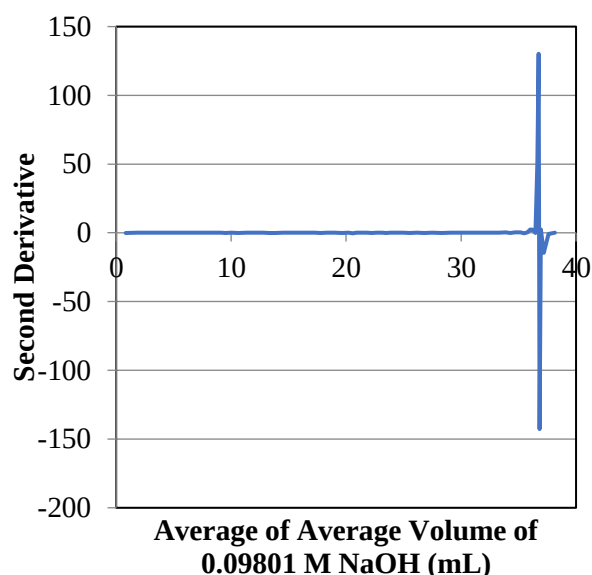


Figure 3. Second derivative of the titration curve of an unknown diprotic acid with 0.09801 M NaOH.

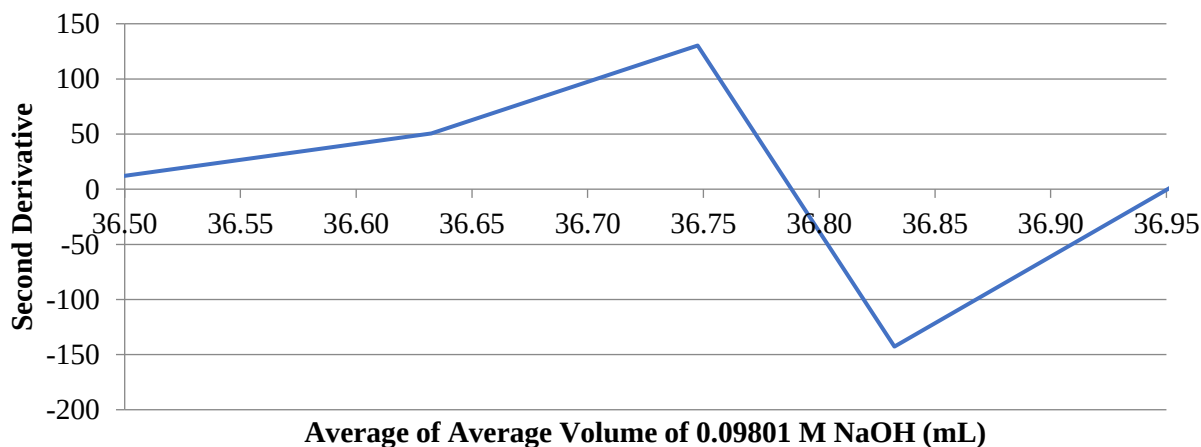


Figure 4. Zoom in of the second derivative of the titration curve of an unknown diprotic acid with 0.09801 M NaOH.

Identifying the Unknown Acid

To identify the second endpoint of the titration curve, look at Figure 4. Find where the biggest jump in second derivative crosses at $y=0$. Record this as your endpoint to the nearest tenth of a milliliter. For example, on Figure 4 the second equivalence volume is 36.79 mL. The first endpoint would be half the volume of the second endpoint (e.g. 18.40 mL).

3. Use the first endpoint volume to calculate the molar mass of the unknown diprotic acid.

$$\frac{0.1940 \text{ g unknown acid}}{18.40 \text{ mL OH}^-} * \frac{1000 \text{ mL OH}^-}{1 \text{ L OH}^-} * \frac{1 \text{ L OH}^-}{0.09801 \text{ mol OH}^-} * \frac{1 \text{ mol OH}^-}{1 \text{ mol unknown acid}} \\ = 107.6 \text{ g/mol}$$

4. Use the first endpoint and second endpoint volumes to determine the volumes at pK_{a1} and pK_{a2} .
a. pK_{a1} is equal to the pH at $\frac{1}{2}V_{e1}$. First, calculate the volume at $\frac{1}{2}V_{e1}$. Then, look at Figure 1 at that volume and estimate the pH at that volume, which is pK_{a1} .

$$pK_{a1} \approx 2.8$$

- b. pK_{a2} is equal to the pH at $\frac{3}{2}V_{e1}$. First, calculate the volume at $\frac{3}{2}V_{e1}$. Then, look at Figure 1 at that volume and estimate the pH at that volume, which is pK_{a2} .

$$pK_{a2} \approx 4.7$$

5. Now, use the molar mass and pK_a values of Jamie's unknown diprotic acid and the information in Table 1 to identify Jamie's unknown diprotic acid.

The calculated molar mass (107.6 g/mol) is closest to the molar masses of malonic acid (104.06 g/mol) and maleic acid (116.07 g/mol). The remaining molar masses are too far away from 107.6 g/mol to be considered.

The estimated pK_a values of 2.8 and 4.7 are closer to the pK_a of malonic acid (2.847 and 5.696) than maleic acid (1.910 and 6.332). While not perfect fits, the most likely candidate for the unknown acid based on molar mass and pK_a values is malonic acid.

Practice with a Second Unknown

Shay conducted a similar experiment. However, Shay dissolved 0.7500 grams of unknown acid into 250.0 mL of water. A 50.00 mL aliquot of the unknown diprotic acid solution was titrated with 0.1000 M NaOH. The titration curve was plotted in Figure 5 and the second derivative was plotted in Figure 6.

6. Using Figure 6, identify the second endpoint to the nearest hundredth of a milliliter.

$$V_{e2} = 32.53 \text{ mL}$$

7. Calculate the first endpoint volume in milliliters. Then, use it to calculate the molar mass of the unknown diprotic acid.

$$V_{e1} = 16.26 \text{ mL}$$

$$\frac{50.00 \text{ mL acid}}{16.26 \text{ mL OH}^-} * \frac{1000 \text{ mL OH}^-}{1 \text{ L OH}^-} * \frac{1 \text{ L OH}^-}{0.1000 \text{ mol OH}^-} * \frac{1 \text{ mol OH}^-}{1 \text{ mol acid}} * \frac{0.7500 \text{ g acid}}{250.0 \text{ mL acid}} = 92.25 \text{ g/mol}$$

8. Use the first endpoint and second endpoint volumes to determine the volumes at pK_{a1} and pK_{a2} and then estimate the pH at those volumes from the titration curve (Figure 5).

$$pK_{a1} \approx 2.0 \text{ and } pK_{a2} = 4.0$$

9. Now, use the molar mass and pK_a values of Shay's unknown diprotic acid and the information in Table 1 to identify Shay's unknown diprotic acid.

The unknown diprotic acid is oxalic acid. The calculated molar mass (92.25 g/mol) is about 2 g/mol of the actual molar mass (90.03 g/mol). The estimate pK_a values of 2.0 and 4.0 from the titration curve are somewhat close to the known values of 1.252 and 4.266, respectively. None of the other unknown diprotic acid options have a combination of molar mass and pK_a values close enough to be the possible unknown.

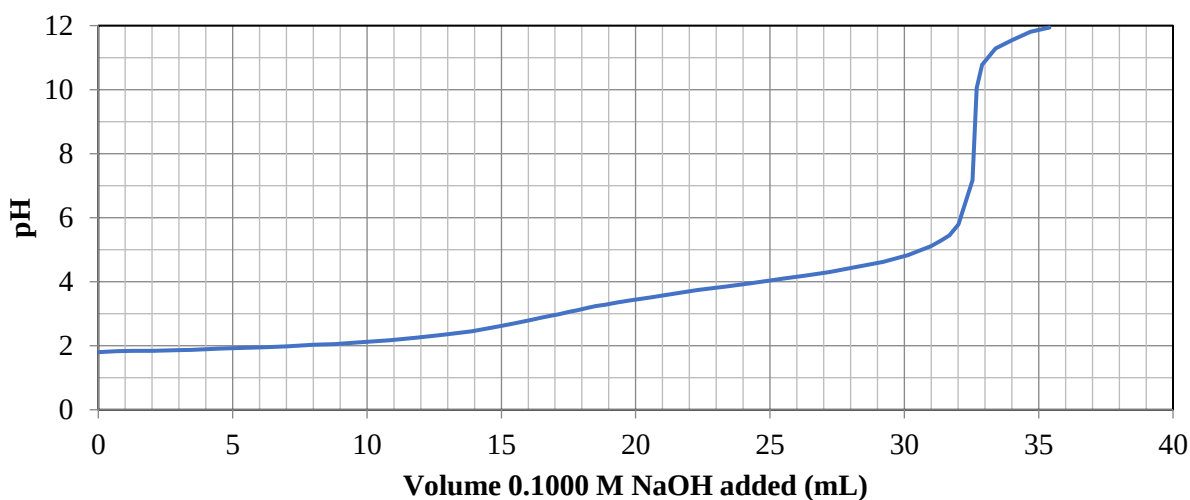


Figure 5. Titration curve of Shay's unknown diprotic acid with 0.1000 M NaOH.

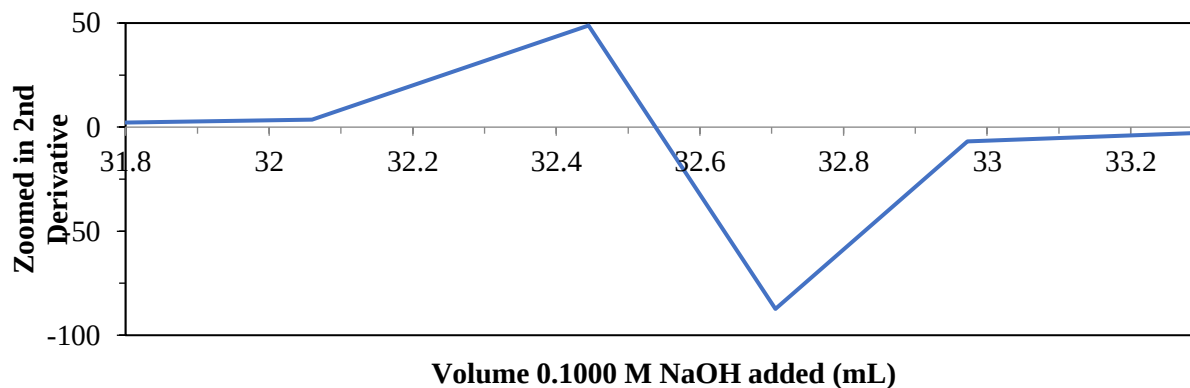


Figure 6. Zoom in of the second derivative of the titration curve of an unknown diprotic acid with 0.1000 M NaOH.

Instructions for Excel Plotting

- **Columns A and B** are the raw data from the titration. Ensure that your volumes used start at 0.00 mL. If your first reading was not at 0.00 mL, subtract your starting volume from all measurements.
- **Column C: Average volumes.** Column C is the average of two volumes. For example, in cell C2, the volumes from A2 and A3 are averaged.
- **Column D: First derivative.** The first derivative is calculated as the change in pH over the change in volume. For example, cell D2 is calculated as “ $=(B3-B2)/(A3-A2)$ ”.
- **Column E: Average of average volumes.** Column E is the average of two average volumes. For example, in cell E2, the volumes from C2 and C3 are averaged.
- **Column F: Second derivative.** The second derivative is calculated as the change in first derivative over the change in average volume. For example, cell F2 is calculated as “ $=(D3-D2)/(C3-C2)$ ”.

Table 2. Example titration data and calculations for the titration of Jamie’s unknown diprotic acid with 0.09801 M NaOH.

	A	B	C	D	E	F
1	Volume 0.10 M NaOH (mL)	pH	Average 0.10 M NaOH Volume (mL)	$\Delta\text{pH}/\Delta\text{mL}$ 1st Derivative	Average 0.10 M NaOH Volume (mL)	2nd Derivative ($\Delta(\Delta\text{pH}/\Delta\text{mL})/\Delta\text{mL}$)
2	36.28	6.69	36.36	1.5625	36.4575	-0.209
3	36.44	6.94	36.555	1.5217	36.6325	50.666
4	36.67	7.29	36.71	9.3750	36.7475	130.238
5	36.75	8.04	36.785	19.1429	36.8325	-142.732
6	36.82	9.38	36.88	5.5833	36.9525	2.468
7	36.94	10.05	37.025	5.9412	37.1950	-14.533
8	37.11	11.06	37.365	1.0000	37.4925	-2.715
9	37.62	11.57	37.62	0.3075	37.6200	0.008

Practice Preparing Titration Graphs in Excel

From the calculations in Excel, you will prepare four figures (examples on the next page).

1. Figure 1. Plot pH as a function of volume of titrant added.
2. Figure 2. Plot first derivative as a function of average volume of titrant added.
3. Figure 3. Plot second derivative as a function of average of average volume of titrant added.
4. Figure 4. Zoom in on the x-axis of Figure 3 to see where the volume changed the most in the second derivative. Your zoomed in figure will likely have the x-axis cover less than 1 mL in volume. For example, in Figure 4, the x axis is zoomed in on 36.50-36.95 mL.

Active Learning Worksheets for Analytical Chemistry

#13 Equilibrium Chemistry: Identifying Principal Species by pH

Faculty Resources

(Delete before sharing with students):

- **Analytical Context and Framing:** I use this worksheet to help students predict what chemical form actually exists in solution before doing calculations or making experimental decisions. The main goal is for students to understand principal species and to use ladder diagrams as a visual tool for connecting pH, pK_a values, and dominant acid–base forms. I emphasize that knowing the formula of the acid is not enough; the pH determines which form is most abundant.
- **Key Concepts and Common Challenges:** Students often treat pK_a values as isolated numbers rather than boundaries between dominant species. Ladder diagrams help them decide which species is most abundant at a given pH, and they can also help students identify the second- and third-most abundant species by looking at which pK_a values are closest. Students may also struggle with distribution plots when the pK_a values are close together. I tell students that an intermediate form only becomes strongly dominant when the neighboring pK_a values are well separated, often by roughly 4–5 pH units. If the pK_a values are closer together, sizable fractions of the more acidic and more basic forms can be present at the same time, so the intermediate form may not rise all the way to the top of the distribution plot.
- **Facilitated Discussion Pauses:** Pause after the adipic acid questions. Ask: “At pH 4.99, why is the intermediate form the principal species, and why does the second-most-abundant species depend on which pK_a value is closer?” This is a good place to connect the ladder diagram to the distribution plot so students see that both representations are telling the same chemical story.
- **Calculation Support:** For the abundance-ratio questions, remind students to start from the Henderson–Hasselbalch equation and identify the two species being compared. Encourage them to check whether their answer makes sense by comparing the pH to the relevant pK_a value.
- **Synthesis and Extension Questions:** Ask students: “If you were trying to separate or detect one form of a polyprotic acid, why would it matter whether the pH is near a pK_a value or far from all pK_a values?” This helps reinforce that speciation is not just a graphing exercise; it affects which chemical form is actually available to measure or separate.
- **Pacing Note:** This activity can stand alone, but it strongly connects with Worksheets #9–11. It is usually a relatively quick worksheet if students understand the idea of principal species and can use a ladder diagram. If time is limited, complete the adipic acid ladder diagram and distribution plot during class, then assign the phosphoric acid ranking and abundance-ratio questions as follow-up practice.

Answer Keys for Active Learning Worksheets for Analytical Chemistry

#13 Equilibrium Chemistry: Identifying Principal Species by pH

Before You Begin

- Read about ladder diagrams: [6.6: Ladder Diagrams](#)
- Read about buffers: [6.8: Buffer Solutions](#)

What You Should Already Know

- Definitions and behavior of weak acids and bases
- pK_a and its relationship to pH
- Equilibrium expressions and ladder diagrams
- Basic stoichiometry and charge balancing

What You'll Learn

- Identify and rank principal species of polyprotic acids and bases at given pH values
- Interpret and construct ladder diagrams to visualize dominant acid–base forms
- Sketch and interpret distribution plots of species across the pH scale
- Estimate fractional abundances using Henderson–Hasselbalch logic

What You'll Practice

- Translate pK_a values into visual and quantitative models
- Use equilibrium logic to justify species predominance
- Apply reasoning to unfamiliar acids and bases based on given data
- Communicate relative abundance and speciation clearly using diagrams and equations

Estimated Completion Time: 45-60 minutes

Author: Rebecca L. Sanders

Active Learning Worksheets for Analytical Chemistry, Version 1.0, 2026

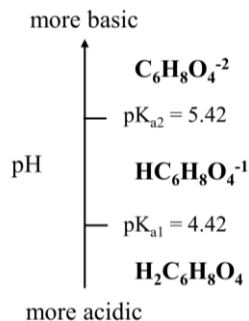
Useful Equation

- $pH = pK_a + \log(\text{base/acid})$

Identifying Principal Species in a Diprotic System

Consider the diprotic acid, adipic acid ($H_2C_6H_8O_4$). It has $pK_{a1} = 4.42$ and $pK_{a2} = 5.42$.

1. Draw a ladder diagram for adipic acid.



2. What is the most abundant species at pH = 3.00?

$H_2C_6H_8O_4$ because pH below 4.42

3. What is the most abundant species at pH = 4.99?

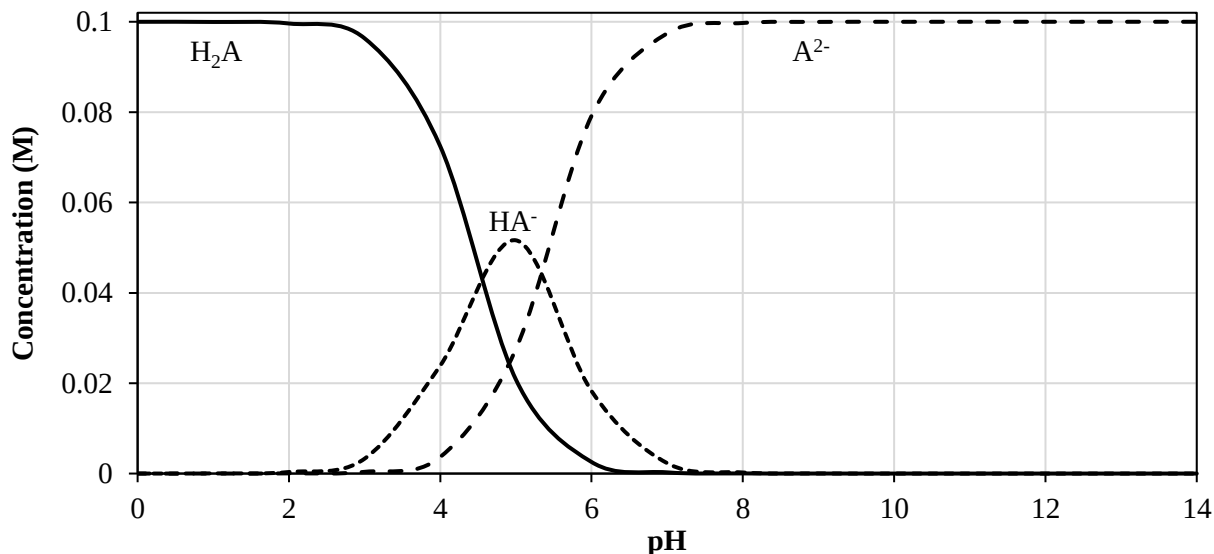
$HC_6H_8O_4^{-}$ because pH is between 4.42 and 5.42

4. What is the second most abundant species at pH = 4.99? Explain your answer.

$C_6H_8O_4^{2-}$ because pH is closer to 5.42 than 4.42

Visualizing Speciation with Distribution Plots

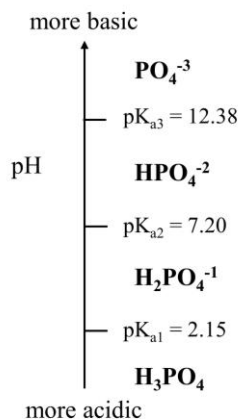
5. Sketch the distribution of species across pH for 0.10 M adipic acid. Label curves for $[H_2A]$, $[HA^-]$, and $[A^{2-}]$. Ensure the total concentration sums to 0.10 M at each point.



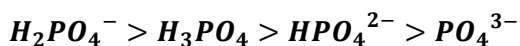
Analyzing Complex Speciation in a Triprotic System

Consider the triprotic acid, H_3PO_4 . H_3PO_4 has $pK_{a1} = 2.15$, $pK_{a2} = 7.20$, and $pK_{a3} = 12.38$.

6. Draw a ladder diagram for phosphoric acid.



7. At pH 3.00, rank the four phosphate species from most to least abundant.

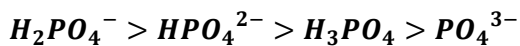


Quantitative Assessment of Abundance Ratios

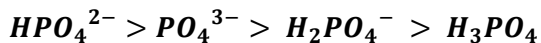
8. What is the ratio of the two most abundant species at pH = 3.00?

$$\begin{aligned} pH &= pK_a + \log \left(\frac{[H_2PO_4^-]}{[H_3PO_4]} \right) \\ 3.00 &= 2.15 + \log \left(\frac{[H_2PO_4^-]}{[H_3PO_4]} \right) \\ 0.85 &= \log \left(\frac{[H_2PO_4^-]}{[H_3PO_4]} \right) \\ 10^{0.85} &= \frac{[H_2PO_4^-]}{[H_3PO_4]} \\ 7.1 &= \frac{[H_2PO_4^-]}{[H_3PO_4]} \end{aligned}$$

9. At pH 5.00, rank the four phosphate species from most to least abundant.



10. At pH 11.00, rank the four phosphate species from most to least abundant.



11. What is the ratio of the two most abundant species at pH = 11.00?

$$pH = pK_a + \log \left(\frac{[PO_4^{3-}]}{[HPO_4^{2-}]} \right)$$

$$11.00 = 12.38 + \log \left(\frac{[PO_4^{3-}]}{[HPO_4^{2-}]} \right)$$

$$-1.38 = \log \left(\frac{[PO_4^{3-}]}{[HPO_4^{2-}]} \right)$$

$$10^{-1.38} = \frac{[PO_4^{3-}]}{[HPO_4^{2-}]}$$

$$0.042 = \frac{[PO_4^{3-}]}{[HPO_4^{2-}]}$$

Active Learning Worksheets for Analytical Chemistry

#14 Equilibrium Chemistry: Titration Curve Calculations and Analysis for Diprotic Systems

Faculty Resources

(Delete before sharing with students):

- **Analytical Context and Framing:** I use this worksheet to show students that diprotic titration curves look intimidating, but they are built from pieces they have already practiced. The worksheet starts conceptually by having students label regions of a titration curve before doing calculations. I emphasize that if students can identify what species are present and what chemistry controls each region, the math becomes much easier. I sometimes describe sketching a titration curve as “chemistry connect the dots”: students will not calculate every point, but they can use chemical intuition to decide how the curve should move between important points.
- **Key Concepts and Common Challenges:** The main challenge is tracking which species controls the pH in each region of a diprotic titration. Students may use the right equation at the wrong point, especially near equivalence points where Henderson–Hasselbalch is no longer appropriate. In the diprotic acid/strong base titration, students often struggle most in the second buffer region because they may not recognize that they need to represent the equilibrium of the intermediate species. At the second equivalence point, they may also have trouble identifying which species is present and which equilibrium calculation controls the pH. In the later questions, students often find it difficult to flip their thinking and start with the basic form of a diprotic system being titrated with strong acid.
- **Facilitated Discussion Pauses:** Pause after students label the first titration curve. If time allows, put the curve on the board and label it as a class or have groups label different copies of the curve on the board at the same time. Ask: “What changes at the half-equivalence points compared with the equivalence points? Which points tell us pK_a values, and which points require equilibrium calculations?” This is a useful place to reinforce that the curve is not just a shape to memorize; each region corresponds to a different dominant species and a different way of calculating pH. Later, before students begin the titration of the basic form with strong acid, it can help to sketch the expected curve qualitatively so they can visualize starting at high pH and moving downward through buffer regions toward the equivalence points.
- **Calculation Support:** Encourage students to track moles before calculating pH. For each volume of titrant, have them identify whether they are before the first equivalence point, at a half-equivalence point, at an equivalence point, between equivalence points, or beyond the second equivalence point. Remind students that there are only a few new ideas in this worksheet; most of the work is pulling together mole tracking, principal species, buffer logic, and equilibrium calculations. For the basic-form titration, students may need extra support switching from K_a thinking to K_b thinking and recognizing that the titration starts at a high pH and moves lower as strong acid is added.
- **Synthesis and Extension Questions:** Ask students: “If you were choosing an indicator for a diprotic titration, what would you need to know besides the fact that the analyte is diprotic?” Push them to connect the indicator range to the actual shape of the titration curve, including whether the relevant endpoint is sharp enough to see clearly. If students sketch curves in groups, use their sketches as a synthesis activity by comparing what each group labeled correctly and what pieces are missing. This can help students see the recurring patterns across diprotic acid/strong base and basic-form/strong acid titrations.
- **Pacing Note:** This activity builds on Worksheets #10–13 and should usually be treated as at least a two-part activity in a 50–60 minute class format. Depending on students’ comfort with titration calculations, it can easily take three class periods. A natural stopping point is after Question 5, after students complete the diprotic acid/strong base titration and sketch the curve. The remaining questions shift to titrating the basic form of a diprotic system with strong acid, which requires students to apply similar reasoning in the reverse direction. If short on time, one option is to split the class so half works on the weak diprotic acid/strong base calculation and half works on the basic-

form/strong acid calculation, then have the class compare the reasoning. The final page can be treated as an optional synthesis if time allows; it is useful for recognizing patterns but may not be necessary if students are already spending significant time on the calculations.

Answer Keys for Active Learning Worksheets for Analytical Chemistry

#14 Equilibrium Chemistry: Titration Curve Calculations and Analysis for Diprotic Systems

Before You Begin

- Read about acid-base reactions: [6.4 Equilibrium Constants for Chemical Reactions](#)
- Read about the method of successive approximations: [6.7: Solving Equilibrium Problems](#)
- Read about buffers: [6.8: Buffer Solutions](#)
- Read about acid-base titrations: [9.2: Acid–Base Titrations](#)

What You Should Already Know

- Titration fundamentals (equivalence points, mole tracking)
- pK_a , strong vs. weak acid/base behavior
- Conjugate acid–base pairs and species transitions
- How to use the Henderson–Hasselbalch equation
- Stoichiometry and molarity conversions

What You'll Learn

- Identify equivalence points and buffer regions in diprotic acid/base titrations
- Determine the dominant species at various pH and titration stages
- Select appropriate indicators based on titration curve shape
- Sketch titration curves and label key chemical events

What You'll Practice

- Apply mole and pH logic to multi-step neutralization reactions
- Translate titration data into visual and quantitative models
- Use the Henderson–Hasselbalch equation where appropriate
- Communicate species changes and pH shifts across titrations clearly

Estimated Completion Time: 90-115 minutes

Author: Rebecca L. Sanders

Active Learning Worksheets for Analytical Chemistry, Version 1.0, 2026

Useful Equations

- $M_A V_A = M_B V_B$
- $pH = -\log[H^+]$
- $pH + pOH = 14.00$
- $[H^+] * [OH^-] = K_a * K_b = K_w = 1.0 * 10^{-14}$
- $pKa = -\log(K_a)$
- $pK_a + pK_b = pK_w = 14.00$
- $pH = pK_a + \log(base/acid)$

Interpreting a Diprotic Acid Titration Curve with a Strong Base

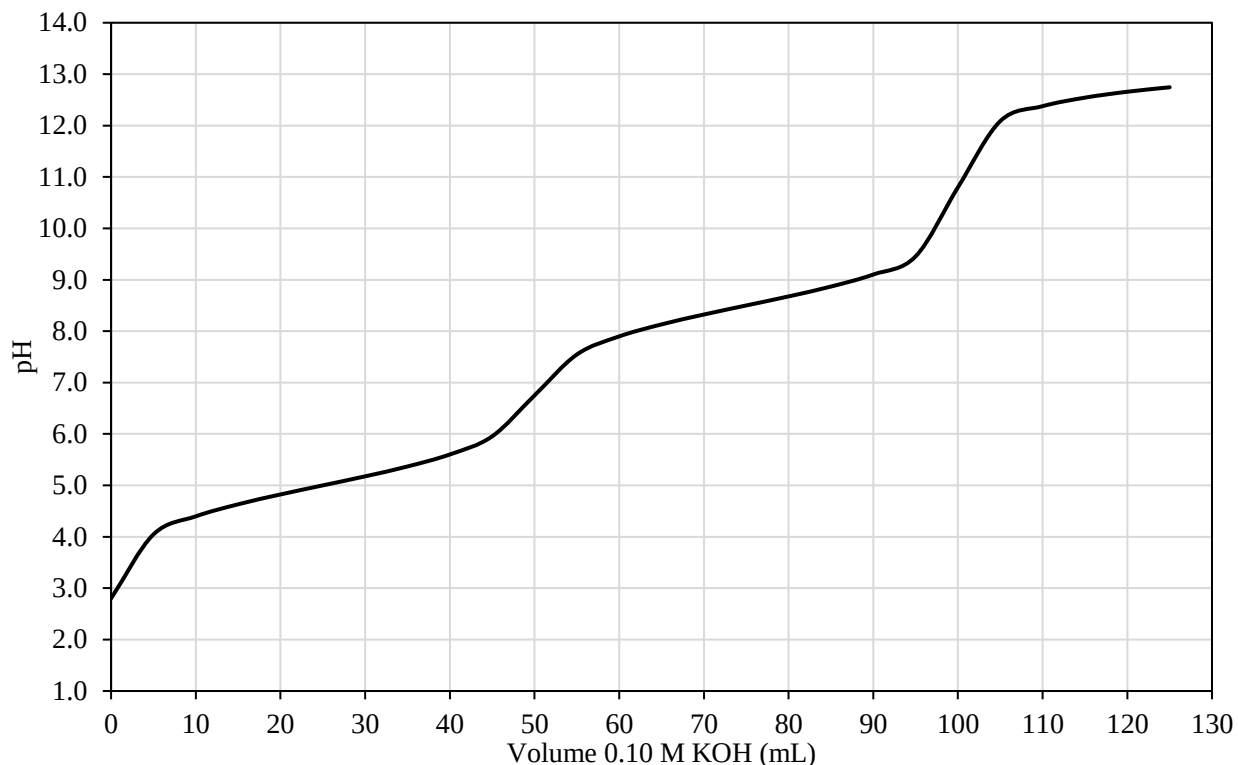


Figure 1. Calculated titration curve of an unknown diprotic acid with 0.10 M KOH.

- Consider the figure of a diprotic acid (H_2A) titration curve. Label the following:
 - the first equivalence volume
50 mL KOH
 - the second equivalence volume
100 mL KOH
 - where there is excess strong base
Beyond 100 mL of KOH added
 - where H_2A is the principal species in solution
Before 25 mL KOH added
 - where HA^- is the principal species in solution
 HA^- is the principal species in solution after 25 mL of KOH and before 75 mL of KOH added
 - where A^{2-} is the principal species in solution
 A^{2-} is the principal species in solution after 75 mL of KOH added
 - where $pH = pK_{a1}$
At 25 mL KOH
 - where $pH = pK_{a2}$
At 75 mL KOH
 - any buffer regions
There are two buffer regions. The first is around pK_{a1} (~5-45 mL KOH) and the second buffer region is around pK_{a2} (~55-95 mL KOH)
- What pH range should an indicator change color to visualize the first equivalence point? The second equivalence point?
To visualize the first endpoint, the indicator should change color around a pH of 6.75. To visualize the second endpoint, the indicator should change color around a pH of 10.80.

Diprotic Acid Titration Calculations with a Strong Base

3. 100.0 mL of 0.250 M diprotic acid (H_2A) is titrated with 0.500 M KOH. What is the 1st equivalence volume? What is the 2nd equivalence volume?

$$M_A V_A = M_B V_B$$

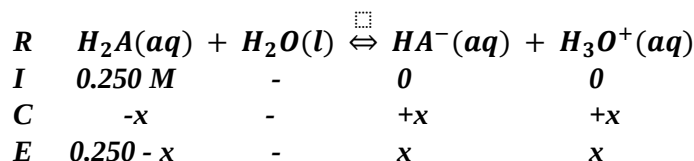
$$(0.250\text{ M})(100.0\text{ mL}) = (0.500\text{ M})V_B$$

$$V_B = 50.0\text{ mL} = V_{e1}$$

$$V_{e2} = 2V_{e1} = 100.0\text{ mL}$$

4. What is the pH after the following volumes of 0.500 M KOH have been added to 100.0 mL of 0.250 M diprotic acid (H_2A)? pK_{a1} of H_2A is 4.50 and pK_{a2} is 10.00.
- 0.00 mL
 - 15.0 mL
 - 25.0 mL
 - 50.0 mL
 - 75.0 mL
 - 85.0 mL
 - 100.0 mL
 - 125.0 mL

a. 0.00 mL



$$K_a = 10^{-4.50} = 3.16 \times 10^{-5} = \frac{x^2}{0.250-x} \text{ assume the } x \text{ in the denominator is small}$$

$$3.16 \times 10^{-5} = \frac{x^2}{0.250}$$

$$x = 2.8 \times 10^{-3}\text{ M} = [H_3O^+]$$

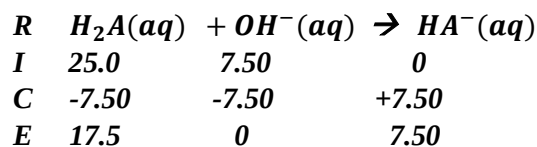
$$\text{Good assumption } \left(\frac{0.0028}{0.250} \times 100 = 1.1\% < 5\% \right)$$

$$pH = -\log(2.8 \times 10^{-3}\text{ M}) = 2.55$$

b. 15.0 mL (Buffer region before first equivalence volume)

$$15.0\text{ mL KOH} \times 0.500\text{ M KOH} = 7.50\text{ mmol KOH} = 7.50\text{ mmol OH}^-$$

$$100.0\text{ mL} \times 0.250\text{ M } H_2A = 25.0\text{ mmol } H_2A$$



$$pH = pK_a + \log\left(\frac{HA^-}{H_2A}\right)$$

$$pH = 4.50 + \log\left(\frac{7.50}{17.5}\right)$$

$$pH = 4.13$$

c. 25.0 mL (One half to first equivalence volume)

$$\text{At } \frac{1}{2} V_{e1}, pH = pK_{a1} = 4.50$$

d. 50.0 mL (First equivalence volume)

$$\text{At } V_{e1}, pH = \frac{1}{2} (pK_{a1} + pK_{a2}) = \frac{1}{2} (4.50 + 10.00) = 7.25$$

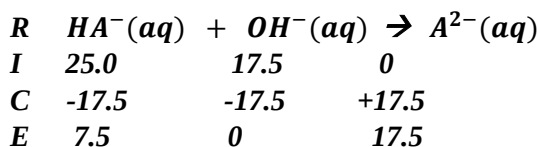
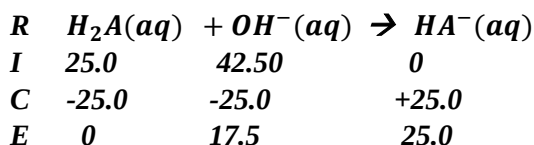
e. 75.0 mL (Halfway between first and second equivalence volumes)

$$\text{At } \frac{3}{2} V_{e1}, pH = pK_{a2} = 10.00$$

f. 85.0 mL (Buffer region between first and second equivalence volumes)

$$85.0 \text{ mL KOH} \times 0.500 \text{ M KOH} = 42.5 \text{ mmol KOH or } 42.50 \text{ mmol OH}^-$$

$$100.0 \text{ mL of } 0.250 \text{ M H}_2\text{A} = 25.0 \text{ mmol H}_2\text{A}$$



$$pH = pK_a + \log \frac{A^{2-}}{HA^-}$$

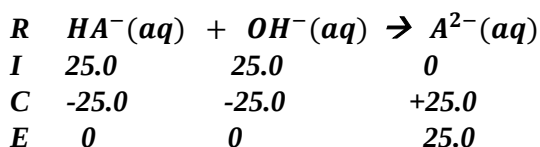
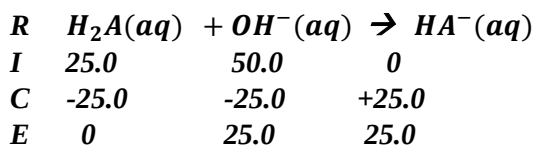
$$pH = 10.00 + \log \frac{17.5}{7.5}$$

$$pH = 10.37$$

g. 100.0 mL (Second equivalence volume)

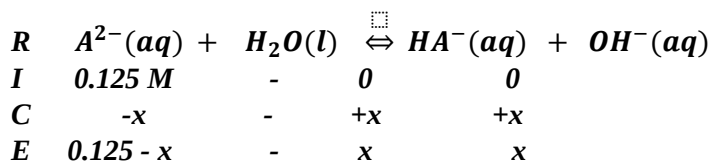
$$100.0 \text{ mL KOH} \times 0.500 \text{ M KOH} = 50.0 \text{ mmol KOH or } 50.0 \text{ mmol OH}^-$$

$$100.0 \text{ mL of } 0.250 \text{ M H}_2\text{A} = 25.0 \text{ mmol H}_2\text{A}$$



All of the H_2A has been converted to A^{2-} . Now, a RICE diagram for the basic form in water is written after finding the diluted concentration of A^{2-} .

$$[A^{2-}] = \frac{25.0 \text{ mmol } A^{2-}}{200.0 \text{ mL}} = 0.125 \text{ M}$$



$$K_{b1} = 10^{-4.00} = 1.0 \times 10^{-4} = \frac{x^2}{0.125 - x} \quad \text{assume the } x \text{ in the denominator is small}$$

$$1.0 \times 10^{-4} = \frac{x^2}{0.125}$$

$$x = 3.5 \times 10^{-3} \text{ M} = [OH^{-}]$$

$$\text{Good assumption } \left(\frac{0.0035}{0.125} \times 100 = 2.8\% < 5\% \right)$$

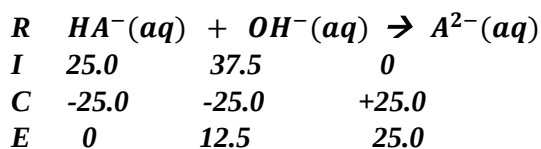
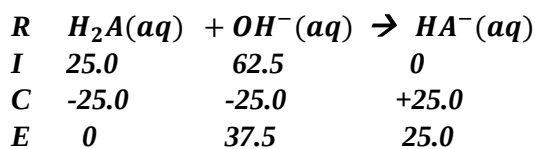
$$pOH = -\log(3.5 \times 10^{-3} \text{ M}) = 2.45$$

$$pH = 14.00 - 2.45 = 11.55$$

h. 125.0 mL (Beyond the second equivalence volume)

$$125.0 \text{ mL KOH} \times 0.500 \text{ M KOH} = 62.5 \text{ mmol KOH or } 62.5 \text{ mmol } OH^{-}$$

$$100.0 \text{ mL of } 0.250 \text{ M } H_2A = 25.0 \text{ mmol } H_2A$$



At this point, there is excess OH^{-} in solution, which dictates the pH of the solution.

$$[OH^{-}] = \frac{12.5 \text{ mmol}}{225.0 \text{ mL}} = 0.0556 \text{ M}$$

$$pOH = -\log(5.56 \times 10^{-2} \text{ M}) = 1.255$$

$$pH = 14.00 - 1.255 = 12.745$$

5. Sketch the titration curve. Label
 - a. the first equivalence volume
50 mL KOH
 - b. the second equivalence volume
100 mL KOH
 - c. where there is excess strong base
Beyond 100 mL of KOH added
 - d. where H_2A is the principal species in solution
Before 25 mL KOH added
 - e. where HA^- is the principal species in solution
 HA^- is the principal species in solution after 25 mL of KOH and before 75 mL of KOH added
 - f. where A^{2-} is the principal species in solution
 A^{2-} is the principal species in solution after 75 mL of KOH added
 - g. where $pH = pK_{a1}$
At 25 mL KOH
 - h. where $pH = pK_{a2}$
At 75 mL KOH
 - i. any buffer regions
There are two buffer regions. The first is around pK_{a1} (~5-45 mL KOH) and the second buffer region is around pK_{a2} (~55-95 mL KOH)

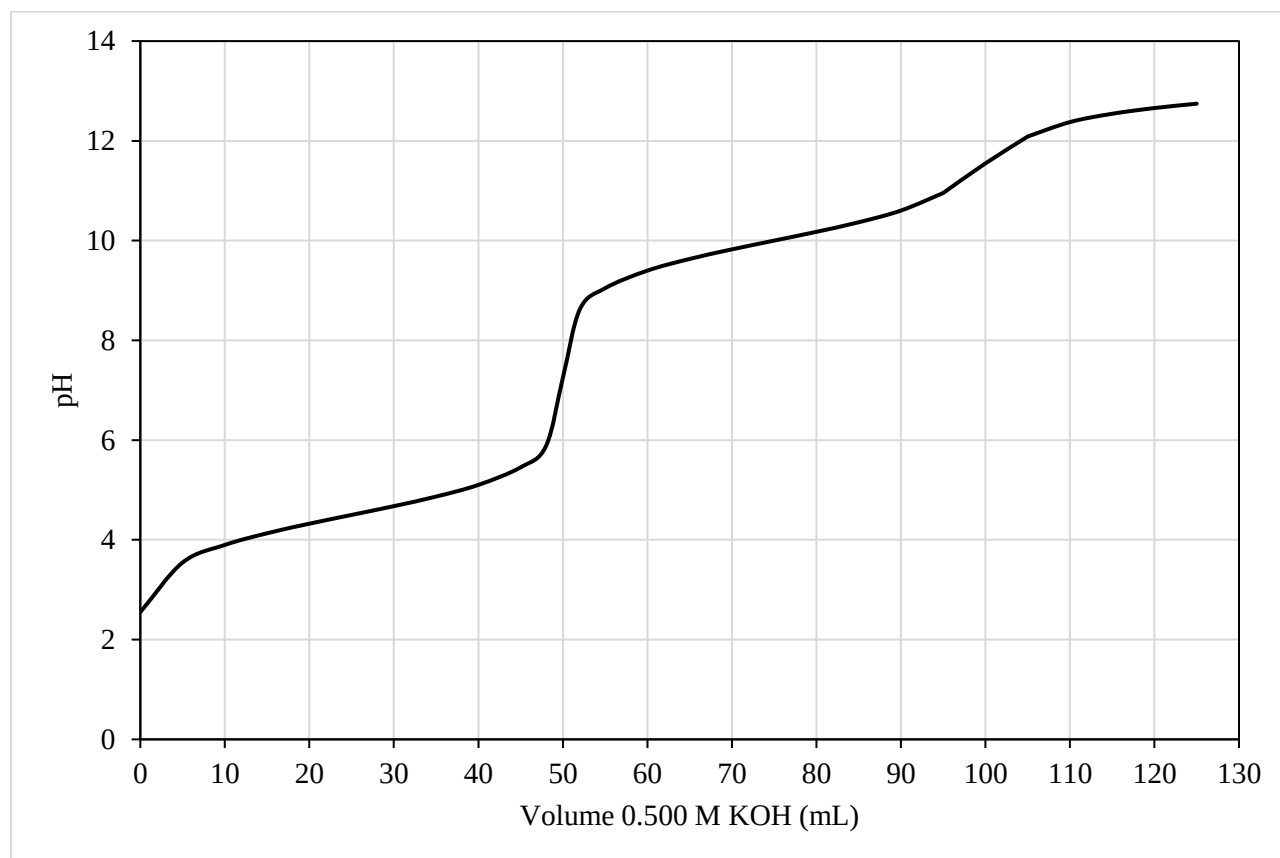


Figure 2. Titration of 100.0 mL of 0.250 M diprotic acid (H_2A) with $pK_{a1} = 4.50$ and $pK_{a2} = 10.00$ using 0.500 M KOH.

Basic Form of a Diprotic Acid Titration Calculations with a Strong Acid

6. 200.0 mL of 0.050 M A^{2-} is titrated with 0.40 M HCl. What is the 1st equivalence volume? What is the 2nd equivalence volume?

$$M_A V_A = M_B V_B$$

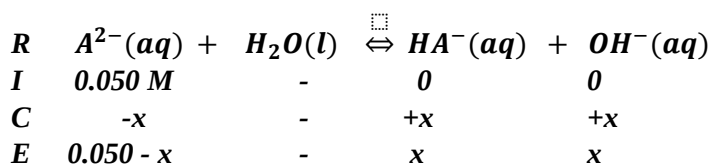
$$(0.40 \text{ M}) V_A = (0.050 \text{ M})(200.0 \text{ mL})$$

$$V_A = 25.0 \text{ mL} = V_{e1}$$

$$V_{e2} = 2V_{e1} = 50.0 \text{ mL}$$

7. What is the pH after the following volumes of 0.40 M HCl have been added to 200.0 mL of 0.050 M A^{2-} ? pK_{a1} of H_2A is 5.00 and pK_{a2} is 8.50.

a. 0.00 mL



$$K_{b1} = 10^{-5.50} = 3.2 * 10^{-6} = \frac{x^2}{0.050-x} \quad \text{assume the x in the denominator is small}$$

$$3.2 * 10^{-6} = \frac{x^2}{0.050}$$

$$x = 4.0 * 10^{-4} \text{ M} = [OH^-]$$

$$\text{Good assumption } \left(\frac{0.0040}{0.050} * 100 = 0.8\% < 5.0\% \right)$$

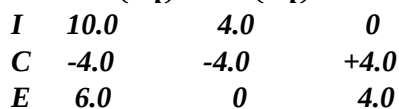
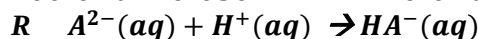
$$pOH = -\log(4.0 * 10^{-4} \text{ M}) = 3.40$$

$$pH = 14.00 - 3.40 = 10.60$$

b. 10.0 mL (Buffer region before first equivalence volume)

$$10.0 \text{ mL HCl} * 0.40 \text{ M HCl} = 4.0 \text{ mmol HCl or } 4.0 \text{ mmol } H^+$$

$$200.0 \text{ mL} * 0.050 \text{ M } A^{2-} = 10.0 \text{ mmol } A^{2-}$$



$$pH = pK_a + \log\left(\frac{A^{2-}}{HA^-}\right)$$

$$pH = 8.50 + \log\left(\frac{6.0}{4.0}\right)$$

$$pH = 8.68$$

c. 12.5 mL (One half to first equivalence volume)

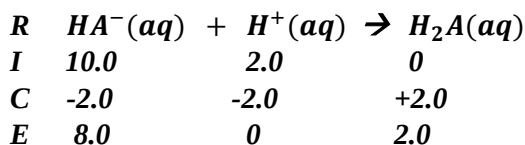
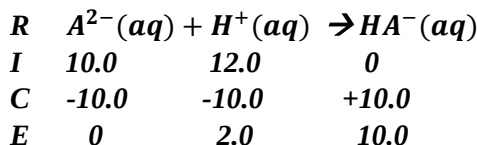
$$\text{At } \frac{1}{2} V_{e1}, pH = pK_{a2} = 8.50$$

d. 25.0 mL (First equivalence volume)

$$\text{At } V_{e1}, pH = \frac{1}{2} (pK_{a1} + pK_{a2}) = \frac{1}{2} (5.00 + 8.50) = 6.75$$

e. 30.0 mL (Buffer region between the first and second equivalence volumes)

$$\begin{aligned} 30.0 \text{ mL HCl} * 0.40 \text{ M HCl} &= 12.0 \text{ mmol HCl} = 12.0 \text{ mmol H}^+ \\ 200.0 \text{ mL of } 0.050 \text{ M A}^{2-} &= 10.0 \text{ mmol A}^{2-} \end{aligned}$$



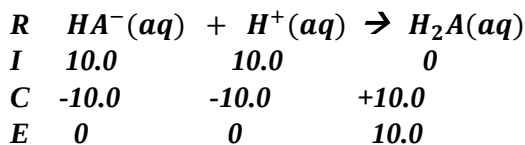
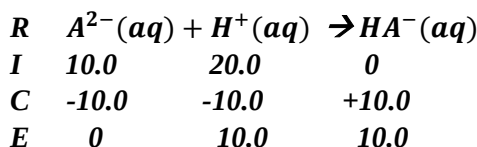
$$pH = pK_{a1} + \log\left(\frac{HA^-}{H_2A}\right)$$
$$pH = 5.00 + \log\left(\frac{8.0}{2.0}\right)$$
$$pH = 5.60$$

f. 37.5 mL (Halfway between first and second equivalence volumes)

$$\text{At } 3/2 V_{e1}, pH = pK_{a1} = 5.00$$

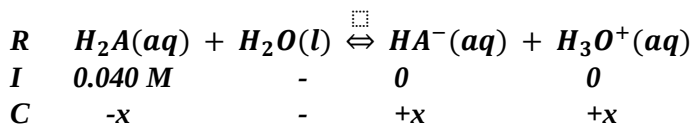
g. 50.0 mL (Second equivalence volume)

$$\begin{aligned} 50.0 \text{ mL HCl} * 0.40 \text{ M HCl} &= 20.0 \text{ mmol HCl} = 20.0 \text{ mmol H}^+ \\ 200.0 \text{ mL} * 0.050 \text{ M A}^{2-} &= 10.0 \text{ mmol A}^{2-} \end{aligned}$$



All of the A^{2-} has been converted to H_2A . Now, a RICE diagram for the basic form in water is written after finding the diluted concentration of H_2A .

$$[H_2A] = \left(\frac{10.0 \text{ mmol } H_2A}{250.0 \text{ mL}} \right) = 0.040 \text{ M}$$



$$E \quad 0.040 - x \quad - \quad x \quad x$$

$$K_{a1} = 10^{-5.00} = 1.0 * 10^{-5} = \frac{x^2}{0.040 - x} \quad \text{assume the } x \text{ in the denominator is small}$$

$$1.0 * 10^{-5} = \frac{x^2}{0.040}$$

$$x = 6.3 * 10^{-4} M = [H_3O^+]$$

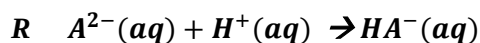
$$\text{Good assumption} \left(\frac{0.00063}{0.040} * 100 = 1.6\% < 5\% \right)$$

$$pH = -\log(6.3 * 10^{-4} M) = 3.20$$

h. 60.0 mL (Beyond the second equivalence volume)

$$60.0 \text{ mL HCl} * 0.40 \text{ M HCl} = 24.0 \text{ mmol HCl} = 24.0 \text{ mmol } H^+$$

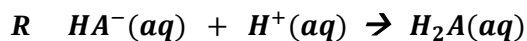
$$200.0 \text{ mL} * 0.050 \text{ M } A^{2-} = 10.0 \text{ mmol } A^{2-}$$



$$I \quad 10.0 \quad 24.0 \quad 0$$

$$C \quad -10.0 \quad -10.0 \quad +10.0$$

$$E \quad 0 \quad 14.0 \quad 10.0$$



$$I \quad 10.0 \quad 14.0 \quad 0$$

$$C \quad -10.0 \quad -10.0 \quad +10.0$$

$$E \quad 0 \quad 4.0 \quad 10.0$$

At this point, there is excess H^+ in solution, which dictates the pH of the solution.

$$[H^+] = \frac{4.0 \text{ mmol}}{260.0 \text{ mL}} = 0.0154 \text{ M}$$

$$pH = -\log(0.0154 \text{ M}) = 1.81$$

8. Sketch the titration curve. Label
 - a. the first equivalence volume
25 mL HCl
 - b. the second equivalence volume
50 mL HCl
 - c. where there is excess strong acid (or base)
Beyond 50 mL HCl added
 - d. where H_2A is the principal species in solution
Beyond 37.5 mL HCl added
 - e. where HA^- is the principal species in solution
After 12.5 mL HCl added and before 37.5 mL HCl added
 - f. where A^{2-} is the principal species in solution
Before 12.5 mL HCl added
 - g. where $pH = pK_{a1}$
37.5 mL HCl added
 - h. where $pH = pK_{a2}$
12.5 mL HCl added
 - i. any buffer regions
There are two buffer regions. The first is around pK_{a2} (~2.5-22.5 mL HCl) and the second buffer region is around pK_{a1} (~27.5-47.5 mL HCl)
9. What pH range should an indicator change color to visualize the first equivalence point? The second equivalence point?
To visualize the first endpoint, the indicator should change color around a pH of 6.75. To visualize the second endpoint, the indicator should change color around a pH of 3.20.

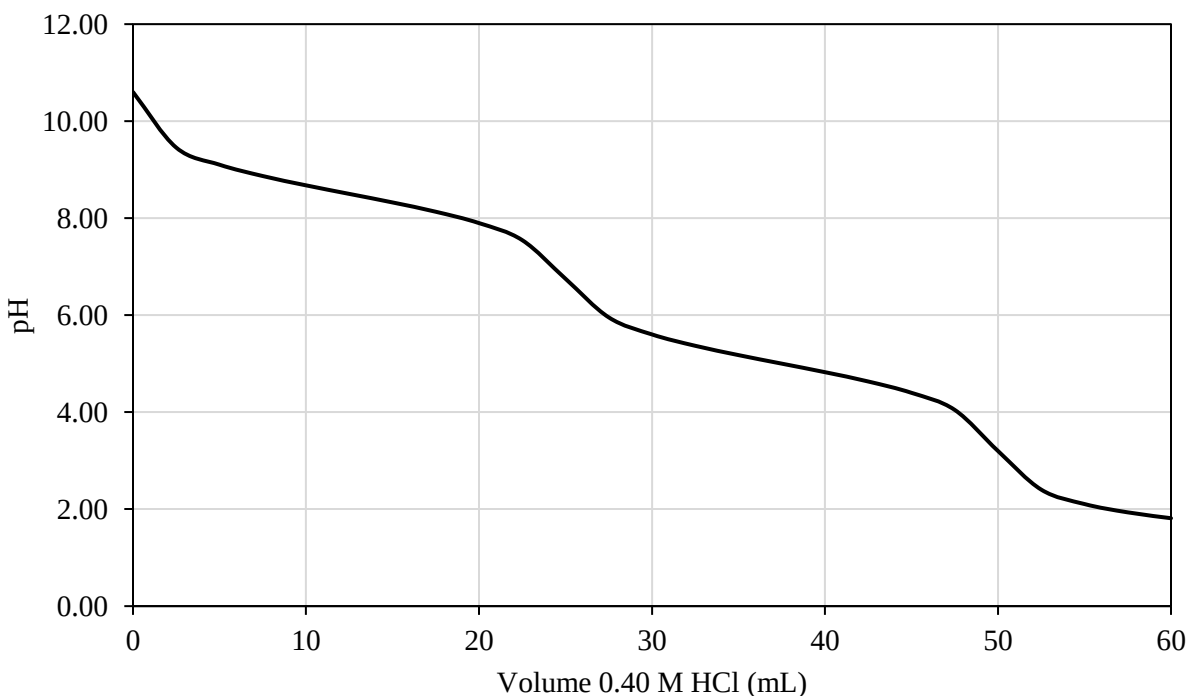


Figure 3. Titration curve of 200.0 mL of 0.050 M basic form of diprotic acid (A^{2-}) with $pK_{a1} = 5.50$ and $pK_{a2} = 8.50$ using 0.40 M HCl.

Summarizing Trends in Polyprotic Titration Curves

10. You have now sketched two diprotic titration curves:

1. A **diprotic acid (H_2A)** titrated with strong base (KOH)
2. A **diprotic base (A^{2-})** titrated with strong acid (HCl)

Complete the table below to compare and contrast these two titration curves. Think about the direction of the titration, species present at each stage, shape of the curve, and pH at key points.

Feature	Diprotic Acid (H_2A) + Strong Base	Diprotic Base (A^{2-}) + Strong Acid
Initial pH	<i>Low (~2–3), due to fully protonated acid (H_2A)</i>	<i>High (~10–11), due to fully deprotonated base (A^{2-})</i>
General shape of curve	<i>pH increases as base is added</i>	<i>pH decreases as acid is added</i>
First equivalence point volume	<i>When 1 mol of OH^- per mol of H_2A is added (e.g., 50 mL in example)</i>	<i>When 1 mol of H^+ per mol of A^{2-} is added (e.g., 25 mL in example)</i>
Second equivalence point volume	<i>When 2 mol of OH^- per mol of H_2A is added (e.g., 100 mL)</i>	<i>When 2 mol of H^+ per mol of A^{2-} is added (e.g., 50 mL)</i>
Species progression	$H_2A \rightarrow HA^- \rightarrow A^{2-}$	$A^{2-} \rightarrow HA^- \rightarrow H_2A$
Location of buffer regions	<i>Between 0–50 mL and 50–100 mL</i>	<i>Between 0–25 mL and 25–50 mL</i>
pH at 1st equivalence point	<i>7.25 (average of pK_{a1} and pK_{a2})</i>	<i>6.75 (average of pK_{a1} and pK_{a2})</i>
pH at 2nd equivalence point	<i>Around 11.55 (due to basic A^{2-} in water)</i>	<i>Around 3.20 (due to acidic H_2A in water)</i>
Where $pH = pK_{a1}$	<i>At 25 mL of KOH added ($\frac{1}{2}$ to 1st eq. pt.)</i>	<i>At 37.5 mL of HCl added ($\frac{3}{4}$ to 2nd eq. pt.)</i>
Where $pH = pK_{a2}$	<i>At 75 mL of KOH added ($\frac{3}{4}$ to 2nd eq. pt.)</i>	<i>At 12.5 mL of HCl added ($\frac{1}{2}$ to 1st eq. pt.)</i>
Final solution species	<i>A^{2-} (fully deprotonated) and excess OH^-</i>	<i>H_2A (fully protonated) and excess H^+</i>

Active Learning Worksheets for Analytical Chemistry

#15 Equilibrium Chemistry: Charge Balance, Mass Balance, Ionic Strength, and Activity

Faculty Resources

(Delete before sharing with students):

- **Analytical Context and Framing:** I think of this worksheet as a bridge between simplified equilibrium calculations and more realistic solution chemistry. It fits naturally with equilibrium, but I often use it later because ideas like charge balance, mass balance, ionic strength, and activity become especially important when students move into electrochemistry. I tell students that real solutions still have to obey conservation of mass and conservation of charge, even when the chemistry looks complicated. I also use this worksheet to introduce the idea that molarity is often a useful simplification, but activity is a better representation of “effective concentration” when ionic strength becomes important.
- **Key Concepts and Common Challenges:** Students often confuse mass balance and charge balance. I remind them that they have already used mass-balance thinking in acid–base problems: the concentrations of the different forms of a compound should add up to the formal concentration they started with. Charge balance is different; it is about counting positive and negative charge so that the solution is electrically neutral. Neutral species belong in mass balances but not charge balances. For ionic strength and activity, the main conceptual hurdle is recognizing that concentration and effective concentration are not always the same thing.
- **Facilitated Discussion Pauses:** Pause after the first few charge-balance and mass-balance questions. Ask: “Why does a species with a -2 charge need a coefficient of 2 in the charge balance, but not necessarily in the mass balance?” This is a good place to slow down and have students explain whether each coefficient is tracking atoms/formula units or charge.
- **Calculation Support:** For ionic strength, remind students that charge is squared in the calculation. This means a small concentration of a highly charged ion can contribute strongly to ionic strength. When introducing activity, I usually describe it as effective concentration: in dilute solutions, molarity may be good enough, but as ionic strength increases, ions do not behave as independently as our simple concentration-based equations assume.
- **Synthesis and Extension Questions:** Ask students: “Why might an equilibrium calculation using concentration work well in a dilute solution but fail in a solution with high ionic strength?” This helps students see why activity is not just a mathematical correction, but a way to make equilibrium calculations better match real solution behavior.
- **Pacing Note:** This worksheet can feel a little less story-driven than some of the others, and I think that is okay. I treat it as a toolbox worksheet. Students may not love charge balance, mass balance, ionic strength, and activity the first time they see them, but I want the ideas to be familiar when they reappear in electrochemistry and in more realistic equilibrium problems. I do not usually dwell on these topics for long. If time is limited, focus class time on Questions 1–5 and assign the ionic strength/activity questions as follow-up work. If splitting across two class periods, a natural stopping point is after Question 5.

Answer Keys for Active Learning Worksheets for Analytical Chemistry

#15 Equilibrium Chemistry: Charge Balance, Mass Balance, Ionic Strength, and Activity

Before You Begin

- Read [6.7: Solving Equilibrium Problems](#)
- Read [6.9: Activity Effects](#)

What You Should Already Know

- Familiarity with solution concentrations and ion notation
- Understanding of strong and weak electrolytes
- General equilibrium principles

What You'll Learn

- Define and construct charge and mass balance equations for ionic solutions
- Calculate ionic strength from ionic concentrations and charges
- Apply activity coefficients to solve equilibrium problems involving solubility and water autoionization

What You'll Practice

- Translate chemical descriptions into quantitative balance expressions
- Distinguish between concentration and activity
- Interpret activity coefficient tables and apply them to equilibrium calculations

Estimated Completion Time: 50-75 minutes

Author: Rebecca L. Sanders

Active Learning Worksheets for Analytical Chemistry, Version 1.0, 2026

Useful Equations

- $\mu = \frac{1}{2} \sum c_i z^2$
- $A_i = \gamma_i [i]$

Mass and Charge Balance

A **mass balance equation** is a statement that matter is conserved during a chemical reaction. For example, a 0.10 M solution of Na_2CO_3 dissociates in water into 0.20 M Na^+ and 0.10 M CO_3^{2-} . We know the CO_3^{2-} can gain a proton or two to form HCO_3^- and H_2CO_3 , therefore the sum of the carbonate species should equal 0.10 M:

$$[H_2CO_3] + [HCO_3^-] + [CO_3^{2-}] = 0.10 M$$

Because the concentration of Na^+ is also 0.20 M, we can set these two equal to one another by multiplying the carbonate species by 2:

$$[Na^+] = 2 \{ [H_2CO_3] + [HCO_3^-] + [CO_3^{2-}] \}$$

A **charge balance equation** requires that the total positive charge from the cations equal the total negative charge from the anions. For a 0.10 M solution of Na_2CO_3 , the charge balance equation would be:

$$[Na^+] + [H^+] = [HCO_3^-] + 2[CO_3^{2-}] + [OH^-]$$

All positive ions are on one side of the equation and all negative ions are on the other side of the equation. Since carbonate has a -2 charge, those charges must be counted twice, which is why there is a coefficient of 2 in front of $[CO_3^{2-}]$. H_2CO_3 is not included in this equation because it has no charge.

-
1. Write a charge balance for a solution of oxalic acid ($H_2C_2O_4$) in water if oxalic acid ionizes to $HC_2O_4^-$, and $C_2O_4^{2-}$.

$$[H^+] = [HC_2O_4^-] + 2[C_2O_4^{2-}] + [OH^-]$$

2. Write a charge balance for a solution containing H^+ , OH^- , Al^{3+} , $Al(OH)^{2+}$, $Al(OH)_2^+$, $Al(OH)_4^-$, NO_3^- , and Na^+ .

$$[H^+] + 3[Al^{3+}] + 2[Al(OH)^{2+}] + [Al(OH)_2^+] + [Na^+] = [OH^-] + [Al(OH)_4^-] + [NO_3^-]$$

3. Suppose that $Ca(NO_3)_2$ dissolves completely to give Ca^{2+} and NO_3^- in aqueous solution.
 - a) Write a charge balance expression for this solution assuming only full dissociation.

$$[H^+] + 2[Ca^{2+}] = [OH^-] + [NO_3^-]$$

- a) What is the charge balance expression if, in addition to Ca^{2+} and NO_3^- , $CaNO_3^+$ also forms in solution?

$$[H^+] + 2[Ca^{2+}] + [CaNO_3^+] = [OH^-] + [NO_3^-]$$

4. Suppose that $Ca(NO_3)_2$ dissolves completely to give Ca^{2+} and NO_3^- in aqueous solution.
- a) Write the mass balance expression for this solution assuming only full dissociation.

$$2[Ca^{2+}] = [NO_3^-]$$

- b) Suppose you have a solution of 0.050 M $Ca(NO_3)_2$. If $[Ca^{2+}] = 0.050\text{ M}$, what is the concentration of NO_3^- ?

$$[NO_3^-] = 0.10\text{ M}$$

- c) Now suppose that $CaNO_3^+$ is formed in addition to Ca^{2+} and NO_3^- . Write the mass balance this aqueous solution.

$$2\{[Ca^{2+}] + [CaNO_3^+]\} = [NO_3^-] + [CaNO_3^+]$$

5. Write a mass balance for an aqueous solution of $Al_2(CO_3)_3$ if the species are Al^{3+} , $Al(OH)^{2+}$, $Al(OH)_2^+$, $AlCO_3^+$, CO_3^{2-} , and HCO_3^- .

$$3([Al^{3+}] + [Al(OH)^{2+}] + [Al(OH)_2^+] + [AlCO_3^+]) = 2([HCO_3^-] + [CO_3^{2-}] + [AlCO_3^+])$$

Ionic Strength and Activity

6. What is the ionic strength of 0.42 M $MnBr_3$?

$$\mu = \frac{1}{2} [(0.42)(+3)^2 + (1.26)(-1)^2] = 2.52\text{ M}$$

7. What is the ionic strength of a solution of 0.20 M KCl and 0.30 M Li_3PO_4 ?

$$\mu = \frac{1}{2} [(0.20)(+1)^2 + (0.20)(-1)^2 + (0.90)(+1)^2 + (0.30)(-3)^2] = 2.00\text{ M}$$

8. Define activity.

Activity is the effective concentration based on the ionic strength of the solution.

Modified Table of Activity Coefficients⁷

	Ion	Ionic Strength (μ , M)				
Ion	Size (pm)	0.001	0.005	0.01	0.05	0.1
Charge = ± 1		Activity coefficient (γ)				
H^+	900	0.967	0.933	0.914	0.86	0.83
Na^+	450	0.964	0.928	0.902	0.82	0.775
OH^-	350	0.964	0.926	0.900	0.81	0.76
Cl^-, NO_3^-	300	0.964	0.925	0.899	0.805	0.755
Ag^+	250	0.964	0.924	0.898	0.80	0.75

9. Find the concentration of Cl^- in 0.10 M $AgNO_3$ saturated with $AgCl$. Include activity coefficients in the solubility-product expression, which are listed above. The K_{sp} of $AgCl$ is 1.8×10^{-10} .

$$\mu = \frac{1}{2} [(0.10)(+1)^2 + (0.10)(-1)^2] = 0.10 \text{ M}$$

Use table to find activity coefficients of Ag^+ and Cl^-

$$K_{sp} = A_{Ag^+}A_{Cl^-} = [Ag^+]\gamma_{Ag^+}[Cl^-]\gamma_{Cl^-}$$

$$1.8 \times 10^{-10} = (0.10 \text{ M})(0.75)[Cl^-](0.755)$$

$$[Cl^-] = 3.2 \times 10^{-9} \text{ M}$$

10. If $K_w = 1.0 \times 10^{-14}$, use activities to determine $[H^+]$ in 0.10 M NaCl?

$$\mu = \frac{1}{2} [(0.10)(+1)^2 + (0.10)(-1)^2] = 0.10 \text{ M}$$

Use table to find activity coefficients of H^+ and OH^-

$$K_w = A_{H^+}A_{OH^-} = [H^+]\gamma_{H^+}[OH^-]\gamma_{OH^-}$$

$$[H^+] = [OH^-] = x$$

$$1.00 \times 10^{-14} = (x)(0.83)(x)(0.76)$$

$$x = [H^+] = 1.3 \times 10^{-7} \text{ M}$$

⁷ Activity Coefficients for Aqueous Solutions at 25 °C. ChemPendix. <https://sites.google.com/site/chempendix/activity-coefficients-for-aqueous-solutions-at-25-c> (accessed July 3, 2025).

Active Learning Worksheets for Analytical Chemistry

#16 Spectroscopic Methods: Concepts and Calculations Associated with Light

Faculty Resources (Delete before sharing with students):

- **Analytical Context and Framing:** I frame this worksheet as the foundation for understanding why spectroscopy works. Some of it may feel like physics review, but students need a basic understanding of light, energy, wavelength, frequency, absorbance, and transmittance before they can make sense of spectroscopic measurements. I remind students that spectroscopy is not just “shining light through a sample”; we are using interactions between light and matter to get chemical information.
- **Key Concepts and Common Challenges:** Students often memorize the electromagnetic spectrum without fully connecting wavelength, frequency, and energy. Emphasize that frequency and energy increase together, while wavelength changes in the opposite direction. Students may also need help connecting energy differences to the spectra shown later in the worksheet: a larger energy jump corresponds to a shorter wavelength, while a smaller energy jump corresponds to a longer wavelength.
- **Facilitated Discussion Pauses:** Pause after the percent transmittance and absorbance questions. Ask: “If absorbance doubles, why does percent transmittance not simply get cut in half?” Students often need help seeing that absorbance and transmittance are related logarithmically, not linearly. I also pause around the Jablonski diagram and spectra to help students connect Figure 1 and Figure 2. Have them compare the size of the energy arrows to where absorbance, fluorescence, and phosphorescence appear in the spectrum.
- **Calculation Support:** Question 3 may require extra support because students have to move between absorbance and percent transmittance. In Question 4, students often need reminders to convert wavelength to meters and to distinguish the energy of one photon from the energy of a mole of photons. Encourage them to track units carefully, especially when converting from single photons to moles of photons. In the multi-step Beer’s Law/dilution problem, have students work backward from the cuvette concentration to the original flask and write each dilution step explicitly before converting to mass.
- **Synthesis and Extension Questions:** Ask students: “If two molecules absorb at the same wavelength but one has a much larger molar absorptivity, how would that affect the concentration range you would choose for an experiment?” This helps students connect the light calculations in this worksheet to later decisions about calibration range, sensitivity, and method design.
- **Pacing Note:** This worksheet may fit within one class period, but Question 4 and the Jablonski diagram section can slow students down. If time is limited, complete Questions 1–5 during class and assign the Jablonski diagram questions as follow-up work or as preparation for the next spectroscopy discussion. If students are struggling with the figures, it is worth spending a few minutes as a class connecting the energy arrows to the relative wavelengths of absorbance, fluorescence, and phosphorescence.

Answer Keys for Active Learning Worksheets for Analytical Chemistry Spectroscopic Methods: Concepts and Calculations Associated with Light

Before You Begin

- Read [10.1: Overview of Spectroscopy](#)
- Read [10.2: Spectroscopy Based on Absorption](#)
- Read [10.5: Emission Spectroscopy](#)
- Read [10.6: Photoluminescent Spectroscopy](#)

What You Should Already Know

- Structure of the electromagnetic spectrum
- Relationship between wavelength, frequency, and energy
- Beer's Law and transmittance
- Units of molarity, absorbance, and dilution

What You'll Learn

- Interpret the electromagnetic spectrum in terms of energy, wavelength, and frequency
- Use absorbance and transmittance relationships to calculate concentration and %T
- Perform dilution and spectroscopic calculations
- Identify physical processes on a Jablonski diagram

What You'll Practice

- Apply Beer's Law to multi-step concentration problems
- Analyze spectral shifts and line broadening in molecular transitions
- Connect observed spectral data to underlying energy transitions

Estimated Completion Time: 60-75 minutes

Author: Rebecca L. Sanders

Active Learning Worksheets for Analytical Chemistry, Version 1.0, 2026

Useful Equations and Conversion Factors

- $E = h\nu$
- $h = 6.626 \times 10^{-34} \text{ J} \cdot \text{s}$
- $c = \lambda\nu = 2.998 \times 10^8 \text{ m/s}$
- $6.022 \times 10^{23} \text{ molecules/mole}$
- $A = \epsilon bc = -\log(T)$

Concepts and Calculations Associated with Light

1. The electromagnetic spectrum contains x-rays, visible light, γ -rays, ultraviolet light, infrared, radio waves, and microwaves.

- a. List these waves in order from lowest to highest energy.

Radio waves < Microwaves < Infrared < Visible light < Ultraviolet < X-rays < Gamma rays

- b. List these waves in order from lowest to highest frequency.

Radio waves < Microwaves < Infrared < Visible light < Ultraviolet < X-rays < Gamma rays

- c. List these waves in order from lowest to highest wavelength.

Gamma rays < X-rays < Ultraviolet < Visible light < Infrared < Microwaves < Radio waves

2. What value of absorbance corresponds to 45.0% T?

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

$$\text{where } T = \frac{\%T}{100} = \frac{45.0}{100} = 0.450$$

$$A = -\log(0.450) = 0.347$$

3. When the concentration of a solution is doubled, the absorbance is doubled. If a 0.010 M solution exhibits 45.0 %T at some wavelength what will be the percent transmittance for a 0.0200 M solution of the same substance?

Since absorbance is proportional to concentration, doubling concentration doubles absorbance. We already found that $A = 0.347$ at 0.010 M. At 0.0200 M, the absorbance doubles to 0.694.

Now calculate %T:

$$T = 10^{-A} = 10^{-0.694} = 0.202$$
$$\%T = T * 100 = 0.202 * 100 = 20.2\%$$

4. Determine the frequency (s^{-1}) and energy (kJ/mol) for light with a wavelength of 340.0 nm.

$$\text{Convert wavelength to meters: } 340.0 \text{ nm} * \frac{1 \text{ m}}{10^9 \text{ nm}} = 3.400 * 10^{-7} \text{ m}$$

$$\nu = \frac{c}{\lambda} = \frac{2.998 \times 10^8 \text{ m/s}}{3.400 \times 10^{-7} \text{ m}} = 8.818 * 10^{14} s^{-1}$$

$$E (\text{single photon}) = h\nu = 6.626 \times 10^{-34} J \cdot s * 8.818 \times 10^{14} s^{-1}$$

$$E (\text{single photon}) = 5.843 \times 10^{-19} J/\text{photon}$$

$$E (\text{per mol}) = \frac{5.843 \times 10^{-19} J}{1 \text{ photon}} * \frac{6.022 \times 10^{23} \text{ photon}}{1 \text{ mol photons}} = 351.9 \text{ kJ/mol}$$

5. A compound with a formula mass of 292.16 g/mol was dissolved in solvent in a 5.00 mL volumetric flask and diluted to the mark. A 1.00 mL aliquot was withdrawn, placed in a 10.0 mL volumetric flask, and diluted to the mark. The absorbance measured at 340 nm was 0.427 in a 1.00 cm cuvette. The molar absorptivity for this compound at 340 nm is $6130 \text{ M}^{-1} \cdot \text{cm}^{-1}$.

- a. Calculate the concentration of compound in the cuvette.

$$A = \epsilon bc$$

$$0.427 = 6130 * 1.00 * c$$

$$c = 6.97 * 10^{-5} \text{ M}$$

- b. Calculate the concentration of compound in the 5.00 mL flask.

$$M_1 V_1 = M_2 V_2$$

$$(6.97 * 10^{-5} \text{ M})(10.0 \text{ mL}) = M_2(1.00 \text{ mL})$$

$$M_2 = 6.97 * 10^{-4} \text{ M}$$

- c. How many milligrams of compound were used to make the 5.00 mL solution?

$$5.00 \text{ mL} * \frac{1 \text{ L}}{1000 \text{ mL}} * \frac{6.97 * 10^{-4} \text{ mol}}{1 \text{ L}} * \frac{292.16 \text{ g}}{1 \text{ mol}} = 1.02 \text{ mg}$$

Jablonski Diagrams

6. Fill out the table below about different physical processes represented on Jablonski diagrams.

Table 1. Different physical processes represented in a Jablonski diagram.

Physical processes	Describe the transition.	Is a photon absorbed, is a photon emitted, or is the process a radiationless transition?
Absorption	S_0 to higher energy state with same spin	A photon is absorbed
Fluorescence	<i>Emission of a photon from a transition between states of the same spin</i>	<i>Emitted</i>
Internal conversion	<i>Radiationless transition between states with the same spin</i>	<i>Radiationless transition</i>
Intersystem crossing	<i>Radiationless transition between states with different spins</i>	<i>Radiationless transition</i>
Phosphorescence	<i>Emission of a photon from a transition between states with different spin</i>	<i>Emitted</i>
Vibrational relaxation	<i>Energy lost through collisions to relax to the lowest energy level in a state.</i>	<i>Radiationless transition</i>

7. On this Jablonski diagram, label each physical process based on one of the six definitions on the previous page.

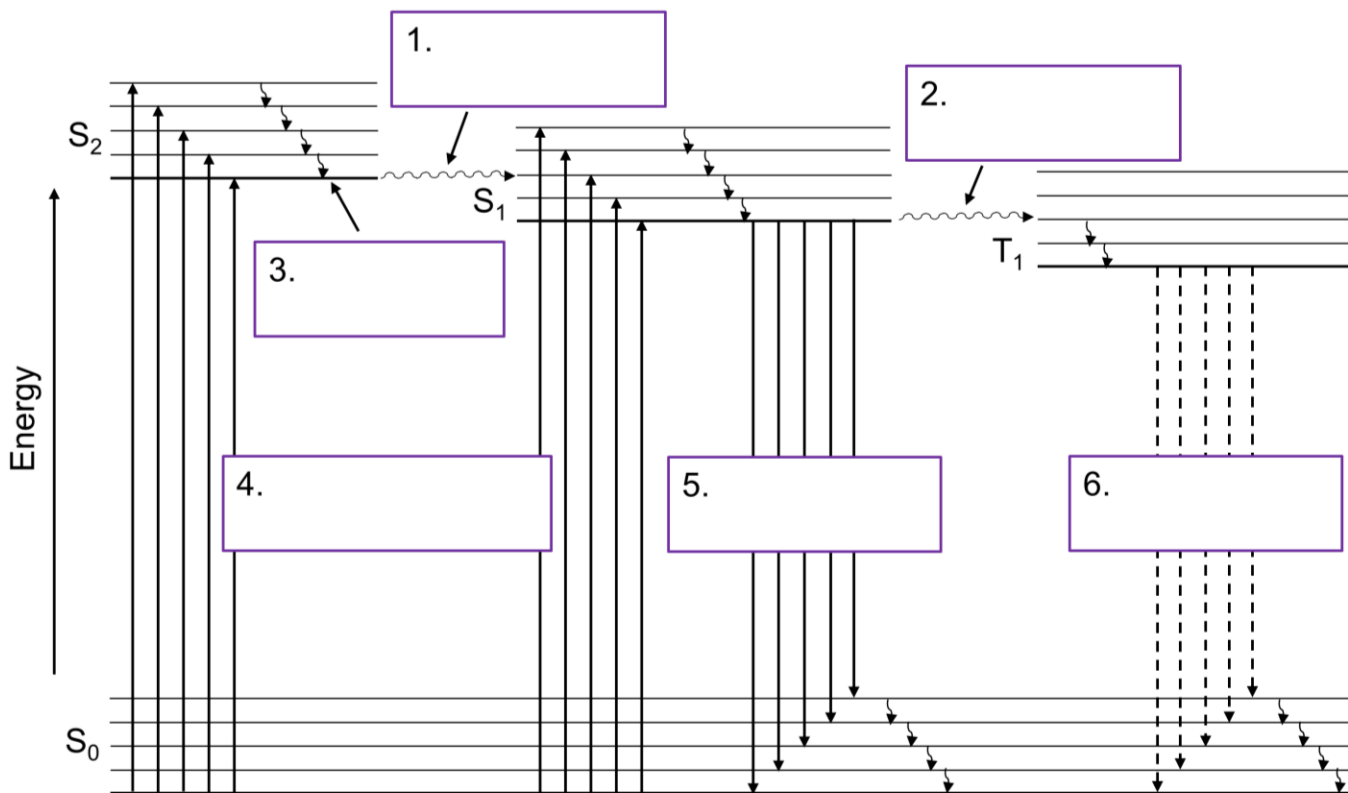


Figure 1. Jablonski diagram to be labeled with the physical processes from Table 1.

Answers: 1 = *Internal conversion*; 2 = *Intersystem crossing*; 3 = *Vibrational relaxation*; 4 = *Absorption*; 5 = *Fluorescence*; 6 = *Phosphorescence*

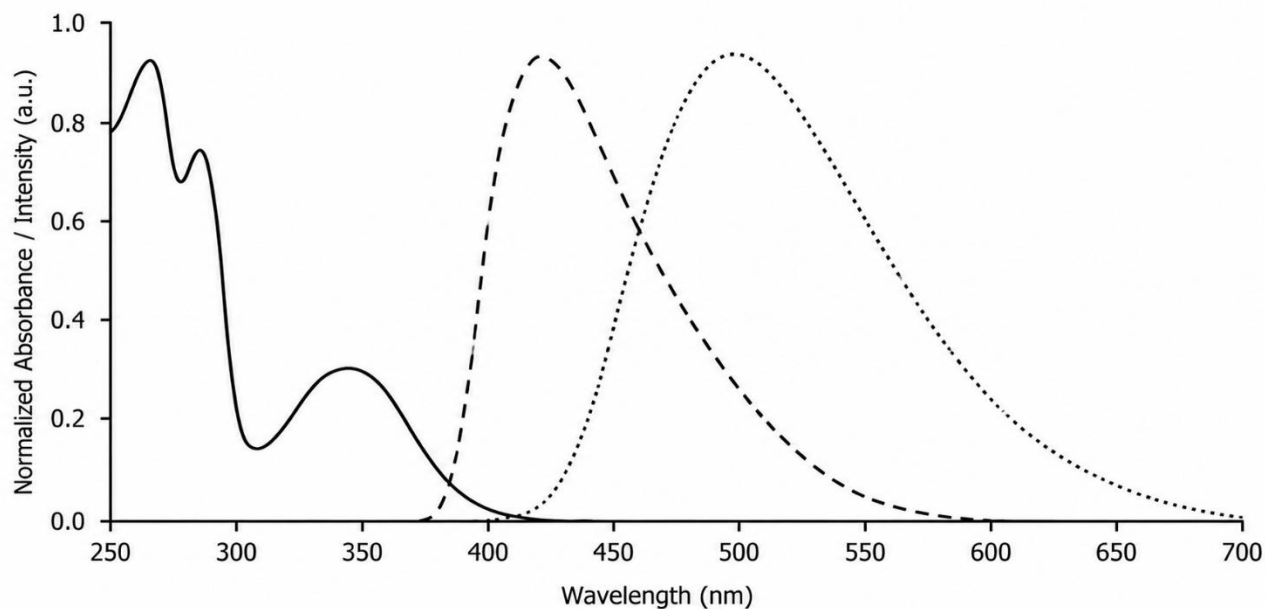


Figure 2. Representative absorbance, fluorescence emission, and phosphorescence emission spectra as a function of wavelength. Image created with ChatGPT (OpenAI).

8. Consider Figure 2, representing spectra for absorbance, fluorescence, and phosphorescence and Figure 1, representing the energy transitions needed for different physical processes represented in a Jablonski diagram. What do you notice about the wavelengths of light from the three spectra associated with absorbance, fluorescence, and phosphorescence in Figure 2? How do these wavelengths correspond to the associated Jablonski diagram (Figure 1)?

Absorbance has the shortest wavelength, so the largest jump in energy whereas phosphorescence has the longest wavelength and the lowest energy transition.

9. The peaks on the spectra are 10s of nm wide. Consider the Jablonski diagrams in this activity. Why do these spectral peaks spread out over several 10s of nanometers?

There are multiple levels within each state allows for a range of energy transitions leading to spectral peaks that are 10s of nanometers wide for molecular spectroscopy.

Answer Keys for Active Learning Worksheets for Analytical Chemistry

#17 Spectroscopic Methods: Spectrophotometer Components and Comparisons

Faculty Resources (Delete before sharing with students):

- **Analytical Context and Framing:** I frame this worksheet as a way to move from “I have used a spectrophotometer” to “I understand what the instrument is actually doing.” Most students have already collected UV-visible spectra or absorbance measurements in lab, but they may still think of the instrument as a black box. I emphasize that a spectrophotometer does not just give an absorbance value. Every component affects what wavelengths reach the sample, how much light reaches the detector, and how reliable the measurement is.
- **Key Concepts and Common Challenges:** Students may know the words source, monochromator, sample, and detector, but still struggle to explain why each component is needed. Push them to connect each part of the instrument to the quality of the analytical signal. Students often need help visualizing the light path through the instrument, understanding what a monochromator actually does, and connecting slit width to the trade-off between signal intensity and resolution. They may also need reinforcement on how to choose an appropriate blank, even if they have used blanks many times in previous lab courses.
- **Facilitated Discussion Pauses:** Pause after the single-beam and double-beam diagrams. Ask: “What problem is the double-beam design trying to solve? Why might continuously comparing the sample and blank improve the measurement?” If an instrument is available, this is a useful place to show students the inside of a spectrophotometer or point out the likely path from the light source to the monochromator, sample, shutter, and detector. A physical instrument can make the diagrams feel much less abstract.
- **Calculation Support:** This worksheet is mostly conceptual, but students may need help interpreting specification tables and instrument descriptions. Encourage them to identify the analytical need first, such as wavelength range, sensitivity, resolution, cost, or throughput, before selecting the instrument. For questions about slit width and resolution, it can help to use a simple spectroscopy or other visual model so students can see the trade-off between how much light enters the instrument and how well closely spaced wavelengths can be distinguished.
- **Synthesis and Extension Questions:** Ask students: “If you were buying an instrument for a teaching lab, would you choose the most sensitive instrument, the cheapest instrument, or the easiest one for students to use? What trade-offs would matter most?” Students often enjoy talking about instrument cost because many have little sense of how expensive lab equipment can be. If your department has instruments students use regularly, consider having them guess the cost or compare approximate prices and features. I use this discussion to move students away from the idea of a single ‘best’ instrument and toward the more realistic question: which instrument fits the analytical goal, budget, and users?
- **Pacing Note:** This worksheet can usually be completed in one class period if students move efficiently through the diagrams. If time is limited, use class time for the instrument flow path, light sources, monochromators, slit width, and wavelength-selection questions, then assign the detector and instrument-comparison questions as follow-up work. Optional demonstrations, such as opening a spectrophotometer or using a simple spectroscopy to explore slit width, are not required but can make the instrument-design choices much more concrete.

Answer Keys for Active Learning Worksheets for Analytical Chemistry

#17 Spectroscopic Methods: Spectrophotometer Components and Comparisons

Before You Begin

- Read [10.1: Overview of Spectroscopy](#)
- Read [10.2: Spectroscopy Based on Absorption](#)
- Read [10.3: UV/Vis and IR Spectroscopy](#)

What You Should Already Know

- General structure of electromagnetic radiation
- Relationship between absorbance and transmittance
- Concept of a blank in spectroscopic measurements

What You'll Learn

- Identify and describe the components of a single- and double-beam spectrophotometer.
- Distinguish between continuum and line light sources.
- Explain the trade-offs involved with monochromator slit width selection.
- Compare photodiode arrays and photomultiplier tubes as detectors.
- Evaluate and compare instrument specifications based on real-world needs.

What You'll Practice

- Interpret technical diagrams of instruments and identify components.
- Analyze how changes in experimental parameters affect measurement outcomes.
- Justify instrument choices based on cost, precision, and application.
- Synthesize data from specification tables to support analytical decisions.

Estimated Completion Time: 60-75 minutes

Author: Rebecca L. Sanders

Active Learning Worksheets for Analytical Chemistry, Version 1.0, 2026

Part I: Basic Instrument Flow Path

Figures 1 and 2 highlight the typical flow path of light in spectrophotometers, starting from the light source, and then to the monochromator, sample, detector, and display. This worksheet will work through the components of typical spectrophotometers.

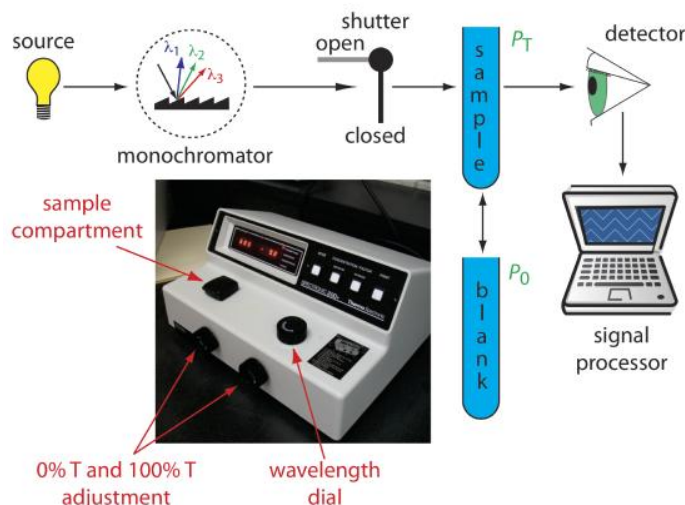


Figure 1. Schematic diagram of a fixed-wavelength, single-beam spectrophotometer. The photographic inset shows a typical instrument. The shutter remains closed until the sample or blank is placed in the sample compartment. The analyst manually selects the wavelength by adjusting the wavelength dial. From Figure 10.3.2 in David Harvey, *Analytical Chemistry 2.1*, licensed under CC BY-NC-SA 4.0.

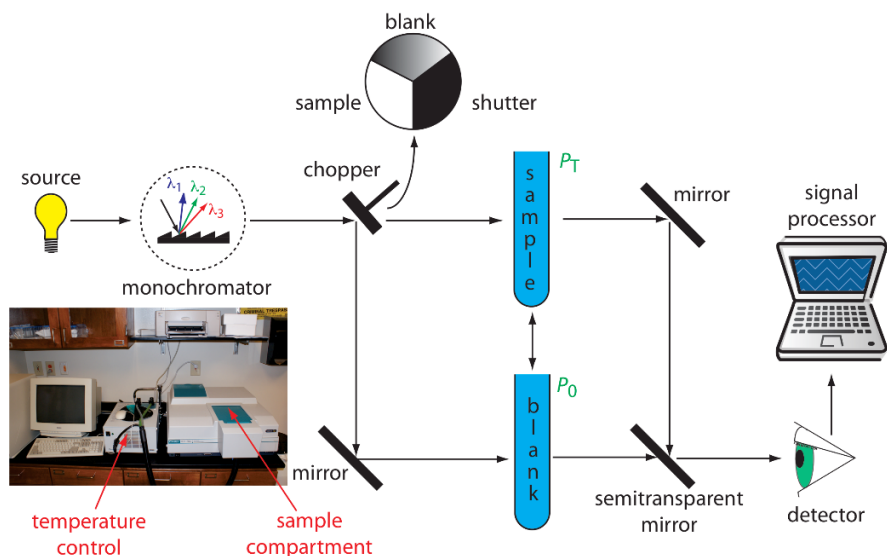


Figure 2. Schematic diagram of a scanning, double-beam spectrophotometer. A chopper directs the source's radiation, using a transparent window to pass radiation to the sample and a mirror to reflect radiation to the blank. The chopper's opaque surface serves as a shutter, which allows for a constant adjustment of the spectrophotometer's 0%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. From Figure 10.3.3 in David Harvey, *Analytical Chemistry 2.1*, licensed under CC BY-NC-SA 4.0.

Part II: Light Sources

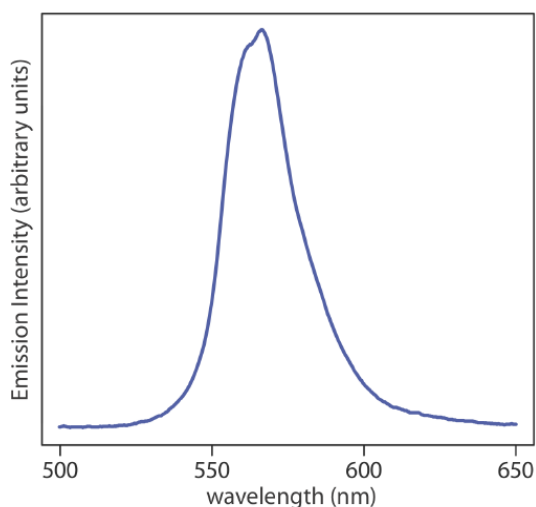


Figure 3. Spectrum showing the emission from a green LED, which provides continuous emission over a wavelength range of approximately 530–640 nm. From Figure 10.1.7 in David Harvey, *Analytical Chemistry 2.1*, licensed under CC BY-NC-SA 4.0.

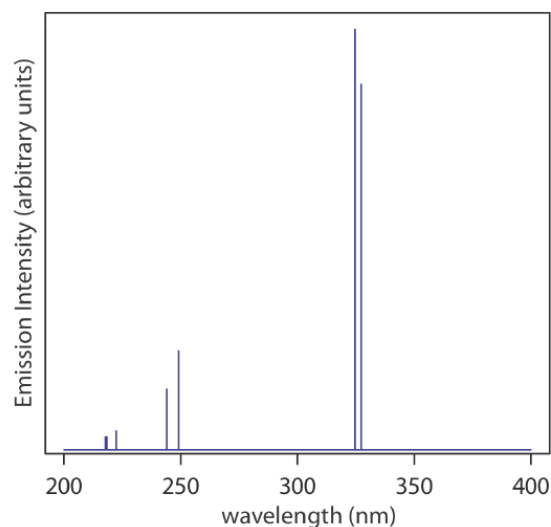


Figure 4. Emission spectrum from a Cu hollow cathode lamp. This spectrum consists of several distinct emission lines. Each emission line has a width of approximately 0.01 nm at one half of its maximum intensity. From Figure 10.1.8 in David Harvey, *Analytical Chemistry 2.1*, licensed under CC BY-NC-SA 4.0.

1. Consider the spectra for two possible line sources used for different spectroscopic techniques (Figures 3 and 4). One is classified as a line source and the other is classified as a continuum source. Identify which figure represents which type of light source. Explain.

The first is a continuum source because it emits radiation over a broad range of wavelengths whereas the second is a line source because it emits radiation at selected wavelengths.

2. There are many possible light sources. A few are summarized in the list on the next page. A visible spectrophotometer contains a tungsten lamp, which is a continuum light source. Why is it important for a visible spectrophotometer to contain a continuum light source?

A continuum light source is needed to produce light at a range of wavelengths covering the entire visible part of the electromagnetic spectrum. This is critical to ensure measurements can be made at any wavelength in that range.

3. Why are both a tungsten halogen and a deuterium lamp included in a UV-Vis spectrophotometer, which is used for measurements in the wavelength range from 190–1100 nm?

The D₂ lamp only covers the UV region up to 380 nm whereas the tungsten lamp focuses on the visible region from 320 nm and above. Two lamps are needed to cover the entire measurement range from 190–1100 nm.

Common Sources of Electromagnetic Radiation

Adapted from Table 10.1.3 in David Harvey, *Analytical Chemistry 2.1*, licensed under CC BY-NC-SA 4.0.

- D_2 lamp
 - Type: Continuum
 - Wavelength Range: 160–380 nm
 - Useful for: Molecular absorption
- Tungsten lamp
 - Type: Continuum
 - Wavelength Range: 320–2400 nm
 - Useful for: Molecular absorption
- Hollow cathode lamp
 - Type: Line
 - Wavelength Range: UV/Vis
 - Useful for: Atomic absorption
- Green LED
 - Type: Continuum
 - Wavelength Range: Visible
 - Useful for: Molecular fluorescence
- Laser
 - Type: Line
 - Wavelength Range: UV/Vis/IR
 - Useful for: Atomic and molecular absorption, fluorescence, and scattering

4. A student wants to measure absorbance at 210 nm with a visible spectrophotometer, which typically only contains a tungsten lamp. Will their experiment work? Explain.

This would not be appropriate as a visible spectrophotometer does not contain a light source that is appropriate to measure in the UV part of the electromagnetic spectrum.

5. Why doesn't all of the light from the source reach the sample in Figure 1? Where does it go?

Light from the source spreads in all directions, but only a portion enters the monochromator through the entrance slit. Inside the monochromator, most wavelengths are blocked so only a narrow range exits toward the sample. The rest is absorbed or reflected internally.

Part III: Selecting Wavelengths with a Monochromator

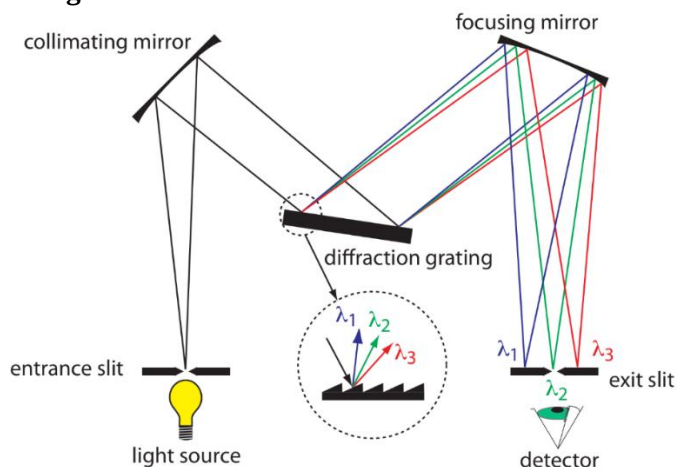


Figure 5. Schematic diagram of a monochromator that uses a diffraction grating to disperse the radiation. From Figure 10.1.12 in David Harvey, *Analytical Chemistry 2.1*, licensed under CC BY-NC-SA 4.0.

6. Explain the purpose of each component of the monochromator.
- Entrance slit

The entrance slit defines the portion of polychromatic light entering the monochromator and shapes the beam that the instrument will disperse and selects spatial coherence.

- Collimating mirror

The collimating mirror captures light emerging from the entrance slit and reflects it as a parallel beam.

- Diffraction grating

The diffraction grating is a reflective surface with many grooves that disperses incoming parallel light into its constituent wavelengths. Rotating the grating steers specific wavelengths toward the exit slit.

- Focusing mirror

The focusing mirror collects the dispersed light from the grating and directs it onto the exit slit plane, ensuring that only the selected wavelengths reach the exit slit.

- Exit slit

The exit slit acts as the final wavelength selector, passing a narrow band of light to the detector.

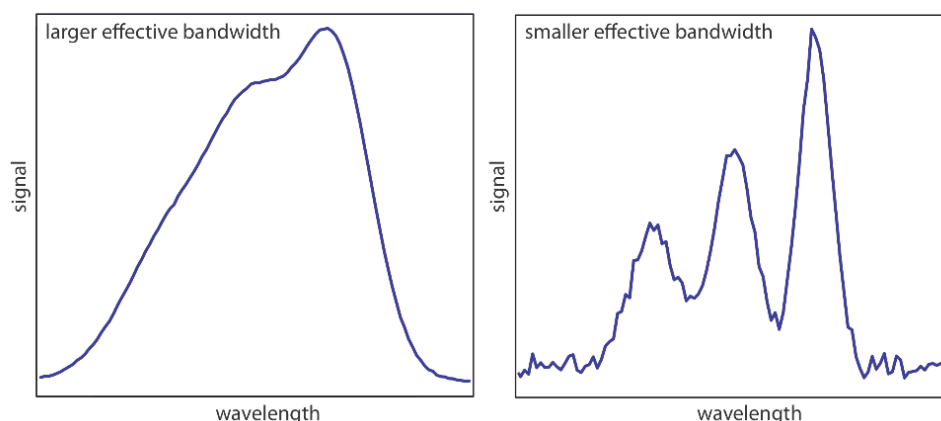


Figure 6. Example showing the effect of the wavelength selector's effective bandwidth on resolution and noise. The spectrum with the smaller effective bandwidth is on the right and the spectrum with the larger effective bandwidth is on the left. From Figure 10.1.11 in David Harvey, *Analytical Chemistry 2.1*, licensed under CC BY-NC-SA 4.0.

7. Slit widths associated with the monochromator have a direct impact on the spectra produced by the spectrophotometer. For example, examine the figure below and answer the following questions. The larger slit width is shown on the left and the smaller slit width is shown on the right.
 - a. Which slit width gives better resolution?

The smaller slit width (right) has better resolution because three distinct peaks are observed. A narrow slit minimizes spectral overlap by reducing the range of wavelengths reaching the detector at each moment, allowing you to resolve closely spaced peaks.

- b. Which slit width allows more light to reach the detector?

The larger slit width (left) allows more light through to reach the detector, increasing signal intensity, but at the cost of resolution.

- c. Which slit width results in a noisier spectrum?

The smaller slit width lets less light reach the detector, causing the spectrum to be noisier (the spectrum is not as smooth).

- d. Summarize the trade-off between slit width size and resolution.

A smaller slit width improves spectral resolution, allowing better separation of closely spaced peaks. However, this also reduces light intensity and increases noise, making it harder to distinguish weak signals.

8. In what scenarios would you prioritize intensity over resolution?

You would prioritize intensity over resolution when detecting low-concentration analytes or when signal strength is more critical than resolving fine spectral features.

Part IV: Sample Considerations

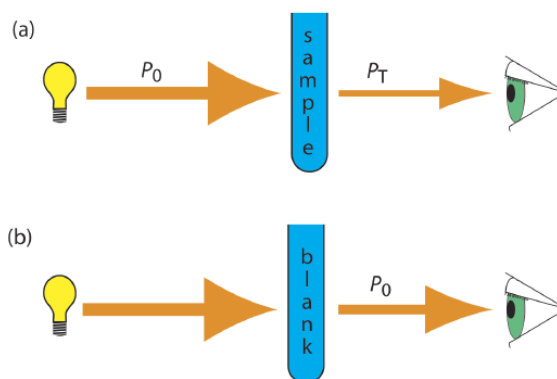


Figure 7. (a) Schematic diagram showing the attenuation of radiation passing through a sample; P_0 is the source's radiant power and P_T is the radiant power transmitted by the sample. (b) Schematic diagram showing how we redefine P_0 as the radiant power transmitted by the blank. From Figure 10.2.7 in David Harvey, *Analytical Chemistry* 2.1, licensed under CC BY-NC-SA 4.0.

9. In the figure above, P_0 is the source's radiant power and P_T is the radiant power transmitted by the sample (a). However, in Figure 7b, P_0 as the radiant power transmitted by the blank.
- a. Why is P_0 redefined as the radiant power transmitted by the blank rather than the source?

Redefining P_0 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.

- b. Does a spectrophotometer measure photons that are absorbed by the sample or transmitted through the sample?

A spectrophotometer measures the amount of light transmitted through the sample.

- c. Dante is conducting an experiment to measure the amount of iron in tap water using a colorimetric assay. To conduct this experiment, Fe^{3+} standards are prepared by adding variable volumes of an iron stock solution to a volumetric flask and then adding fixed volumes of citrate to pH adjust the solution, hydroquinone to reduce Fe^{3+} to Fe^{2+} , and o-phenanthroline to complex the Fe^{2+} . The volumetric flasks are filled to the mark with DI water. What would be an appropriate blank for this experiment?

The blank should be prepared in the same way as the standards and samples and it should contain everything except the analyte of interest. In this example, the blank should be made by adding the fixed volumes citrate, hydroquinone, and o-phenanthroline to the same size volumetric flask and then filling to the mark with DI water.

- d. Dante's solutions have a reddish orange color. What wavelength of light would be best to measure absorbance of those solutions? Hint: Use the visible spectrum of light shown to the right to answer the question.

A solution with a reddish-orange color would absorb light best with the complementary color of green-cyan, which is around 500-520 nm.

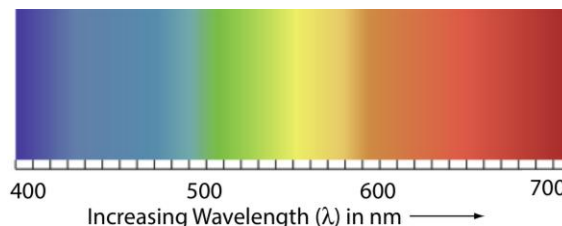


Figure 8. The visible spectrum of light. Adapted from Figure 10.1.3 in David Harvey, *Analytical Chemistry 2.1*, licensed under CC BY-NC-SA 4.0.

10. Consider the path of light in a typical spectrophotometer. For each scenario, explain if the amount of light reaching the detector would increase, decrease, or stay the same.

- a. Increasing the sample's concentration

As sample concentration increases, transmittance of light goes down and absorbance goes up.

- b. Using a longer path length cuvette

An increased cell path length would let less light to be transmitted and therefore, less light would reach the detector.

- c. Using a light source with lower intensity

A lower intensity light source would reduce P_0 and therefore would reduce the amount of light that reaches the detector.

- d. Using a scratched cuvette

A scratched cuvette scatters or reflects light, reducing the amount of light that reaches the detector.

Part V: Detectors

There are a few different detectors present in a spectrophotometer including a photomultiplier tube (Figure 9) and a diode array (Figure 10).

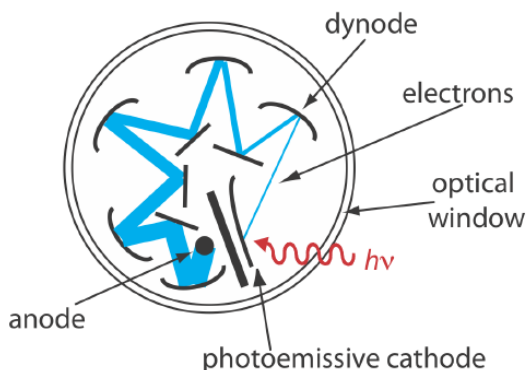


Figure 9. Schematic of a photomultiplier. A photon strikes the photoemissive cathode producing electrons, which accelerate toward a positively charged dynode. Collision of these electrons with the dynode generates additional electrons, which accelerate toward the next dynode. A total of 10^6 – 10^7 electrons per photon eventually reach the anode, generating an electrical current. From Figure 10.1.14 in David Harvey, *Analytical Chemistry 2.1*, licensed under CC BY-NC-SA 4.0.

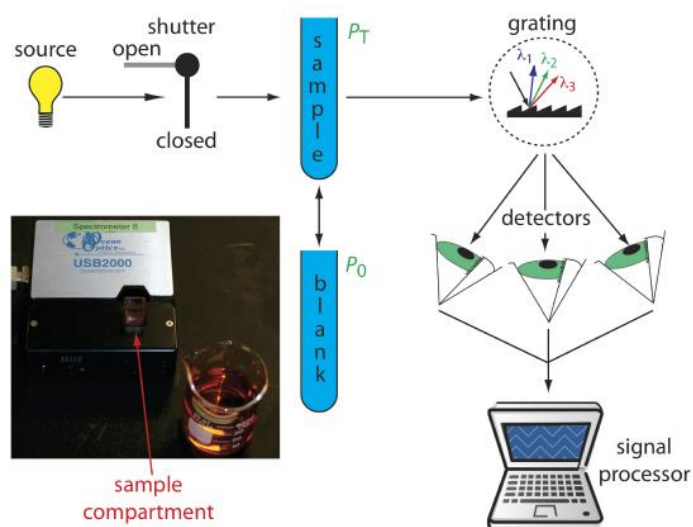


Figure 10. Schematic diagram of a diode array spectrophotometer. The photographic insert shows a typical instrument. Because the spectrometer is small and compact, it is easy to transport into the field. From Figure 10.3.4 in David Harvey, *Analytical Chemistry 2.1*, licensed under CC BY-NC-SA 4.0.

11. A PMT converts photons into electrons and uses a cascade of dynodes to amplify the signal dramatically ($\times 10^6$ – 10^7 gain) before reaching the detector anode. Explain how the design of a PMT enables detection of extremely weak light signals.

Dynode chain amplifies each photoelectron ($\sim 10^6$ – 10^7) so even single-photon events generate detectable current

12. A PDA consists of a linear array of several hundred to a few thousand individual silicon photodiodes placed along the focal plane of a monochromator. What is the primary advantage of using a PDA over a single-element detector like a PMT in absorbance spectroscopy?

PDA's can measure an entire spectrum at once, enabling rapid data collection without mechanical scanning.

13. Although PDA's are less sensitive than PMT's, they offer improved usability in modern spectrometers. List **two** benefits of PDA's related to **speed** and **mechanical reliability**.

They are fast, capturing full spectra in milliseconds, ideal for kinetics and real-time monitoring. They are also reliable because they don't have moving parts, resulting in low maintenance.

Part VI: Comparing Spectrophotometers

Below lists the specifications for three different spectrophotometers: a simple visible-only spectrophotometer¹, an entry-level UV-vis spectrophotometer², and a more sophisticated UV-vis spectrophotometer³. Use these lists to compare and contrast the different instruments:

V-1200 Visible-Only Spectrophotometer

- Light Source: Tungsten
- Wavelength Range: 325–1000 nm
- Beam Type: Single
- Accuracy: ± 2 nm
- Reproducibility: ± 0.8 nm
- Slit Width: 4 nm
- Detector: Silicon photodiode
- Cost: Low

UV-2650 UV-Vis Spectrophotometer

- Light Source: Tungsten + D_2
- Wavelength Range: 190–1100 nm
- Beam Type: Single
- Accuracy: ± 0.5 nm
- Reproducibility: ± 0.2 nm
- Slit Width: 2 nm
- Detector: Silicon photodiode
- Cost: Medium

Cary 3500 UV-Vis Spectrophotometer

- Light Source: Xenon flash lamp
- Wavelength Range: 190–1100 nm
- Beam Type: Double
- Accuracy: ± 0.2 nm
- Reproducibility: < 0.025 nm
- Slit Width: 0.1–5.0 nm (adjustable)
- Detector: Silicon photodiode
- Cost: High

¹ **VWR International, LLC.** VWR V-1200 & UV-1600PC Spectrophotometers: Basic VIS or UV/VIS; Avantor. <https://www.avantorsciences.com/us/en/product/12243123/vwr-v-1200-uv-1600pc-spectrophotometers-basic-vis-or-uv-vis> (accessed July 23, 2025).

² **Labomed, Inc.** Spectro UV-2650 UV-VIS Scanning Spectrophotometer, Model UV-2650. <http://www.spectrophotometer.com/pdf/UV-2650.pdf> (accessed September 3, 2026).

³ **Agilent Technologies.** Cary 3500 Flexible UV-Vis Spectrophotometer Specifications; Publication No. 5994-5844EN; August 2023. <https://www.agilent.com/en/product/molecular-spectroscopy/uv-vis-uv-vis-nir-spectroscopy/uv-vis-uv-vis-nir-systems/cary-3500-flexible-uv-vis-spectrophotometer> (accessed September 3, 2026).

14. Based on the specifications of three spectrometers listed above, two of the spectrophotometers are single beam and one is a double beam spectrophotometer. Using Figures 1 and 2, explain what the difference is between the two types of spectrophotometers.

Single-beam uses one light path; you measure a blank, then the sample sequentially. It's simple and low-cost, but prone to drift and light-source fluctuations because there's no automatic reference path. Double-beam splits light into two simultaneous paths—one for the sample and one for the reference (blank). This design automatically compensates for lamp instability and improves accuracy and reproducibility, albeit with higher cost and complexity

15. Which instrument is least expensive? Suggest why.

The visible-only spectrophotometer is least expensive because it uses a single-beam design, a simple tungsten lamp, lower accuracy (± 2 nm), fixed slit width, and a basic silicon photodiode detector, all of which reduce cost by simplifying components and eliminating beam-splitting mechanics.

16. Which instrument is most expensive? Suggest why.

The Cary 3500 UV-Vis Spectrophotometer is most expensive because it features a double-beam design, high-precision optics (accuracy ± 0.2 nm, reproducibility < 0.025 nm), variable slit width, xenon flash lamp with long lifespan, and robust software, all of which add to manufacturing, maintenance, and performance costs.

17. Which instrument would you recommend for quantitative trace analysis? Why?

The Cary 3500 UV-Vis has a double-beam configuration, which offers excellent stability and drift compensation. The xenon flash lamp allows rapid, precise measurements even at very low light levels. The high wavelength accuracy and reproducibility make it ideal for detecting trace analytes with confidence.

18. In which real-world scenarios would it make more sense to use the cheaper visible-only spectrophotometer instead of the Cary 3500? Provide two examples and justify your choices in terms of cost, speed, sensitivity, and ease of use.

For routine applications in teaching laboratories or undergraduate settings, the visible-only spectrophotometer is often the smarter choice. Its low cost and simple single-beam design with fewer components and no split-beam optics make it both budget-friendly and easy to operate for students. Although this setup is more susceptible to drift than double-beam systems, regular blanking keeps measurements reliable, and its performance is more than adequate for most classroom experiments. Similarly, for standard colorimetric analyses such as nutrient testing in water samples or dye concentration assays, the visible-only instrument performs well. It provides sufficient sensitivity thanks to the high-energy throughput of its optical path and delivers fast, straightforward measurements. This is perfect for high-volume, lower-stakes testing where ultra-high precision isn't needed.

In both scenarios, the trade-off in accuracy and stability is outweighed by the advantages of affordability, simplicity, and speed, making the visible-only spectrophotometer a practical and effective tool for many routine lab applications.

Active Learning Worksheets for Analytical Chemistry

#18 Spectroscopic Methods: Beer's Law, Mixtures, and Deviations

Faculty Resources (Delete before sharing with students):

- **Analytical Context and Framing:** I frame this worksheet around the idea that Beer's Law is powerful, but only when the assumptions are reasonable. Students often come in thinking that if a spectrophotometer gives a number, the number must be useful. I emphasize that a straight calibration curve is not guaranteed, and part of analytical chemistry is knowing when the simple model has stopped working. Students should leave this worksheet understanding that concentration range matters because absorbance and percent transmittance determine whether the measurement is reliable.
- **Key Concepts and Common Challenges:** Students often think deviations from Beer's Law are just "bad data." Emphasize that deviations can come from real chemical behavior, instrumental limitations, stray light, polychromatic radiation, cuvette material, or experimental conditions. Students especially need to understand that very high absorbance values are not useful just because the instrument still displays a numerical reading. They may also need help recognizing that cuvette choice depends on wavelength range; quartz is needed for many UV measurements, but inexpensive plastic cuvettes can be appropriate for visible measurements if they transmit the wavelengths being used.
- **Facilitated Discussion Pauses:** Pause after the high-absorbance/low-transmittance question. Ask: "Why is an absorbance of 2.8 a problem even if it still gives a numerical reading?" This is an important place to reinforce that too little light reaching the detector makes the measurement unreliable. Question 3 may also need some discussion with individual groups because students can struggle to give a complete explanation of why deviations occur rather than simply saying the data are "wrong."
- **Calculation Support:** For the mixture problem, help students set up the two Beer's Law equations before solving. Remind them that the total absorbance at each wavelength is the sum of the absorbance contributions from each absorbing species. Some students will move through this calculation quickly, while others may need help seeing why measurements at two wavelengths allow them to solve for two unknown concentrations.
- **Synthesis and Extension Questions:** Ask students: "If your calibration curve bends at high concentration, would you dilute only the unknown or redesign the full standard range? What makes one choice more defensible than the other?" If time allows, this worksheet also pairs well with a quick discussion or demonstration of different cuvette materials. Showing students plastic, glass, and quartz cuvettes, or comparing transmission spectra for different cuvette materials, can help them understand why the cuvette has to be compatible with the wavelength range.
- **Pacing Note:** This worksheet builds on Worksheets #16–17 and is usually a quicker one-class activity. Students generally move through much of the conceptual work efficiently, but Questions 3–5 may need some instructor support. If time is limited, focus class discussion on Beer's Law deviations, high absorbance/low transmittance, and the concentration range needed for useful spectrophotometric data. The mixture calculation can be assigned as follow-up practice if needed.

Answer Keys for Active Learning Worksheets for Analytical Chemistry

#18 Spectroscopic Methods: Beer's Law, Mixtures, and Deviations

Before You Begin

- Read [10.1: Overview of Spectroscopy](#)
- Read [10.2: Spectroscopy Based on Absorption](#)
- Read [10.3: UV/Vis and IR Spectroscopy](#)

What You Should Already Know

- Beer's Law and its derivation
- Concept of molar absorptivity and λ_{max}
- Linear regression and use of calibration curves
- Basic algebra for solving simultaneous equations

What You'll Learn

- Define and interpret the variables in Beer's Law.
- Identify and explain deviations from linearity.
- Use absorbance data at multiple wavelengths to determine concentrations in a mixture.
- Select appropriate cuvette material based on wavelength requirements.

What You'll Practice

- Solve systems of equations derived from Beer's Law for multicomponent analysis.
- Analyze and troubleshoot real-world deviations from expected spectroscopic behavior.
- Interpret graphical representations of data (e.g., absorbance vs. concentration).
- Make evidence-based recommendations for experimental design (e.g., cuvette selection).

Estimated Completion Time: 45-60 minutes

Author: Rebecca L. Sanders

Active Learning Worksheets for Analytical Chemistry, Version 1.0, 2026

Useful Equations and Conversion Factors

- $A = \epsilon bc = -\log(T)$

Part I: Beer's Law Refresher and Limitations

1. Define the terms in the equation $A = \epsilon bc$ and describe how each impacts absorbance.

ϵ : Molar absorptivity constant, greater value increases A . However, this value is typically constant in an experiment

b : Pathlength. A longer pathlength increase absorbance. In most experiments, this value is also held constant.

c : Concentration. Absorbance increases with increasing concentration.

2. There are several reasons why a Beer's law might deviate from linearity (Figure 1). In which of the following cases is Beer's Law likely to fail?
 - a. Highly concentrated solutions
 - b. Polychromatic light
 - c. Reaction between solutes
 - d. Stray light reaches the detector
 - e. Temperature changes throughout the experiment
 - f. All of the above

Explain why each causes deviation:

All of the above

- a. Highly concentrated solutions: At high analyte concentrations, molecules do not act independently. Inter-particle interactions and changes in refractive index cause ϵ and b to vary, leading to fundamental deviations from linearity.***
- b. Polychromatic light: If the instrument passes a finite wavelength range (not strictly monochromatic light), ϵ may vary across that bandwidth, causing a negative deviation as absorbance is measured off the peak.***
- c. Reaction between solutes: For chemical systems in equilibrium (e.g., $HA \rightleftharpoons H^+ + A^-$), both species can absorb light differently causing a chemical deviation from linearity occurs.***
- d. Stray light reaches the detector: Light that bypasses the sample (stray light) adds to the detected signal, causing absorbance to be underestimated, especially at high concentrations, resulting in instrumental negative deviation.***
- e. Temperature changes throughout the experiment: Temperature shifts can change solution refractive index, solute equilibria, instrumental response, and ϵ , leading to deviations.***

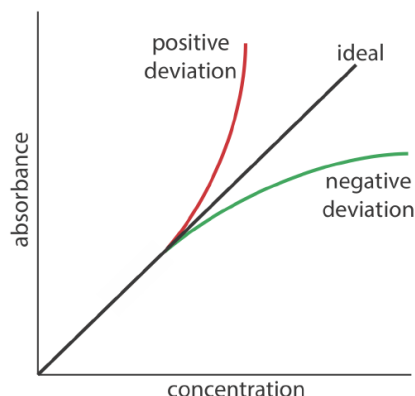


Figure 1. Plots of absorbance vs. concentration showing positive and negative deviations from the ideal Beer's law relationship, which is a straight line. From Figure 10.2.9 in David Harvey, *Analytical Chemistry 2.1*, licensed under CC BY-NC-SA 4.0.

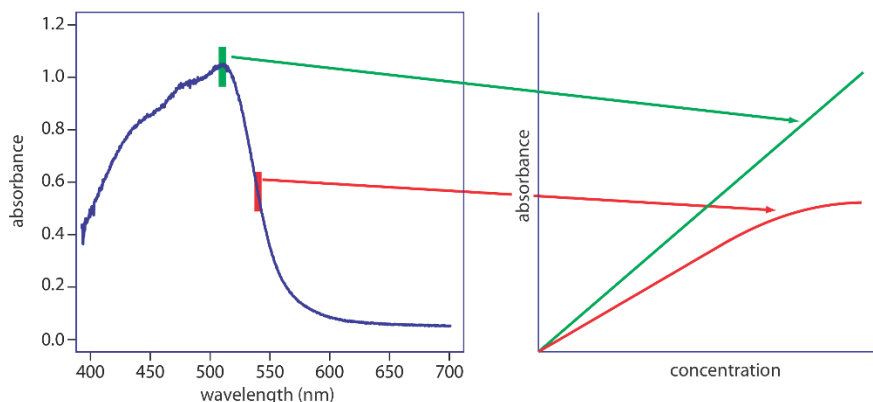


Figure 2. Effect of wavelength selection on the linearity of a Beer's law plot. From Figure 10.2.10 in David Harvey, *Analytical Chemistry 2.1*, licensed under CC BY-NC-SA 4.0.

3. Using Figure 2, explain why absorbance of a sample should be collected at its wavelength of maximum absorbance (λ_{max}).

Beer's law assumes that radiation reaching the sample is a purely monochromatic source of radiation. However, even the best wavelength selector passes radiation with a small, but finite effective bandwidth. Polychromatic radiation always gives a negative deviation from Beer's law. Therefore, it is better to make absorbance measurements at λ_{max} . Another reason for measuring absorbance at λ_{max} 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 in the Figure above.

4. A student prepares five standards, and her highest concentration gives an absorbance of 2.8.
 - a. Calculate the percent transmittance (%T) for this standard. Based on this value, why might the detector have trouble producing a reliable reading?

$$T = 10^{-A}$$

$$T = 10^{-2.8} = 0.0016$$

$$T * 100 = \%T$$

$$\%T = 0.0016 * 100 = 0.16\%$$

At this level, 99.84% of the light is blocked by the sample. Because so few photons reach the detector, even a tiny amount of stray light (light leaking into the instrument) will make the detector "think" the sample is more transparent than it actually is, causing a negative deviation from Beer's Law.

- b. As the designer of this experiment, do you: A) proceed with the measurement, B) dilute only the unknown, or C) redefine the concentration range of your standards? Justify your choice based on the relationship between stray light and %T.

The best choice is C) Redefine the concentration range of your standards. Choices A and B are scientifically indefensible because the calibration curve is already broken at the high end. If the highest standard is non-linear due to stray light, your slope is inaccurate, and every concentration you calculate, even for diluted unknowns, will be wrong. An analyst ensures the standards fall within the linear range to guarantee data integrity.

Part II: Calculation of Components in a Mixture

5. Molar absorptivities of compounds X and Y were measured with pure sample of each. The molar absorptivity constants for X were $16740\text{ M}^{-1}\text{cm}^{-1}$ and $3890\text{ M}^{-1}\text{cm}^{-1}$ at 230 and 273 nm, respectively. The molar absorptivity constants for Y were $3720\text{ M}^{-1}\text{cm}^{-1}$ and $7420\text{ M}^{-1}\text{cm}^{-1}$ at 230 and 273 nm, respectively.
- a. A mixture of X and Y in a 1.000 cm cell had an absorbance of $A_{230} = 0.957$ and $A_{273} = 0.559$. Find the concentrations of X and Y in the mixture.

Use two equations and two unknowns:

$$1. A_{230} = 0.957 = 16740C_X + 3720C_Y$$

$$2. A_{273} = 0.559 = 3890C_X + 7420C_Y$$

Rearrange equation 2: $C_X = \frac{0.559 - 7420C_Y}{3890}$

$$0.0001437 - 1.907C_Y = C_X$$

Plug C_X into equation 1

$$0.957 = 16740 * (0.0001437 - 1.907C_Y) + 3720C_Y$$

$$0.957 = 2.4055 - 31931C_Y + 3720C_Y$$

$$-1.4485 = -28211C_Y$$

$$C_Y = 5.13 * 10^{-5}\text{ M}$$

Plug C_Y value into equation 1 to solve for C_X

$$0.957 = 16740C_X + 3720 * 5.13 * 10^{-5}$$

$$0.957 = 16740C_X + 0.1910$$

$$0.7660 = 16740C_X$$

$$C_X = 4.58 * 10^{-5}\text{ M}$$

- b. The mixture measured in part a was a 1:200 dilution of an original sample. Calculate the original concentration of X and Y.

$$C_X = 4.58 * 10^{-5}\text{ M} * 200 = 9.16 * 10^{-3}\text{ M}$$

$$C_Y = 5.13 * 10^{-5}\text{ M} * 200 = 1.03 * 10^{-2}\text{ M}$$

Part III: Cuvette Materials and Instrument Limitations

Cuvettes can only transmit light at certain wavelengths. Outside of those wavelengths, the cuvette absorbs too much light and cannot be used for spectroscopic measurements. Below is a list of common cuvette types and their measurement wavelength ranges¹¹:

- Glass cell (G cell): 320-2500 nm
- Quartz cell (S cell): 190-2500 nm
- Disposable polystyrene (PS) cell: 340-750 nm
- Disposable polymethylmethacrylate (PMMA) cell: 285-750 nm

6. Examine the list of cuvettes.

- a. Which type of cuvette was likely used for the sample in Part II, which had λ_{max} values of 230 and 273 nm? Why?

Quartz is the only option that has a reasonable transmittance of light at 230 nm. The rest of the cuvettes absorb most of the light at 230 nm.

- b. Which cuvette(s) would be acceptable for visible measurements? Which is most likely to be used? Why?

Any of them can be used. However, plastic cuvettes tend to be much less expensive and not easily broken, so are more likely to be used.

- c. Your friend is conducting an experiment at 254 nm. They are making a set of standards and are confident they made their dilutions correctly, but the absorbance values they collected aren't making sense. They are measuring transmittance using disposable PS cells. Explain what is going wrong with their experiment.

Disposable PS cells absorb most light at 254 nm, so there is no light reaching the detector. They should switch to quartz cuvettes, which transmit most light at 254 nm.

¹¹ Shimadzu Scientific Instruments. What Is the Light Transmittance of Glass, Quartz, and Plastic Cuvettes? <https://www.ssi.shimadzu.com/service-support/faq/uv-vis/cuvettes/3/index.html> (accessed July 23, 2025).

Active Learning Worksheets for Analytical Chemistry

#19 Spectroscopic Methods: Atomic Spectroscopy Part 1: Fundamentals and Flame AA Instrumentation

Faculty Resources (Delete before sharing with students):

- **Analytical Context and Framing:** I frame this worksheet as preparation for using atomic absorption spectroscopy in lab. Students may have used UV-visible spectroscopy before, but atomic spectroscopy requires a different way of thinking about the analyte and the instrument. For metals, the sample has to be converted into free atoms before the measurement makes sense, so sample preparation, sample introduction, atomization, wavelength selection, and calibration all matter. I emphasize that the goal is not just to understand the instrument diagram, but to make better decisions when preparing stock solutions, standards, and samples for AA analysis.
- **Key Concepts and Common Challenges:** Students may assume atomic and molecular absorption spectra look similar because both involve absorbance. They are often more comfortable with broad molecular spectra than with narrow atomic lines, so Question 2 may need extra discussion. It can help to connect back to the Jablonski diagram and remind students that molecules have electronic, vibrational, and rotational contributions, while free atoms have much more discrete electronic transitions. Students may also need help understanding how samples are introduced into the instrument and why sample introduction affects the kind of sample preparation needed.
- **Facilitated Discussion Pauses:** Pause after students compare the flame AA and UV/Vis schematics. Ask: “Which parts of these instruments are doing similar jobs, and which parts are fundamentally different because flame AA measures atoms instead of molecules?” This is a useful place to talk through how the sample gets into the flame and why the flame is not just a heat source, but part of the measurement process because it produces free atoms. If students have not used AA before, spend a few minutes connecting the schematic to what they will physically do in lab. I also show them the instrument to have them connect the flow schematic in Figure 3 to a real-world instrument.
- **Calculation Support:** When students interpret detection limits, linear ranges, sensitivity, and wavelength-selection information, remind them to connect the numbers back to the analytical goal. Students may not be familiar with sensitivity as an analytical term, so Question 9 may need some explicit discussion. A wavelength with strong sensitivity may not always be the best choice if interferences or concentration range create problems. Question 13 can be treated as optional if time is limited, but it is a useful way to revisit dilution planning and connect the worksheet to preparing stock solutions, standards, and samples for an AA experiment.
- **Synthesis and Extension Questions:** Ask students: “If Lucia is measuring iron in tap water, what decisions need to be made before she ever runs the sample?” Have them consider sample preparation, how the sample enters the instrument, lamp choice, wavelength, flame conditions, calibration range, and whether dilution is needed. This helps students see that AA analysis starts before the instrument is turned on.
- **Pacing Note:** This worksheet will likely fit within one class period, but it can run long if students spend significant time interpreting instrument manual information or planning dilutions. If time is limited, prioritize the atomic vs. molecular spectra comparison, the UV/Vis versus AA instrument comparison, sample introduction, and wavelength/sensitivity decisions. The dilution-planning question can be assigned as follow-up work or skipped if students will get similar practice while preparing standards and samples in lab.

Answer Keys for Active Learning Worksheets for Analytical Chemistry

#19 Spectroscopic Methods: Atomic Spectroscopy Part 1: Fundamentals and Flame AA Instrumentation

Before You Begin

- Read [4.7: Detection Limits](#)
- Read [10.1: Overview of Spectroscopy](#)
- Read [10.2: Spectroscopy Based on Absorption](#)
- Read [10.3: UV/Vis Spectroscopy](#)
- Read [10.4: Atomic Absorption Spectroscopy](#)

What You Should Already Know

- Basic concepts of electromagnetic radiation and absorbance
- The difference between atomic and molecular species
- General components of a spectrophotometer (light source, sample holder, detector)
- Concepts like detection limit, linear range, and calibration

What You'll Learn

- Differentiate between atomic and molecular absorption spectra
- Label and explain the function of key components in a flame atomic absorption spectrometer
- Compare a flame AA spectrometer with a typical UV/Vis instrument
- Describe how samples are introduced into flame AA and what sample prep may be required
- Interpret instrumental parameters (e.g., light source, slit width, wavelength selection) for Fe analysis

What You'll Practice

- Use visual figures and schematics to interpret instrument design
- Compare experimental setups across different spectroscopic methods
- Evaluate detection limits and linear ranges based on manufacturer data
- Make evidence-based decisions about wavelength selection and sample preparation

Estimated Completion Time: 60-70 minutes

Author: Rebecca L. Sanders

Active Learning Worksheets for Analytical Chemistry, Version 1.0, 2026

Atomic Spectroscopy Compared to Molecular Spectroscopy

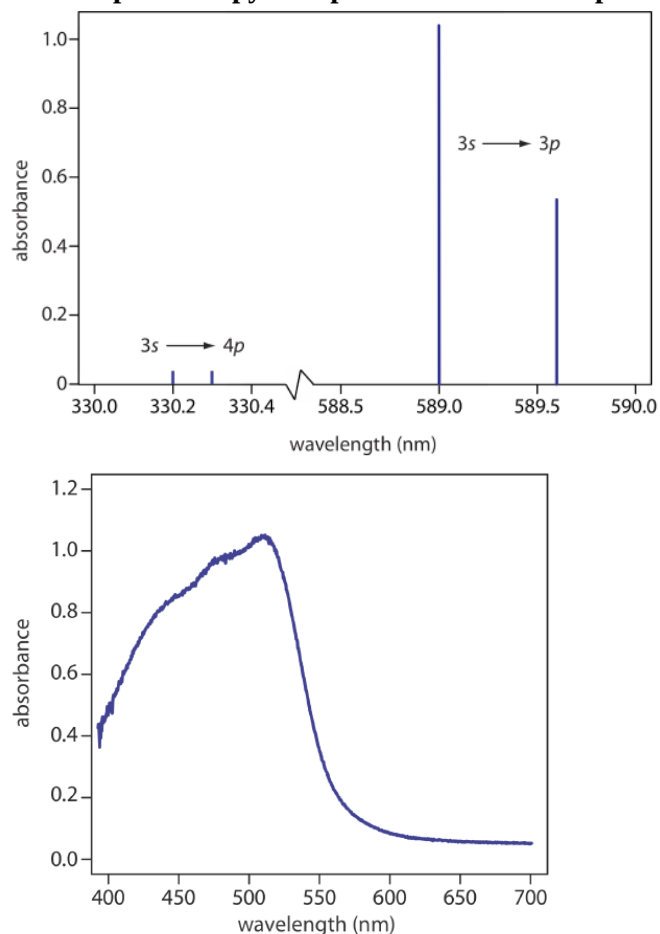


Figure 1. Atomic absorption spectrum for sodium. Note that the scale on the x-axis includes a break. From Figure 10.2.6 in David Harvey, *Analytical Chemistry 2.1*, licensed under CC BY-NC-SA 4.0.

spectra.

Figure 2. UV/Vis spectrum for the metal-ligand complex $Fe(phen)_3^{2+}$, where phen is the ligand o-phenanthroline. From Figure 10.2.4 in David Harvey, *Analytical Chemistry 2.1*, licensed under CC BY-NC-SA 4.0.

- The figures above represent an atomic spectrum of sodium (left) and a molecular spectrum of the metal-ligand complex $Fe(phen)_3^{2+}$ (right). Fill out the table below to compare and contrast these

	Atomic Spectrum	Molecular Spectrum
Graph's y-axis	<i>Absorbance</i>	<i>Absorbance</i>
Graph's x-axis	<i>Wavelength (nm)</i>	<i>Wavelength (nm)</i>
Types of light (UV, Vis, IR, etc)?	<i>Typically visible or UV</i>	<i>UV or visible</i>

	Atomic Spectrum	Molecular Spectrum
Width of absorbance peaks	<i>Very narrow so sharp lines produced</i>	<i>Broad bands</i>

2. Why is there a difference in peak widths between atomic spectra and molecular spectra?

Atomic transitions involve only electronic energy levels, which are discrete and not influenced by bonding or molecular motion. This results in very sharp, narrow absorption lines. Molecular spectra involve not only electronic transitions but also vibrational and rotational energy levels. These additional transitions broaden the peaks, producing wider bands.

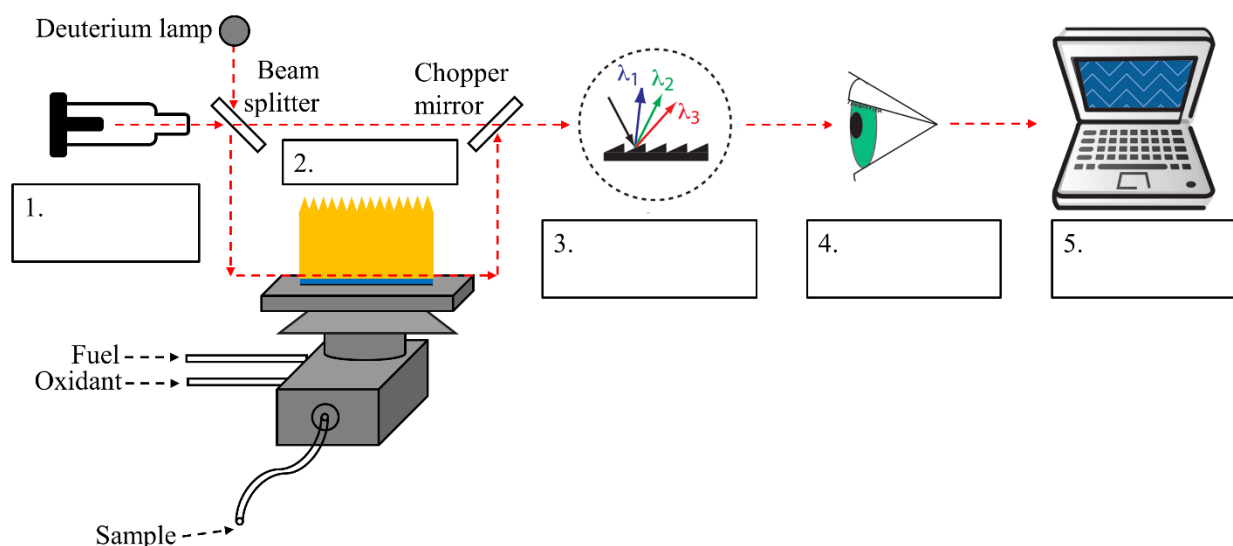


Figure 3. Flame atomic absorption spectrometer schematic. Adapted in part from Figure 10.3.2 in David Harvey, *Analytical Chemistry 2.1*, licensed under CC BY-NC-SA 4.0. Additional components and labels were added for this worksheet.

3. Label the five numbered components on the flame AA spectrometer schematic above.

1 – hollow cathode lamp; 2 – flame; 3 – monochromator; 4 – detector; 5 – signal processor

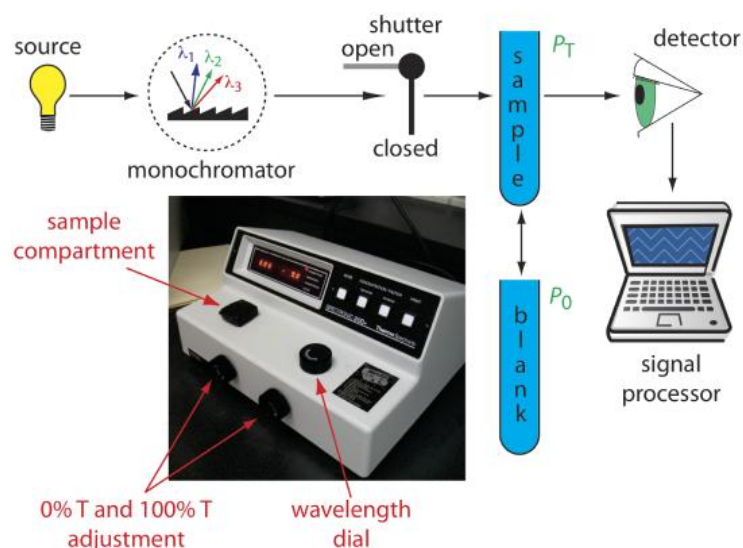


Figure 4. Schematic diagram of a fixed-wavelength, single-beam spectrophotometer. From Figure 10.3.2 in David Harvey, *Analytical Chemistry 2.1*, licensed under CC BY-NC-SA 4.0.

4. Consider the schematic for a flame AA compared to the schematic for a UV/Vis spectrophotometer. What's similar? What's different?

Both use light sources, monochromators, and detectors and both measure absorbance.

Flame AA uses a hollow cathode lamp and flame atomizer. UV/Vis uses broadband light and does not atomize samples. Flame AA is element-specific; UV/Vis can analyze broader molecules.

5. How are samples introduced into these instruments?

Flame AA uses a liquid sample that is nebulized into a fine mist and aspirated into the flame. UV/Vis uses a liquid sample that is placed in a cuvette.

Sample Considerations and Experimental Parameters for Atomic Spectroscopy

6. What types of sample preparation steps are generally required for atomic spectroscopy techniques such as flame AA or ICP-AES?

There are several possible steps necessary to measure concentrations with flame AA or ICP-AES. For example, solids might need to be digested with acid (e.g. nitric acid). Filtration or centrifugation might be needed to remove particulates. Dilution may be needed to reach a measurable concentration range. Acidification may be necessary to stabilize metals in solution.

Lucia wants to determine the amount of iron in tap water using flame atomic absorption spectroscopy. In her lab, she has access to an Agilent Technologies hollow cathode lamps¹² and a flame atomic absorption spectrometer. She uses the manual for the instrument to set up her experiment.

¹² Agilent Technologies. *Hollow Cathode Lamp Operating Conditions*; Agilent Technologies. https://www.agilent.com/Library/posters/Public/31-725_Hollow_Cathode_Lamp_Operating_Conditions.pdf (accessed 2026-05-11).

7. The flame is listed as air- C_2H_2 . What purpose does the air serve? What is C_2H_2 ? What is it used for and why not another chemical?

Air is the oxidant and supplies oxygen for combustion. C_2H_2 is acetylene, the fuel. An air-acetylene flame provides a suitable temperature and stable atomization environment for Fe. Changing the fuel or oxidant would change flame temperature and atomization efficiency, so the method is optimized for this flame.

8. To look at Fe in a sample with flame AA spectroscopy, what kind of light source is needed? What would the cathode be made of?

A hollow cathode lamp specific to iron (Fe) is required. The cathode would be made of elemental iron, producing element-specific emission lines.

9. The handbook for the instrument states there are three possible wavelengths that can be used for Fe. They are at 248.3, 372.0, and 386.0 nm, with sensitivity ratios of 15%, 100%, and 50%, respectively. Which wavelength would be the best to start with when designing the experiment? Why?

372.0 nm is best to use because it has the highest sensitivity and would be used unless there's interference at that wavelength.

10. The suggested slit width for the experiment is 0.2 nm. Is this a small or large slit width? How does slit width affect resolution and signal-to-noise ratio? Why might that slit width be suggested for a flame AA spectroscopy experiment?

0.2 nm is small (narrow). Signal-to-noise ratio decreases with narrower slits due to reduced light intensity. Narrow slits improve resolution because there is better separation of close spectral lines. However, narrow slits reduce signal intensity. A narrow slit width is chosen for flame AA to isolate narrow atomic lines and reduce interferences.

11. For the experiment, D_2 background correction is suggested. Why is a D_2 lamp used for background correction?

The deuterium lamp corrects for background absorbance from the flame, solvent, or matrix, providing a continuum of UV light. The system subtracts background signal from the atomic absorption signal.

12. An AA handbook suggested a calibration curve using 0, 0.5, 1.0, 1.5, and 2 ppm Fe standards. The linear range of concentrations is fairly small (0-2 ppm). Why is this a challenge for flame AA experiments?

A narrow linear range (0–2 ppm) means that absorbance no longer increases proportionally with concentration beyond this range. This makes it easier to exceed the linear range, which can lead to inaccurate results. It also requires careful preparation of standards to ensure that all measurements fall within the range where the instrument response is reliable.

13. Lucia wants to make a similar calibration curve using 0, 0.5, 1.0, 1.5, and 2 ppm Fe standards. The manual suggests starting with a 1000. mg Fe/L standard that can be prepared by measuring 1.000 g of pure iron (99.9%). Then, it is heated and dissolved with 20 mL of aqua regia, which is a 1:3 molar ratio of nitric acid to hydrochloric acid. Once cooled, the solution is diluted to 1.000 L accurately. Using the 1000. ppm Fe stock solution, propose how Lucia could make a set of standards for her experiment. Each standard, including the blank, should be prepared with the same acid concentration/matrix as the samples when possible.

There are many options to make a set of standards. One option would be to first prepare 100.0 mL of a 10 ppm intermediate stock solution by diluting 1.00 mL of 1000 ppm stock to 100.0 mL. Then a series of standards could be made with the 10 ppm intermediate stock solution. For example:

<i>Final Concentration (ppm)</i>	<i>Volume of 10 ppm stock (mL)</i>	<i>Volume of concentrated nitric acid (mL)</i>	<i>Final Volume (mL)</i>
<i>0.5</i>	<i>5.00</i>	<i>1.00</i>	<i>100.0</i>
<i>1.0</i>	<i>10.00</i>	<i>1.00</i>	<i>100.0</i>
<i>1.5</i>	<i>15.00</i>	<i>1.00</i>	<i>100.0</i>
<i>2.0</i>	<i>20.00</i>	<i>1.00</i>	<i>100.0</i>

Lucia prepared the tap water sample by diluting 1.00 mL of original sample with some DI water. Then, she added 1.00 mL concentrated nitric acid and filled to the line in a 100.0 mL volumetric flask with DI water. The diluted tap water's absorbance was measured, resulting in diluted concentration measured as 0.08 ppm Fe.

14. The handbook listed the limit of detection (LOD) of Fe as 0.06 ppm and the limit of quantification (LOQ) for Fe as 0.2 ppm. LOD and LOQ are determined as the standard deviation of the absorbance of several blank solution measurements times three or ten, respectively (e.g., $LOD = 3\sigma$ and $LOQ = 10\sigma$). Lucia measured a diluted sample of tap water and calculated the diluted sample contained 0.08 ppm Fe. Is that measurement accurate? Why or why not?

The measurement of 0.08 ppm Fe is detectable but not accurate or reliably quantifiable. It falls above the limit of detection ($LOD = 0.06$ ppm), meaning the instrument can distinguish it from background noise, but it is below the limit of quantification ($LOQ = 0.2$ ppm), which is the lowest concentration that can be measured with acceptable precision and accuracy. Since it does not meet the minimum threshold for quantitative confidence, the result suggests Fe is present but cannot be reported as an accurate concentration.

15. Since she only measured a concentration of 0.08 ppm Fe from this dilution, propose another way she could prepare the tap water sample to give a more accurate reading?

The 1:100 dilution gave 0.08 ppm, so the original sample was about 8 ppm Fe. A 1:10 dilution would give about 0.8 ppm, and a 1:5 dilution would give about 1.6 ppm, both within the 0–2 ppm calibration range and above the LOQ.

Active Learning Worksheets for Analytical Chemistry

#20 Spectroscopic Methods: Atomic Spectroscopy Part 2: Interferences, Calibration, and Sensitivity

Faculty Resources (Delete before sharing with students):

- **Analytical Context and Framing:** I frame this worksheet around the idea that atomic spectroscopy works only when several practical conditions are met. The instrument does not measure the analyte in isolation; the sample matrix, interferences, atomization conditions, detection limits, and instrument choice all affect the quality of the result. I tell students that good analytical chemists need to recognize when the sample or method is causing problems and choose a strategy to deal with those constraints.
- **Key Concepts and Common Challenges:** Students should recognize that different atomic spectroscopy techniques solve different analytical problems. Flame AA, furnace AA, and ICP-AES differ in sensitivity, cost, sample throughput, number of elements measured, and tolerance for complex matrices. I usually stop to ask not which technique is best, but which technique fits the sample, concentration range, matrix, budget, and number of elements being measured. If students have also encountered ICP-MS, this is a good place to briefly point out that it is another important elemental analysis technique, especially for trace and ultratrace concentrations. I would not dwell on the instrument details here unless ICP-MS is part of your course emphasis; the main goal is for students to recognize that very low elemental concentrations may require a more sensitive and more expensive technique than flame AA or ICP-AES.
- **Facilitated Discussion Pauses:** Pause after the standard addition questions. Ask: “Why does adding known amounts of standard directly to the sample help when the sample matrix is difficult to reproduce?” This is a useful place to reinforce that standard addition is not just another calibration option; it is a strategy for dealing with matrix effects. Later, when comparing atomic spectroscopy techniques, ask students what they would need to know before choosing an instrument: concentration range, number of samples, number of elements, available budget, detection limits, and whether the sample matrix is likely to interfere.
- **Calculation Support:** For standard addition calculations, remind students that the x-intercept is used to determine the original analyte concentration, but dilution factors and sample volumes still matter. Encourage them to write what each volume represents before substituting into equations. Question 14 may require substantial support. Students often struggle with converting energy values to joules per atom or per photon and with deciding what values to use in the Boltzmann calculations. If time is limited, the detailed calculations can be shortened, but students should still compare the ratios in part D so they understand why absorbance methods depend mostly on ground-state atoms, while emission methods are much more sensitive to temperature.
- **Synthesis and Extension Questions:** Ask students: “If your lab could use flame AA, furnace AA, or ICP-AES for this analysis, what information would you need before deciding which method is worth using?” Have them consider detection limits, sample volume, number of samples, number of elements, cost, throughput, and whether the sample matrix is likely to cause problems. If your department has any of these instruments, students often enjoy discussing approximate cost, maintenance, and practical throughput. This helps them see that instrument choice is not just about analytical performance, but also about resources and scale.
- **Pacing Note:** This worksheet works best after students have completed or reviewed Worksheet #19, which introduces atomic spectra and flame atomic absorption instrumentation. It can be completed in one longer class period or split across two shorter class periods. A natural stopping point is after Question 6, after students have worked through interferences and standard addition. The remaining sections shift to comparing atomic spectroscopy techniques and interpreting temperature effects using the Boltzmann distribution. If short on time, prioritize the interference/standard addition sections and the qualitative comparison of atomic spectroscopy

techniques. The Boltzmann calculation can be reduced or assigned as follow-up, but the conceptual comparison between absorbance and emission should still be discussed.

Answer Keys for Active Learning Worksheets for Analytical Chemistry

#20 Spectroscopic Methods: Atomic Spectroscopy Part 2: Interferences, Calibration, and Sensitivity

Before You Begin

- Read [5.2: Calibrating the Signal](#)
- Read [5.3: Determining the Sensitivity](#)
- Read [5.4: Linear Regression and Calibration Curves](#)
- Read [10.4: Atomic Absorption Spectroscopy](#)
- Read [10.5: Emission Spectroscopy](#)
- Read [10.7: Atomic Emission Spectroscopy](#)

What You Should Already Know

- The basic operating principles of atomic absorption spectroscopy
- How to use calibration data to determine unknown concentrations
- What detection limits and interferences are, conceptually

What You'll Learn

- Identify and describe common interferences in flame AA and how to mitigate them
- Explain how and when to use the method of standard additions
- Compare flame AA, furnace AA, and ICP-AES in terms of cost, sensitivity, and throughput
- Describe how temperature affects atom populations using the Boltzmann distribution
- Calculate analyte concentrations from standard additions or calibration data

What You'll Practice

- Analyze real or simulated calibration data to extract meaningful quantitative results
- Apply correction strategies for chemical and spectral interferences
- Justify selection of atomic spectroscopy techniques based on experimental context
- Perform multi-step calculations using logarithms, calibration equations, and statistical tools

Estimated Completion Time: 60-90 minutes

Author: Rebecca L. Sanders, *Active Learning Worksheets for Analytical Chemistry*, Version 1.0, 2026

Useful Equations and Conversion Factors

- $E = h\nu$
- $h = 6.626 \times 10^{-34} \text{ J} \cdot \text{s}$
- $c = \lambda\nu = 2.998 \times 10^8 \text{ m/s}$
- $\frac{S_{\text{samp}}}{C_{A\frac{V_o}{V_f}}} = \frac{S_{\text{spike}}}{C_{A\frac{V_o}{V_f}} + C_{\text{std}} \frac{V_{\text{std}}}{V_f}}$
- $\frac{N^*}{N} = \left(\frac{g^*}{g_0}\right) e^{-E_i/kT}$
- $k = 1.381 \times 10^{-23} \text{ J/K}$

Interferences

A flame AA handbook¹³ gives information about each element that can be analyzed. For each remark, explain the type of interference and the purpose behind the suggested fix.

1. Cesium: Addition of ~0.1-0.5% Na, K or Rb as ionization suppressant is recommended.

Ionization interference. Ionized atoms (e.g. Cs^+) and neutral atoms (e.g. Cs) do not give the same analytical signal. In this case, adding an easily ionized element suppresses ionization of the analyte and shifts the equilibrium toward neutral Cs atoms, which produce the atomic absorption signal.

2. Magnesium: Addition of ~0.1-0.5% $LaCl_3$ or $SrCl_2$ would prevent chemical interference by Al or Si or phosphoric acid.

If chemicals like Al, Si, or H_3PO_4 are present, they can react with Mg, which would reduce the concentration of the analyte. Lanthanum or strontium acts as a releasing agent by preferentially reacting with phosphate or other interferents, leaving more Mg available for atomization.

3. Zinc: Coexistence of several thousand ppm Fe would give spectral interference.

Fe and Zn have characteristic wavelengths that may overlap when Fe is present at a high concentration. If the sample contains a lot of Fe, another wavelength for Zn should be selected.

Method of Standard Addition

4. What is the method of standard addition and why is it necessary?

In the method of standard addition, a calibration curve is prepared by adding known amounts of standard directly to aliquots of the unknown sample. This is typically used when a sample has a complex sample matrix that is not easily replicated when making a standard curve.

¹³ Agilent Technologies. *Flame Atomic Absorption Spectroscopy: Method Development ePrimer*; Agilent Technologies: Santa Clara, CA, 2023. <https://www.agilent.com/cs/library/brochures/AA-5991-8145EN-methods-primer.pdf> (accessed 2026-06-08).

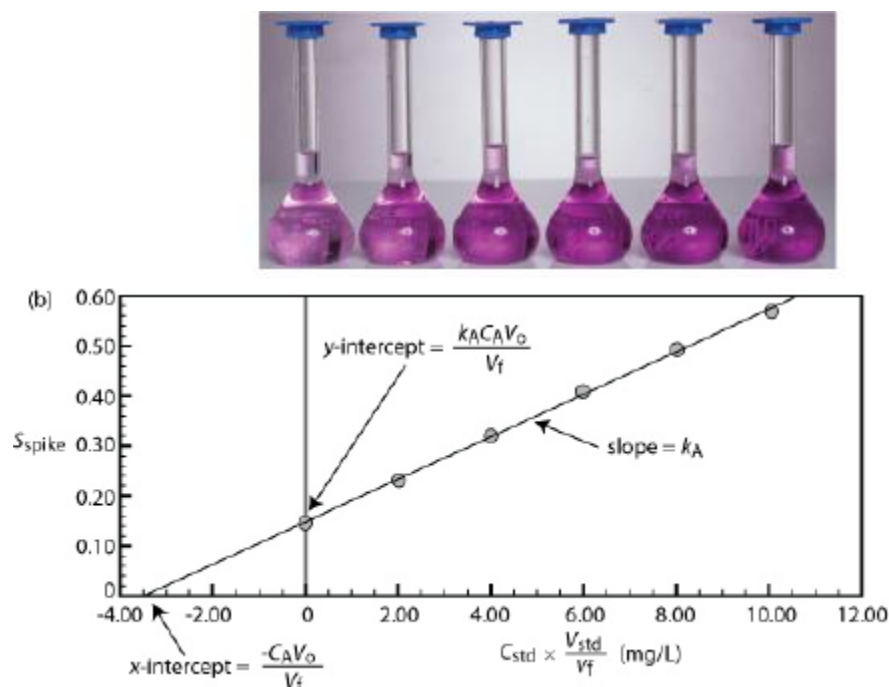


Figure 1. Shown at the top of the figure is a set of six standard additions for the determination of Mn^{2+} . The absorbance for each standard addition, S_{spike} , is shown by the filled circles. From Figure 5.3.6 in David Harvey, *Analytical Chemistry 2.1*, licensed under CC BY-NC-SA 4.0.

5. Figure 1 shows a standard addition calibration curve for the quantitative analysis of Mn^{2+} . Each solution contains 5.00 mL of the original sample and either 0, 1.00, 2.00, 3.00, 4.00, or 5.00 mL of a 50.0 mg/L external standard of Mn^{2+} . All standard addition samples were diluted to 25.0 mL with water before reading the absorbance. The equation for the calibration curve is $y = 0.0425x + 0.1478$. What is the concentration of Mn^{2+} in this sample? Problem adapted from Exercise 5.3.3 in David Harvey, *Analytical Chemistry 2.1*, Section 5.3, licensed under CC BY-NC-SA 4.0.

$$y = 0.0425x + 0.1478$$

$$0 = 0.0425x + 0.1478$$

$$x = -3.47 \text{ mg/L} \quad \text{Drop the negative sign}$$

$$3.47 \text{ mg/L} \times \frac{25.0 \text{ mL}}{5.00 \text{ mL}} = 17.4 \text{ mg/L } Mn^{2+} \text{ in the unknown}$$

6. A spectrophotometric method for the quantitative analysis of Pb^{2+} in blood yields an S_{samp} of 0.136 when a 1.00 mL sample of blood is diluted to 10.00 mL. A second 1.00 mL sample of blood is spiked with 0.500 mL of a 250.0 ppb Pb^{2+} external standard and diluted to 10.00 mL, yielding an S_{spike} of 0.320. What is the concentration of Pb^{2+} in the original sample of blood?

$$\text{Hint: Use } \frac{S_{\text{samp}}}{C_A \frac{V_o}{V_f}} = \frac{S_{\text{spike}}}{C_A \frac{V_o}{V_f} + C_{\text{std}} \frac{V_{\text{std}}}{V_f}}$$

$$\frac{0.136}{C_A \frac{1.00 \text{ mL}}{10.00 \text{ mL}}} = \frac{0.320}{C_A \frac{1.00 \text{ mL}}{10.00 \text{ mL}} + (250.0 \text{ ppb}) \frac{0.500 \text{ mL}}{10.00 \text{ mL}}}$$

$$\frac{0.136}{0.100C_A} = \frac{0.320}{0.100C_A + 12.5}$$

$$0.136(0.100C_A + 12.5) = 0.320(0.100C_A)$$

$$0.0136C_A + 1.7 = 0.0320C_A$$

$$1.7 = 0.0184C_A$$

$$C_A = 92.4 \text{ ppb Pb}^{2+}$$

Comparing Different Atomic Spectroscopy Techniques

Table 1. Features of flame and furnace atomic absorption (AA) spectroscopy and inductively coupled plasma atomic emission spectroscopy (ICP-AES).

Feature	Flame AA	Furnace AA	ICP-AES
Scale of Operation	Trace to major analytes; macro/meso samples; microsampling possible	Trace to ultratrace; very small sample volumes (micro/ultramicro)	Trace to ultratrace analytes; macro/meso samples
Accuracy	0.5–5% if interferences are minimized	Typically worse than flame AA due to stronger matrix effects	1–5% if interferences are minimal; stray radiation from overlapping lines can reduce accuracy
Precision (RSD)	0.3–1% for absorbance > 0.1–0.2	1–5%; affected by sample injection consistency	1–5%; affected by plasma/flame temperature stability; improved with internal standards
Sensitivity	Moderate; depends on flame conditions and matrix	Very high; optimized by temperature and time control in drying/ashing stages	High; less matrix-dependent than AA; optimized by plasma settings
Detection Limits	Moderate (trace level)	Very low (ultratrace level detection)	Low, but not as low as furnace AA
Dynamic Range / Flexibility	Moderate range; sample often diluted	Narrow range; optimized for trace analysis	Wide range; can handle trace to major analytes with dilution
Selectivity	Excellent due to narrow absorption lines	Excellent; same as flame AA	Good, but may suffer from spectral overlap due to many emission lines
Sample Throughput	Fast (250–350 samples/hour with automation)	Slower (20–30 samples/hour)	Very high (up to 3000/hour with multichannel ICP; ~300/hour for sequential)
Instrument Cost	\$10,000–\$50,000	\$18,000–\$70,000	\$55,000–\$300,000 depending on configuration; argon gas adds to operating cost

7. Which technique is the most sensitive?

Furnace AA: It provides the highest sensitivity and the lowest detection limits, making it ideal for ultratrace analysis.

8. Which technique is the least expensive? Most expensive?

Least expensive: Flame AA

Most expensive: ICP-AES — especially due to equipment cost and argon gas consumption.

9. Which technique can detect the smallest concentrations of analyte?

Furnace AA: It has the lowest detection limits.

Limitation: Matrix interferences and variability in sample injection can reduce accuracy and precision.

10. Which technique is most appropriate for measuring analytes present at a wide range of concentrations?

ICP-AES: It has the widest dynamic range and can handle both trace and major analytes, especially with dilution.

11. Which technique allows for the fastest sample throughput?

ICP-AES: Up to 3000 determinations/hour with multichannel instruments.

12. Which technique can measure the most elements at once?

ICP-AES: It allows simultaneous multielement detection using optical emission from plasma and multichannel detectors.

13. The table notes that ICP-AES can suffer from overlapping emission lines. Why might ICP-AES be more prone to spectral interference than atomic absorption techniques?

Because plasma produces many emission lines from multiple elements, overlapping signals (stray radiation) are more likely. In contrast, AA uses narrow absorption lines and a single-element lamp for each analysis, minimizing overlap.

Note: Another important elemental analysis technique is inductively coupled plasma mass spectrometry, or ICP-MS. ICP-MS also uses a plasma, but instead of measuring light emitted from excited atoms and ions, it uses a mass spectrometer to separate ions by mass-to-charge ratio. ICP-MS is especially useful for measuring trace or ultratrace elements at very low concentrations, but it is typically more expensive and more complex than the atomic spectroscopy methods discussed here.

Effects of Temperature on Atomic Spectroscopy

Temperature determines how much of a sample breaks down and how much of the atom is found in the ground, excited, or ionized states. If you consider two states E_0 and three at E^* , the number of states at each energy is called degeneracy (g_0 and g^* in this case). They are separated by E_i , the energy of the excited state relative to the ground state energy, E_0 . The Boltzmann distribution describes the relative populations of different states at thermal equilibrium is defined as: $\frac{N^*}{N} = \left(\frac{g^*}{g_0}\right)e^{-E_i/kT}$

where $\frac{N^*}{N}$ is the relative population at any two states, T is temperature in K, k is the Boltzmann constant ($1.381 \times 10^{-23} \text{ J/K}$), and e is base of the natural log (2.718...).

14. Consider an element that reaches its first excited state by absorption of 310.8 nm light.

a. Determine the energy difference (J/atom) between the ground state and the first excited state.

$$\begin{aligned} 310.8 \text{ nm} * \frac{1 \text{ m}}{10^9 \text{ nm}} &= 3.108 * 10^{-7} \text{ m} \\ c = \lambda \nu &= 2.998 * 10^8 \text{ m/s} \\ E = h\nu &= \frac{hc}{\lambda} = \frac{(6.626 * 10^{-34} \text{ J}\cdot\text{s}) * (2.998 * 10^8 \text{ m/s})}{3.108 * 10^{-7} \text{ m}} \\ E &= 6.391 * 10^{-19} \text{ J/atom} \end{aligned}$$

b. If the degeneracies of the two states for the element are $\frac{g^*}{g_0} = 2$, determine $\frac{N^*}{N}$ at 2010 K.

$$\frac{N^*}{N} = \left(\frac{g^*}{g_0}\right)e^{-\frac{E_i}{kT}} = (2)e^{-\frac{6.391 \times 10^{-19}}{(1.381 \times 10^{-23})(2010)}} = 2.00 \times 10^{-10}$$

c. By what percentage does $\frac{N^*}{N}$ change if the temperature is raised by 50 K?

$$\frac{N^*}{N} = \left(\frac{g^*}{g_0}\right)e^{-\frac{E_i}{kT}} = (2)e^{-\frac{6.391 \times 10^{-19}}{(1.381 \times 10^{-23})(2060)}} = 3.50 \times 10^{-10}$$

$$\frac{3.50 \times 10^{-10} - 2.00 \times 10^{-10}}{2.00 \times 10^{-10}} * 100 = 74.9\% \text{ increase}$$

Answers can vary based on if students rounded throughout the calculations.

- d. Based on the $\frac{N^*}{N}$ values you calculated, are more atoms in the ground state or the excited state?

More atoms are in the ground state because the $\frac{N^*}{N}$ ratio is much less than 1. The ratio shows for every atom in the excited state, there are billions of atoms in the ground state.

- e. The excited-state population increased by about 75% when the temperature increased by 50 K. Explain if this a large change in overall population distribution between the ground and excited states or not.

The 50 K temperature increase causes a large relative increase (~75%) in the excited-state population, but the overall population distribution does not change very much because the vast majority of atoms remain in the ground state. Even at 2060 K, there are billions of atoms in the ground state for every one atom in the excited state.

- f. How does this population distribution affect the signals measured in atomic absorption, atomic emission, and ICP-MS?

Most atoms are in the ground state, so atomic absorption signals are often strong because absorption depends on ground-state atoms absorbing photons. Atomic emission signals depend on atoms or ions reaching excited states and then emitting light as they relax, so emission intensity is more sensitive to temperature. ICP-MS does not measure emitted light, but plasma conditions still matter because the plasma controls how efficiently atoms are converted into ions for mass-spectrometric detection.

Active Learning Worksheets for Analytical Chemistry

#21 External Calibration, Standard Addition, and Uncertainty Calculations in Standard Curves

Faculty Resources (Delete before sharing with students):

- **Analytical Context and Framing:** I use this worksheet to give students practice working with a larger calibration data set and comparing external calibration with standard addition. The drinking water scenario helps emphasize why calibration choice matters. Two methods can both give precise-looking numbers, but if the sample matrix changes the signal, the more precise method may not be the more accurate one. I also use this worksheet to prepare students for analyzing their own lab data, where they need to calculate a result, estimate uncertainty, and decide how much confidence they have in the method.
- **Key Concepts and Common Challenges:** Students often equate smaller uncertainty with a better result. Emphasize that external calibration may give smaller uncertainty because the standards are cleaner and the procedure is simpler, while standard addition may be more appropriate when matrix effects are important. The specific uncertainty values in this worksheet should be treated as examples from this data set, not as universal rules about which method always has better precision or accuracy. Students should focus on using the data to decide what the results suggest in this particular case.
- **Facilitated Discussion Pauses:** Pause after students compare the external calibration and standard addition slopes. Ask: “If the slopes are different, what does that tell us about how the sample matrix affects the analytical signal?” This is a good place to reinforce that standard addition is not just a more complicated calibration curve; it is designed to account for matrix effects by adding standard directly to the sample.
- **Calculation Support:** Students may need support using LINEST and interpreting the Excel output. If using the provided template, have students open the template at the start and walk them through which cells they should change and which cells contain formulas. Students can often enter the standard concentrations and absorbances correctly, but may need help identifying the slope, intercept, standard deviation about the regression, and unknown absorbance before using the uncertainty equation. If students need to re-enter the LINEST calculation, remind them that they may need to clear the full output range before entering the function again. The final dilution step is a common sticking point: students need to keep the diluted concentration and original concentration separate and then use the dilution factor to convert back to the original sample concentration and uncertainty.
- **Synthesis and Extension Questions:** Ask students: “If you were reporting this result to someone concerned about drinking water quality, which method would you trust more, and how would you explain the trade-off between precision, accuracy, and matrix effects?” A useful follow-up is: “Based on these results, what would you change if you were designing the next experiment?” This helps students move from carrying out the calculations to making decisions about calibration strategy, dilution, matrix effects, and experimental design.
- **Pacing Note:** This activity works best after students have completed or reviewed Worksheet #20 and earlier calibration-curve material. It can vary substantially in length depending on students’ comfort with Excel, LINEST, dilution calculations, and uncertainty propagation. If the main goal is conceptual comparison, the instructor can provide some of the regression and uncertainty values and focus class time on interpretation. If the goal is lab preparation, students should work through the provided template themselves so they know how to analyze their own data later. If time is short, or if students will not be using this type of uncertainty calculation in lab, this worksheet can be skipped more easily than some of the core concept-building worksheets. A natural stopping point is after Question 4, after the external calibration calculation. The standard addition section can be completed in a second class period or assigned as follow-up work. Another option is to complete both calculations in class and assign the comparison and interpretation questions as a written reflection.

Answer Keys for Active Learning Worksheets for Analytical Chemistry

#21 External Calibration, Standard Addition, and Uncertainty Calculations in Standard Curves

Before You Begin

- Read [5.4: Linear Regression and Calibration Curves](#)

What You Should Already Know

- Explain the purpose of a calibration curve in quantitative analysis
- Distinguish between external calibration and standard addition
- Interpret a linear relationship in the context of analytical data
- Perform dilution calculations and apply dilution factors
- Calculate averages and recognize sources of experimental variability
- Use Excel to create plots and perform basic data analysis

What You'll Learn

- Construct calibration curves using linear regression
- Use LINEST to obtain slope, intercept, and uncertainties
- Determine the concentration of an unknown using both calibration methods
- Calculate the uncertainty in concentration from a calibration curve
- Explain how matrix effects influence analytical results
- Compare the accuracy and precision of external calibration and standard addition

What You'll Practice

- Use Excel to perform least-squares regression
- Analyze replicate measurements to obtain mean values
- Carry uncertainty through multi-step calculations
- Clearly organize and present calculations and results
- Interpret data and justify conclusions using chemical reasoning

Estimated Completion Time: 50-75 minutes

Author: Rebecca L. Sanders

Active Learning Worksheets for Analytical Chemistry, Version 1.0, 2026

Background

You are analyzing Pb^{2+} in a drinking water sample collected from an older home. The sample may contain high levels of Ca^{2+}/Mg^{2+} (hardness), dissolved solids, and trace organics. These components can alter the analytical signal in flame atomic absorption (AA), leading to matrix effects. Two calibration approaches are used: external calibration (standards in DI water) and standard addition (standards added directly to the sample). Your goal is to determine the Pb^{2+} concentration using both methods and evaluate which result is more reliable. You can use Microsoft Excel to analyze this data. A template Excel file has been provided with a file name of 21_LINEST_uncertainty_template.xlsx.

Part I. External Calibration

A set of Pb^{2+} standards was prepared and then 10.00 mL aliquots of original water sample were diluted to 20.00 mL. Absorbance data is listed below.

	Pb^{2+} (ppb)	Absorbance
Standards	0.0	0.001
	5.0	0.113
	10.0	0.222
	15.0	0.334
	20.0	0.454
Unknowns	1:2 dilution of unknown 1	0.287
	1:2 dilution of unknown 2	0.294
	1:2 dilution of unknown 3	0.290

- Plot the standard curve of absorbance as a function of concentration.
- Using Excel, use the LINEST function to perform a linear regression of absorbance vs. concentration. Report:
 - slope (m): **0.02254**
 - intercept (b): **-0.0006**
 - standard deviation about the regression (s_y): **0.003554**
 - calculate the mean absorbance of the diluted unknown samples
- Determine the concentration of Pb^{2+} in the diluted unknown. Then use the following equation to find the uncertainty in the diluted concentration: **12.9 ± 0.1 ppb**

$$s_x = \frac{s_y}{|m|} \sqrt{\frac{1}{k} + \frac{1}{n} + \frac{(y - \bar{y})^2}{m^2 \sum_{i=1}^n (x_i - \bar{x})^2}}$$

- s_y = the standard deviation of y
 - m = absolute value of the slope of the least-squares line
 - K = number of replicates
 - n = number of data points
 - y = average unknown y measurement
 - $\bar{y}$ = the mean value of y for the standards
 - x_i = the individual values of x for the standards
 - $\bar{x}$ = the mean value of x for the standards
- Now determine the original concentration of Pb^{2+} and its uncertainty in ppb: **25.8 ± 0.2 ppb**

Part II. Standard Addition

A second set of Pb^{2+} standards was prepared using the method of standard addition with each containing 10.00 mL aliquots of original water sample. All samples were diluted to 20.00 mL. Absorbance data is listed below.

	Added Pb^{2+} (ppb)	Absorbance
Standards	0.0	0.210
	5.0	0.300
	10.0	0.388
	15.0	0.475
	20.0	0.558

- Plot the standard curve of absorbance as a function of added concentration.
- Using Excel, use the LINEST function to perform a linear regression of absorbance vs. concentration. Report:
 - slope (m): **0.01742**
 - intercept (b): **0.212**
 - standard deviation about the regression (s_y): **0.002359**
- Determine the concentration of Pb^{2+} in the diluted unknown. Then use the following equation to find the uncertainty in the diluted concentration: **12.2 ± 0.2 ppb**

$$s_x = \frac{s_y}{|m|} \sqrt{\frac{1}{n} + \frac{\bar{y}^2}{m^2 \sum_{i=1}^n (x_i - \bar{x})^2}}$$

- s_y = the standard deviation of y
 - m = slope of the least-squares line
 - n = number of data points
 - $\bar{y}$ = the mean value of y for the samples run
 - x_i = the individual values of x for the samples run
 - $\bar{x}$ = the mean value of x for the samples run
- Now determine the original concentration of Pb^{2+} and its uncertainty in ppb: **24.3 ± 0.4 ppb**

Part III. Comparison and Interpretation

- Compare your results:

External Calibration: **25.8 ± 0.2 ppb**

Standard Addition: **24.3 ± 0.4 ppb**

Which method gives a higher concentration?

External calibration

10. Compare uncertainties: Which method has the smaller uncertainty? Why?

The external calibration produced a smaller uncertainty because the regression had lower scatter and the sample preparation is simpler. Standard addition introduces error from additional volumetric operations and incorporates matrix variability into the calibration, both of which can increase uncertainty.

11. Matrix Effects: Compare the slopes from Parts I and II. Are they the same or different? What does this indicate about matrix effects in this sample?

The slopes are not the same. The external calibration method has a slope of 0.0225 ± 0.0002 and the standard addition method has a slope of 0.01742 ± 0.00015 . The matrix of the unknown changes the slope of the Beer's Law relationship.

12. Accuracy vs. Precision: Which method is more precise? Which method is more accurate for this sample? Why?

The external calibration method is more precise because it has the lower percent relative standard deviation. Given the information provided at this point in the worksheet, there is no way to know which technique is more accurate. However, because standard addition tends to incorporate matrix effects, it is expected to give a more accurate result.

Part IV. Evaluating Accuracy

A second water sample from the same home is analyzed. The true Pb^{2+} concentration (determined by ICP-MS) is 24.4 ppb. ICP-MS is often used as a highly sensitive reference technique for trace elemental analysis.

13. Calculate percent error for each method. Which method produced a value closer to the true concentration?

The percent errors for the external calibration and standard addition methods were 5.7% and 0.4%, respectively. The standard addition method produced a value closer to the true value.

14. If you were analyzing a new unknown sample from this same source, which method would you choose? Provide a justification that includes matrix effects, uncertainty, and accuracy.

For a new unknown, standard addition would likely be preferred even if it has a larger uncertainty. The different slopes between the two methods suggest significant matrix effects, meaning external calibration may produce results with systematic error. Although external calibration is more precise, standard addition produced a result closer to the true concentration and is therefore more accurate for this sample matrix.

15. Final Reflection. Support or refute this statement: External calibration is more precise because it uses cleaner standards and fewer experimental steps, which minimizes random error, but it can be less accurate if the sample matrix affects the signal.

For this data set, this statement is supported.

Active Learning Worksheets for Analytical Chemistry

#22 Electrochemical Methods: Electrochemistry Basics

Faculty Resources (Delete before sharing with students):

- **Analytical Context and Framing:** Frame electrochemistry as a way to connect electron transfer, thermodynamics, and measurable electrical work. This worksheet focuses on the electrochemistry foundations students need before considering analytical electrochemical techniques. Later in the course, these ideas can be connected to methods such as potentiometry, electrogravimetric analysis, amperometry, and voltammetry, where measured potential, current, or mass change provides chemical information. Tell students: “If electrons are moving, we can often measure that movement and use it to learn something quantitative about a chemical system.”
- **Key Concepts and Common Challenges:** Students often struggle to connect oxidation/reduction language with balanced half-reactions, cell notation, and electrochemical calculations. Emphasize that oxidation occurs at the anode, reduction occurs at the cathode, and the line notation should reflect the physical organization of the cell. Students may also need substantial review on assigning oxidation numbers, identifying what is oxidized and reduced, splitting a reaction into half-reactions, and balancing under acidic or basic conditions.
- **Facilitated Discussion Pauses:** Pause during or after Question 1 if students seem rusty. It may be worth working through that question as a class, especially if it has been a long time since students have balanced redox reactions. Talk through the strategy: assign oxidation numbers, identify the oxidation and reduction processes, split the reaction into half-reactions, balance atoms and charge, and then combine the half-reactions. Later, after the first line-notation problem, ask: “How do we know which species belongs on the anode side and which belongs on the cathode side? What information does the line notation give us that a balanced equation alone does not?”
- **Calculation Support:** For the E_{cell}° , ΔG° , and K calculations, encourage students to identify n before substituting into equations. Students often use the correct equation but the wrong number of electrons, especially when the half-reactions must be multiplied to balance the overall reaction. For Faraday’s Law calculations, remind students to connect charge, moles of electrons, and moles of product rather than treating the equation as a plug-in formula.
- **Synthesis and Extension Questions:** Ask students: “If you were designing an electroplating experiment, what would you need to control to make the actual plated mass match the theoretical mass as closely as possible?” This helps students connect the review calculations to a measurable analytical or experimental outcome.
- **Pacing Note:** This worksheet can move quickly if students have a strong and recent general chemistry background, but it can also take much longer if redox balancing and cell notation feel unfamiliar. If students are rusty, it is helpful to work through Question 1 together before having them continue in groups. This worksheet contains a lot of review, but students need the grounding before moving into later electrochemistry topics. If time is limited, complete the redox balancing and cell notation questions during class, then assign the Faraday’s Law and cell potential/equilibrium questions as follow-up work. If splitting across two class periods, a natural stopping point is after Question 4.

Answer Keys for Active Learning Worksheets for Analytical Chemistry

#23 Electrochemical Methods: Electrochemistry Basics

Before You Begin

- Review balancing redox reactions in acid and base, cell diagrams and line notation, and Nernst equation and Faraday's laws: [17.1 Review of Redox Chemistry](#), [17.2 Galvanic Cells](#), [17.3 Electrode and Cell Potentials](#), [17.4 Potential, Free Energy, and Equilibrium](#)

What You Should Already Know

- Assign oxidation states to atoms in compounds
- Balance simple redox reactions
- Use logarithms and basic algebra
- Understand the role of electrons in redox reactions

What You'll Learn

- Identify oxidation and reduction components of a redox reaction
- Balance redox reactions in both acidic and basic media
- Use standard reduction potentials to determine spontaneity
- Write line notation for galvanic cells
- Calculate Gibbs free energy and equilibrium constants from electrochemical data
- Use Faraday's Law to calculate plating mass and efficiency

What You'll Practice

- Setting up and balancing redox half-reactions
- Interpreting and constructing line notation
- Applying thermodynamic and electrochemical equations
- Sketching and annotating electrochemical cells
- Analyzing data from electroplating experiments

Estimated Completion Time: 60-80 minutes

Author: Rebecca L. Sanders, *Active Learning Worksheets for Analytical Chemistry*, Version 1.0, 2026

Select Standard Reaction Potentials from [Appendix 16.13: Standard Reduction Potentials](#).

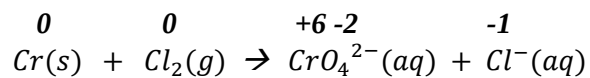
- $\text{BrO}_3^-(aq) + 6\text{H}^+(aq) + 6e^- \rightarrow \text{Br}^-(aq) + 3\text{H}_2\text{O}(l)$ 1.478 V
- $\text{Cr}_2\text{O}_7^{2-}(aq) + 14\text{H}^+(aq) + 6e^- \rightarrow 2\text{Cr}^{3+}(aq) + 7\text{H}_2\text{O}(l)$ 1.36 V
- $\text{I}_2(s) + 2e^- \rightarrow 2\text{I}^-(aq)$ 0.5355 V
- $\text{Cu}^{2+}(aq) + 2e^- \rightarrow \text{Cu}(s)$ 0.3419 V

Useful Equations

- $E_{\text{cell}}^{\circ} = \frac{RT}{nF} \ln K$
- At 25 °C, $E_{\text{cell}} = E_{\text{cell}}^{\circ} - \frac{0.0592}{n} \log Q$
- At 25 °C, $E_{\text{cell}}^{\circ} = \frac{0.0592}{n} \log K$
- $E_{\text{cell}}^{\circ} = E_{\text{cathode}}^{\circ} - E_{\text{anode}}^{\circ}$
- $R = 8.314 \frac{\text{J}}{\text{mol} \cdot \text{K}}$
- $F = 96,485 \frac{\text{C}}{\text{mol } e^-}$
- $1 \text{ A} = 1 \frac{\text{C}}{\text{s}}$

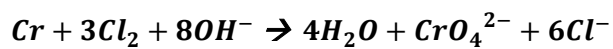
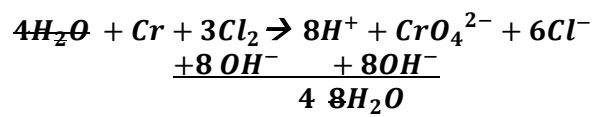
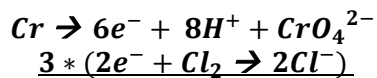
Part 1: Balancing Redox Reactions and Identifying Oxidation–Reduction Processes

- Determine the oxidation state of each element in the following reaction and then balance it in a base. What is being oxidized? Reduced? What is the oxidizing agent? Reducing agent?

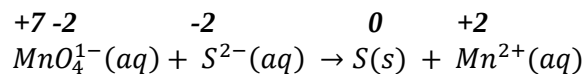


Cr(s): Oxidized, reducing agent

Cl₂(g): Reduced, oxidizing agent

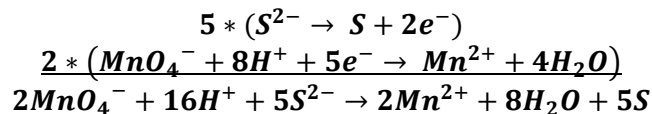


2. Determine the oxidation state of each element in the following reaction and then balance it in an acid. What is being oxidized? Reduced? What is the oxidizing agent? Reducing agent?



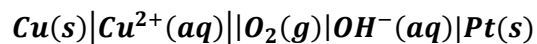
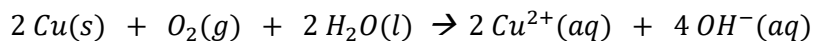
S²⁻: Oxidized, reducing agent

MnO₄⁻: Reduced, oxidizing agent

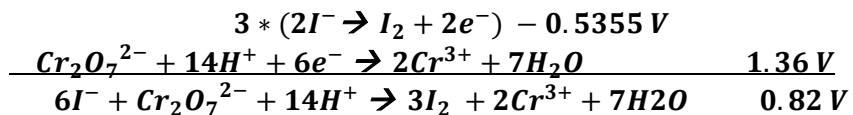
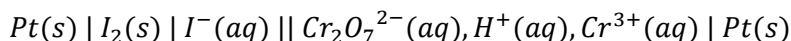


Part 2: Cell Notation and Electrochemical Reactions

3. Write the line notation for the following reaction.



4. Using the line notation below, derive a balanced redox reaction in acid. Determine E_{cell}° for the reaction and list if the reaction is spontaneous.



Spontaneous

Part 3: Electroplating and Faraday's Law

5. A current of 4.77 A is passed through a $SnSO_4$ solution for 2.00 h.
- Theoretically, how much tin is plated out of the solution? The atomic mass of Sn is 118.71 g/mol.

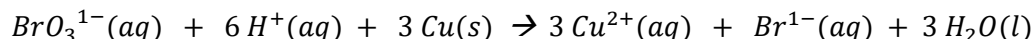
$$2.00 \text{ h} * \frac{3600 \text{ s}}{1 \text{ h}} * \frac{4.77 \text{ C}}{1 \text{ s}} * \frac{1 \text{ mol } e^{-}}{96485 \text{ C}} * \frac{1 \text{ mol Sn}}{2 \text{ mol } e^{-}} * \frac{118.71 \text{ g Sn}}{1 \text{ mol Sn}} = 21.1 \text{ g Sn}$$

- If 19.0 g of Sn was plated, what was the efficiency of plating in the experiment?

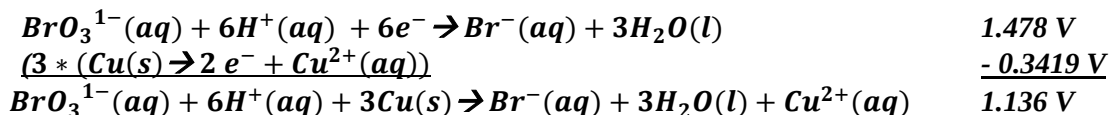
$$\frac{19.0 \text{ g Sn}}{21.1 \text{ g Sn}} * 100 = 90.0\% \text{ efficiency}$$

Part 4: Cell Potentials, Equilibrium, and Cell Design

6. A voltaic cell is based on the reaction



a. Calculate E°_{cell} .



b. Calculate K for the reaction at 25 °C.

$$E^\circ_{\text{cell}} = \frac{0.0592}{n} \log K$$

$$1.136 \text{ V} = \frac{0.0592}{6} \log K$$

$$115.1 = \log K$$

$$10^{115.1} = K$$

$$K = 1.259 * 10^{115}$$

c. If $[\text{Cu}^{2+}] = 0.100 \text{ M}$, $[\text{BrO}_3^{1-}] = 0.600 \text{ M}$, $[\text{H}^+] = 0.800 \text{ M}$, and $[\text{Br}^{1-}] = 0.0500 \text{ M}$, what is E_{cell} ?

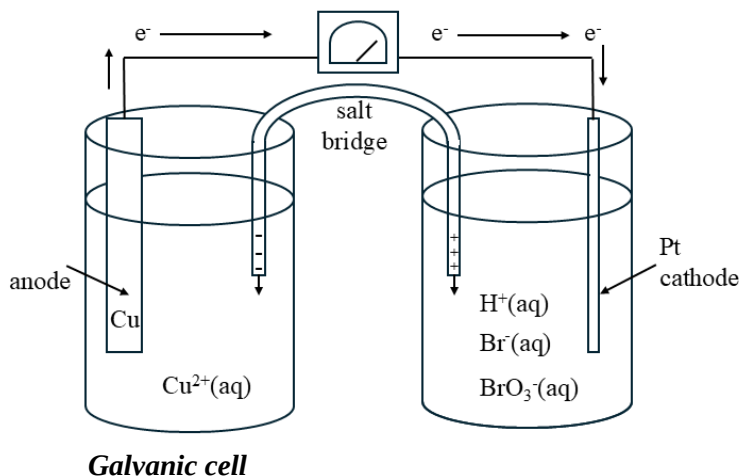
$$E_{\text{cell}} = E^\circ_{\text{cell}} - \frac{0.0592}{n} \log Q$$

$$E_{\text{cell}} = 1.136 \text{ V} - \frac{0.0592}{n} \log \left(\frac{[\text{Br}^-][\text{Cu}^{2+}]^3}{[\text{BrO}_3^-][\text{H}^+]^6} \right)$$

$$E_{\text{cell}} = 1.136 \text{ V} - \frac{0.0592}{6} \log \left(\frac{[0.0500][0.100]^3}{[0.600][0.800]^6} \right)$$

$$E_{\text{cell}} = 1.171 \text{ V}$$

d. Sketch the cell of the reaction listed above, and label the anode and cathode, the direction of electron flow in the external circuit, and the direction of flow of cations and anions through the salt bridge. Is it an electrolytic or galvanic cell?



Active Learning Worksheets for Analytical Chemistry

#23 Electrochemical Methods: Exploring Ion-Selective Electrodes

Faculty Resources (Delete before sharing with students):

- **Analytical Context and Framing:** I start with the pH electrode as the familiar example. Most students have used a pH probe many times, but they may not have thought carefully about what part of the probe is actually sensing the ion or why the electrode has to be stored and maintained in a particular way. I frame this worksheet as a chance to understand the chemistry behind an instrument they already know and then extend that thinking to other ion-selective electrodes. As students compare different ISEs, I want them to ask what stays the same, what changes, and how the electrode is designed to respond selectively to one ion.
- **Key Concepts and Common Challenges:** Students often think the entire electrode is “selective,” rather than recognizing that selectivity comes mainly from the sensing membrane. Emphasize that many ISEs share similar structural features, but the membrane chemistry determines the target ion. Students may also need help seeing why ISEs are useful for ions that are difficult to measure by other methods. Atomic spectroscopy works well for many metals, but it is not appropriate for every ion of interest; molecular spectroscopy may require a color-forming reaction; and some ions are not easily measured using redox-based techniques. An ISE can be useful when the target ion can be measured directly under appropriate conditions.
- **Facilitated Discussion Pauses:** Pause after students compare the pH, chloride, and calcium electrodes. Ask: “What parts stayed the same across the electrodes, and what changed? What does that tell us about how an ISE is designed?” If ion-selective electrodes are available, bring them in so students can see and handle them. Having students handle the probes makes the diagrams more concrete, especially for students who have only used a pH electrode without thinking about its internal structure. This is also a good place to connect electrode structure to practical issues such as storage, hydration, selectivity, and junction potentials.
- **Calculation Support:** Although this worksheet is mostly conceptual, students may need help interpreting the logarithmic relationship between potential and ion activity. Connect this back to the Nernst equation and remind students that ISE response is linear with the logarithm of activity, not concentration directly. This helps explain why ISEs can be useful over a wide concentration range. It can also be helpful to compare this large linear range with the narrower working ranges students have seen in UV-visible and atomic spectroscopy.
- **Synthesis and Extension Questions:** Ask students: “If you had to choose between an ISE and flame AA for a water-quality measurement, what would you consider?” Have them think about the target ion, detection limits, sample matrix, speed, cost, calibration, linear range, and possible interferences. This worksheet can also serve as early preparation for the later instrument-selection synthesis activity, where students compare techniques based on what the sample is, what the analyte is, and what practical constraints matter.
- **Pacing Note:** This activity works best after students have completed or reviewed Worksheet #22 or have otherwise reviewed basic electrochemical cells and electrode potentials. The worksheet is somewhat long, but several figures and tables take up space, so it may not take as long as the page count suggests. If time is limited, complete the pH electrode and ISE structure questions during class, then assign the performance-specification and technique-comparison questions as follow-up work. Sections can also be cut or shortened if the goal is only to introduce ISE structure and practical use rather than explore all of the performance details.

Answer Keys for Active Learning Worksheets for Analytical Chemistry

#23 Electrochemical Methods: Exploring Ion-selective Electrodes

Before You Begin

- Read [11.2: Potentiometric Methods](#)

What You Should Already Know

- The concept of electrochemical cells and how potentials are measured
- The role of reference electrodes (e.g., Ag/AgCl)
- Basic understanding of equilibrium and concentration gradients
- The relationship between ion charge, mobility, and diffusion
- General acid–base chemistry, including the definition and measurement of pH

What You'll Learn

- Explain how junction potentials arise and how they impact electrochemical measurements
- Describe the structure and function of a pH electrode, including the role of the glass membrane
- Explain how ion-selective electrodes (ISEs) generate a measurable potential
- Interpret instrument specifications (e.g., concentration range, pH range, precision) for ISEs
- Identify and explain the impact of interfering ions on ISE measurements
- Compare different electrochemical methods (e.g., ISE vs. flame AA) for real-world analysis

What You'll Practice

- Interpreting tables and figures to extract chemical meaning
- Applying conceptual understanding to predict electrochemical behavior
- Evaluating instrument performance and limitations
- Making evidence-based comparisons between analytical techniques
- Connecting instrument design to function and measurement outcomes

Estimated Completion Time: 55-70 minutes

Author: Rebecca L. Sanders

Active Learning Worksheets for Analytical Chemistry, Version 1.0, 2026

pH electrode

A combination pH electrode (Figure 1) contains two electrochemical cells housed within a single probe. One serves as a reference electrode with a stable potential, while the other contains a glass membrane that responds selectively to H^+ . A porous wick serves as a salt bridge between the sample and its reference electrode. The meter measures the potential difference between these two electrodes and relates that potential to pH. Use Figure 1 to connect each physical part of the probe to its role in the measurement.

1. What parts of this probe act as reference electrodes?

The Ag/AgCl electrodes act as the reference electrodes. One Ag/AgCl electrode is inside the pH-sensitive glass electrode, and the other Ag/AgCl reference electrode is in the sample-side reference compartment. The glass membrane is not a reference electrode; it is the sensing/indicator membrane.

2. What part responds to H^+ ?

The pH-sensitive glass membrane responds to H^+ . The hydrated surface of the glass membrane exchanges H^+ with the solution, creating a potential that depends on the difference in H^+ activity between the internal solution and the sample solution.

3. Why does the electrode contain an internal HCl solution with a fixed H^+ activity?

The internal HCl solution provides a constant, known H^+ activity on the inside of the glass membrane. Because the internal H^+ activity is fixed, changes in the measured potential mainly reflect changes in the H^+ activity of the sample solution.

4. Why do we calibrate with buffers?

Calibration with buffers relates the measured potential to known pH values. Calibration corrects for the actual electrode slope and intercept, which may differ from ideal behavior because of temperature, electrode aging, junction potentials, and nonideal solution behavior. Buffers provide stable, known pH values for this calibration.

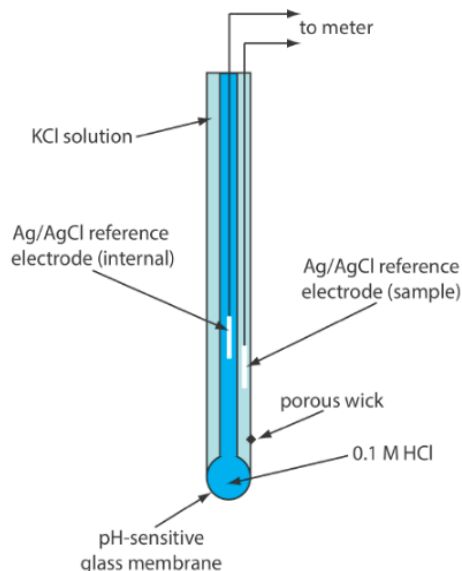


Figure 1. Schematic diagram showing a combination glass electrode for measuring pH. From Figure 11.2.10 in David Harvey, *Analytical Chemistry* 2.1, licensed under CC BY-NC-SA 4.0.

5. How must the probe be stored? Why?

The probe should be stored in aqueous solution (usually KCl or storage solution). Hydration is required for proper ion exchange at the membrane and for stable, reproducible potentials.

6. The typical range a pH probe can measure is from pH 0 to 14. Why is this range sufficient for most aqueous acid–base systems?

Most aqueous systems fall within this range due to autoionization of water and practical limits of strong acid/base concentrations. Extreme pH values are rare and difficult to measure reliably.

Ion-Selective Electrodes

7. A pH electrode responds selectively to H^+ because of its membrane. If you wanted to build an electrode that measured a different ion, such as chloride or calcium, which component of the electrode would most likely need to change? Explain your reasoning.

The sensing membrane would need to change. The membrane determines which ion the electrode responds to. Other parts of the electrode, such as the reference electrode, internal solution, and meter, may still be present, but the membrane chemistry must be selective for the target ion.

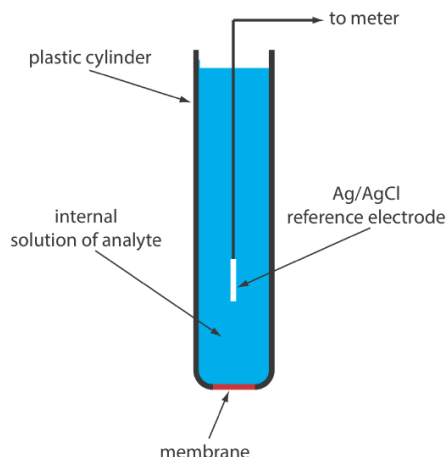


Figure 2. Schematic diagram of a solid-state electrode for ions such as Cl^- . The internal solution contains a solution of analyte of fixed activity. From Figure 11.2.11 in David Harvey, *Analytical Chemistry 2.1*, licensed under CC BY-NC-SA 4.0.

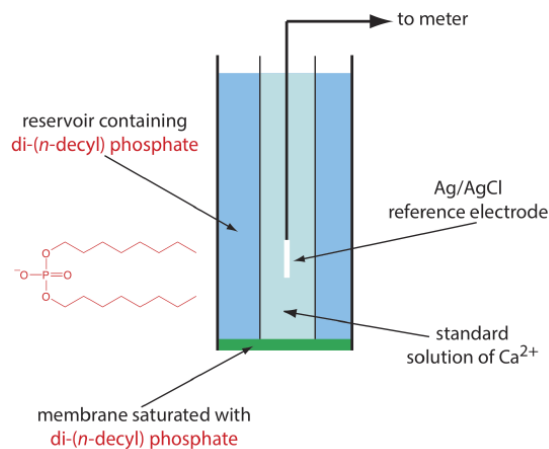


Figure 3. Schematic diagram showing a liquid-based ion-selective electrode for Ca^{2+} . The structure of the cation exchanger, di-(*n*-decyl) phosphate, is shown in red. From Figure 11.2.12 in David Harvey, *Analytical Chemistry 2.1*, licensed under CC BY-NC-SA 4.0.

8. Compare Figures 1, 2, and 3. Each shows a different ion-selective electrode.
- Do all three ISEs have reference electrodes?

Yes. All three include a reference electrode made of Ag/AgCl.

- Do all three electrodes contain an internal solution?

Yes. Each electrode contains an internal solution of known composition or fixed analyte activity.

- What type of membrane is used in each ISE?

The pH electrode uses a pH-sensitive glass membrane. The chloride electrode uses a solid-state membrane, such as an inorganic salt membrane. The calcium electrode uses a liquid-based membrane containing an ion exchanger.

- What ion does each electrode measure?

The pH electrode measures H^+ activity. The solid-state electrode shown is an example of an electrode for ions such as Cl^- . The liquid-based electrode shown measures Ca^{2+} .

- Does your answer to Question 7 appear to be supported by these examples? Explain.

Yes. The electrodes have similar overall structures, including reference electrodes, internal solutions, and meters, but they use different membranes. The different membranes correspond to different target ions, supporting the idea that membrane chemistry determines ion selectivity.

9. Some ISEs are designed to measure monatomic ions (e.g. H^+ , Ca^{2+} , K^+ , and Cl^-) while other ion-selective electrodes can measure polyatomic ions (NH_4^+ and NO_3^-). Why might it be especially useful to have ISEs that can measure polyatomic ions (e.g., NH_4^+ , NO_3^-) in environmental or biological systems?

Polyatomic ions like ammonium and nitrate are common in environmental systems (water quality, agriculture) and important in biological systems (nitrogen cycle, metabolism).

Direct measurement avoids conversion steps or indirect methods and can be faster and more practical. Methods like flame AA spectroscopy are not able to quantify some elements like nitrogen.

10. Based on Figures 1–3, what would need to be different about a nitrate ISE compared with a pH electrode? What parts would likely remain the same?

A nitrate ISE would need a membrane that responds selectively to NO_3^- instead of H^+ . The internal solution would also likely contain nitrate at a fixed activity. The general design would remain similar: a sensing membrane, a reference electrode, an internal solution, and a meter that measures the potential difference.

Ion-Selective Electrodes and Concentration

The potential measured by an ISE changes predictably with the logarithm of ion concentration. An example of this relationship is plotted in Figure 4.

11. Is the electrode potential linear with concentration or linear with the natural log of concentration?

The electrode potential is linear with the natural log of concentration, or more precisely with the logarithm of ion activity.

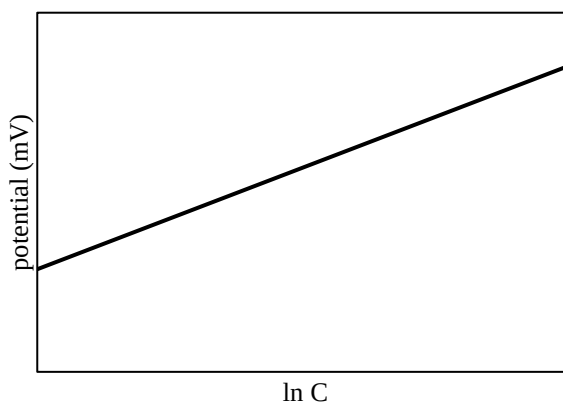


Figure 4. The electrode response of an ISE is represented by the Nernst equation with potential plotted as a function of natural log of concentration.

12. Why is a logarithmic response advantageous when measuring concentrations that span several orders of magnitude?

A logarithmic response allows a very wide range of concentrations to be represented with a manageable range of potential values. Each tenfold change in concentration produces a predictable change in potential, so ISEs can often be calibrated across several orders of magnitude. This is one reason ISEs can have broad working ranges.

The Pros and Cons of Ion-Selective Electrodes

Below are some specifications about the Vernier Ion-Selective Electrodes:

Table 1. Specifications for individual ion-selective electrodes from Vernier.¹⁴

Ion	K^+	Ca^{2+}	Cl^-	NO_3^-	NH_4^+
Concentration range (ppm)	1-39000	1-40000	1-35000	1-14000	1-18000
Reproducibility (precision)	±11% of full scale (calibrated 10 to 1000 mg/L)	±10% of full scale (calibrated 10 to 1000 mg/L)	±10% of full scale (calibrated 10 to 1000 mg/L)	±10% of full scale (calibrated 1 to 100 mg/L)	±10% of full scale (calibrated 1 to 100 mg/L)
pH range	2-12	2-8	2-12	2-11	4-7.5
Interfering ions	Cs^+ , NH_4^+ , H^+ , Ag^+ , Li^+ , Na^+	Pb^{2+} , Hg^{2+} , Fe^{2+} , Cu^{2+} , Ni^{2+} , NH_3 , $Tris^+$, K^+ , Ba^{2+} , Zn^{2+} , Mg^{2+}	CN^- , Br^- , I^- , OH^- , S^{2-} , NH_3	ClO_4^- , I^- , ClO_4^- , CN^- , BF_4^-	K^+ , Li^+ , Na^+ , Cs^+ , Mg^{2+} , Ca^{2+} , Sr^{2+} , Ba^{2+}

13. In table 1, concentration ranges for each ISE are listed.

- a. For example, a potassium electrode measures approximately 1–39,000 ppm. Over how many orders of magnitude does this measurement range extend?

The range spans about 4.6 orders of magnitude because $\log(39,000) \approx 4.6$.

- b. Is this broader or narrower than most Beer's Law calibrations?

This is broader than most Beer's Law calibrations. Spectrophotometric Beer's Law calibrations are usually linear over a more limited concentration range, often one to two orders of magnitude, before deviating from linearity.

14. Some ISEs operate over broad pH ranges, while others are limited to narrower pH ranges. Based on Table 1, identify one ISE with a broad pH range and one with a narrow pH range. What factors might explain these differences, and how would pH limitations affect practical use?

¹⁴Vernier Software & Technology. Nitrate Ion-Selective Electrode. <https://www.vernier.com/product/nitrate-ion-selective-electrode/> (accessed July 18, 2025).

Vernier Software & Technology. Chloride Ion-Selective Electrode. <https://www.vernier.com/product/chloride-ion-selective-electrode/> (accessed July 18, 2025).

Vernier Software & Technology. Ammonium Ion-Selective Electrode. <https://www.vernier.com/product/ammonium-ion-selective-electrode/> (accessed July 18, 2025).

Vernier Software & Technology. Potassium Ion-Selective Electrode. <https://www.vernier.com/product/potassium-ion-selective-electrode/> (accessed July 18, 2025).

Vernier Software & Technology. Calcium Ion-Selective Electrode. <https://www.vernier.com/product/calcium-ion-selective-electrode/> (accessed July 18, 2025).

An example of a broad pH range is K^+ or Cl^- , which operate over about pH 2–12. An example of a narrower pH range is NH_4^+ , which operates over about pH 4–7.5, or Ca^{2+} , which operates over about pH 2–8.

Differences in pH range may arise from changes in analyte speciation, competition from H^+ or OH^- , membrane stability, formation of precipitates or complexes, or chemical reactions involving the target ion. For example, NH_4^+ can convert to NH_3 at higher pH, which changes the form of the analyte being measured. Practically, pH limitations mean the sample may need to be adjusted or buffered before measurement, and some samples may be outside the usable range for a particular ISE.

15. Each ion-selective electrode has interfering ions. Based on the list of interfering ions, what general properties appear to predict whether an ion will interfere with the measurement.

Interfering ions often have similar charge, size, hydration behavior, or chemical properties to the target ion. They may interact with the membrane or ion exchanger in a similar way. Interference can also occur if an ion forms similar precipitates, has similar binding affinity, or is present at high concentration relative to the analyte.

16. A chloride ion-selective electrode has interfering ions such as I^- , Br^- , and OH^- . The selectivity coefficients for these three interfering ions are 10^6 , 100, and 0.01, respectively. Which of the three ions interferes the most?

I^- interferes the most because it has the largest selectivity coefficient.

In a solid-state chloride ISE, iodide is a particularly strong interferent because $AgCl$ is much more soluble than AgI . As a result, the chloride-selective membrane is actually much more sensitive to I^- than to Cl^- , and sufficiently high iodide concentrations can irreversibly damage the electrode. Br^- also interferes strongly, but the effect of I^- is greater because of the especially low solubility of AgI . (Nico2000. Chloride Ion-Selective Electrode.

<https://www.nico2000.net/analytical/chloride.htm> (accessed 2026-09-03).)

17. Based on Table 1, why might chemists prefer an ISE, such as the ones listed in Table 1, for some measurements? What limitations would they need to consider before choosing this method?

Chemists might prefer an ISE because it can provide a direct, relatively fast, inexpensive, and portable measurement. ISEs can also cover broad concentration ranges and may require less sample preparation than some instrumental methods.

However, chemists must consider limitations such as pH range, interfering ions, moderate precision, calibration requirements, ionic strength effects, temperature effects, junction potentials, membrane maintenance, and whether the analyte concentration falls within the working range of the electrode.

One limitation of ion-selective electrodes is the presence of junction potentials. Junction potentials arise whenever two ionic solutions of different composition come into contact. Because ions move at different rates through solution, a small electrical potential can develop at the interface (Figure 5). Consider a porous membrane separating 0.1 M HCl from 0.01 M HCl. Both H^+ and Cl^- diffuse from the more concentrated solution toward the less concentrated solution. However, H^+ moves through water much faster than Cl^- , as shown in Table 2.

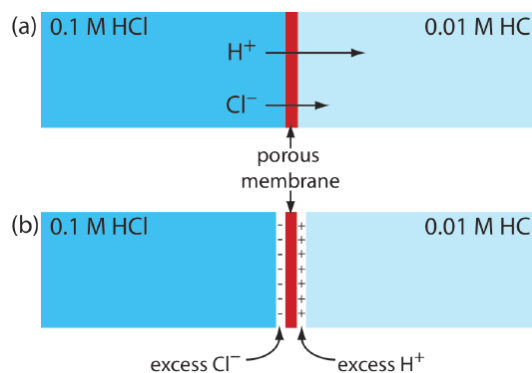


Figure 5. Origin of the junction potential between a solution of 0.1 M HCl and a solution of 0.01 M HCl. From Figure 11.2.3 in David Harvey, *Analytical Chemistry 2.1*, licensed under CC BY-NC-SA 4.0.

18. Consider the liquid junction of made of 0.1 M KNO_3 |0.1 M KBr . Assume the first solution is on the left side of the junction and the second solution is on the right side.
- You can ignore the potassium cation on each side because they will diffuse at the same rates. Using Table 2, which anion diffuses more rapidly across the junction?

Br^- diffuses more rapidly than NO_3^- because Br^- has the larger mobility value.

Table 2. Mobility of common ions in water at 298 K.¹

Ion	Mobility ($m^2s^{-1}V^{-1}$)
H^+	$36.23 * 10^{-8}$
K^+	$7.62 * 10^{-8}$
NO_3^-	$7.41 * 10^{-8}$
Br^-	$8.09 * 10^{-8}$
Cl^-	$7.92 * 10^{-8}$

- Which side of the liquid junction becomes positive as diffusion begins? Justify your response based on ion mobilities.

The KBr side becomes positive. Br^- is more mobile than NO_3^- , so Br^- diffuses from the KBr side toward the KNO_3 side faster than NO_3^- diffuses in the opposite direction. This creates excess negative charge on the KNO_3 side and excess positive charge on the KBr side.

19. In practice, junction potentials are usually small and often remain nearly constant during a measurement. A junction potential introduces a small systematic error into a pH measurement. Why does this error affect the absolute pH value measured by a pH electrode but have little effect on locating the endpoint of an acid-base titration?

A direct pH measurement depends on the absolute measured potential, so a junction potential can shift the measured voltage and lead to a slightly inaccurate pH value. In an acid-base titration, the endpoint is located from the large change in potential or pH as titrant is added. If the junction potential is small and approximately constant, it shifts the entire curve but does not greatly change the volume at which the sharp change or inflection point occurs. Therefore, it affects the absolute pH value more than the endpoint location.

20. You have two days to measure calcium concentration in 50 lake samples and a limited budget. You could use flame AA spectroscopy or a Ca^{2+} ISE. Compare and contrast flame AA spectroscopy and a Ca^{2+} ISE in terms of cost, detection limits, selectivity, portability, and sample preparation.

A Ca^{2+} ISE would likely be cheaper, faster, and more portable than flame AA. It could potentially be used in the field or with minimal sample preparation, making it useful for analyzing many lake samples quickly. It would be a good choice if the calcium concentrations are within the electrode's working range and if the sample matrix does not contain serious interfering ions.

Flame AA spectroscopy would generally require a more expensive laboratory instrument, fuel/oxidant gases, standards, and more formal sample handling. It is less portable and less convenient for field work. However, flame AA is often more sensitive and more selective for elemental calcium than an ISE, and it may provide better precision and lower detection limits. It may be preferred if accurate trace-level calcium measurements are needed or if matrix effects and interferences make the ISE unreliable.

A strong answer should conclude that the best method depends on the goal. For rapid, low-cost screening of many lake samples, the Ca^{2+} ISE may be more practical. For more accurate or lower-level calcium analysis, flame AA may be the better choice.

Active Learning Worksheets for Analytical Chemistry

#24 Chromatographic Methods: Chromatography Basics

Faculty Resources (Delete before sharing with students):

- **Analytical Context and Framing:** I use this worksheet to set the foundation for the chromatography unit. Most students have seen chromatography before, often through GC in organic chemistry or a simple column in general chemistry, but they may not have thought carefully about what is happening at the molecular level. I frame chromatography as controlled competition between two phases. A compound only moves through the column when it is in the mobile phase, and separation happens because different compounds spend different amounts of time associated with the stationary phase versus the mobile phase.
- **Key Concepts and Common Challenges:** Students often confuse retention time, void time, and retention factor. Emphasize that void time is the time for a nonretained solute, retention time is when the analyte peak appears, and retention factor compares time spent in the stationary phase to time spent in the mobile phase. Students may also get overly focused on counting dots in the microscopic partitioning figure. The dot counting is useful, but the larger goal is for students to connect the microscopic picture to the movement of solutes through the column and then to the peaks that appear in the chromatogram.
- **Facilitated Discussion Pauses:** Pause after the microscopic partitioning snapshot. Ask: “Which solute should elute first, and how can you tell from how many molecules are in the mobile phase versus the stationary phase?” Then help students connect that figure to the next column diagram and finally to the chromatogram. This figure-to-figure connection is important because students need to see how molecular-level partitioning becomes macroscopic separation and then a measurable signal.
- **Calculation Support:** For retention factor, resolution, and theoretical plates, have students write the meaning of each variable before substituting values. Students may need help connecting peak width to band broadening and recognizing that better resolution depends on both separation of peak centers and peak width. Check in on the resolution calculations if students are moving quickly, since small setup errors can change the interpretation. For Question 10, students may need help connecting column composition, mobile-phase composition, polarity, and which species should be retained more strongly.
- **Synthesis and Extension Questions:** Ask students: “If two peaks overlap, what would you try first: changing the method so the peaks come out farther apart, or changing the method so the peaks become narrower?” I want students to see that improving resolution can come from more than one place. The best choice depends on whether the problem is mainly selectivity, band broadening, run time, or practical method constraints. Question 11 is a good place to check in with groups because students are beginning to strategize how to shorten analysis time while keeping peaks resolved. For Questions 12–16, I usually let students work individually or in groups first, then bring the class back together to discuss the answers. Question 14 may need extra attention because students can struggle with ranking calcium versus magnesium.
- **Pacing Note:** This worksheet may fit in one class period, but it can run long if students are new to chromatogram interpretation or if you spend time discussing method-improvement strategies. If time is limited, focus class time on the microscopic partitioning, retention-time concepts, and how those ideas connect to the chromatogram. The resolution/theoretical plate calculations and later method-improvement questions can be assigned as follow-up work. This worksheet is meant to build the basic chromatography intuition students need before comparing GC and HPLC and before moving into HPLC method-development decisions.

Answer Keys for Active Learning Worksheets for Analytical Chemistry

#24 Chromatographic Methods: Chromatography Basics

Before You Begin

- Read [12.1: Overview of Analytical Separations](#)
- Read [12.2: General Theory of Column Chromatography](#)

What You Should Already Know

- Types of intermolecular forces and general polarity trends
- Partitioning behavior between two phases
- Basic understanding of physical separation techniques (e.g., filtration, distillation)
- Familiarity with interpreting simple plots or experimental data

What You'll Learn

- Define and apply key chromatographic terms including retention time, void time, retention factor, resolution, and number of theoretical plates
- Explain the impact of stationary and mobile phase polarity on analyte separation
- Describe how experimental parameters influence separation efficiency and speed
- Classify chromatography methods by mechanism (e.g., partition, ion exchange, affinity)

What You'll Practice

- Interpret chromatographic data to calculate and compare separation parameters
- Predict elution order based on molecular structure and stationary/mobile phase polarity
- Propose experimental changes to improve resolution or decrease retention time
- Compare different chromatographic setups based on separation goals

Estimated Completion Time: 50-65 minutes

Author: Rebecca L. Sanders

Active Learning Worksheets for Analytical Chemistry, Version 1.0, 2026

Useful Equations

- $k = \frac{t_r - t_m}{t_m}$
- $R_s = \frac{\Delta t_r}{w_{av}} = \frac{0.589 \Delta t_r}{w_{1/2av}}$
- $N = \frac{5.55 t_r^2}{w_{1/2}^2}$
- $H = L/N$

Chromatography Basics

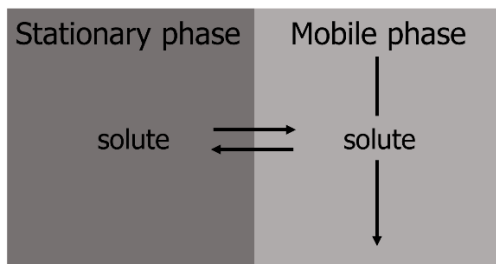


Figure 1. In chromatography, we pass a mobile phase over a stationary phase. When we inject a sample into the mobile phase, the sample's components both move with the mobile phase and partition into the stationary phase. The solute that spends the most time in the stationary phase takes the longest time to move through the system.

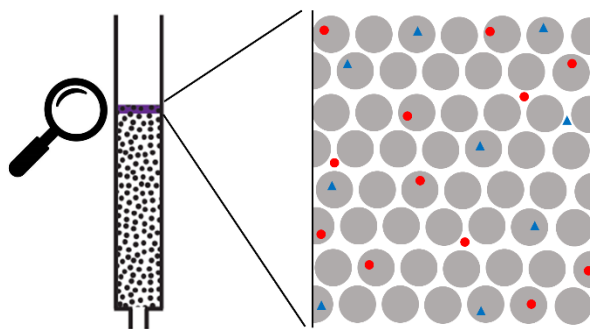


Figure 2. Example of a column with a microscopic view of the packed stationary phase (grey circles) and mobile phase (white space). In the microscopic snapshot, two solutes (red circles and blue triangles) are either associated with the mobile phase or stationary phase.

In **chromatography** we pass a sample-free phase, which we call the **mobile phase**, over a second sample-free **stationary phase** that remains fixed in space (Figure 1). We inject or place the sample into the mobile phase. As the sample moves with the mobile phase, its components partition between the mobile phase and the stationary phase. A component whose distribution ratio favors the stationary phase requires more time to pass through the system. Given sufficient time and sufficient stationary and mobile phase, we can separate solutes even if they have similar distribution ratios. One common method for bringing the mobile phase and the stationary phase into contact is **column chromatography**, where the stationary phase is packed into a narrow column and the mobile phase passes through the column using gravity or by applying pressure. The stationary phase is a solid particle or a thin liquid film coated on either a solid particulate packing material or on the column's walls. In **adsorption chromatography**, solutes separate based on their ability to adsorb, or stick, to a solid stationary phase (Figure 2).

1. Using Figure 1, do solutes move through the column when attached to the stationary phase?

No

2. Using Figure 1, do solutes move through the column when dissolved in the mobile phase?

Yes

3. In Figure 2, a microscopic view of a column chromatography experiment is given shortly after a mix of red and blue solutes are added to the column. In this snapshot, there are nine blue solute molecules. One of the blue solute molecules is on the white space, representing it being dissolved in the mobile phase and eight of the blue solute molecules are on grey circles, representing them being associated with the stationary phase.
 - a. Consider the red solute molecules. In the snapshot, how many are associated with the mobile phase? The stationary phase?

Nine red solute molecules are on the stationary phase and three are in the mobile phase.

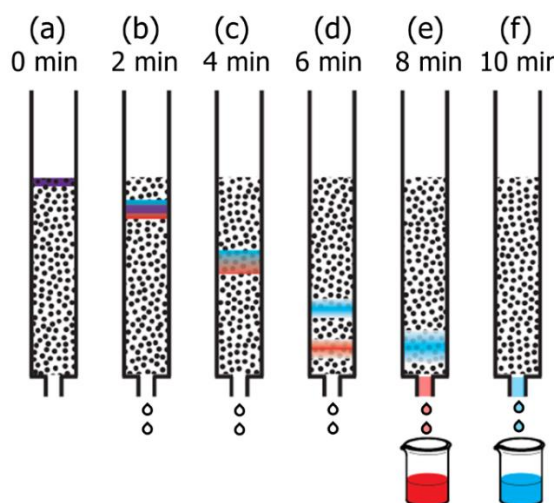


Figure 3. Progress of a column chromatographic separation of a two-component mixture. Adapted from Figure 12.2.1 in David Harvey, *Analytical Chemistry 2.1*, licensed under CC BY-NC-SA 4.0.

- b. How long it takes for solute molecules to make their way through the column depends on how much time they are retained on the stationary phase over how much time they move with the mobile phase. This ratio is referred to as a **retention factor**. In the microscopic snapshot, we can estimate this retention factor by dividing the number of molecules on the stationary phase by the number of molecules dissolved in the mobile phase. Estimate the retention factors of the red and blue solute molecules.

Red: $\frac{9}{3} = 3$; **Blue:** $\frac{8}{1} = 8$

- c. The larger the retention factor, the longer it takes for the solute to come out of, or elute from, the column. Based on your estimated retention factors, predict which solute molecules will elute from the column first.

The red molecules should elute first.

4. We can now explore a simple view of the liquid–solid column chromatography experiment started in Figure 2. The sample containing red and blue solute molecules is introduced as a narrow band at the top of the column (a) (Figure 3).

- a. Why is it important for all the solute to be introduced at once as a narrow band at the top of the column?

It is important to introduce all the solute at once as a narrow band at the top of the column to ensure good separation and sharp peaks. If the solute is added over a longer period or as a wide band, the sample starts spreading before separation begins, leading to broader, overlapping peaks and poor resolution between compounds.

- b. Some solutes may not interact with the column. Even without interacting with the column, nonretained solutes still have to travel through the column, moving at the same rate as the mobile phase. The time required to elute the nonretained solutes is called the column's **void time**, t_m . In Figure 3, what is the void time for the column?

2 minutes

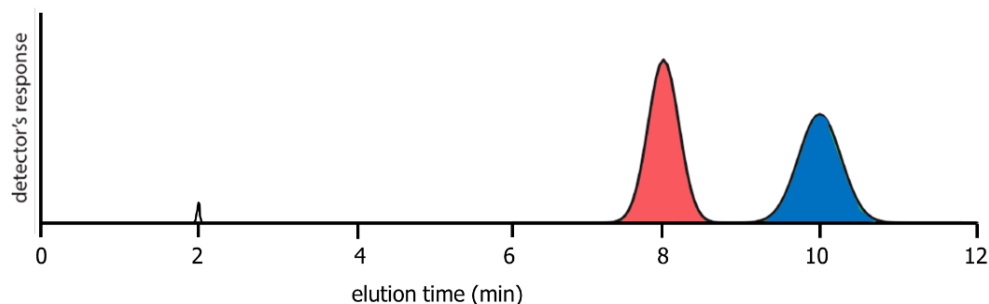


Figure 4. Chromatogram for the separation shown in Figure 3, showing the detector response as a function of elution time. Adapted from Figure 12.2.3 in David Harvey, *Analytical Chemistry 2.1*, licensed under CC BY-NC-SA 4.0.

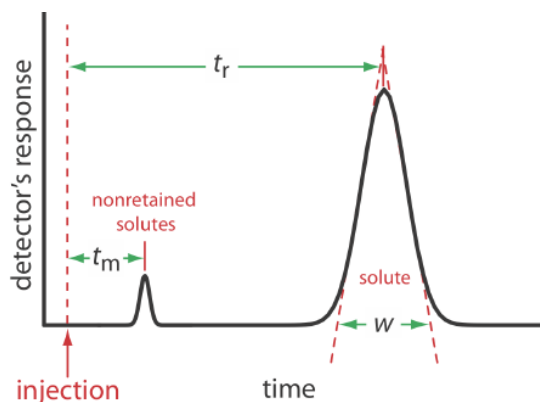


Figure 5. Chromatogram showing a solute's retention time, t_r , and baseline width, w , and the column's void time, t_m , for nonretained solutes. From Figure 12.2.4 in David Harvey, *Analytical Chemistry 2.1*, licensed under CC BY-NC-SA 4.0.

- c. What happens to the colored solutes in the column in steps b-d?

They start to separate and spread out. With more time, they separate and spread more.

- d. What happens to the size of the colored bands as time progresses?

The colored bands widen as time progresses.

- e. The red band at 6 mins is in a similar location in the column as the blue band at 8 min. Which band is wider? Why?

The blue band at 8 minutes is wider because there has been more time passed, giving more time for the blue molecules to spread out more.

- f. We can follow the progress of the separation by collecting fractions as they elute from the column or by placing a suitable detector at the end of the column. What time does the red solute elute? What time does the blue solute elute?

The red solute comes out around 8 minutes and the blue solute comes out around 10 minutes.

5. A plot of the detector's response as a function of elution time, or as a function of the volume of mobile phase, is known as a **chromatogram**, and consists of a peak for each solute. An example from the experiment in Figure 3 is shown in Figure 4. We can characterize a chromatographic peak's properties in several ways, two of which are shown in Figure 5. **Retention time**, t_r , is the time between the sample's injection and the maximum response for the solute's peak.

- a. What is the retention time for each colored solute in Figure 4? How does the correspond to Figure 3?

Red: 8 minutes

Blue: 10 minutes

Both times correspond to when the colored solution was coming out of the column.

- b. Why is the peak maximum used to determine the retention time and not another time point on each solute peak?

The peak maximum is used to determine retention time because it provides a clear, reproducible point that corresponds to the highest concentration of the solute reaching the detector. Unlike the start or end of a peak, which can be affected by baseline noise or peak tailing, the maximum is typically sharp and well-defined, making it less sensitive to variations in peak shape. This consistency is important for accurately identifying and comparing solutes across chromatograms.

- c. Label the void time peak on the chromatogram in Figure 4 and list the void time below.

2 minutes

- d. Retention factor (k) can be calculated as the time spent in the stationary phase divided by time spent in the mobile phase. Because retention time is the sum of the time spent with both phases, the time spent in the stationary phase is found between the difference between retention time and void time:

$$k = \frac{t_r - t_m}{t_m}$$

Calculate the retention factors for the red and the blue solution. Compare your results to the theoretical retention factors calculated in question 3b.

$$\text{Red: } k = \frac{t_r - t_m}{t_m} = \frac{8 - 2}{2} = 3$$

$$\text{Blue: } k = \frac{t_r - t_m}{t_m} = \frac{10 - 2}{2} = 4$$

6. A chromatographic peak's **baseline width**, w , as shown in Figure 5, is determined by extending tangent lines from the inflection points on either side of the peak through the baseline. We can report t_r and w using units of time. Estimate the baseline width for each colored solute from Figure 4 in units of minutes.

The retention times for the first and second peaks are approximately 8 and 10 minutes, respectively. The average width for each peak is about 1 minute.

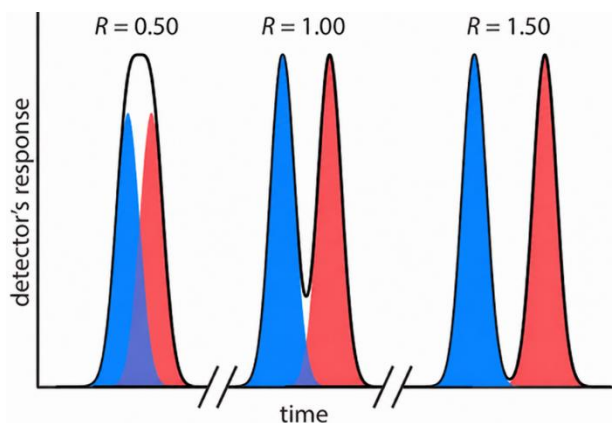


Figure 6. Three examples showing the relationship between resolution and the separation of a two-component mixture. The blue peak and the red peak are the elution profiles for the two components. The chromatographic peak, which is the sum of the two elution profiles, is shown by the solid black line.

7. The goal of chromatography is to separate a mixture into a series of chromatographic peaks, each of which constitutes a single component of the mixture. The **resolution** between two chromatographic peaks, R_s , is a quantitative measure of their separation, and is defined as the difference between retention time divided by the average baseline width

$$R_s = \frac{\Delta t_r}{w_{av}}$$

- a. Compare Figures 4 and 6. Predict if the resolution of the two peaks in Figure 4 less than, equal to, or greater than 1.5?

The resolution is greater than 1.5 because there is baseline separation between the two peaks

- b. Using the retention times and baseline widths from Figure 4 of the two colored peaks, calculate their resolution.

$$R_s = \frac{\Delta t_r}{w_{av}} = \frac{10 - 8}{1} \cong 2$$

- c. Alternatively, if baseline width is difficult to estimate, the widths at half the maximum peak height can be estimated instead and used to calculate resolution.

$$R_s = \frac{\Delta t_r}{w_{av}} = \frac{0.589 \Delta t_r}{w_{1/2av}}$$

If the red peak had a $w_{1/2}$ of 0.50 min and the blue peak had a $w_{1/2}$ of 0.70 min, calculate resolution.

$$R_s = \frac{\Delta t_r}{w_{av}} = \frac{0.589 \Delta t_r}{w_{1/2av}} = \frac{0.589 (2)}{0.6} \cong 2$$

8. Calculate the number of theoretical plates for the red solute. What does the number of theoretical plates represent? Is it better for the number to be large or small?

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

$$N = \frac{5.55(8)^2}{(0.5)^2} = 1420.8 \text{ (rounded to one significant figure, } N = 1 \times 10^3 \text{ theoretical plates)}$$

It is better to have a large number of theoretical plates, which represents high column efficiency and column separation.

9. Calculate the plate height assuming the column was 10.0 cm long. What does plate height represent? Is it better for plate height to be large or small?

$$H = L/N$$

$$H = \frac{10.0 \text{ cm}}{1420.8} = 0.007 \text{ cm}$$

It's better for the plate height to be a smaller value.

10. The stationary phase from Figure 3 was made of SiO_2 .
a. Is this column a polar or nonpolar column?

Relatively polar

- b. The solvent used for the experiment was 95:5 hexane:ethyl acetate? Is this mobile phase relatively polar or nonpolar?

Relatively nonpolar

- c. Normal phase chromatography has a stationary phase that is relatively polar and a mobile phase that is relatively nonpolar whereas reverse phase chromatography has a stationary phase that is relatively nonpolar and a mobile phase that is relatively polar. Is this experiment normal or reverse phase chromatography?

Normal phase chromatography

- d. Which solute in the experiment – the red or the blue – was more polar? Explain how you know.

The blue solute molecules are more polar because they are retained on the polar column longer.

- e. Suppose the mobile phase was changed to 50:50 hexane:ethyl acetate. How would that change the retention time of the solutes? Why?

With more ethyl acetate in the mobile phase, the mobile phase is more polar. This decreases the retention time because the polar solutes have more affinity for the mobile phase and spend less time on the stationary phase.

11. Suppose you wanted to shorten the experiment from Figures 3 and 4. Propose three changes that could be made to speed up the reaction while ensuring the two peaks are still resolved.

There are several options including decreasing the column length, increasing the flow rate, making the mobile phase more polar, and increasing the column temperature.

Identifying Chromatography Type and Elution Orders

For each experiment, identify the type of chromatography being used from the following options: adsorption chromatography, size exclusion chromatography, ion exchange chromatography, partition chromatography, and affinity chromatography. Then, predict the elution order of the compounds or proteins involved, from first to last.

12. A mixture of small organic compounds – caffeine (a relatively nonpolar compound), acetone (a polar compound), and benzene (a nonpolar compound) – is loaded onto a column packed with a C_{18} stationary phase. The mobile phase is 50:50 water:methanol.

Adsorption chromatography. Order: acetone, caffeine, benzene

13. A mixture of proteins – insulin (a small peptide hormone), albumin (a large, abundant protein), and glucagon (another peptide hormone with structural similarities to insulin) – is loaded onto a column with a stationary phase containing insulin receptors. The mobile phase is a buffer solution that helps elute the proteins by competing for binding sites on the stationary phase.

Affinity chromatography. Order: albumin, glucagon, insulin

14. A mixture of small metal ions – Ca^{2+} (a divalent cation), Na^+ (a monovalent cation), and Mg^{2+} (a divalent cation) – is loaded onto a column with a charged stationary phase. The mobile phase is a buffered solution that helps elute the metal ions by competing for binding sites on the column

Ion exchange chromatography. Order: Na^+ , Ca^{2+} , Mg^{2+}

15. A mixture of small organic compounds — acetone (a polar solvent), benzene (a nonpolar solvent), and ethanol (a moderately polar solvent) – is loaded onto a column with a stationary phase made of water that is coated on a solid support. The mobile phase is a solvent mixture of 50:50 hexane:ethyl acetate that moves through the column, carrying the sample with it.

Partition chromatography. Order: benzene, ethanol, acetone

16. A mixture of three proteins – albumin (a large protein), lysozyme (a medium-sized protein), and insulin (a small protein) – is loaded onto a column packed with porous beads that separates solutes based on size. The column is then eluted with a buffer solution.

Size exclusion chromatography. Order: albumin, lysozyme, insulin

Active Learning Worksheets for Analytical Chemistry

#25 Chromatographic Methods: Comparison of GC and HPLC

Faculty Resources (Delete before sharing with students):

- **Analytical Context and Framing:** I talk about GC and HPLC as two solutions to the same general problem: separating mixtures before detection. Both techniques use a mobile phase and a stationary phase, but the sample has to be compatible with the instrument. This worksheet helps students move from “chromatography separates mixtures” to “which chromatographic method makes sense for this analyte, sample, and analytical goal?” I use it especially to build conceptual understanding of HPLC and to compare it with the GC experience many students may already have from organic chemistry.
- **Key Concepts and Common Challenges:** Students may focus on instrument parts without connecting them to sample compatibility. Emphasize that GC is best for volatile, thermally stable compounds, while HPLC is better for nonvolatile, polar, larger, or thermally sensitive compounds that can be dissolved in a suitable mobile phase. Students should also connect method choice to practical considerations such as cost, analysis time, resolution, detection method, sample preparation, and the level of separation needed.
- **Facilitated Discussion Pauses:** Pause after students compare the GC and HPLC schematics. Ask: “Which parts do both instruments need, and which parts are different because one mobile phase is a gas and the other is a liquid?” If GC and HPLC instruments are available, this is a good worksheet to pair with a short lab tour. Pointing out the flow path on the actual instruments can help students connect the schematic to the real system. It is also helpful to show students a GC column and an HPLC column side by side so they can see that the columns differ in material, length, diameter, and whether the stationary phase is coated or packed.
- **Calculation Support:** This worksheet is primarily conceptual, but students may need support using experimental parameters to justify method choice. Encourage them to look for evidence such as temperature, mobile phase, column type, detector, sample preparation, analyte volatility, thermal stability, polarity, and solubility. Table 1 can take longer than expected, so it may be useful to complete part of it together or provide a partially completed version if time is limited. The goal is not for students to get lost in every parameter, but to use the parameters as evidence for comparing GC and HPLC.
- **Synthesis and Extension Questions:** Ask students: “If a method works beautifully on paper but requires the analyte to survive a hot injection port, what sample properties would make you switch from GC to HPLC?” Question 7 works well as a group activity followed by whole-class discussion. Let students make an initial technique choice in groups, then discuss the reasoning as a class so they can see how sample properties, instrument capabilities, and practical constraints all affect method selection.
- **Pacing Note:** This activity works best after students have completed or reviewed Worksheet #24. It is likely to fit within one class period if students have completed the readings, but it can run longer if Table 1 is completed fully in class or if you include a lab tour/instrument demonstration. If time is limited, use class time for identifying instrument components, comparing GC/HPLC features, and discussing technique selection. The detailed table-completion or application questions can be assigned as follow-up work. This worksheet is less calculation-focused than some others in the unit; its main purpose is to build the conceptual basis for choosing between chromatographic methods.

Answer Keys for Active Learning Worksheets for Analytical Chemistry

#25 Chromatographic Methods: Comparison of GC and HPLC

Before You Begin

- Read [12.4: Gas Chromatography](#)
- Read [12.5: High-Performance Liquid Chromatography](#)

What You Should Already Know

- General structure and function of chromatographic systems
- Concepts of polarity, volatility, and intermolecular forces
- Purpose of stationary and mobile phases
- Basics of detector types (UV-Vis, MS)

What You'll Learn

- Describe and compare the instrumentation and theory behind GC and HPLC
- Identify key parameters that influence retention, resolution, and sample compatibility
- Distinguish between when GC and HPLC are appropriate based on compound properties
- Analyze experimental setups to interpret how columns, mobile phases, and detection vary

What You'll Practice

- Interpret schematics and instrument components for GC and HPLC systems
- Synthesize reading and experimental data to complete a comparison table
- Apply chromatographic principles to real-world analytical problems
- Justify technique selection based on sample type, compound properties, and method limitations

Estimated Completion Time: 50-65 minutes

Author: Rebecca L. Sanders

Active Learning Worksheets for Analytical Chemistry, Version 1.0, 2026

Gas Chromatography (GC) and High-Performance Liquid Chromatography (HPLC)

GC and HPLC are both widely used techniques for separating and analyzing mixtures. While both methods involve a stationary phase and a mobile phase, they differ in their phases and sample types. GC typically uses a gas as the mobile phase and is ideal for analyzing volatile compounds, while HPLC uses a liquid mobile phase to analyze a broader range of compounds. Both techniques are widely used in areas such as environmental testing, pharmaceutical analysis, and food analysis. In this worksheet, you will compare GC and HPLC.

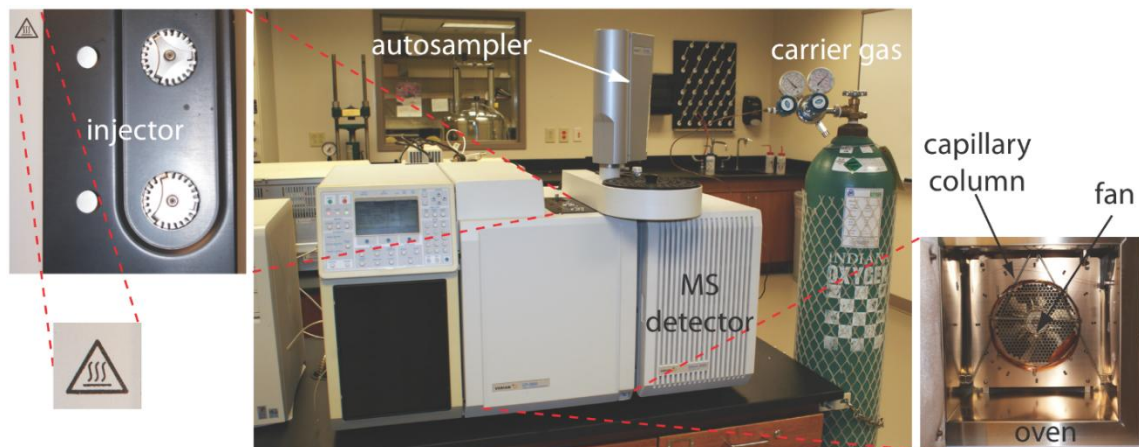


Figure 1. Example of a typical gas chromatograph, with insets showing the heated injection ports and the oven that houses the column. This instrument is equipped with an autosampler for injecting samples, a capillary column, and a mass spectrometer as the detector. From Figure 12.4.1 in David Harvey, *Analytical Chemistry 2.1*, licensed under CC BY-NC-SA 4.0.

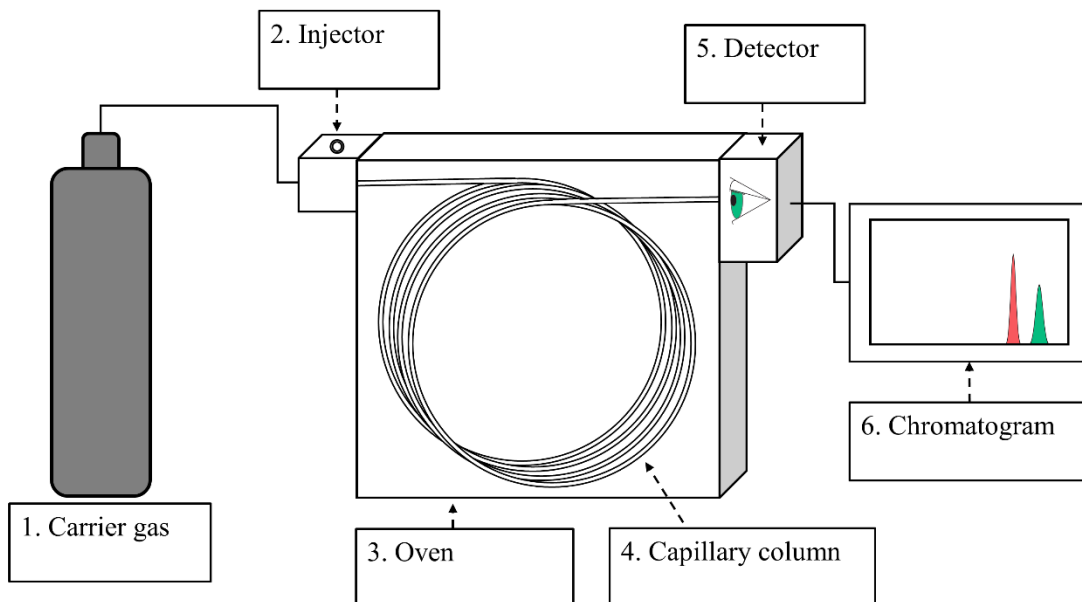


Figure 2. Schematic diagram of a gas chromatograph.

1. Consider the image of a GC in Figure 1. Identify each component on the picture of the gas chromatograph in Figure 2.

See labeled Figure 2.

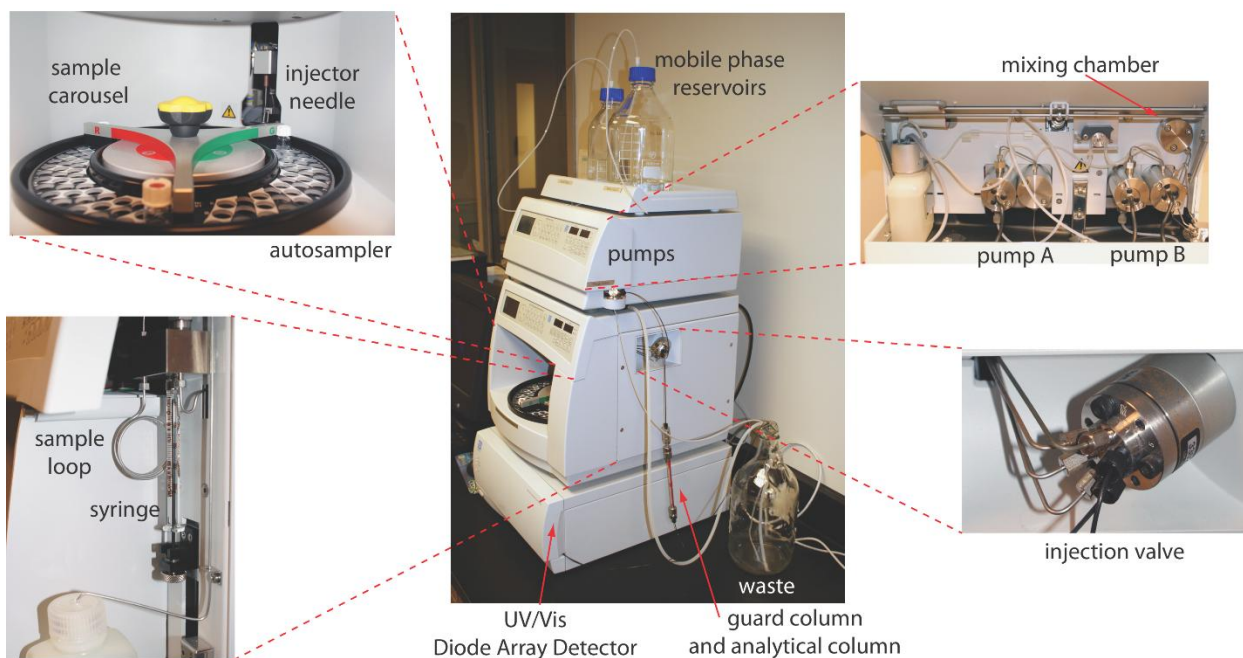


Figure 3. Example of a typical high-performance liquid chromatograph, with insets showing the pumps that move the mobile phase through the system and the plumbing used to inject the sample into the mobile phase. From Figure 12.5.1 in David Harvey, *Analytical Chemistry 2.1*, licensed under CC BY-NC-SA 4.0.

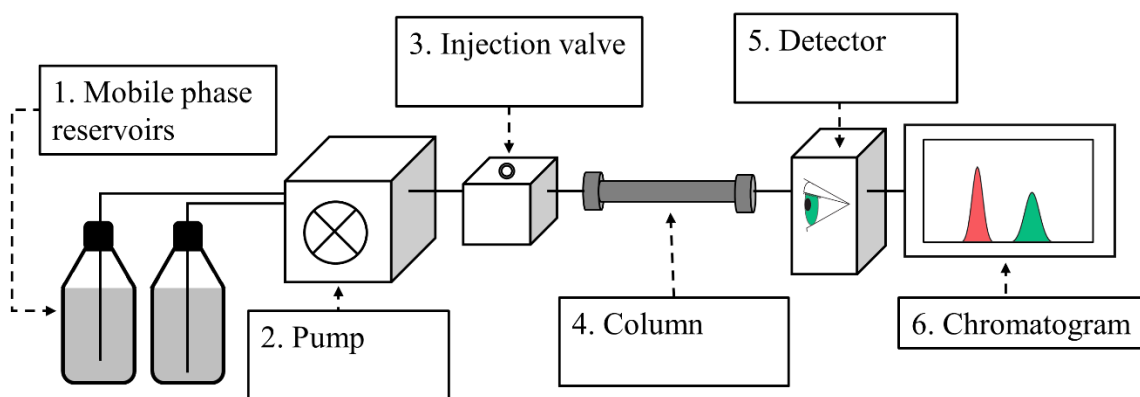


Figure 4. Schematic diagram of a high-performance liquid chromatograph.

2. Consider the image of a HPLC in Figure 3. Identify each component on the picture of the high-performance liquid chromatograph in Figure 4.

See labeled Figure 4.

3. Examine the schematics of the GC (Figures 1 and 2) and the HPLC (Figures 3-4). Discuss with your group some similarities and differences between GC and HPLC.

Expected student response: GC columns are long, narrow capillary tubes with stationary phase coated on the inner wall. HPLC columns are shorter and wider and are packed with small particles containing the stationary phase.

Additional instructor context: GC capillary columns are often many meters long and have very small internal diameters, which contributes to their high efficiency. HPLC columns are typically 10–30 cm long and packed with micron-sized particles; the packed bed requires the liquid mobile phase to be pushed through under high pressure.



Figure 5. Typical example of a capillary column for gas chromatography. This column is 30 m long, has an internal diameter of 247 μm , and has a 0.25 μm coating of liquid stationary phase on its interior surface. From Figure 12.4.3 in David Harvey, *Analytical Chemistry 2.1*, licensed under CC BY-NC-SA 4.0.



Figure 6. Typical packed column for HPLC. This column has an internal diameter of 4.6 mm, is 150 mm long, and is packed with 5 μm particles coated with stationary phase. From Figure 12.5.2 in David Harvey, *Analytical Chemistry 2.1*, licensed under CC BY-NC-SA 4.0.

4. At the heart of all chromatography experiments is the column used for separation. Compare and contrast a capillary column for GC and a packed column of HPLC.

In GC, a capillary column is typically a long, narrow tube, often several meters long and less than 1 cm in diameter. The capillary column is coated on the inside with a thin film of stationary phase coated along the inner wall. These columns provide high efficiency and resolution due to their long length and small internal diameter, and are ideal for separating volatile, low molecular weight compounds.

In HPLC, a packed column is much shorter (usually 10–30 cm) and wider (about 4.6 mm internal diameter), and is filled with small solid particles (commonly 3–5 μm in size) coated with stationary phase. Packed columns allow for high-pressure flow of liquid mobile phase and are well-suited for nonvolatile, polar, and larger molecules.

5. There are many similarities and differences between GC and HPLC experiments. Read the two sections on Gas Chromatography and High-Performance Liquid Chromatography from the LibreTexts website: [12.4: Gas Chromatography](#) and [12.5: High-Performance Liquid Chromatography](#). You can also consult the example experimental parameters on the next page. Based on your readings, fill out the table below comparing the key components and features of each technique. Make sure to include the following in your answers:

Table 1. Gas Chromatography (GC) vs. High-Performance Liquid Chromatography (HPLC)

Feature	GC	HPLC
Mobile phase	<i>Inert gas (e.g., He, N₂)</i>	<i>Liquid (often water/organic solvent mix)</i>
Stationary phase	<i>Liquid/polymer on inert solid or solid support in capillary</i>	<i>Silica-based solid packing (can be modified with polar/nonpolar groups)</i>
Types of samples analyzed	<i>Volatile, thermally stable, small molecules</i>	<i>Larger, polar, nonvolatile, thermally sensitive compounds</i>
Sample injection	<i>Heated gas-phase injection</i>	<i>Liquid sample injection via loop or autosampler</i>
Experiment temperatures	<i>Oven used to run at elevated temperatures</i>	<i>Usually run at room or moderate temps</i>
Detection method	<i>MS, Flame Ionization Detector (FID), Thermal Conductivity Detector (TCD)</i>	<i>UV-Vis, MS, Refractive Index (RI)</i>
Separation mechanism	<i>Partitioning between gas and stationary liquid/solid</i>	<i>Partitioning, adsorption, ion exchange, size exclusion, etc.</i>

Example Experiments with Experimental Parameters for GC and HPLC

Gas Chromatography-Mass Spectrometry Method for Analysis of Fatty Acid Methyl Esters (FAMES) in Biodiesel

1. Sample Preparation:
 - Sample Type: Biodiesel (fatty acid methyl esters from vegetable oils or animal fats)
 - Preparation:
 - Dilute biodiesel sample (1:10) in hexane
 - Pass through 0.45 µm filter to remove particulates
 - Inject directly into GC-MS
2. GC-MS Experimental Parameters:
 - Injection Parameters:
 - Injection Mode: Split 50:1 (to prevent column overload)
 - Injection Temperature: 250 °C
 - Injection Volume: 1 µL
 - Carrier Gas: Helium
 - Flow Rate: 1.2 mL/min
 - Column Parameters:
 - Column Type: Capillary column
 - Stationary Phase: 5% Phenyl / 95% Dimethylpolysiloxane
 - Column Length: 30 m
 - Internal Diameter: 0.25 mm
 - Film Thickness: 0.25 µm
- Oven Temperature Program:
 - Initial Temperature: 50 °C, hold for 1 min
 - Ramp Rate: 10 °C/min
 - Final Temperature: 320 °C, hold for 5 min
 - Total Run Time: ~30 min
3. Mass Spectrometry (MS) Detection Parameters:
 - Ionization Mode: EI at 70 eV
 - Mass Range: 50–550 m/z
4. Expected Retention Times for Common FAMES:

Compound	Retention Time (min)
• Methyl Palmitate (C16:0)	16.5
• Methyl Stearate (C18:0)	18.0
• Methyl Oleate (C18:1)	18.5
• Methyl Linoleate (C18:2)	19.0
• Methyl Linolenate (C18:3)	19.5
5. Data Analysis:
 - Identification: Compare spectra with NIST MS library
 - Quantification: Calibration Curve: FAME standards in hexane at 1–100 mg/mL

HPLC Method for Analysis of Pharmaceutical Compounds in Pain Relief Tablets (Paracetamol, Caffeine, and Ibuprofen)

1. Sample Preparation:
 - Sample Type: Commercial pain relief tablets
 - Preparation Steps:
 - Weigh and finely grind the tablet
 - Dissolve in methanol:water (50:50, v/v)
 - Filter through a 0.45 µm membrane filter before injection
2. HPLC Experimental Parameters:
 - Mobile Phase Parameters:
 - Solvent A: 0.1% formic acid in water
 - Solvent B: Acetonitrile
 - Gradient Elution:
 - 0 min: 5% B
 - 10 min: 50% B
 - 20 min: 95% B
 - 25 min: 95% B (hold)
 - 30 min: 5% B (re-equilibration)
 - Flow Rate: 1.0 mL/min
 - Column Parameters:
 - Column Type: C18 (Octadecyl Silica, ODS)
 - Column Length: 150 mm
- Internal Diameter: 4.6 mm
- Particle Size: 5 µm
- Pore Size: 100 Å
- Injection Volume: 10 µL
- Detection Parameters:
 - Detector Type: UV-Vis or Diode Array Detector (DAD)
 - Detection Wavelengths:
 - 254 nm
 - Flow Cell Path Length: 10 mm
- Column Temperature: 30–40 °C
3. Expected Retention Times:

Compound	Retention Time (min)
• Paracetamol	4.5
• Caffeine	7.0
• Ibuprofen	13.5
4. Data Analysis:
 - Identification: Compare retention times with reference standards
 - Quantification with calibration curve: Standard solutions in 1–100 µg/mL range

6. Summarize

- a. What are 2-3 advantages of GC? Of HPLC?

GC advantages: High efficiency and resolution for small, volatile analytes; fast analysis times; widely coupled with MS for powerful ID

HPLC advantages: Handles a wide range of compounds (nonvolatile, polar, large); compatible with aqueous systems and biomolecules; precise control over separation conditions (pH, polarity, gradients)

- b. What are 2-3 disadvantages of GC? Of HPLC?

GC disadvantages: Only suitable for volatile and thermally stable compounds; not compatible with large or polar biomolecules; requires vaporization step.

HPLC disadvantages: Slower than GC for small analytes; higher operating costs (pumps, solvents, maintenance); sometimes less resolution for small volatile compounds than GC

- c. Based on the table, when would you use GC versus HPLC? What factors would influence your decision?

Use GC when analyzing small, volatile, and thermally stable compounds such as solvents, fragrance molecules, or gases. GC is often faster and provides better resolution for these analytes. Use HPLC for larger, polar, nonvolatile, or thermally labile compounds such as pharmaceuticals, biomolecules, or environmental contaminants. The choice depends on volatility, thermal stability, polarity, and the sample matrix.

- d. What are some limitations of each technique in terms of sample size, type, and sensitivity?

GC is only suitable for volatile, thermally stable analytes. It requires sample vaporization and may have difficulty handling aqueous or large biological samples. Sensitivity can be high with MS detectors but depends on analyte properties.

HPLC requires careful sample preparation and can have lower resolution for small volatile molecules. It uses larger sample volumes and more solvents. The sensitivity depends on detection method (e.g., UV-Vis vs. MS).

7. Real-World Scenarios: Identify whether GC or HPLC would most likely be used to analyze the sample, and explain why in 1-2 sentence(s).

- a. Environmental Monitoring of Air Pollution: A laboratory is tasked with analyzing volatile organic compounds (VOCs) in air samples to assess pollution levels.

Use GC: VOCs (volatile organic compounds) are, by definition, volatile and thermally stable, making them well-suited for gas-phase separation.

- b. Pharmaceutical Quality Control: A pharmaceutical company needs to analyze a new drug product for residual organic solvents remaining from the manufacturing process before it goes to market.

Use GC: Residual solvents are generally small, volatile organic compounds making GC well suited for their separation and analysis.

- c. Food and Beverage Industry: A food manufacturer wants to quantify the amount of caffeine in a line of energy drinks.

Use HPLC: Caffeine is polar and nonvolatile. HPLC with UV-Vis detection is a standard method.

- d. Forensic Science: A forensic lab needs to analyze blood samples to detect alcohol content.

Use GC: Ethanol is a small, volatile molecule that can be easily separated and quantified by gas chromatography.

- e. Biotechnology Research: Researchers are working to purify proteins and peptides from a complex mixture for further studies.

Use HPLC: Peptides are large and thermally unstable. HPLC (possibly reverse phase) is ideal, often coupled with MS.

Active Learning Worksheets for Analytical Chemistry

#26 Chromatographic Methods: HPLC Simulations

Faculty Resources (Delete before sharing with students):

- **Analytical Context and Framing:** I frame the simulator as a low-risk version of HPLC method development. In a real HPLC method, changing one variable can cost time, solvent, sample, and instrument availability. The simulator lets students test those choices quickly and see how changes in experimental parameters affect the resulting chromatogram. I want students to see that method development is not about finding one magic setting; it is about understanding how flow rate, mobile-phase composition, gradient elution, column properties, resolution, and analysis time all interact.
- **Key Concepts and Common Challenges:** Students often look for a single “best” condition, but chromatographic optimization requires balancing resolution, analysis time, peak shape, solvent use, and practical constraints. Emphasize that faster is not always better if resolution is lost. Students should also recognize that some changes are easier to make in a real lab than others. Changing mobile-phase composition or flow rate is usually much simpler than changing the column, even though column properties can strongly affect the separation.
- **Facilitated Discussion Pauses:** Before students begin, it is helpful to project the simulator and orient them to the interface. Show them where to change variables, where to find the chromatogram, and where to get the data they will need. Pause after students examine the flow-rate/van Deemter section. Ask: “Why does the best flow rate occur at the bottom of the curve rather than at the fastest possible flow rate?” Later, when students change mobile-phase composition, ask them to describe not just what changed in the chromatogram, but why the elution order, retention times, or resolution changed.
- **Calculation Support:** For theoretical plates, plate height, and resolution calculations, make sure students distinguish between quantities that improve efficiency and quantities that only make the run faster. If students use the simulator in groups, ask them to record not just the final condition, but also what they changed and why. Questions 3, 9, and 10 can be shortened or skipped if the goal is primarily conceptual exploration, but the calculations are useful if students need practice connecting chromatographic parameters to the visual changes in the chromatogram.
- **Synthesis and Extension Questions:** Ask students: “If two conditions both separate the peaks, how would you choose the better method?” Have them consider resolution, run time, solvent use, reproducibility, ease of changing the method, and whether the method would be practical in a real lab. Question 15 can be treated as an optional method-development challenge. I like to frame it almost like a game: can students adjust the parameters to get the needed separation in the shortest reasonable run time? This can become a low-stakes competition, as long as students remember that the goal is not just speed, but speed while maintaining acceptable resolution.
- **Pacing Note:** This worksheet works best after students have completed or reviewed Worksheets #24-25, since students use chromatography terminology and compare HPLC method-development choices. It works best if students have individual laptops, shared devices, or access to a computer lab. If students do not all have access to computers, the activity can be adapted into a guided class demonstration using a projected version of the simulator. This is likely less effective than having students test conditions themselves, but it can still work if students are asked to predict what will happen before each parameter is changed and then interpret the resulting chromatogram as a class. This activity is best treated as a longer or two-part activity. A natural stopping point is after the flow-rate and van Deemter section. The remaining sections shift toward mobile-phase composition, elution order, gradient reasoning, and broader method optimization. If class time is limited, have students complete the simulator exploration in class and assign the written interpretation questions as follow-up work. The worksheet depends on the current version of the HPLC simulator, so instructors may need to adjust prompts if the simulator interface or available parameters change.

Answer Keys for Active Learning Worksheets for Analytical Chemistry #26 Chromatographic Methods: HPLC Simulations

Before You Begin

- Read [12.3: Optimizing Chromatographic Separations](#)
- Read [12.5: High-Performance Liquid Chromatography](#)

What You Should Already Know

- Define retention time, retention factor (k), and resolution (R_s)
- Explain general principles of chromatographic separation
- Describe the differences between normal and reverse phase chromatography
- Interpret Gaussian peak shape and related parameters (e.g., σ , $w_{1/2}$)

What You'll Learn

- Identify key parameters influencing resolution in HPLC
- Analyze how flow rate affects efficiency using van Deemter relationships
- Predict elution order in reverse phase HPLC based on polarity and structure
- Optimize separations using changes in mobile phase composition and gradient programming

What You'll Practice

- Using an interactive simulator to explore chromatographic method development
- Reading and interpreting chromatograms and output tables
- Applying critical thinking to optimize multivariable experimental conditions
- Collaborating to evaluate trade-offs in method performance

Estimated Completion Time: 75-90 minutes

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Active Learning Worksheets for Analytical Chemistry, Version 1.0, 2026

Useful Equations

- $R_s = \frac{\Delta t_r}{w_{av}} = \frac{0.589 \Delta t_r}{w_{1/2av}}$
- $N = \frac{5.55 t_R^2}{w_{1/2}^2} = \frac{16 t_R^2}{w^2}$
- $H = \frac{L}{N}$

HPLC Simulations

High-performance liquid chromatography (HPLC) is a powerful analytical tool. However, optimizing experiments can be time consuming. To experience this, we will use an online HPLC simulator to learn how different experimental parameters influence chromatograms and a chemist's ability to identify and quantify components of a sample. Visit <http://www.multidlc.org/hplcsim/hplcsim.html>. This website hosts a Multi-Dimensional Separations HPLC Simulator.

1. First, the default setting in the simulator is a C_{18} column as the stationary phase and a mobile phase made of 60% water and 40% acetonitrile (CH_3CN) as the mobile phase. Is this default setting representative of normal phase or reverse phase chromatography? Explain.

This is reverse phase chromatography because the mobile phase is polar and the stationary phase is relatively nonpolar.

2. The default setting has six compounds included in the simulator: uracil, phenol, benzonitrile, p-chlorophenol, acetophenone, and nitrobenzene. You will see a chromatogram with only five peaks. Below the simulated chromatogram, a table lists important variables for each component such as k (retention factor), t_R (retention time), and σ (variance).

- a. If there are six components in the mixture, why are only five peaks observed in the chromatogram?

Two of the components (benzonitrile and p-chlorophenol) have very similar retention times (~2.2 min) so their peaks are overlapping, resulting in insufficient resolution.

- b. As retention time increases (t_R), variance (σ) also increases. Variance is related to peak width. Assuming the peak is a Gaussian shape, the width at the baseline (w) equation is $w = 4\sigma$ and the width at half the maximum peak height ($w_{1/2}$) is $w_{1/2} = 2.35\sigma$. Why does variance increase with longer retention times?

Components spread out more in the column when they have longer retention times.

- c. Click on "Manage Compounds." Adjust the concentration of uracil by making it 25, 50, and 100. What happens to the peak height and peak area as concentration increases? What happens to the retention time as concentration increases?

As the concentration increases, both the peak height and peak area increase. The retention time remains essentially unchanged because concentration affects the amount of analyte detected, not how strongly it interacts with the stationary and mobile phases under these conditions.

- d. Deselect p-chlorophenol. Then, set the remaining five compounds to a concentration of 100. While all five compounds have the same concentration, they have different peak heights. Why? How do their peak areas compare, and why is peak area generally preferred over peak height for quantitative analysis?

Although all five compounds have the same concentration, compounds with longer retention times experience more band broadening, producing wider, shorter peaks. Peak area is less affected by this broadening and remains proportional to the amount of analyte present, so it is generally preferred over peak height for quantitative analysis.

Flow Rate

One variable that can be optimized in an HPLC experiment is flow rate. Under “Manage Compounds”, deselect all compounds and add anisole (concentration defaults as 80.16). Under “Chromatographic Properties”, the flow rate can be changed to rate between 0.05 and 8 mL/min. Experimental variables are shown in Table 1 for a 5 µL injection of anisole in a 100x4.6 mm C₁₈ column with a 3.0 µm particle size using a 40% water/60% acetonitrile mobile phase at 40 °C at variable flow rates.

Table 1. Retention time, variance, peak width at half height, number of plates, and plate height for 5 µL of anisole injected at variable flow rates into a 100x4.6 mm C₁₈ column with a 3.0 µm particle size using a 40% water/60% acetonitrile mobile phase at 40 °C.

Flow rate (mL/ min)	t_R (min)	Variance (σ)	$w_{1/2}$ (s)	$w_{1/2}$ (min)	N (number of plates)	Calculated Plate Height H (mm)
0.1	77.6104	75.6763	177.8	2.964	3805	2.628E-02
0.5	15.5221	8.4074	19.76	0.3293	12332	8.109E-03
1.0	7.761	3.6974	8.689	0.1448	15941	6.273E-03
1.5	5.174	2.4078	5.658	0.09431	16706	5.986E-03
2.0	3.8805	1.8211	4.280	0.07133	16427	6.087E-03
3.0	2.5870	1.2722	2.990	0.04983	14960	6.684E-03
4.0	1.9403	1.0096	2.373	0.03954	13363	7.483E-03
5.0	1.5522	0.8541	2.007	0.03345	11949	8.369E-03
6.0	1.2935	0.7504	1.763	0.02939	10750	9.302E-03
7.0	1.1087	0.6758	1.588	0.02647	9738	1.027E-02
8.0	0.9701	0.6191	1.455	0.02425	8882	1.126E-02

3. Calculate theoretical plates (N) and plate height (H) for the 8.0 mL/min flow rate.

$$N = \frac{5.55t_R^2}{w_{1/2}^2} = \frac{5.55(0.9701)^2}{(0.02425)^2} = 8882 \text{ plates}$$

$$H = \frac{L}{N} = \frac{100.0 \text{ mm}}{8882} = 0.01126 \text{ mm}$$

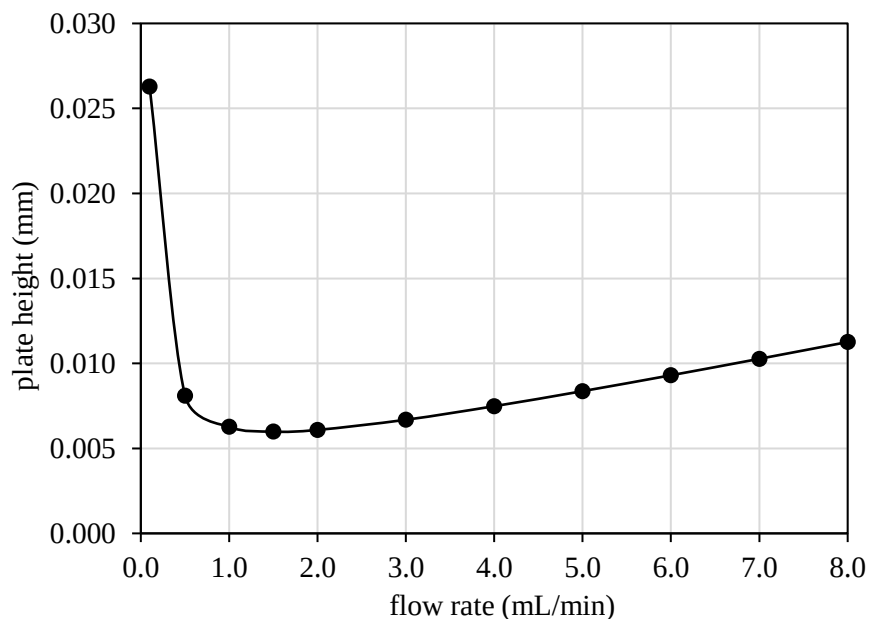


Figure 1. van Deemter graph of 5 μL of anisole in a 100x4.6 mm C_{18} column with a 3.0 μm particle size using a 40% water/60%acetonitrile mobile phase at 40 $^{\circ}\text{C}$ at variable flow rates.

4. To select the optimal flow rate, a van Deemter graph of plate height as a function of flow rate was made (Figure 1).

a. Why does the graph never reach a plate height of 0.000 mm?

Compounds have multiple paths through the column that always add to H regardless of flow rate.

- b. What mechanism caused an increase of plate height at very low flow rates? Using the Figure, list the range of flow rates where this is occurring.

Longitudinal diffusion is the mechanism that dominates at slow flow rates (below 1.0 mL/min).

- c. What mechanism caused an increase of plate height at very fast flow rates? Using the Figure, list the range of flow rates where this is occurring.

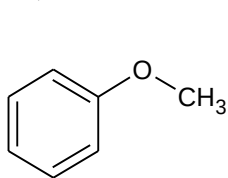
Mass transfer is the mechanism that dominates at fast flow rates (above 2.0 mL/min)

- d. What flow rate is optimal for anisole based on Figure 1?

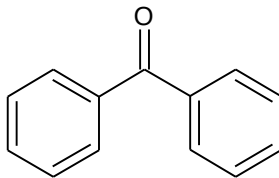
~1.5 mL/min because that has the lowest H .

Mobile Phase Composition

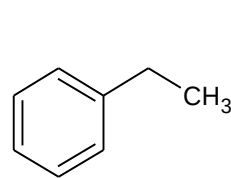
Consider the chemical structures for anisole, ethylbenzene, and benzophenone where anisole consists of a benzene ring attached to a methoxy group ($-\text{OCH}_3$), benzophenone contains two benzene rings connected by a carbonyl group ($\text{C}=\text{O}$), and ethylbenzene consists of a benzene ring attached to an ethyl group ($-\text{CH}_2\text{CH}_3$).



anisole



benzophenone



ethylbenzene

5. Predict the elution of the three components sketched above based on a reverse phase chromatography.

Order: anisole, benzophenone, ethylbenzene. Anisole is the smallest and contains an oxygen atom so has the greatest affinity for the mobile phase. Benzophenone has one oxygen atom and two benzene rings. It's larger than anisole and is more nonpolar than anisole. Ethylbenzene has no heteroatoms so it is more nonpolar and has a greater affinity for the column.

6. Under "Manage Compounds", select the three compounds (anisole, ethylbenzene, and benzophenone). Set the concentration to 100 for all three compounds. Make sure the flow rate is set to 2.0 mL/min under "Chromatographic Properties." Look at the retention times for the three compounds. Did they elute in the order that you predicted? If not, explain why your prediction may have differed from the simulated result.

Yes.

7. The default has the mobile phase composition of 60% water (solvent A) and 40% (solvent B: either acetonitrile or methanol) that stays constant throughout the experiment. Using the simulator, compare 60% water/40% acetonitrile with 60% water/40% methanol. Which is the better choice for solvent B for this separation? Explain using the simulated chromatograms and retention times.

Acetonitrile is the better choice because there is still separation and the retention times are shorter (longest retention time around 13 min for acetonitrile and 35 min for methanol).

8. Set solvent B to acetonitrile for isocratic elution mode. Increase and decrease the fraction of acetonitrile to observe its effect on the separation.
- a. As the fraction of acetonitrile is increased, what happens to the polarity of the mobile phase? What happens to the retention times and the distance between peaks?

With more acetonitrile, the mobile phase is less polar. Retention times decrease and peaks get closer together.

- b. As the fraction of acetonitrile is decreased, what happens to the polarity of the mobile phase? What happens to the retention times and the distance between peaks?

With less acetonitrile, the mobile phase is more polar. Retention times increase and peaks get further apart.

- c. What is an ideal percentage of water and acetonitrile? Why?

The ideal percentage is the shortest retention times while still having a resolution greater than 2 between peaks, which is about 28% water and 72% acetonitrile.

Table 2. Retention times, variance, and widths as half maximum peak height for a three-component separation.

Compound	t_R (min)	Variance (σ , s)	$w_{1/2}$ (s)	$w_{1/2}$ (min)
Anisole	1.0107	0.4828	1.135	0.01891
Benzophenone	1.0853	0.5167	1.214	0.02024
Ethylbenzene	1.4812	0.6978	1.640	0.02733

9. Set the mobile phase to the ideal percentage of water and acetonitrile you identified in the previous question. Make sure all three components have concentrations of 100. Record the retention times and variance for each compound in the table above. Then, calculate $w_{1/2}$ in seconds and minutes using the equation: $w_{1/2} = 2.35\sigma$. Record the $w_{1/2}$ in seconds and minutes in the table above.

Example calculation: $w_{1/2} = 2.35\sigma = 2.35 * 0.4828 = 1.135$ s

10. Calculate the resolution between anisole and benzophenone.

$$R_s = \frac{0.589 \Delta t_r}{w_{1/2av}} = \frac{0.589*(1.0853-1.0107)}{0.5*(0.02024+0.01891)} = 2.245$$

11. How does your calculated resolution compare to the R_s value listed in the simulator for anisole and benzophenone?

It is similar.

12. Is your calculated resolution representative of a quantitative separation between the two peaks? Explain.

Yes. $R_s > 2$ so quantitative separation was achieved.

Column Properties

Set the mobile phase conditions to 70% acetonitrile in isocratic elution mode. Next, examine the conditions under the heading “Column Properties.”

13. The default particle size is 3 μm . Reduce the particle size to 1.5 μm . Then, increase it to 6.0 μm .

- a. What happens to the three peaks in the chromatogram as particle size changes?

As particle size decreases, resolution between peaks increases.

- b. What happens to the back pressure (listed under “Chromatographic Properties”) when the particle size is changed?

As particle size decreases, back pressure increases.

14. Decrease and increase the column length. What is the advantage of a shorter column? A longer column?

A shorter column has shorter retention times and a longer column increases separation.

Complex Mixture Separations

HPLC can be used to separate complex mixtures. For your final section, you will attempt to identify a set of successful experimental parameters that can separate a complex mixture.

Refresh the website to reset all components. Under “Manage Compounds”, select the following seven compounds: anisole, benzophenone, ethylbenzene, butylparaben, propiophenone, butyrophenone, and heptanophenone. You’ll notice immediately that the default settings in the simulator lead to a long experiment time (almost 30 minutes). Your goal is to find a set of parameters that can quantitatively separate all components, ideally in less than 8 minutes if possible.

There are a range of changes that can be made to try to achieve this goal:

- Altering the mobile phase composition in isocratic elution mode (acetonitrile vs. methanol as solvent B)
 - Using gradient elution mode. In the previous activities associated with this worksheet, the mobile phase was set to isocratic elution mode, where the mobile phase composition stays the same throughout the separation. Gradient elution mode offers an alternative where the mobile phase composition changes over time to increase the fraction of solvent B. This helps speed up the elution of compounds that stick to the column. Select “Gradient Elution Mode” under “Mobile Phase Composition.” You can change the starting and ending fractions of solvent B as well as the duration of time with which the fraction of solvents changes in the experiment.
 - Changing the flow rate
 - Changing the column length
 - Changing the particle size of the stationary phase.
15. Were you able to achieve quantitative separation of the seven components? Report the final experimental parameters you used and the total amount of time the experiment would take. Continue to tweak the parameters to see if you can shorten the experiment anymore while still maintaining quantitative separation.

There are likely several options to achieve quantitative separation. For example, the column length and particle size can remain the same with a gradient elution starting with 50% water and 50% methanol. By 8 min, the mobile phase is 26% water and 74% methanol. Total time < 7 min.

Active Learning Worksheets for Analytical Chemistry #27 Answers

#27 Comparing Methods: Selecting an Instrument

Faculty Resources for Worksheet 27 (Delete before sharing with students):

- **Analytical Context and Framing:** I frame this worksheet as the course-level synthesis activity. By this point, students have seen many individual techniques, but the larger analytical skill is deciding which tool fits the question. I tell students that analytical chemistry is not just knowing how each instrument works; it is knowing how to choose from the tools available. Because I do not spend as much time on electroanalytical techniques earlier in the course, the opening review gives students enough background on techniques such as potentiometry, electrogravimetric analysis, amperometry, and voltammetry to use them in the method-selection scenarios.
- **Key Concepts and Common Challenges:** Students often want one correct answer for each scenario, but real method selection usually involves trade-offs. Emphasize that sensitivity, selectivity, sample preparation, cost, matrix effects, analyte type, concentration range, and available instrumentation all matter. Students can become overwhelmed when they have a dozen or more possible techniques in front of them, so it helps to give them a triage strategy: first decide whether they are measuring an atom, an ion, a molecule, or a mixture; then consider whether the analyte is present at high or low concentration; then think about whether the method needs a separation step, an electroactive species, a metal atom, or a molecule that absorbs light.
- **Facilitated Discussion Pauses:** Pause after students complete the technique summary in Question 4. Ask: “Which techniques are mainly for metals? Which are best for organic molecules? Which require a separation step? Which are useful for ions in solution? Which require the analyte to be oxidized or reduced?” I use this pause as a sorting step before the scenarios. Once students group the techniques by analyte type, signal, and major limitation, the method-selection questions feel much less overwhelming. For Question 5, I usually let students work through the scenarios in groups, then reserve the last 15 minutes of class to go through the scenarios one by one. Have students suggest possible techniques, then discuss which answers are strong, which are possible but less ideal, and which do not really fit the analytical problem.
- **Calculation Support:** This worksheet is not calculation-heavy, but students may need help making evidence-based choices. Encourage them to justify each selected technique with at least one sample constraint and one instrument capability.
- **Synthesis and Extension Questions:** Ask students: “If two or more methods could work, what additional information would you want before choosing?” Push them to consider whether their answer would change if the lab needed the cheapest method, the fastest method, the lowest detection limit, the least sample preparation, or the best selectivity in a complex matrix. I also use this worksheet to emphasize that more than one answer may be defensible if the reasoning is sound. On exams, I often accept multiple answers for method-selection questions when students justify the choice appropriately, even if I had one preferred technique in mind.
- **Pacing Note:** This worksheet is designed to fit within one class period when some portions are treated as review rather than discussed in detail. If time is limited, have students complete the technique summary in Question 4 before class. In class, focus discussion on the more detailed method-selection scenarios in Question 5. Question 6 provides additional rapid method-selection practice and does not need to be reviewed item-by-item; instructors can focus discussion on scenarios that generate disagreement or uncertainty.

Active Learning Worksheets for Analytical Chemistry

#27 Comparing Methods: Selecting an Instrument

Before You Begin

- Review previous worksheets on spectroscopy, electrochemistry, and chromatography. Focus on the types of analytes and samples each technique can measure, as well as major strengths, limitations, interferences, and practical considerations.
- Read [11.2: Potentiometric Methods](#)
- Read [11.3: Coulometric Methods](#)
- Read [11.4: Voltammetric and Amperometric Methods](#)

What You Should Already Know

- Distinguish between qualitative and quantitative analysis.
- Explain the basic operating principles of spectroscopy, chromatography, and electrochemistry.
- Recognize the types of analytes that different techniques can measure.
- Recall detection limits and sample type compatibility for key instruments discussed in earlier weeks.

What You'll Learn

- Differentiate between different types of electrochemical methods
- Match an analytical problem to the most suitable instrumental method.
- Identify alternate viable techniques for a given analysis and explain trade-offs.
- Explain the type of sample each major technique can analyze.
- Recognize when a separation step is needed before analysis.

What You'll Practice

- Applying knowledge of instrument capabilities to real-world sample scenarios.
- Comparing multiple analytical approaches to determine the most appropriate one.
- Justifying your choice of method based on sensitivity, selectivity, and sample constraints.
- Collaborating with peers to critically evaluate different analysis strategies.

Estimated Completion Time: 60-80 minutes

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Quick Review: Additional Electrochemical Methods

Throughout the semester, you have worked with a range of spectroscopic, chromatographic, and electrochemical methods. Before comparing all of these techniques, use the table below to review several electrochemical methods that were covered in less detail earlier in the course and to help decide when an electrochemical method is appropriate for a particular analytical problem.

Table 1. Summary of common electrochemical methods.

Technique	What is controlled or varied?	What is measured?	What does the signal tell us?	Common examples or uses
Potentiometry	The cell is measured under static conditions; little or no current flows.	Potential, (E)	The potential is related to the activity or concentration of an analyte through the Nernst equation.	pH electrodes, ion-selective electrodes
Electrogravimetric analysis	A potential is applied so the analyte is deposited on an electrode.	Change in electrode mass	The mass gained by the electrode is used to determine the amount of analyte.	Deposition of metals such as copper onto an electrode
Voltammetry	The applied potential is varied with time.	Current as a function of applied potential	The current can be related to concentration, while the potential where current changes can help identify the analyte or redox process.	Polarography, stripping voltammetry, cyclic voltammetry
Amperometry	The potential is held constant.	Current	The current is related to the concentration of an electroactive species.	Dissolved oxygen sensors, glucose sensors

1. A dissolved metal ion can be reduced and plated onto an electrode. After the experiment, the electrode is dried and weighed, and the increase in mass is used to determine how much metal was originally present. Which electrochemical technique does this describe? What is the main analytical signal?

Electrogravimetric analysis. The main analytical signal is mass, specifically the mass gained by the electrode.

2. A sensor is placed in a flowing stream and gives a current that changes as the analyte concentration changes. Which electrochemical technique does this most closely match? What is being held constant, and what is being measured?

Amperometry. The potential is held constant and the current is measured.

3. In another experiment, the applied potential is changed over time while the current is measured. The potential where the current changes gives information about the redox behavior of the analyte, and the

size of the current can be related to concentration. Which electrochemical technique does this describe?

Voltammetry. The potential is varied and the current is measured as a function of potential.

Review of Techniques Learned Throughout the Semester

4. Throughout the semester, you have used or discussed the following analytical techniques. Complete the missing information in the table to summarize the types of analytes each technique is best suited for and whether the technique provides a separation. Information about sample requirements and typical sensitivity is provided to help you compare the methods.

Technique	Best suited for...	Sample requirements / form	Typical sensitivity / concentration	Separates components?
UV-vis Spectroscopy	<i>Molecules or ions that absorb UV or visible light; often colored or UV-active species</i>	Usually a solution; analyte must absorb at an appropriate wavelength	Depends strongly on analyte and molar absorptivity; generally less sensitive than fluorescence	No
Fluorescence Spectroscopy	<i>Species that naturally fluoresce or can be made fluorescent</i>	Usually a solution; analyte must fluoresce or be derivatized/tagged	Generally high sensitivity; depends strongly on analyte	No
Flame Atomic Absorption (AA) Spectroscopy	<i>Individual metals/metalloids</i>	Sample must be introduced as a solution; solid samples generally require dissolution/digestion	Typically ppm	No
ICP-AES	<i>Metals/metalloids, especially when several elements need to be measured</i>	Usually a liquid solution; solid samples generally require dissolution/digestion	Typically ppm–ppb	No
ICP-MS	<i>Trace and ultratrace metals/metalloids; multielement analysis</i>	Usually a liquid solution; solid samples generally require dissolution/digestion	Typically ppb–ppt	No
Electrogravimetric Analysis	<i>Metal ions that can be deposited quantitatively on an electrode</i>	Analyte must be in solution and capable of electrodeposition	Best suited for concentrations large enough to produce a measurable mass change	No

Technique	Best suited for...	Sample requirements / form	Typical sensitivity / concentration	Separates components?
Affinity Chromatography	<i>Biological molecules with a specific binding partner, such as proteins or antibodies</i>	Sample must be compatible with the binding system and mobile phase	Depends on analyte and detection method	Yes
Ion Exchange Chromatography	<i>Charged species such as inorganic ions, amino acids, and proteins</i>	Sample is generally in solution; analyte must carry charge under the separation conditions	Depends on analyte and detector	Yes
Molecular (Size) Exclusion Chromatography	<i>Molecules that differ in size, especially proteins, polymers, and polysaccharides</i>	Sample must be dissolved and compatible with the column/mobile phase	Depends on analyte and detector	Yes
Gas Chromatography (GC)	<i>Volatile, thermally stable compounds, especially small organic molecules</i>	Sample must be volatile or able to be vaporized without decomposition	Depends strongly on detector and analyte	Yes
High-Performance Liquid Chromatography (HPLC)	<i>Nonvolatile or thermally unstable compounds; many organic and biological molecules</i>	Sample must be soluble in an appropriate solvent/mobile phase	Depends strongly on detector and analyte	Yes
Ion-Selective Electrode (ISE)	<i>A specific ion in solution, such as Na^+, K^+, Cl^-, or F^-</i>	Ion must be dissolved; an appropriate selective electrode must exist	Depends on the ion and electrode	No
Amperometry	<i>Electroactive species whose oxidation or reduction produces measurable current</i>	Usually in solution; analyte must be electroactive at the selected potential	Depends on analyte, electrode, and conditions	No
Voltammetry	<i>Electroactive/redox-active analytes, including some metals, vitamins, and drugs</i>	Usually in solution; analyte must undergo an accessible redox process	Can be sensitive, but depends strongly on analyte and voltammetric method	No

Select a Technique for Analysis

5. Using the information summarized in Question 4, select the analytical technique from Question 4 you think would be most appropriate for each scenario below. More than one technique may be reasonable. For each scenario:

- Select the technique you would use.
 - Identify one additional piece of information about the sample or analysis that would help you determine whether this is the best choice.
 - Identify one advantage or trade-off associated with your choice.
- a. You want to measure chloride concentrations in the DuPage River.
- **Technique:** *Ion-selective electrode*
 - **Additional information needed:** *What concentration range of chloride is expected, and are there ions present that might interfere with the electrode?*
 - **Trade-off:** *ISE is simple and direct for chloride in solution, but selectivity and matrix effects can limit accuracy.*
- b. You want to separate one protein in a large mixture of proteins.
- **Technique:** *Affinity chromatography*
 - **Additional information needed:** *Is there a known binding partner, antibody, or ligand that selectively binds the target protein?*
 - **Trade-off:** *Affinity chromatography can be highly selective, but it requires a suitable specific binding interaction and may be more expensive or specialized than other chromatographic methods.*
- c. You want to analyze the concentrations of various drugs in a blood sample.
- **Technique:** *HPLC*
 - **Additional information needed:** *What drugs are present, what concentration range is expected, and what detector is available?*
 - **Trade-off:** *HPLC can separate a complex mixture, but method development and sample preparation may be substantial.*
- d. You want to measure the concentration of Vitamin C in grapefruit juice.
- **Technique:** *Voltammetry*
 - **Additional information needed:** *Are there other electroactive compounds in the juice that could interfere with the Vitamin C signal?*
 - **Trade-off:** *Voltammetry can directly measure an electroactive analyte such as Vitamin C, but other electroactive species in the sample matrix may interfere. HPLC or UV-vis could also be reasonable depending on the available instrumentation and sample preparation.*
- e. You want to determine how much chromium is in a sample of stainless steel.

- *Technique: ICP-AES*
- *Additional information needed: What concentration of chromium is expected, and how will the solid stainless-steel sample be dissolved or digested before analysis?*
- *Trade-off: ICP-AES is well suited for metal analysis and can measure multiple elements, but the solid sample must first be converted to a solution. Flame AA or ICP-MS could also be appropriate depending on the concentration and required detection limit.*

6. For each of the following analytical problems, select the technique you think would be most appropriate. More than one answer may be reasonable depending on the analytical conditions.
- a. You want to quantify the concentration of Na^+ in salt water.

*Best technique: **Flame AA***

*Other possible techniques: **ICP-AES***

- b. You want to measure parts per trillion concentrations of mercury in Lake Michigan.

*Best technique: **ICP-MS***

*Other possible techniques: **ICP-AES***

- c. You want to measure the concentration of several common metal ions in soil.

*Best technique: **ICP-AES***

*Other possible techniques: **Flame AA***

- d. You want to measure the concentration of greenhouse gases in the atmosphere.

*Best technique: **GC***

*Other possible techniques: **GC-MS***

- e. You want to determine the concentration of ammonium ions in fertilizer.

*Best technique: **Ion-selective electrode***

*Other possible techniques: **Ion exchange chromatography***

- f. You want to determine an unknown number of waters of hydration after 0.4902 g of $\text{CuCl}_2 \cdot x\text{H}_2\text{O}$ are dissolved in 100.0 mL of water.

*Best technique: **ICP-AES or Flame AA***

*Other possible techniques: **Electrogravimetric analysis***

- g. You want to test the blood sugar for a woman with gestational diabetes.

Best technique: *Amperometry*

Other possible technique from this list: *None*

- h. You want to determine the concentration of tin found in canned tuna.

Best technique: *ICP-MS (assuming low concentration)*

Other possible techniques: *ICP-AES, Flame AA*

- i. You want to determine the concentration of Li^+ in brine from an oil field.

Best technique: *Flame AA*

Other possible techniques: *ICP-AES, ICP-MS*

- j. You want to quantify the amount of nicotine in a smoker's urine.

Best technique: *HPLC*

Other possible techniques: *GC-MS*

- k. You want to detect and quantify chlorophyll concentrations in algae samples from a lake.

Best technique: *Fluorescence spectroscopy*

Other possible techniques: *HPLC with fluorescence detection, GC-MS*