Spectroscopy Lab Manual

Spectroscopy Lab Manual

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FANSHAWE COLLEGE PRESSBOOKS
LONDON, ON, CA

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Acknowledgments

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Prompt: Generate a cover for a spectroscopy lab manual, scientific illustration, realistic and modern.

Collaborators

This project was a collaboration between the author and the team in the OER Design Studio at Fanshawe. The following staff and students were involved in the creation of this project:

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- Wilson Poulter, *Copyright*
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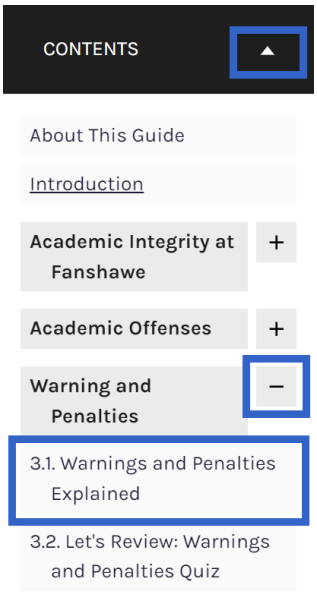
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CHAPTER 1: GENERAL SAFETY RULES & GOOD LAB PRACTICES

Chapter Outline

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1.1 General Laboratory Safety Rules

Analytical Instrumentation Laboratory (D3008)

Safe and professional conduct is essential in all laboratory environments. Analytical instrumentation labs often involve electrical equipment, pressurized gases, hazardous chemicals, and open flames. The following rules are mandatory and designed to protect students, instructors, and equipment while ensuring high-quality analytical results.

General Conduct and Supervision

Never Work Alone: Laboratory work must always be carried out under the supervision of an instructor or technician.

Authorized Experiments Only: Perform only those experiments assigned or approved by your instructor. Unauthorized work is strictly prohibited.

No Food or Drink: Eating, drinking, or chewing gum in the lab is strictly prohibited to prevent chemical ingestion or contamination.

No Personal Items on Benchtops: Bags, coats, and personal belongings must be stored in lockers or designated areas.

Phones and Electronic Devices: Use of phones or personal electronic devices is strictly prohibited during lab sessions. All devices must be silenced and stored to maintain safety, focus, and professionalism.

Professional Behaviour: Show respect for others and maintain a quiet, focused work environment. Avoid distractions-lab work requires full attention and precision.

Required Materials and Personal Storage

Students must arrive on time for every laboratory session with:

- CSA-approved laboratory goggles.
- White laboratory coat (buttoned up at all times; stored in a resealable zip-top plastic bag when not in use).
- Hardcover laboratory notebook.

Lockers

- Personal items must be stored in lockers located near the lab.

- Lockers are available through the Security Office (D1018) at a cost of $12 per term.

Personal Protective Equipment (PPE) and Clothing

Lab Coats: Must be worn at all times. Handle coats carefully to avoid contact with contaminated outer surfaces.

Eye Protection: Always wear CSA-approved safety glasses or goggles when performing experiments or cleaning.

Gloves:

- Disposable gloves must be worn whenever handling chemicals, reagents, or samples.
- Replace gloves immediately if torn or contaminated.
- Remove gloves aseptically and discard them in the appropriate waste bins.
- Wash your hands thoroughly after removing gloves.

Footwear: Closed-toe shoes are mandatory; sandals or open footwear are not permitted.

Hair and Accessories: Tie back long hair and remove loose jewelry. Avoid scarves or clothing that may catch on equipment.

No PPE Outside the Lab: Lab coats, gloves, and goggles must not be worn in hallways or public areas.

Preparation Before Entering the Laboratory

Pre-Read Procedures: Review all experimental instructions, safety notes, and chemical hazards before the session.

Gather Materials: Assemble required glassware, reagents, and waste containers before starting work.

Inspect Equipment:

- Ensure glassware is free of cracks or chips.
- Confirm that balances, pipettes, and instruments are clean and functioning.
- Report all malfunctions to your instructor immediately.

Know Safety Equipment Locations:

- Eyewash stations.
- Fire extinguishers.
- First Aid kit.
- Emergency exits and evacuation routes.
- Emergency phone (dial 9, then 9-1-1, or x4242 for campus response).

Chemical Handling and Labelling

Read All Labels Carefully: Confirm reagent name, concentration, and hazard classification before use.

Label All Containers Clearly with:

- Chemical name.
- Concentration.
- Date prepared.
- Analyst's name.
- Expiry date.
- WHMIS / hazard information (if applicable).

Dispense Small Quantities: Take only the amount of reagent required for your procedure.

Avoid Contamination: Never return unused chemicals to stock bottles.

Use Appropriate Tools: Always use pipettes, scoops, or funnels—not hands—for transferring materials.

Proper Storage: Keep reagent containers closed when not in use and return them to their designated shelves promptly.

1.2 Good Laboratory Practices in Chemistry

To ensure safe and efficient laboratory operation:

1. **Maintain Cleanliness**: Keep your workspace clean and free of unnecessary materials.
2. **Avoid Cross-Contamination**: Handle only one reagent at a time; use separate spatulas and droppers.
3. **Handle Glassware Safely**: Never use cracked or chipped glassware; dispose of damaged pieces immediately in the glass waste container.
4. **Avoid Working When Fatigued**: Concentration is essential to avoid accidents and ensure data accuracy.
5. **Use Fume Hoods**: Conduct procedures that involve volatile or corrosive chemicals inside a fume hood with proper ventilation.
6. **Prevent Fires**:
    - Keep flammable solvents away from open flames or hot surfaces.
    - Never leave a heat source unattended.
7. **Protect Documentation**: Keep lab notebooks and papers away from wet areas or use protective covers.
8. **Avoid Earphones**: Headphones are prohibited in the lab to ensure you can hear instructions and alarms.
9. **Avoid Mouth Contact**: Never pipette by mouth or place pens or caps in your mouth.

Special Precautions in Analytical Instrumentation

- **Instruments (UV-Vis, AAS, AES, and FTIR)**:
    - Follow all Standard Operating Procedures (SOPs).
    - Never operate or repair instruments without supervision.
    - Handle cuvettes, test tubes, and cells carefully; clean with lint-free wipes.
- **Electrical Equipment**: Ensure power cords and plugs are intact and dry.
- **Gas Lines**: Inspect connections for leaks before igniting flames.
- **Hotplates and Heating Devices**: Assume all surfaces are hot; use tongs or heat-resistant gloves.

Instrument Logbook Policy

To ensure accountability, traceability, and instrument integrity, a logbook is maintained for each analytical instrument in Laboratory D3008. Every student/group must complete an entry each time they use an instrument.

Each entry must include the following information:

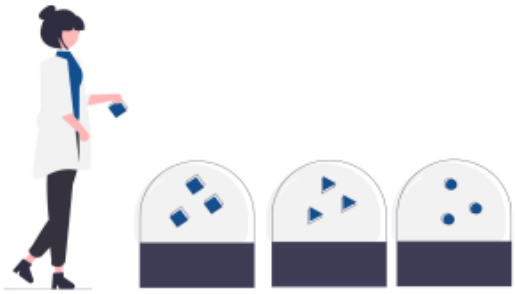
1. Date and Time of Use
2. Name of Student(s) operating the instrument
3. Instructor or Supervisor's Name
4. Number of Samples Tested
5. Analytical Method Title (e.g., "Tartrazine Analysis – UV–Vis Calibration")
6. Any Observations, Issues, or Comments (e.g., instrument malfunction, unusual readings, cleaning performed)

Cleaning and Decontamination

- **Before and After Each Lab**: Wipe benches with 70% reagent alcohol or isopropyl alcohol.
- **Glassware**: After each use, wash all glassware thoroughly with lab-grade soap and rinse with deionized water. Dry completely and return all items to their designated storage locations.
- **Equipment**: Follow the manufacturer's or instructor's cleaning protocols after using instruments.
- **End of Session**:
  - Disinfect the work area.
  - Remove gloves and wash hands.
  - Store PPE properly.

Waste Management and Disposal

All waste generated in the chemistry lab must be disposed of properly.



- **Solid Waste**: Place in labelled solid waste containers (not regular garbage).
- **Liquid Waste**: Dispose of in designated chemical waste containers by type (inorganics and organics).
- **Broken Glass**: Place broken glass in the designated broken glass waste bin (white metal bin) located in the lab. Do not dispose of broken glass in regular garbage.
- **Contaminated Materials**: Dispose of gloves, wipes, and contaminated consumables in the appropriate waste bins.

1.3 Core Safety Principles & Enforcement

Emergency Procedures

In case of accidents, spills, or exposure:

- Alert the Instructor and Nearby Students Immediately.
- Turn Off Equipment or Heat Sources only if safe to do so.
- Evacuate the Area if required.
- Contact Emergency Services – Dial 9-1-1 or campus line x4242.
- Use Safety Equipment: Eyewash or fire extinguisher as appropriate.
- Report All Incidents—including minor spills, cuts, or exposures—to the instructor.

Enforcement and Consequences

Violation of any laboratory rule may result in:

- Immediate removal from the lab.
- Loss of laboratory marks for that session.
- Disciplinary action for repeated or serious offences.
- Safety violations, including failure to wear PPE, unsafe handling of chemicals, or unauthorized experiments, are taken seriously and can lead to suspension of lab privileges.

Summary of Core Safety Principles

> **Always Plan Ahead** — Know what you are doing before you begin.
>
> **Always Protect Yourself** — Wear PPE and handle chemicals safely.
>
> **Always Prevent Contamination** — Clean your workspace before and after each experiment.
>
> **Always Practice Responsibility** — Dispose of waste properly and respect others' work.
>
> **Always Prepare for Emergencies** — Know the location and function of all safety equipment.

Remember!

Laboratory safety is not a checklist but a mindset.

Every student, at every bench, contributes to a safe, respectful, and professional scientific environment.

CHAPTER 2: LAB NOTEBOOK PREPARATION & DOCUMENTATION STANDARDS

Chapter Outline

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2.1 Lab Notebook Preparation & Documentation Standards

Purpose of a Laboratory Notebook

A laboratory notebook is an official, permanent record of all laboratory work performed. It documents experimental design, procedures, observations, results, and data interpretation. In both academic and professional laboratories, it serves as:

- A legal document that demonstrates scientific integrity and reproducibility.
- A communication tool that enables others to understand and replicate your work.
- A quality assurance record, compliant with recognized standards such as ISO/IEC 17025, USP, and GLP.

General Requirements

Table 2.1.1. General Laboratory Notebook Requirements and Specifications

Requirement	Specification
Notebook Type	Hard-bound laboratory notebook (no spiral binding).
Writing Tool	Blue or black ink only.
Page Use	Write neatly and legibly on each page. You may use both sides of the page for recording data, observations, and calculations.
Page Numbering	Pages must be pre-numbered or manually numbered consecutively.
Table of Contents (TOC)	Reserve the first 1–2 pages for a TOC or index. Keep it up to date.
Personal Information	Include your full name, course code, section number, and contact information on the front cover.
Supervisor Signature	Every session must be reviewed and signed by your instructor/supervisor before leaving the lab. Unsigned notebooks will not be graded.

Notebook Structure

Each experiment must follow this standardized format:

A. Header Information

- Page Number.
- Date (DD/MM/YYYY).
- Experiment Title – include the analyte, matrix, and instrument (e.g., *Determination of Iron in Soil Using Atomic Absorption Spectrophotometry*).
- Your Name, Lab Partner(s), and Group Code (e.g. A1, B3).
- Purpose/Objective – a short statement explaining *why* the experiment is performed.

B. Procedure Reference

- Cite the official method or lab manual (e.g., “Spectroscopy Lab from FOL Contents, CHEM5005, F2025, labs, LC Experiment 1”).
- If any modifications are made, record changes clearly in the notebook during the lab session.

C. Experimental Work and Observations

- Enter the experimental procedure steps into your lab notebook before coming to the lab.
- Redraw the tables provided in the instructions in your lab notebook to record measurements and data, if applicable.
- Record:
  - Amounts of reagents used and units of measurement.
  - Instrument model and settings used.
  - File names and storage locations of instrument data.
  - Qualitative observations (colour change, precipitation, gas evolution, etc.).

D. Data Presentation

- Use properly labelled tables, graphs, and units.
- Show sample calculations where applicable.
- Record results to the correct number of significant figures.

Notes

All data must be written clearly and directly into your lab notebook at the time the experiment is performed. Do not copy data from rough notes later—record it in real time as the original entry.

Keep your notebook clean, organized, and readable. Avoid contact with wet surfaces, spills, or chemical residues to maintain a professional appearance and preserve your records.

E. Error Corrections and Empty Spaces Handling Procedures

Table 2.1.2. Error Corrections and Empty Spaces Handling Procedures

Type	Action Required
Error or correction	Cross out with a single line, write the correction nearby, and initial + date the change. No white-out.
Blank spaces	Draw a diagonal line through the unused area and initial + date.

F. Digital Data

- If files are saved on a USB drive, clearly note the full path or folder name (e.g., “C:\FANSHAWE USB\CHEM3004\Lab 2\Group 5\AAS Results. Abs”).
- Ensure files are organized and accessible for verification if requested.
- Attach spectra, chromatograms, or other printouts to the end of your report.

G. Post-Lab Documentation

- Have your instructor review and sign your lab notebook at the end of the experiment.
- Scan or photograph the signed notebook pages clearly.
- Attach the scanned pages before the instrument printouts in your lab report and upload them together ***as one PDF*** file to the Fanshawe Online (FOL) submission folder.

2.2 Example Page Layout

Page #: 15

Date: 25/10/2025

Title: Determination of Phosphate in Soil by UV-Vis

Name: Tara Sarchami Partner: Mo Delavar Group: B1

Purpose:

To determine the concentration of phosphate in soil using the molybdenum-blue colorimetric method and UV-Vis analysis.

Reference:

Fanshawe Online Tool → CHEM3004 →W2025→ Labs → UV-Vis Experiment#2

Procedure:

Enter the experimental procedure ***step by step*** in this section

Observations:

- Solution turned deep blue upon adding stannous chloride.
- No visible precipitate.

Data Table: (Add a proper, descriptive caption)

Sample ID	**Absorbance**	**Concentration (mg/L)**
Std 1	0.124	0.5
Std 2	0.256	1.0
Soil	0.217	0.84

Results & Calculations:

$$C_{soil} = \frac{(A - b)}{m}$$

$$= \frac{(0.217 - 0.05)}{0.20}$$

$$= 0.84 mg/L$$

Instructor Signature: _____

Date: _____

2.3 Evaluation Criteria

Common Errors to Avoid

- Writing data after leaving the lab.
- Using loose sheets or erasable ink.
- Leaving blank pages or sections unmarked.
- Forgetting to include instrument name/model or file names.
- Submitting a report without signed notebook pages.

Evaluation Criteria

- 1 mark will be deducted if the lab notebook pages are not attached to your report.
  - Notebook pages must include the instructor's signature and date.
  - Attach the signed notebook pages at the end of your report (before instrument printouts).
  - Only pages signed by the lab instructor will be evaluated.
- 0.25 marks will be deducted for each missing item listed below:
  - Page number.
  - Date.
  - Title of experiment.
  - Student name.
  - Lab partner name(s).
  - Group number.
  - Purpose.
  - Reference the procedure.
  - Detailed procedure.
  - Instrument used.
  - Method used and file location where results are saved.
  - Lab instructor's signature and date.
  - Empty spaces not crossed out (must have a single diagonal line drawn through the unused area, then initialled and dated).
  - Errors not corrected properly (must be crossed out with a single line, then initialled and dated).

Final Reminder:

Your lab notebook is not just a record—it is part of your professional identity as a laboratory technologist. Keep it accurate, organized, and honest.

CHAPTER 3: GENERAL LAB REPORT FORMAT, SUBMISSION, & EVALUATION GUIDELINES

Chapter Outline

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3.1 General Lab Report Format, Submission, & Evaluation Guidelines

A well-structured laboratory report demonstrates not only your understanding of the analytical method but also your ability to communicate results in a clear, organized, and professional manner. The following formatting, submission, and evaluation standards apply to *all laboratory experiments* in this manual. Unless otherwise noted in a specific experiment, these rules are mandatory and will be used for grading consistency.

Report Structure and Order

All laboratory reports must be typewritten, 1.5-line spaced, and submitted electronically as a *single PDF file* through Fanshawe Online (FOL).

The report must follow this order:

1. Title Page.
2. Introduction / Theory.
3. Data and Calculations.
4. Results and Discussion.
5. References (APA format).
6. Signed Laboratory Notebook Pages.
7. Instrument Printouts (if applicable).

Each section must begin on a new page. Tables and figures should be clearly labelled, formatted professionally, and kept together on one page whenever possible.

Title Page

Every report must include a properly formatted title page containing the following information:

- Course number and section.
- Title of experiment.
- Student's name and lab partner's name(s).
- Group number.
- Lab instructor's name.
- Date the experiment was performed.
- Indication of whether it is an *individual* or *group* report.

Mark Deductions:
0.25 marks will be deducted for each missing or incorrect item.

Introduction/Theory, Data & Calculations, and Results & Discussion

Detailed expectations for the Introduction/Theory, Data and Calculations, and Results and Discussion sections—along with their corresponding marking rubrics—are specific to each experiment. These can be found at the end of each lab under the section titled "*Experiment-Specific Lab Report Guidelines and Evaluation Criteria.*"

Students must review that section before preparing their report to ensure all required questions, calculations, and discussion points are addressed accurately and meet the evaluation criteria.

Note: Experiment-specific rubrics complement (*not replace*) the general grading deductions outlined in this section of the manual.

References

All external sources (manuals, websites, textbooks, journal articles, etc.) used in your report must be cited using APA referencing style. Refer to the Learning Centre's APA guidelines [PDF], available through MyFanshawe, for details.

Mark Deductions:
0.5 marks will be deducted for missing or incorrect reference formatting.

Notebook Attachment

Your signed laboratory notebook pages are a required component of every submission.

- Include the scanned notebook pages *before* any instrument printouts at the end of the report.
- Only *signed and dated* pages will be evaluated.
- Follow notebook documentation standards outlined in the Lab Notebook Preparation and Documentation section.

Mark Deductions:
1.0 mark will be deducted if notebook pages are missing.
0.1 mark will be deducted for each missing detail (title, date, partner name, purpose, instrument used, etc.).

Instrument Printouts

When applicable, include the electronic printouts of your instrument data at the end of the report. UV-Vis experiments (Experiment #1 and Experiment #2) without software do not require this section.

Mark Deductions:
0.5 marks will be deducted for missing instrument printouts.

Report Appearance and Formatting

Reports are expected to meet professional standards of written communication and scientific presentation.

- 0.25 Marks will be deducted for poor report appearance, including:
  - Spelling or grammar errors.
  - Report sections are presented in the incorrect order.
  - Missing or unlabeled tables and figures.
  - Incorrect significant figures or units.

- Missing subscripts/superscripts in chemical formulas.
- Tables not fitting on one page.
- Use of first-person language (I, we, our).
- Failure to use the past tense when describing procedures or observations
- Missing or incomplete title page.

Note: All reports should be written in *third person* and *past tense* (e.g., "The solution was prepared…").

Lab Performance and Professional Conduct

Performance during lab sessions is part of the evaluation.

- 0.25 Marks will be deducted for poor lab performance, including:
  - Late arrival or leaving early without permission.
  - Failure to follow safety rules or PPE requirements.
  - Using a cell phone or other devices during lab work.
  - Ignoring instructor directions.
  - Unprofessional or disruptive behaviour.

Submission Notes

- Only one submission per group is allowed unless otherwise indicated.
- All uploaded files must be named clearly using the following format: CourseCode_Lab#_First&Last-Name(s).pdf
  - Example: CHEM3004_UV-Vis Lab1_Tara Sarchami & Mo Delavar.pdf.
- A submission folder has been set up for each instrument under:
  - FOL → Evaluation → Submission.

Report Submission Timeline

- **Due Date:** Lab reports are due four (4) days after the lab session in which the experiment was completed, by 9:00 PM.
  - (For example, if you complete your experiment on a Monday, your report is due on Friday by 9:00 PM.)
- **Submission Platform:** All reports must be submitted *online* through Fanshawe Online (FOL) using the appropriate instrument folder under *Evaluation → Submission*.
- **Late Policy:** 25% deduction if submitted within *24 hours* of the due date.
  - *No grade* will be assigned if submitted more than 24 hours late.
- **Marked Reports:** Reports will be returned *only after all groups have submitted* their results for that

experiment.

Summary of Common Deductions

Table 3.1.1. Typical Report Mistakes and Corresponding Mark Deductions

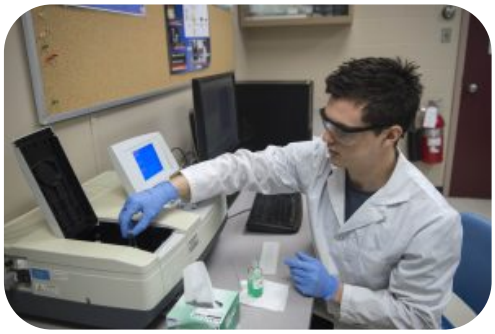
Category	Typical Deductions
Missing title page items	−0.25 each
Missing notebook pages	−1.0
Missing reference or APA error	−0.5
Poor report appearance	−0.25 each
Missing instrument printout	−0.5
Poor lab performance (e.g, Late arrival or unsafe behaviour)	−0.5 each
Late report submission (≤24 hrs)	−25%
Late report submission (>24 hrs)	No grade

Final Reminder

Laboratory reports are not merely assignments—they are professional scientific documents. Adhering to consistent formatting, organization, and referencing standards is essential for clear communication and for maintaining data integrity across all analytical techniques.

CHAPTER 4: UV-VIS SPECTROSCOPY

Chapter Outline



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4.1 Theory: UV-VIS Spectroscopy

Ultraviolet-visible (UV-Vis) spectrophotometry is a common analytical technique used to measure how much light a chemical substance absorbs within the ultraviolet and visible regions of the electromagnetic spectrum. When light passes through a sample, certain wavelengths are absorbed as molecules undergo electronic transitions from their ground to excited states. The amount of light absorbed is proportional to the concentration of the absorbing species, according to Beer–Lambert's Law:

$$A = \varepsilon b c$$

where

A is absorbance,
ε is molar absorpitivty ($L \cdot mol^{-1} \cdot cm^{-1}$),
b is the path length (cm),
and c is the analyte concentration ($mol \cdot L^{-1}$).

Instrument Components

A typical UV-Vis spectrophotometer consists of the following key components:

- **Light Source:** Two lamps provide illumination — a deuterium lamp for UV and a tungsten–halogen lamp for visible light. The instrument switches automatically based on the selected wavelength.
- **Monochromator:** Disperses light into its component wavelengths using a prism or diffraction grating and isolates a narrow wavelength band for measurement.
- **Sample Holder:** The sample is placed in a quartz cuvette (for UV and visible light) or a glass/plastic cuvette (for visible light only). The cuvette must be clean, clear, and properly aligned. Single-beam spectrophotometers have one sample holder, while double-beam instruments have two—one for the sample and one for the reference (blank) solution.
- **Detector:** Measures the transmitted light and converts it to an electrical signal. Common detectors include photodiodes, photomultiplier tubes (PMTs), or diode arrays.
- **Computer and Display:** Processes the detector signal, displays absorbance or transmittance data, and allows quantitative calibration.

Types of UV-Vis Spectrophotometers

Single-Beam Instruments

A single-beam spectrophotometer directs all light through the monochromator and then through the sample before reaching the detector. Measurements of blank and sample are taken separately.

Advantages:	Simple, low-cost, compact design.
Limitations:	Susceptible to lamp intensity drift between blank and sample readings.

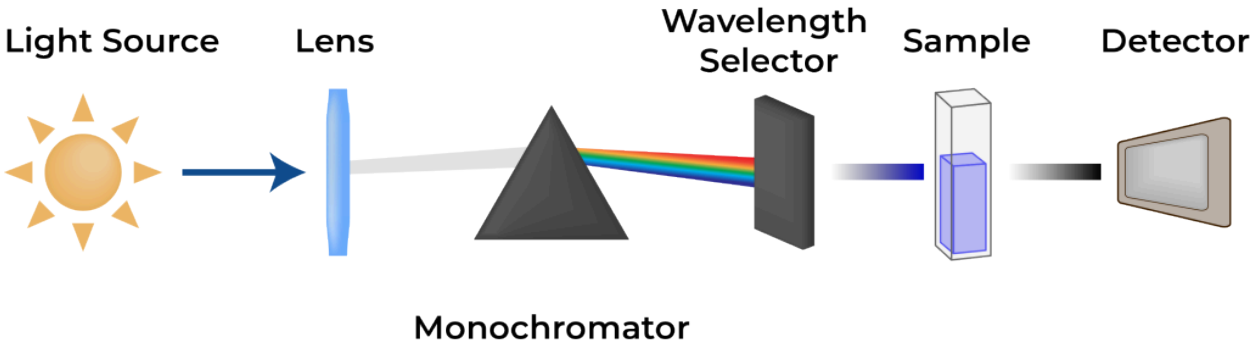

Figure 4.1.1. "Schematic Diagram of a Single Beam UV-Vis Spectrophotometer" by Koen Liddard, CC BY-NC-SA 4.0. Source: "Single Beam Spectrophotometer" by Science Vivid, FDEd.

Figure 4.1.2: Scientific Spectronic 200 Single Beam UV–Vis Spectrophotometer. Image by T. Sarchami, CC BY-NC-ND 4.0.

Double-Beam Instruments

A double-beam spectrophotometer splits the light into two paths-one through the reference (blank) and one through the sample. The instrument compares both signals simultaneously or alternately.

Advantages:	Compensates for lamp fluctuations and improves measurement stability.
Limitations:	More complex and expensive.

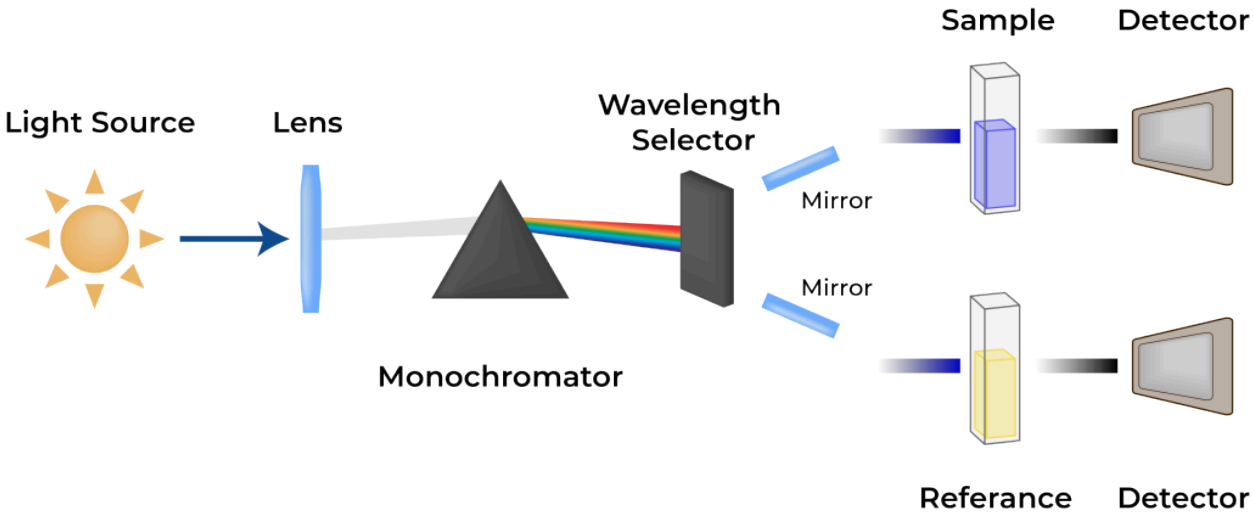

Figure 4.1.3. "Schematic Diagram of a Double Beam UV-Vis Spectrophotometer" by Koen Liddard, CC BY-NC-SA 4.0. Source: "Double Beam Spectrophotometer" by Science Vivid, FDEd.

Figure 4.1.4a: Shimadzu UV-1700, Double-Beam UV-Vis Spectrophotometer. Image by T. Sarchami, CC BY-NC-ND 4.0.

Figure 4.1.4b: Agilent Cary 60, UV-Vis Spectrophotometer. Image by T. Sarchami, CC BY-NC-ND 4.0.

Multichannel (Diode-Array) Instruments

Multichannel or diode-array spectrophotometers use a stationary diffraction grating and a photodiode or CCD detector array to record all wavelengths at once.

Advantages:	Rapid full-spectrum acquisition, high reproducibility, and ideal for kinetic or multi-component studies.
Limitations:	Slightly lower spectral resolution compared to scanning instruments.

Operating Tips for Accurate UV-Vis Measurements

To ensure precision and consistency, always follow these operating guidelines:

Instrument Preparation:

- Turn on the instrument 15–30 minutes before use to allow the lamps and electronics to warm up and stabilize.

Sample Cell Handling:

- Rinse the sample cell (cuvette or test tube) with deionized (DI) water three times, then rinse it three times with your sample solution before filling it for measurement.
- Ensure there are no fingerprints, scratches, or watermarks on the sample cell.
- Clean the outer surface of the sample cells with a Kimwipe before analysis.
- Cuvettes have two frosted and two clear sides—make sure the clear sides face the light path inside the instrument. Handle cuvettes by the frosted sides only.
- Avoid air bubbles or suspended particles in the solution, as they scatter light and affect readings.
- If visible particles or impurities are present, remove them using filtration or wet-chemistry cleanup before analysis.
- Use the same type of cuvette or test tube for all measurements to ensure consistent light transmission and minimize instrumental error.
- Keep the sample chamber closed during measurements to minimize stray light.
- If readings drift, verify warm-up time and re-zero before continuing.

Measurement Tips:

- In % Transmittance mode, set 0%T (no light) and 100%T (blank) using calibration controls.
- In Absorbance mode, zero the instrument with the blank before measuring samples.
- For single-beam instruments, re-blank every 3–4 samples, after wavelength changes, or if readings drift.
- Negative absorbance has no physical meaning; if observed, re-run the blank and re-zero before continuing.

Good Laboratory Practice:

- Record wavelength settings, cuvette type, and blank composition in your lab notebook.
- Close sample compartments during measurements to avoid stray light.
- Dispose of chemical waste properly after analysis.

4.2 Experiment #1

Analysis of Nickel Nitrate Hexahydrate and Cobalt Nitrate Hexahydrate Using Single Beam UV/VIS Spectrophotometer

Background

Nickel(II) and cobalt(II) nitrate solutions exhibit distinct colours—green for Ni^{2+} and pink for Co^{2+}—due to d–d electronic transitions within their partially filled d-orbitals. These transitions make them ideal for quantitative measurement by UV-Visible spectrophotometry, where absorbance at specific wavelengths can be related to concentration through Beer–Lambert's Law.

In this experiment, students measure the absorbance of standard and mixed nickel and cobalt nitrate solutions at 394 nm and 511 nm to calculate molar absorptivity coefficients and, using simultaneous equations, determine the concentrations of each metal in a mixed solution.

Monitoring nickel and cobalt levels in water is essential because both metals, while useful industrially, can be harmful at elevated concentrations. Nickel is widely used in electroplating, stainless steel, batteries, and pigments, and may leach into drinking water through corrosion of plumbing or industrial discharge. Chronic exposure can cause skin sensitization (nickel dermatitis), respiratory irritation, and systemic toxicity. Cobalt, although an essential trace nutrient involved in vitamin B_{12} synthesis, can cause cardiac, thyroid, and neurological disorders when present in excess.

From an environmental standpoint, both metals can accumulate in sediments and aquatic organisms, leading to long-term ecological impacts and biomagnification through the food chain. Industrial effluents from metal finishing, mining, and battery manufacturing are major sources of nickel and cobalt contamination. Regulatory bodies such as Health Canada and the U.S. EPA set strict limits on their concentrations in drinking and surface waters (typically below 0.02–0.1 mg/L) to prevent adverse health and environmental effects.

This experiment demonstrates how UV-Vis spectrophotometry provides a simple, cost-effective, and sensitive method for monitoring trace levels of transition metals, supporting both environmental compliance and industrial quality control.

Learning Objectives

After completing this experiment, students will be able to:

1. Explain the operating principles and key components of a UV-Vis spectrophotometer.
2. Apply Beer–Lambert's Law to determine molar absorptivity and quantify Ni^{2+} and Co^{2+} concentrations.
3. Perform dual-wavelength, multicomponent analysis to resolve individual metal ion concentrations in a mixture.

Safety Precautions

1. Nickel and cobalt nitrate hexahydrates are oxidizers and skin/eye irritants; nickel is a known sensitizer. Avoid inhalation/ingestion; solutions can stain skin and surfaces.
2. Collect all Ni/Co solutions, rinses, and contaminated disposables in the labelled heavy-metal waste. Do not pour to drain.

Procedure

A. Standard Preparation

1. Prepare a solution of nickel nitrate hexahydrate by dissolving 4.7±0.1 g in distilled water and diluting to volume in a 50 mL volumetric flask. Record the actual weight of powder used to 4 decimal places. Calculate the concentration of your standard and express the results in terms of molarity (M).
2. Prepare a solution of cobalt nitrate hexahydrate by dissolving 4.6±0.1 g in distilled water and diluting to volume in a 50 mL volumetric flask. Record the actual weight of powder used to 4 decimal places. Calculate the concentration of your standard and express the results in terms of molarity (M).

B. Sample Analysis: Instrument & Software Setup

1. Confirm that the instrument and power adapter are properly connected to a grounded electrical outlet.
2. Locate the power switch on the back of the instrument and turn it ON.
3. The display will show the Thermo Scientific splash screen followed by self-diagnostic checks (memory test, lamp test, dark current, and auto-zero).
4. When prompted, ensure the sample chamber lid is closed, and no cuvettes or test tubes are inserted.

5. Wait until the initialization completes and the Home Menu appears.
6. Leave the instrument powered ON for 15–30 minutes to allow the lamp to stabilize before collecting data.
7. On the Home Menu, the SPEC 200E Modern Interface is highlighted. Press "*Enter*" to select it.
8. On the next screen, choose "*Live Display*" mode — this displays real-time absorbance or % transmittance readings.
9. Use the arrow keys to highlight "*Measurement Mode*."
10. Press Enter, then toggle to ABS (Absorbance).
    - If %T appears, toggle again until ABS is displayed in green.
11. Highlight "*Measurement λ (Wavelength)*" and rotate the λ knob to set the desired wavelength.
    - Rotate normally → changes by 10 nm increments
    - Press down and rotate the λ knob or use left and right arrow → changes by 1 nm for fine adjustment

Wavelength #1 – 394 nm Setup and Calibration

12. Set the wavelength to 394 nm.
13. Place a clean test tube (the same type to be used for all measurements) into the holder to ensure it fits securely.
14. Measure and record the path length of the test tube to ± 0.1 cm for Beer's Law calculations.
15. Fill a clean test tube about ¾ full with distilled water (blank).
16. Wipe the outside with a Kimwipe to remove droplets or fingerprints.
17. Insert the test tube into the sample holder designed for test tubes, and close the lid.
18. Press the Auto Zero (0.00) button.
    - The display will show "*Performing Auto Zero...*"
    - Wait until the screen returns to 0.000 A.
    - This sets 100 % T (0.000 A) for all subsequent readings at 394 nm.
19. Leave the blank in place during setup; remove it only when ready to measure standards.

Measuring Standards and Unknowns (394 nm)

20. Remove the blank test tube and insert your first standard solution (Ni nitrate hexahydrate).
21. Close the lid — the absorbance reading will update automatically.
22. Record the absorbance value in your laboratory notebook.
23. Repeat for all remaining standard solutions.
24. Measure and record absorbance at 394 nm for:
    - Unknown #1 (Ni only)
    - Unknown #2 (Ni + Co)

Wavelength #2 – 511 nm Setup and Measurement

25. Rotate the λ knob to set the wavelength to 511 nm.
26. Insert the blank (distilled water) and press Auto Zero (0.00) again to re-establish the baseline at the new wavelength.
27. Measure and record absorbance for:
    - Ni standard
    - Co standard
    - Unknown #2 (Ni + Co) only
28. Record all readings in your lab notebook and data table.

29. When all data are collected, press the "*Home*" key to return to the main menu.
30. Remove all test tubes from the holder.
31. Inspect the sample compartment for any spills or moisture:
    - Wipe gently with a clean, lint-free cloth.
    - If necessary, remove the compartment floor for cleaning (use water or ethanol — never acetone).
32. Turn OFF the instrument using the rear power switch.
    Leave the lid open for several minutes to allow any moisture to evaporate.
33. Rinse all test tubes thoroughly. Wash with a few drops of lab-grade detergent, then rinse with deionized water, and dry with Kimwipes or lens tissue.

Experiment-Specific Lab Report Guidelines and Evaluation Criteria (10 marks)

Introduction (3 marks)

- Describe the function of each component of the instrument (source, wavelength selector, sample compartment, detector), as well as the setup required before sample readings can be taken on this single-beam instrument.

Data and Calculations (5 marks)

- Complete the following table and put a copy of it in your report. (1 mark)

Standard Preparation and Analysis				
	Weight (g)	**Concentration (M)**	**Abs.@394nm**	**Abs.@511nm**
Nickel Nitrate Hexahydrate				
Cobalt Nitrate Hexahydrate				
Sample Analysis				
	Abs.@394m		**Abs.@511nm**	
Unknown #1				
Unknown #2				
Path Length of TestTube (cm)				

- Using the data from your standard solution and the path length of the test tube used in your experiment for Part B, calculate the molar absorptivity of each salt at each wavelength. (1 mark)
- Use Beer's Law to determine the concentration of the nickel nitrate hexahydrate in unknown#1. Express your answer in (M). (1 mark)
- Determine the amount of cobalt nitrate hexahydrate and nickel nitrate hexahydrate in unknown#2. Express your answers in (M). (2 marks).

Results and Discussion (2 marks)

- Give an example of a deviation to Beer's Law that could have affected this experiment and discuss how it could have affected your results. Your discussion should include whether the reported concentration would be higher or lower than the true value and an explanation for your conclusion.

4.3 Experiment #2

Quantitative Determination of Soil Phosphate by the Molybdenum Blue Method Using a Double-Beam UV–Vis Spectrophotometer (Shimadzu UV-1700 PharmaSpec)

Background

Phosphorus is one of the essential macronutrients required for plant growth, playing a crucial role in energy transfer (ATP), root development, and overall crop productivity. In soils, phosphorus exists in various chemical forms—organic, inorganic, and adsorbed phases—many of which are not immediately available to plants. The term “available phosphate” refers to the soluble and easily extractable forms that plants can uptake, whereas “total phosphate” includes all forms, including those bound to soil minerals or organic matter.

Excessive phosphate fertilization can lead to leaching and runoff into nearby water bodies, contributing to eutrophication, a process where nutrient enrichment stimulates algal blooms and depletes oxygen levels in aquatic systems. Monitoring phosphate levels in soil is therefore essential to balance crop nutrient management with environmental protection.

The molybdenum-blue method is a widely used colorimetric technique for determining phosphate concentrations in environmental and agricultural samples. In this method, orthophosphate reacts with ammonium molybdate under acidic conditions to form phosphomolybdic acid, which is subsequently reduced by stannous chloride ($SnCl_2$) to produce a blue-colored complex known as molybdenum blue. The intensity of the blue colour, measured spectrophotometrically at 650 nm, is directly proportional to the phosphate concentration in the sample.

Learning Objectives

After completing this experiment, students will be able to:

1. Explain the principle of colorimetric determination of phosphate using the molybdenum-blue method and UV–Vis spectrophotometry.
2. Prepare soil leachate samples, construct a calibration curve, and determine phosphate concentration from experimental data.
3. Analyze results for accuracy and precision by calculating mean, standard deviation, %RSD, and identifying experimental errors.

Safety Precautions

1. Sulfuric acid is highly corrosive and generates heat when mixed with water. Always add acid to water slowly while stirring, and work under a fume hood.
2. Stannous chloride is toxic and a strong reducing agent; avoid skin contact and inhalation. Prepare and handle in a fume hood whenever possible.
3. Ammonium molybdate is an irritant; wear gloves and avoid inhaling dust.
4. Collect all waste solutions in a clearly labelled “Phosphate Colorimetric Waste” container. Do not pour any chemical waste down the drain.

Procedure

Preparation of Reagents

a) Ammonium Molybdate Reagent

1. Dissolve 2.5 g of ammonium molybdate tetrahydrate in 30 mL of deionized water in a 150 mL beaker.
2. Place the beaker in an ice bath and allow it to cool.
3. Gradually add 28 mL of concentrated sulfuric acid to the solution while stirring. This step generates significant heat; keep the beaker on ice to prevent splattering.
4. Once cooled, add the molybdate solution to the acid mixture and stir until uniform.

b) Stannous Chloride Reagent

1. Dissolve 1.25 g of stannous chloride dihydrate ($SnCl_2 \cdot 2H_2O$) in 50 mL of glycerine.
2. Gentle heating may be required to ensure complete dissolution.

Preparation of Standard Solutions

a) Stock Phosphate Standard

1. Weigh out approximately 25.0 mg of potassium dihydrogen phosphate (KH_2PO_4) and record the exact mass.
2. Transfer the solid to a 50 mL volumetric flask, dissolve with deionized water, and make up to the mark.
3. Calculate the exact phosphate concentration in mg/L based on the precise mass used.

b) Working Phosphate Standard

Pipette 5.0 mL of the stock solution into a 50.0 mL volumetric flask and dilute to the mark with deionized water. Mix well.

c) Calibration Standards

Prepare a series of standards using the table below by diluting the given volumes of the working standard to 50.0 mL with deionized water. Compute the phosphate concentration for each.

Standard	Volume of Working Standard (mL)	Phosphate Concentration (mg/L)
A	0.5	
B	1.0	
C	2.0	
D	5.0	

Leachate Sample Preparation

1. Weigh three separate 1.0 g portions of potting soil.
2. Transfer each to a glass funnel lined with filter paper.
3. Pour deionized water through each funnel and collect the filtrate in a 50.0 mL volumetric flask.
4. Stop filtration once the flask reaches the mark. These solutions will serve as your leachate samples.

Colour Development

1. To each flask (standards and samples), add 1.0 mL of ammonium molybdate reagent.
2. Add 2 drops of stannous chloride reagent.
3. Mix thoroughly and note the time.
4. The colour must develop for exactly 10 minutes before measurement.

Sample Analysis: Instrument and Software Setup

1. Confirm the instrument and display unit are properly connected to power.
2. Locate the power switch on the left-hand side of the instrument and turn it ON.
3. Allow the instrument to complete its self-check and initialization process (approximately 3 minutes).
4. Keep the sample chamber lid closed during this period.
5. After initialization, leave the instrument powered on for 15–30 minutes to allow the light source to stabilize before taking measurements.
6. Once initialization is complete, the main menu will appear on the display.
7. Press "*1*" to select Photometric Mode.
8. Confirm that the instrument is set to Absorbance (ABS) mode.
9. If the display shows % Transmittance (%T) instead, press the F1 key on the control panel to switch to Absorbance (ABS).
10. Press the "*Go To WL*" (Go to Wavelength) button.
11. Enter the wavelength 650 nm using the keypad.
12. Press "*Enter*" and wait for the display to update to the new wavelength.
13. Prepare two identical cuvettes filled with the blank solution (contains all reagents except the analyte).
14. Insert one blank cuvette into the Reference Cell (rear position) and the other into the Sample Cell (front position).
15. Close the sample chamber lid.
16. Press the "*Auto Zero*" button.
17. Wait until the display indicates Absorbance = 0.000.
18. Remove the blank from the Sample Cell (front) but leave the blank in the Reference Cell (rear) for the remainder of your measurements.
19. Fill a clean plastic cuvette about three-quarters full with the first standard solution.
20. Wipe the cuvette with a Kimwipe to remove any droplets or fingerprints.
21. Insert the cuvette into the Sample Cell (front position) with the transparent sides facing the light path.
22. Close the sample chamber lid.
23. The absorbance reading will appear automatically on the display—no additional buttons need to be pressed.
24. Record the absorbance value to three decimal places in your laboratory notebook.
25. Repeat steps 18–23 for each remaining standard and sample solutions.
26. Once all sample readings have been recorded, press the "*Mode*" button to return to the Main Menu.
27. Carefully remove both the sample and reference cuvettes from their holders.
28. Check that the sample compartment is clean, dry, and free of spills or fingerprints.
29. Turn off the instrument using the power switch located on the left-hand side of the spectrophotometer.
30. Leave the sample chamber lid open for several minutes to allow air circulation and evaporation of any residual moisture before closing or covering the instrument.
31. Rinse each cuvette thoroughly with deionized water to remove any remaining solution.
32. Dry the cuvettes gently using clean lens tissue or Kimwipes, ensuring that no streaks or residue remain on the optical faces.

Experiment-Specific Lab Report Guidelines and Evaluation Criteria (10 marks)

Introduction (3 marks)

- Explain why measuring the available phosphate concentration is more meaningful for agricultural management than measuring total phosphate. (1 mark)
- Phosphorus in soil can exist in several chemical forms. Based on your understanding of soil chemistry, why might certain phosphate compounds remain unavailable to plants even when total phosphorus levels are high? (Hint: consider chemical bonding, mineral interactions, or pH effects.) (1 mark)
- Excess phosphate leaching from agricultural soils can lead to algal blooms in lakes and rivers. Suggest one effective preventive measure to minimize algal bloom formation caused by phosphate runoff and briefly explain how it helps reduce nutrient loading in aquatic systems. (1 mark)

Data and Calculations (5 marks)

- Plot absorbance (y-axis) vs phosphate concentration (x-axis) for the standard solutions. Use Excel or similar software to determine the equation of the best-fit line and the regression coefficient. Use this equation to calculate the phosphate concentration (mg/mL) for each leachate sample. (1 mark)
- Express phosphate content as mg phosphate per gram of soil. (1 mark)
- Determine the mean, standard deviation, and %RSD for your results. (3 marks)

Results and Discussion (2 marks)

- Identify at least two possible sources of error in this experiment (e.g., instrumental or procedural) and classify each as random or systematic. Explain your reasoning. (1 mark)
- The blue complex forms as a result of a redox process. Predict what might happen to the observed colour intensity if the reducing agent ($SnCl_2$) were omitted or added in excess. Explain your answer. (1 mark)

4.4 Experiment #3

Determination of Tartrazine in Yellow M&M Using a Double-Beam UV-VIS Spectrophotometer (Agilent Cary 60)

Background

Tartrazine (FD&C Yellow No. 5) is a synthetic lemon-yellow azo dye commonly used in foods, beverages, and pharmaceuticals to impart colour. It is one of the most widely used food colourants worldwide due to its bright hue and low cost. However, its use is regulated because excessive intake may cause allergic reactions, hyperactivity in children, or intolerance in sensitive individuals. Determining the concentration of tartrazine in foods ensures compliance with safety limits established by regulatory agencies such as Health Canada, the U.S. FDA, and the European Food Safety Authority (EFSA).

In this experiment, the quantitative determination of tartrazine in yellow M&M candies is performed using Cary 60 UV-Visible spectrophotometry, a technique that measures the absorbance of visible light by coloured solutions. Tartrazine strongly absorbs light in the blue-violet region of the visible spectrum, with a maximum absorbance (λmax) around 425 nm. By comparing the absorbance of candy extracts to a series of prepared standards, the concentration of tartrazine in the sample can be calculated using the calibration curve derived from the Beer-Lambert Law.

Learning Objectives

After completing this experiment, students will be able to:

1. Quantitatively determine tartrazine concentration in yellow M&M samples using Cary 60 UV-Vis spectrophotometry at 425 nm.
2. Construct and evaluate a calibration curve using Beer–Lambert Law to determine sample concentration.
3. Assess method accuracy and precision using statistical tools (%RSD, relative error, Q-test).
4. Demonstrate good analytical technique, instrument calibration, and proper data recording for quality control applications.

Safety Precautions

1. Tartrazine solutions are low-toxicity but may stain skin and clothing. Handle with care.
2. Waste solutions should be collected in a labelled “Food Dye Waste” container and not poured down the drain.
3. Glassware and cuvettes should be rinsed with DI water immediately after use to prevent residue buildup.
4. Avoid direct contact with chemical reagents and solvents; handle all solutions carefully near the instrument to prevent spills.

Procedure

Standard Preparation

- Tartrazine solution was purchased from Sigma-Aldrich with 0.25% (w/v) solution in water.
- Stock standard (provided by instructor) prepared by diluting 7.5 mL of the tartrazine solution from the supplier to 1.0 L in water. Instrument standards are prepared by pipetting known amounts of stock solution (see table below) into 25.0 mL volumetric flasks and diluting to volume with water.
- Prepare the following standard solutions and determine their concentration.

Standard	mLs stock in 25mL	Standard Concentration (µg/mL)
A	1	
B	5	
C	10	
D	15	

- **Check standard preparation**: prepare three additional solutions of Standard C and measure its absorbance to be used as a check standard for accuracy determination.
- **Reproducibility**: prepare three additional solutions of Standard B and measure their absorbances to be used to calculate standard reproducibility.

Sample Preparation

1. Place one yellow M&M into a small beaker and add 5 mL of distilled water. Gently swirl the beaker until the colored coating is completely dissolved, ensuring the chocolate core remains intact. The solution will turn yellow.
2. Filter the yellow solution through a Whatman No. 31 filter paper to remove insoluble particles. If the filtrate remains cloudy, further filter it through a 0.4 µm PVDF syringe filter to obtain a clear solution.
3. Pipette 1.0 mL of the clear filtrate into a 10.0 mL volumetric flask, then dilute to the mark with distilled water. Mix thoroughly to ensure homogeneity.

4. Repeat Steps 1–3 to prepare two additional sample solutions.
5. Transfer the blank, standard, and sample solutions into 1.0 cm pathlength plastic cuvettes for measurement.

Sample Analysis: Instrument and Software Setup

1. Ensure the spectrophotometer, computer, and monitor are properly connected to power and that all communication cables are securely attached.
2. Verify that the sample compartment is empty and the lid is closed before powering on the instrument.
3. Locate the power switch on the front right-hand side of the Cary 60 and turn it ON.
4. The instrument will complete its initialization in approximately 2.5 minutes. Keep the sample chamber lid closed during this process.
5. Turn on the connected computer and log in using the username and password provided on the instrument case.
6. Open the Cary Win UV folder on the desktop and double-click the "*Concentration*" icon to launch the software.
7. A successful connection is indicated when the two grey numbers in the upper left and upper right corners of the software interface turn red.
8. Click the "*Setup*" button located in the upper left corner of the software window.
    - Under the ***Cary tab***:
        - Enter the desired wavelength (425 nm).
    - Under the ***Standard tab***:
        - Select "*Calibrating during run*."
        - Select the concentration unit (e.g., mg/L).
        - Specify the number of standard solutions to be analyzed and enter their concentrations into the table.
    - Under the ***Samples tab***:
        - Enter the number of samples to be analyzed and, optionally, assign names to each sample.
    - Click OK to save settings and exit the setup window.
9. Place the blank solution into the sample compartment.
    - Press "*Zero*" (top left of the screen under the Setup icon) to set the baseline absorbance.
10. Remove the blank from the compartment and insert the first standard solution into the sample compartment.
11. Click on "*Reread*" on the top left of the screen.
12. In the pop-up window, select the standards or samples to analyze and move them to the left-hand side ("Solutions available") using the double-left arrow.
13. Click OK to start the reading.
14. When prompted by the pop-up window, remove the current solution and replace it with the next one.
15. Click OK after each replacement to continue measurements.
16. Repeat this step for all remaining standards and samples.
17. After completing all measurements, click "*Print*" in the lower left corner of the screen.
18. Choose *Save Report* to store the output file to a USB.
19. The report will include:
    - Absorbance readings (ABS) for all standard and sample solutions.
    - The calibration curve generated from the standards.
    - The regression equation of the calibration line used for concentration calculations.
20. Remove all cuvettes from the sample compartment.
21. Ensure the sample chamber is clean and dry before closing the lid.

22. When finished, exit the Cary Win UV software, turn off the computer monitor, and finally, turn off the spectrophotometer using the front power switch.
23. Rinse cuvettes with deionized water and gently dry with lint-free tissue.

Sample	Absorbance @ 425 nm
Blank	
Std A	
Std B	
Std C	
Std D	
Std C	
Std C	
Std C	
Std B	
Std B	
Std B	
Yellow M&M sample 1	
Yellow M&M sample 2	
Yellow M&M sample 3	

Experiment-Specific Lab Report Guidelines and Evaluation Criteria (10 marks)

Introduction (4 marks)

- Describe how the molecular structure of tartrazine allows it to absorb visible light and explain why UV–Visible spectrophotometry is an appropriate method for its quantitative analysis. (2 marks)
- Perform an online search and determine what is the maximum allowable concentration or acceptable daily intake of tartrazine in foods and beverages according to Health Canada, and what natural colourants can be used as alternatives to tartrazine? (2 marks)

Data and Calculation (4 marks)

- Calculate mass of Tartrazine in your M&M candy, µg Tartrazine/g of original sample from package. (1 mark)
- Determine accuracy using relative error of check standard (Std C). Calculation (µg/mL) determined by its absorbance (average of triplicate readings) and the equation for your calibration curve vs. the theoretical calculation based on the amounts you used to prepare it. (1 mark)
- Determine Standard Reproducibility: Standard reproducibility determined by %RSD of the absorbance readings of the three Standard B measurements. (1 mark)
- Determine Sample Reproducibility: Sample reproducibility determined by %RSD of the final concentration (µg/mL) of your three samples. (1 mark)

Results and Discussion (2 marks)

- Evaluate the quality of your calibration curve. Discuss the linearity (R^2 value) and explain what this indicates about the reliability of your quantitative measurements. (1 mark)
- Identify and discuss at least two possible sources of error that could affect the absorbance readings or concentration calculations. Suggest how these could be minimized in future analysis. (1 mark)

CHAPTER 5: ATOMIC ABSORPTION SPECTROSCOPY

Chapter Outline

Image by Fanshawe College. © All Rights Reserved.

5.1 Theory: Flame Atomic Absorption Spectroscopy

Flame Atomic Absorption Spectroscopy (FAAS) is a quantitative technique used to determine metal concentrations in liquid samples by measuring the light absorbed by free atoms. In FAAS, the sample solution is aspirated into a flame, where it is atomized. A hollow cathode lamp specific to the target element emits light at its characteristic wavelength. As this light passes through the flame, part of it is absorbed by ground-state atoms. The absorbance, according to Beer–Lambert's law, is directly proportional to the element's concentration in the sample.

Instrument Components

A typical Flame Atomic Absorption Spectrometer (FAAS) consists of the following key components:

Radiation Source (Hollow Cathode Lamp)

Emits sharp, element-specific spectral lines that match the analyte's absorption wavelength.

- Our instrument uses a deuterium lamp for background correction, compensating for light scattering from particulates or flame gases.

Nebulizer

Converts the liquid sample into a fine aerosol (mist) for introduction into the flame.

- A pneumatic nebulizer is used in our lab, relying on compressed gas (air or acetylene) to produce a stable, reproducible mist.

Atomizer (Flame System)

The aerosol enters the burner, where the solvent evaporates and solute dissociates into free atoms.

- An air–acetylene flame is used in our instrument—air serves as the oxidizer and acetylene as the fuel—producing a stable flame around 2300 °C, suitable for most elements.

Monochromator

Isolates the analyte's characteristic wavelength and removes stray or scattered light.

Detector

Measures the reduction in light intensity caused by atomic absorption and converts it into an electrical signal.

Our instrument uses a CMOS detector for high sensitivity, low noise, and excellent stability.

Readout/Computer System

Processes the detector signal, displays absorbance, and calculates concentration using calibration curves.

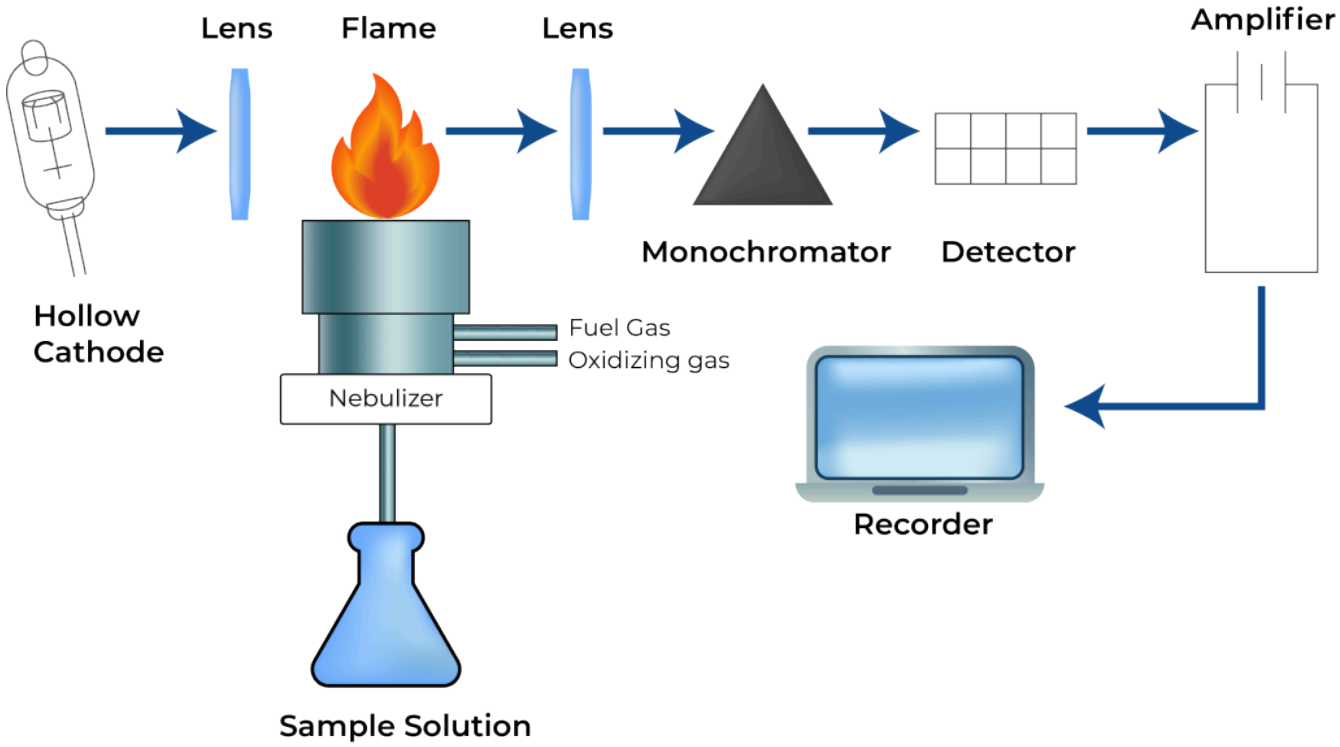

Figure 5.1.1. "Schematic Diagram of Flame Atomic Absorption Spectroscopy" by Koen Liddiard, CC BY-NC-SA 4.0. Source: "Atomic Absorption Spectroscopy" in Learning Chemistry by Priyam Study Centre, FDEd.

Image Description

A diagram of a flame atomic absorption spectroscopy (FAAS) setup. On the left, a sample solution enters a nebulizer connected to a flask. The nebulizer sprays the sample into a burner that produces a tall flame. Light from a hollow-cathode lamp passes through the flame and then through a series of components: focusing lenses, a monochromator (shown as a triangular prism), and a detector grid. The detected signal is sent to a computer, which displays the measurement results. Arrows indicate the direction of light and data flow throughout the system.

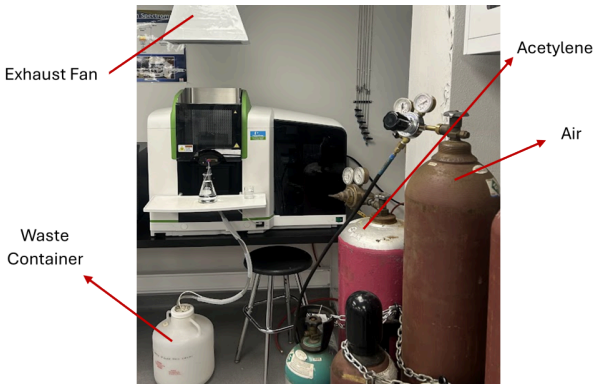

Figure 5.1.2. PinAAcle 900F Perkin Elmer Flame Atomic Absorption Spectrometer. Image by T. Sarchami, CC BY-NC-ND 4.0. Click to enlarge.

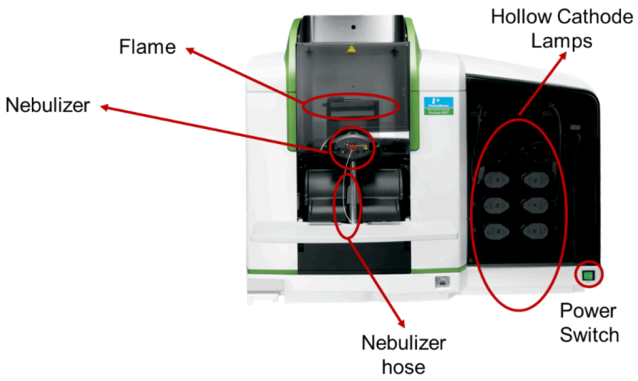

Figure 5.1.3. Main Components of PinAAcle 900F Perkin Elmier Flame Atomic Absorption Spectrometer. Image by T. Sarchami, CC BY-NC-ND 4.0. Click to enlarge.

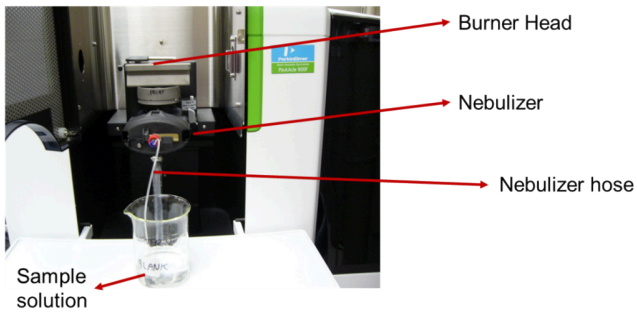

Figure 5.1.4. Sample Introduction System of PinAAcle 900F Perkin Elmer Flame Atomic Absorption Spectrometer. Image by T. Sarchami, CC BY-NC-ND 4.0. Click to enlarge.

Figure 5.1.5. Hollow Cathode Lamp Compartment of PinAAcle 900F Perkin Elmer Flame Atomic Absorption Spectrometer. Image by T. Sarchami, CC BY-NC-ND 4.0. Click to enlarge.

Image Descriptions

Figure 5.1.2: Photograph of a flame atomic absorption spectrometer on a lab bench. An exhaust fan hood hangs above the instrument. A white plastic waste container with tubing attached sits on the floor in front of the bench. On the right side of the image, large compressed-gas cylinders for acetylene and air stand secured with chains and fitted with regulators and gauges.

Figure 5.1.3: Front view of a PerkinElmer flame atomic absorption spectrometer. The central compartment, where the flame forms, is open and highlighted, with a nebulizer and nebulizer hose leading into it. On the right side, the door to the hollow cathode lamp compartment is circled, and a small green power switch is indicated at the bottom right of the instrument.

Figure 5.1.4: Close-up of the sample introduction area of the flame atomic absorption spectrometer. The rectangular metal burner head is at the top, with the nebulizer positioned just below it. A clear nebulizer hose

runs from the nebulizer down into a beaker of clear liquid labelled “BLANK,” representing the sample or blank solution sitting on the instrument tray.

Figure 5.1.5: Close-up of the open hollow cathode lamp compartment on the right side of the atomic absorption spectrometer. The compartment door is open to reveal multiple circular lamp sockets arranged in two vertical columns, with one hollow cathode lamp installed in an upper socket and several empty positions available for additional lamps.

Operating Tips for Accurate FAAS Measurements

To ensure safe, precise, and efficient operation, follow these steps carefully. Instrument operation and shutdown must always be performed under instructor supervision.

Instrument Overview

Before operating the instrument, the instructor will point out the location and function of the following key components:

- Acetylene gas tank and air gas tank
- Burner head assembly
- Nebulizer tube
- Hollow cathode lamps

Sample Preparation

- Use clean, acid-washed glassware to prevent contamination.
- Prepare all standards and samples in the same matrix (same acid type and concentration) to minimize matrix effects
- Filter samples to remove particulates or precipitates that may clog the nebulizer or scatter light.
- Filter any turbid solution before running the analysis.
- Avoid introducing samples with high total dissolved solids (TDS), as they can block the nebulizer and affect signal stability.

Flame Operation

- Ensure the nebulizer and burner head are clean and properly aligned.
- Ignite the flame and ensure a stable, blue flame with minimal noise.
- Adjust the fuel-to-oxidant ratio for optimal stability and sensitivity.

- Never leave the instrument unattended while the flame is lit.
- Keep burner slot and nebulizer clean-carbon deposits can distort readings.

Measurement Procedure

- Begin each run by aspirating the blank to zero the instrument.
- Aspirate standards in order of increasing concentration to build a calibration curve.
- Aspirate sample solutions.
- Between samples, aspirate deionized water to rinse the nebulizer and prevent carry-over.
- If readings drift or fluctuate, re-run the blank and check the burner head and nebulizer for cleanliness and blockages.

Quality Checks and Troubleshooting

- Negative absorbance values are nonphysical; if observed, re-zero with the blank.
- Baseline drift or excessive noise may indicate that the lamp has not fully warmed up, the flame is unstable, or there are air bubbles or blockages in the nebulizer line.
- If the burner slot becomes dirty, extinguish the flame, allow it to cool, and gently clean using a soft brush or compressed air.

Shutdown Procedure

- Rinse the system: Aspirate deionized water for several minutes to clean the nebulizer and burner.
- Extinguish the flame: Turn off the flame using the switch icon in the software.
- Depressurize gas lines: Close both gas tanks, then click “Bleed Lines” to release any residual pressure.
- Save data: Export results as a PDF file and save them to a USB drive or shared folder.
- Close software: Exit WinLab 32 completely before powering down.
- Power down: Switch off the instrument power, then turn off the ventilation system.
- Cool down: Allow the burner head and components to cool completely before cleaning or disassembly

5.2 Experiment #1

Analysis of Copper in Water by External Standard Calibration by Flame Atomic Absorption Spectroscopy

Background

Copper is an essential trace element, but can become toxic when present in elevated concentrations. It commonly enters water systems through the corrosion of household plumbing, industrial effluents, and runoff from mining or agricultural activities. Monitoring copper levels in drinking and environmental waters is therefore critical for protecting public health and ensuring compliance with regulatory standards such as those set by Health Canada and the U.S. Environmental Protection Agency (EPA).

Excess copper can cause unpleasant taste, staining of plumbing fixtures, and, at high levels, gastrointestinal and liver toxicity. Accurate determination of copper concentrations in water supports environmental monitoring, industrial quality control, and assessment of treatment efficiency in water purification systems. This experiment demonstrates a quantitative analytical approach for trace metal assessment in real-world water samples.

Learning Objectives

After completing this experiment, students will be able to:

1. Prepare calibration standards and construct a calibration curve for copper determination.
2. Quantify copper concentration in unknown water samples using external standard calibration.
3. Evaluate accuracy and detection limits using calibration data and statistical tools.
4. Identify potential interferences affecting analytical results.

Safety Precautions

- Operate the flame AAS only under instructor supervision.
- Ensure ventilation is on before lighting the flame and during all measurements.
- Handle nitric acid solutions with care—avoid skin or eye contact and clean spills immediately.
- Dispose of acid and metal waste in the inorganic waste container; do not pour into the sink.

Procedure

Note: All glassware used in this experiment must be acid-washed with 1% nitric acid before use to prevent contamination.

A. Standard & Sample Preparation

1. Using the provided 1000 ppm Cu stock standard, prepare a 10 ppm intermediate standard. Accurately pipette 0.50 mL of the 1000 ppm Cu solution into a 50.0 mL volumetric flask, then fill to the calibration mark with 1% nitric acid solution (provided by the instructor). Mix thoroughly to ensure uniform concentration.
2. Using the intermediate standard, prepare 50.0 mL of each of the following working standards: 0.5 ppm, 1.0 ppm, 2.0 ppm, and 4.0 ppm. Dilute each solution to the mark with 1% nitric acid.
3. Two unknown water samples will be provided by the instructor. One of the samples (Unknown #2) has a high copper concentration and must be diluted. Pipette 5.0 mL of Unknown #2 into a 25.0 mL volumetric flask, then dilute to the mark with 1% nitric acid.

B. AAS Instrument Setup, Operation, and Shutdown

1. **The instructor will indicate the location of the following components:**
   a. Acetylene gas tank and air gas tank
   b. Burner head assembly
   c. Nebulizer tube
   d. Lamps
2. **Instrument Operation** – ***only to be performed with instructor's supervision***
   a. Turn on the ventilation.
   b. Turn on gases – check levels in tanks.
   c. Check liquid level in waste container and water trap within lid of waste container.
   d. Turn on power switch and give 2-3 minutes for it to initialize.
   e. Open WinLab 32 software –takes a few minutes to completely load.
   f. Click the Wkspc icon and open the workspace for the element you wish to analyze (copper in this case).

g. Click on the lamp icon and turn the lamp on, then click on background correction.
h. Make sure that the nebulizer is only in air – not sitting in a solution.
i. Click on the flame switch button and turn the flame on.
j. Click on File, then Open and open the appropriate method (Copper in Water) and sample information file (Copper in Water), which acts as a "job list."
k. In the Manual Analysis Control panel, click on the desired sample and take your measurements (blank, standard, or sample). The instrument will automatically come up with the next sample or standard in the pull-down menu beside the corresponding button once the previous sample has been analyzed. Results will appear in the Results pane.
l. Use deionized water to "zero" the instrument.

3. **Instrument Shutdown** – ***only to be performed with instructor's supervision***
   a. Aspirate deionized water for several minutes to rinse the nebulizer and burner system.
   b. Turn off the flame using the switch icon in the software.
   c. Turn off the gases at the tanks and then click on the Bleed Lines button.
   d. Click on the results window and print the data to a .pdf file, save on a memory stick.
   e. Close the software completely to ensure the program is fully shut down, then turn off the instrument using the main power switch.
   f. Turn off ventilation.

Experiment-Specific Lab Report Guidelines and Evaluation Criteria (10 marks)

Introduction (4 marks)

- Explain the function of each component of the Atomic Absorption Spectroscopy (AAS) instrument and describe what happens to the sample as it passes through each stage. You may include a block diagram to support your explanation. Your discussion should address the operation of the radiation source, sample compartment (Nebulizer-atomizer), wavelength selection system (monochromator), and detector.

Data and calculation (3 marks)

- Determine the amount of copper present in each of your unknown samples. To quantify the copper concentration, construct a calibration curve using the absorbance values and known concentrations of the standard solutions. Use the calibration equation obtained from this curve to calculate the copper content in each unknown sample. If any sample was diluted, be sure to calculate and report its original concentration before dilution. (1 mark)
- Calculate the relative error for the copper concentration in Unknown #2 by comparing the experimental value (obtained from the instrument report) with the theoretical value (calculated using the calibration equation). (1 mark)
- Calculate the detection limit (DL) of copper from your results. (1 mark):

$$DL = \frac{(k \times s_b)}{m}$$

Where

k = statistical constant = 3
m = slope of calibration curve
s_b = standard deviation of blank readings

Results and Discussion (3 marks)

- Give an example of an interference that could affect the results of this experiment and classify it as a physical or chemical interference. (1 mark)
- Would your interference cause your concentration reading to be higher or lower than its true value? Explain why. (2 marks)

5.3 Experiment #2

Analysis of Metals in Air by Flame Atomic Absorption Spectroscopy

Background

Airborne metals such as copper (Cu), iron (Fe), and zinc (Zn) are common by-products of industrial activities, including metal processing, combustion, welding, and manufacturing. When released into the atmosphere, these metals can attach to airborne particulates and pose serious environmental and health risks. Prolonged exposure may lead to respiratory irritation, oxidative stress, and accumulation of toxic metals in tissues, while deposition of these metals can contaminate soil and water systems.

Measuring trace metals in air particulates is essential for assessing air quality, ensuring compliance with environmental regulations, and evaluating the effectiveness of emission control systems. In this experiment, the collected air filter samples are extracted, digested, and analyzed for Cu, Fe, and Zn concentrations using atomic absorption spectroscopy (AAS). The data obtained provide insight into both the composition of air-borne particulates and the recovery efficiency of analytical procedures used in environmental monitoring.

Learning Objectives

After completing this experiment, students will be able to:

1. Perform acid extraction and sample preparation of airborne particulate filters for metal analysis.
2. Prepare and dilute multielement standard solutions for calibration.
3. Quantify Cu, Fe, and Zn concentrations in air samples using AAS.
4. Calculate air-borne metal concentrations (μg/m³) and evaluate analytical accuracy through blank and recovery checks.

Safety Precautions

- Perform all acid digestion and heating steps inside a fume hood; nitric acid vapours are corrosive and toxic.
- During digestion process keep watch glasses in place to minimize splashing.
- Allow hot samples to cool completely before filtering to prevent burns and glass breakage.
- Operate the flame AAS only under instructor supervision and ensure ventilation is on before ignition.
- Dispose of acidic waste and rinses in the inorganic waste container; never pour into the sink.

Procedure

Note: All glassware used in this experiment must be acid-washed with 1% nitric acid before use, to prevent contamination.

A. Sample Preparation

1. Obtain four clean 100 mL beakers and label them appropriately.

   Beaker 1 – add 30 mL of deionized water (**Extraction Blank**).

   Beaker 2 – cut out a toonie-sized piece of unexposed filter sheet and place it in the beaker. Add 30 mL of deionized water
   (**Filter Blank**).

   Beaker 3 – cut out a toonie-sized piece of exposed filter sheet and place it in the beaker, add 30 mL of deionized water
   (**Sample**).

   Beaker 4 – cut out a toonie-sized piece from the unexposed filter sheet and place it in the beaker, add 30 mL of deionized water, then use an adjustable micropipette to spike in 50 µL portions of each of the following solutions (**Recovery Check**).

   1000 ppm Cu standard
   1000 ppm Fe standard
   1000 ppm Zn standard

2. **In a Fumehood**, add 5 mL of concentrated nitric acid (10% Nitric acid) to each beaker.
3. **In a Fumehood**, cover each beaker with a watch glass and boil for 15 minutes on a hot plate. Check the beakers frequently and do not allow them to boil dry. If the liquid level is getting too low, add a small portion of deionized water.
4. Remove the breakers from the fumehood and allow them to cool to room temperature.
5. Quantitatively transfer the contents of each beaker into a separate 50.0 mL volumetric flask, then dilute to

the calibration mark with deionized water. Mix thoroughly to ensure a uniform solution.

6. Set up a rack with four glass funnels and place a piece of ashless Whatman No. 42 filter paper in each funnel. Position a clean, acid-washed 100 mL beaker beneath each funnel to collect the filtrate. Filter the contents of each flask by gravity filtration, ensuring the paper is properly seated in the funnel. Collect the filtrates in the corresponding labelled beakers.

B. Standard Preparation

You will be provided with Standard Solution A, which contains the following analyte concentrations in 1% nitric acid:

Analyte	Concentration (ppm)
Fe	5
Zn	2
Cu	4

Prepare the following dilutions, then calculate the concentration of each element in each solution and complete the table.

- *Solution B*: Pipette 25.0 mL of Solution A into a 50.0 mL volumetric flask and dilute to the mark with 1% nitric acid. Mix well.
- *Solution C:* Pipette 12.5 mL of Solution A into a 50.0 mL volumetric flask and dilute to the mark with 1% nitric acid. Mix well.
- *Solution D:* Pipette 6.25 mL of Solution A into a 50.0 mL volumetric flask and dilute to the mark with 1% nitric acid. Mix well.

Standard	Fe (ppm)	Zn (ppm)	Cu (ppm)
A	5	2	4
B			
C			
D			

C. AAS Instrument Setup, Operation, and Shutdown

1. **The instructor will indicate the location of the following components:**
   a. Acetylene gas tank and air gas tank
   b. Burner head assembly
   c. Nebulizer tube
   d. Lamps
2. **Instrument Operation** – ***only to be performed with instructor's supervision***
   a. Turn on the ventilation.
   b. Turn on gases – check levels in tanks.
   c. Check liquid level in waste container and water trap within lid of waste container.
   d. Turn on power switch and give 2-3 minutes for it to initialize.
   e. Open WinLab 32 software –takes a few minutes to completely load.
   f. Click the Wkspc icon and open the workspace for the element you wish to analyze (copper in this

case).

g. Click on the lamp icon and turn the lamp on, then click on background correction.
h. Make sure that the nebulizer is only in air – not sitting in a solution.
i. Click on the flame switch button and turn the flame on.
j. Click on File, then Open and open the appropriate method (e.g. Zn in Air) and sample information file (e.g. Zn in Air), which acts as a "job list."
k. In the Manual Analysis Control panel, click on the desired sample and take your measurements (blank, standard, or sample). The instrument will automatically come up with the next sample or standard in the pull-down menu beside the corresponding button once the previous sample has been analyzed. Results will appear in the Results pane.
l. Use deionized water to "zero" the instrument.
m. Repeat the analysis process for the other two elements.

3. **Instrument Shutdown** – ***only to be performed with instructor's supervision***
   a. Aspirate deionized water for several minutes to rinse the nebulizer and burner system.
   b. Turn off the flame using the switch icon in the software
   c. Turn off the gases at the tanks and then click on the Bleed Lines button
   d. Click on the results window and print the data to a .pdf file, save on a memory stick
   e. Close the software completely to ensure the program is fully shut down, then turn off the instrument using the main power switch.
   f. Turn off ventilation.

Experiment-Specific Lab Report Guidelines and Evaluation Criteria (10 marks)

Introduction (3 marks)

- After deposition, metals such as Cu, Fe, and Zn can undergo complex interactions in soil and water. Perform an online search to determine how these interactions determine their bioavailability, toxicity, and potential to enter food chains.

Data and Calculations (5 marks)

- Based on your unknown sample results, what would be the concentration of each element for the air sample that passed through the whole rectangular filter sheet (answer should be in ug/m^3)? (3 marks)
- Assume that 73.91 circles can be cut out of one large rectangular sheet.
- You will need the total volume of air that passed through the filter in m^3. This can be calculated with the following information:
  - Flow rate of air sampling apparatus: 43.96 ft^3/min. (1ft^3 = 0.0283m^3).
  - Length of time that air was sampled: 2955 minutes (48hr:55min).
- Calculate the % of the residue collected on an exposed filter due to each analyte. Assume that 0.450 g of residue was collected on the exposed filter. Use your unknown sample results to determine what percentage of this mass is due to each element. Your reported results should be based on a whole sheet of filter paper. (2 marks)

Results and Discussion (2 marks)

- What values did you expect for the extraction blank, filter blank, and recovery check? Do your actual results agree with your expectations – why or why not? (2 marks)

5.4 Experiment #3

Determination of Iron in Vitamin Capsules and Soil by Flame Atomic Absorption Spectroscopy

Background

Iron (Fe) is an essential trace element required for numerous biological and environmental processes. In humans, iron plays a vital role in oxygen transport (as a component of hemoglobin), cellular respiration, and enzyme catalysis. Deficiency can lead to anemia and fatigue, whereas excessive intake can cause toxicity and oxidative stress. Iron is also a key component of soils, influencing plant nutrition and overall soil fertility. Its availability depends on pH, redox potential, and the presence of organic matter or chelating agents.

Accurate determination of iron concentration in pharmaceutical products and environmental samples is crucial for both quality assurance and regulatory compliance. This experiment uses flame atomic absorption spectroscopy (FAAS) to measure iron content in two distinct sample types: commercial vitamin capsules and soil extracts. By comparing results from an external standard calibration and a standard addition method, students will explore how matrix composition affects quantitative accuracy in complex samples.

Prior to analysis, each sample is subjected to acid digestion using concentrated nitric acid. Because nitric acid vapours are corrosive and toxic, the digestion process must be conducted in a fumehood.

Through this experiment, students will learn how AAS can be used to quantify metal ions at trace levels, and how calibration strategies, background correction, and instrumental optimization contribute to analytical accuracy and precision.

Learning Objectives

After completing this experiment, students will be able to:

1. Prepare and digest complex solid samples (vitamin and soil) using nitric acid for metal analysis.
2. Construct and apply external standard and standard addition calibration curves for iron determination.
3. Calculate iron concentration, relative standard deviation (%RSD), and relative error from experimental data.
4. Perform a one-sample t-test to compare experimental and theoretical iron values statistically.
5. Explain the principle of background correction and identify parameters affecting AAS sensitivity.

Safety Precautions

- Perform all acid digestion and heating steps inside a fume hood; nitric acid vapours are corrosive and toxic.
- During digestion process keep watch glasses in place to minimize splashing.
- Allow hot samples to cool completely before filtering to prevent burns and glass breakage.
- Operate the flame AAS only under instructor supervision and ensure ventilation is on before ignition.
- Dispose of acidic waste and rinses in the inorganic waste container; never pour into the sink.

Procedure

Note: All glassware used in this experiment must be acid-washed with 1% nitric acid before use to prevent contamination.

A. Sample Preparation

1. Obtain seven clean 100 mL breakers and label them appropriately.

   Label seven 100 mL breakers:

   Beaker 1: Extraction Blank
   Beaker 3: Vitamin Sample 2
   Beaker 4: Vitamin Sample 3
   Beaker 5: Soil Sample 1
   Beaker 6: Soil Sample 2
   Beaker 7: Soil Sample 3

   Extraction Blank (Beaker 1): Add 30 mL of distilled water to the beaker.

   Vitamin Samples (Beakers 2, 3, and 4): For each vitamin sample

   i. Take 1 iron vitamin capsule.
   ii. Carefully empty the contents of the capsule into the beaker.
   iii. Record the weight of the content of each capsule to 4 decimal places.
   iv. Add approximately 30 mL of distilled water to the beaker to dissolve the capsule contents.
   v. Record the theoretical amount of iron per capsule as listed on the product label.

   Soil Samples (Beakers 5, 6, and 7): For each soil sample:

   i. Add 0.5 g of the soil to each beaker.
   ii. Record the mass of the soil to 4 decimal places.
   iii. Add approximately 30 mL of distilled water to the beaker and stir gently to suspend the soil and facilitate extraction of soluble components.

2. **In a Fumehood**, add 5 mL of concentrated nitric acid (10% nitric acid) to each beaker.
3. **In a Fumehood**, cover each beaker with a watch glass and boil for 15 minutes on a hot plate. Check the beakers frequently and do not allow them to boil dry. If the liquid level is getting too low, add a small portion of deionized water.
4. Remove the beakers from the fumehood and allow them to cool to room temperature.
5. Set up a rack with 4 funnels (acid-washed). Put a piece of Ashless Whatman#42 filter paper in each funnel and set up a 50 mL volumetric flask to collect the filtrates. Quantitatively transfer the contents of each beaker to its own funnel. Use small portions of deionized water to quantitatively transfer the sample, taking care so that the amount of filtrate does not surpass the 50 mL mark on the collection flask. If necessary, add deionized water to bring the flask up to the mark. Cap each flask and mix well. **These are your stock sample solutions**.

B. External Standard & Sample Preparation

1. Prepare 1L of a 1% nitric acid solution (10mLs HNO3 in 1.0L of deionized water).
2. A 1000 ppm Fe stock solution will be provided by the instructor. Prepare the working standards shown in the table below. Make each standard in a 50.0 mL volumetric flask, then dilute to the mark with 1% nitric acid.

Standard	Preparation	Std. Conc. Fe (ppm)
A	Pipette 1.0 mL of the 1000 ppm Fe stock std. into a 50.0 mL volumetric flask. Dilute to volume with 1% nitric acid and mix well.	
B	Pipette 10.0 mL of Std. A into a 50.0 mL volumetric flask. Dilute to volume with 1% nitric acid and mix well.	
C	Pipette 5.0 mL of Std. A into a 50.0 mL volumetric flask. Dilute to volume with 1% nitric acid and mix well.	
D	Pipette 2.0 mL of Std. A into a 50.0 mL volumetric flask. Dilute to volume with 1% nitric acid and mix well.	
E	Pipette 1.0 mL of Std. A into a 50.0 mL volumetric flask. Dilute to volume with 1% nitric acid and mix well.	

3. Follow the sample preparation steps for each beaker:

Extraction Blank Sample – transfer 0.1 mL of the stock blank sample by pipette into a 100.0 mL volumetric flask and dilute to volume with 1% nitric acid.

Vitamin Samples 1, 2 and 3 – transfer 0.1 mL of the stock vitamin extract sample by pipette into a 100.0 mL volumetric flask and dilute to volume with 1% nitric acid.

Soil Samples 1, 2 and 3 – transfer 0.1 mL of the stock soil extract sample by pipette into a 100.0 mL volumetric flask and dilute to volume with 1% nitric acid.

C. Standard Preparation for Standard Additional Analysis (*Use vitamin sample#1 only*)

Vitamin Sample – Standard Solution
Pipette 0.1 mL of the stock vitamin extract into a 100 mL volumetric flask. Add 2.0 mL of Std. A from section B. Bring the flask up to volume with 1% nitric acid and mix well.

D. AAS Instrument Setup, Operation, and Shutdown

1. **The instructor will indicate the location of the following components:**
   a. Acetylene gas tank and air gas tank
   b. Burner head assembly
   c. Nebulizer tube
   d. Lamps
2. **Instrument Operation** – ***only to be performed with instructor's supervision***
   a. Turn on the ventilation.
   b. Turn on gases – check levels in tanks.
   c. Check liquid level in waste container and water trap within lid of waste container.
   d. Turn on power switch and give 2-3 minutes for it to initialize.
   e. Open WinLab 32 software –takes a few minutes to completely load.
   f. Click the Wkspc icon and open the workspace for the element you wish to analyze (copper in this case).
   g. Click on the lamp icon and turn the lamp on, then click on background correction.
   h. Make sure that the nebulizer is only in air – not sitting in a solution.
   i. Click on the flame switch button and turn the flame on.
   j. Click on File, then Open, and open the appropriate method (Fe in Vitamin and Soil) and sample information file (Fe in Vitamin and Soil), which acts as a "job list."
   k. In the Manual Analysis Control panel, click on the desired sample and take your measurements (blank, standard, or sample), the instrument will automatically come up with the next sample or standard in the pull-down menu beside the corresponding button once the previous sample has been analyzed. Results will appear in the Results pane.
   l. Use deionized water to "zero" the instrument.
3. **Instrument Shutdown** – ***only to be performed with instructor's supervision***
   a. Aspirate deionized water for several minutes to rinse the nebulizer and burner system.
   b. Turn off the flame using the switch icon in the software.
   c. Turn off the gases at the tanks and then click on the Bleed Lines button.
   d. Click on the results window and print the data to a .pdf file, save on a memory stick.
   e. Close the software completely to ensure the program is fully shut down, then turn off the instrument using the main power switch.
   f. Turn off ventilation.

Experiment-Specific Lab Report Guidelines and Evaluation Criteria (20 marks)

Introduction (4 marks)

- Explain, using the principle of electronic transitions, how atoms in the flame produce an absorption signal in atomic absorption spectroscopy (AAS). (2 marks)
- Explain why nitric acid digestion and subsequent filtration are essential to preparing representative, interference-free samples for AAS measurement. (2 marks)

Data and Calculations (12 marks)

- What is the average concentration (mg iron per capsule) of the vitamin sample and (mg iron per gram of soil) of the soil samples using your result from the external standard calibration method? Keep in mind that the results on the instrument reports are only the concentrations for the solutions that were analyzed – you will also need to take account your sample preparation steps. (2 marks)
- Calculate the %RSD of your three trials for both vitamin and soil samples? (2 marks)
- The theoretical amount of iron per vitamin capsule is listed on the product label. Perform a one-sample t-test at the 95% confidence level to determine if there is a statistically significant difference between your experimentally determined mean value and the manufacturer's claimed value. Show your calculations (mean, standard deviation, t~calculated~) and state your conclusion. (3 marks)
- What is the concentration (mg iron/vitamin capsule) using the standard addition method? (2 marks)
- Calculate the relative error vs. the external standard result of vitamin samples. Assume that the standard addition result is the "theoretical value" and that the external standard result is the "experimental value". Give an explanation as to why the results differ based on the calibration methods used. (3 marks)

Result and Discussion (4 marks)

- List two factors which might affect the reproducibility of the sample preparation step in this experiment and explain the importance of each. (2 marks)
- What type of background correction is used in this experiment? Describe its principle of operation. (2 marks)
- Give an example of an instrument parameter that could be changed to optimize the absorption of your samples in this experiment, and discuss why your absorbance readings would increase. (1 mark)

CHAPTER 6: ATOMIC EMISSION SPECTROSCOPY

Chapter Outline

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6.1 Theory: Flame Atomic Emission Spectroscopy

Flame Atomic Emission Spectroscopy (FAES) is a quantitative analytical technique used to measure the concentration of metals in a sample by detecting the light emitted from excited atoms. In flame AES, a solution containing the analyte is aspirated into a flame, where the solvent evaporates, and the dissolved salts decompose into free atoms. The high flame temperature excites some of these atoms to higher electronic energy levels. When the excited atoms return to their ground state, they emit light at characteristic wavelengths unique to each element.

The intensity of the emitted light is measured by a detector and is directly related to the number of emitting atoms in the flame, and therefore to the concentration of the element in the sample. Unlike Atomic Absorption Spectroscopy (AAS), which measures the amount of light absorbed by ground-state atoms, AES measures the light emitted by excited atoms.

Instrument Components

A typical Flame Atomic Emission Spectrometer (FAES) consists of the following key components:

Excitation Source (Flame)

FAES uses the flame itself as the excitation source—no external light source is required. When the sample enters the hot flame, atoms are excited and emit light as they return to their ground state

- Our instrument uses an air–acetylene flame, where air serves as the oxidizer and acetylene as the fuel, producing a stable flame (~2300 °C) suitable for elements such as K.

Nebulizer

Converts the liquid sample into a fine aerosol (mist) for introduction into the flame.

- A pneumatic nebulizer is used in our lab; it relies on compressed air or gas to create a stable, reproducible mist for consistent signal output.

Atomizer (Flame System)

The aerosol enters the burner head, where the solvent evaporates, and solute dissociates into free atoms. These atoms are excited by the high-temperature flame and emit light at element-specific wavelengths.

- The air–acetylene flame provides sufficient excitation energy for most metals analyzed by flame AES.

Monochromator

Disperses the emitted light into its component wavelengths and isolates the analyte's characteristic wavelength, minimizing spectral interference.

Detector

Measures the intensity of emitted light and converts it into an electrical signal proportional to emission strength.

- Our instrument uses a CMOS detector, offering high sensitivity, low noise, and excellent stability.

Readout/Computer System:

Processes the detector signal, displays emission intensity, and calculates analyte concentrations from calibration curves. It also manages instrument control and data storage.

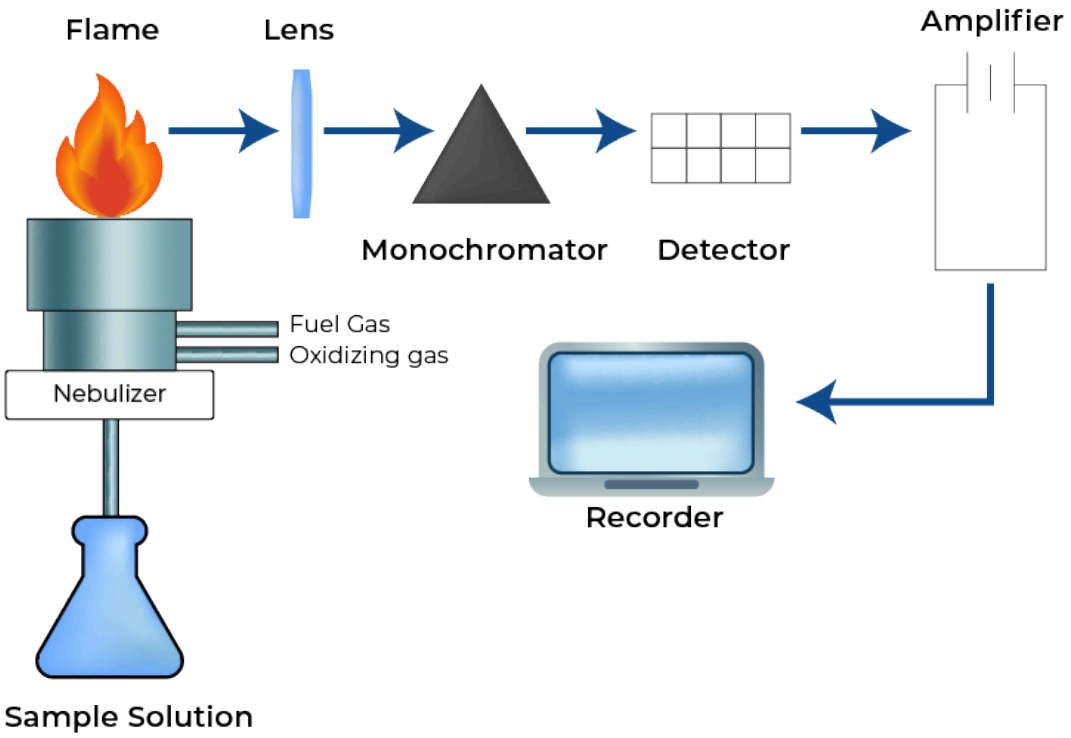

Figure 6.1.1. "Schematic Diagram of Flame Atomic Emission Spectroscopy" by Koen Liddiard, CC BY-NC-SA 4.0. Source: "Atomic Emission Spectroscopy" in Learning Chemistry by Priyam Study Centre, FDEd.

Image Description

At the left, a sample solution in a flask is drawn up through a nebulizer into a burner supplied with fuel gas and oxidizing gas, producing a flame. Light from the flame passes through a lens and then into a triangular monochromator, which isolates a single wavelength. The beam then reaches a grid-like detector, whose signal is sent to an amplifier and finally to a computer recorder that displays the measurement.

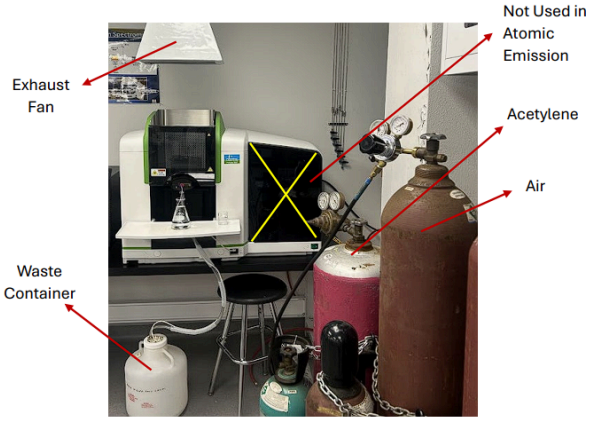

Figure 6.1.2. PinAAcle 900F Perkin Elmer Flame Atomic Emission Spectrometer. Image by T. Sarchami, CC BY-NC-ND 4.0. Click to enlarge.

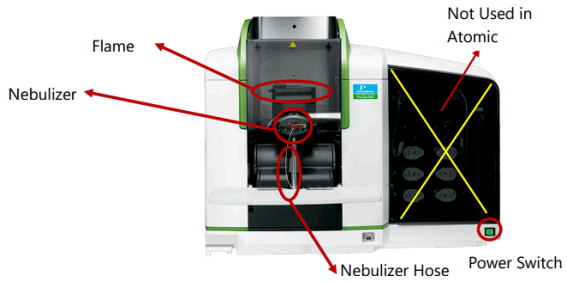

Figure 6.1.3. Main Components of PinAAcle 900F Perkin Elmer Flame Emission Spectrometer. Image by T. Sarchami, CC BY-NC-ND 4.0. Click to enlarge.

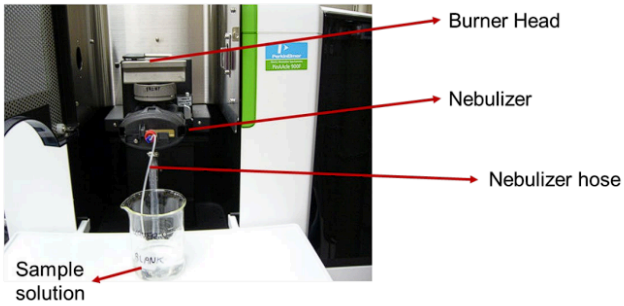

Figure 6.1.4. Sample Introduction System of PinAAcle 900F Perkin Elmer Flame Emission Spectrometer. Image by T. Sarchami, CC BY-NC-ND 4.0. Click to enlarge.

Image Descriptions

Figure 6.1.2: Photograph of the PinAAcle 900F instrument on a lab bench. An exhaust fan is positioned above the instrument, a white waste container sits on the floor, and acetylene and air gas cylinders stand to the right, secured with chains and fitted with regulators.

Figure 6.1.3: Front view of the PinAAcle 900F. The central compartment where the flame forms is highlighted,

with the nebulizer and nebulizer hose leading into it. The right-hand section of the instrument (lamp compartment) is marked as not used in atomic emission, and the power switch is indicated at the lower right.

Figure 6.1.4: Close-up of the sample introduction area. The burner head is at the top, with the nebulizer positioned just below and a nebulizer hose running down into a beaker labelled “Sample solution.”

Operating Tips for Accurate FAES Measurements

To ensure safe, precise, and efficient operation, follow these steps carefully. Instrument operation and shutdown must always be performed under instructor supervision.

Instrument Overview

Before operating the instrument, the instructor will point out the location and function of the following key components:

- Acetylene gas tank and air gas tank.
- Burner head assembly.
- Nebulizer tube.

Sample Preparation

- Use clean, acid-washed glassware to prevent contamination.
- Prepare all standards and samples in the same matrix (same acid type and concentration) to minimize matrix effects.
- Filter samples to remove particulates or precipitates that may clog the nebulizer or scatter light.
- Filter any turbid solution before running the analysis.
- Avoid introducing samples with high total dissolved solids (TDS), as they can block the nebulizer and affect signal stability.

Flame Operation

- Ensure the nebulizer and burner head are clean and properly aligned.
- Ignite the flame and ensure a stable, blue flame with minimal noise.
- Adjust the fuel-to-oxidant ratio for optimal stability and sensitivity.
- Never leave the instrument unattended while the flame is lit.

- Keep burner slot and nebulizer clean-carbon deposits can distort readings.

Measurement Procedure

- Begin each run by aspirating the blank to zero the instrument.
- Aspirate standards in order of increasing concentration to build a calibration curve.
- Aspirate sample solutions.
- Between samples, aspirate deionized water to rinse the nebulizer and prevent carry-over.
- If readings drift or fluctuate, re-run the blank and check the burner head and nebulizer for cleanliness and blockages.

Quality Checks and Troubleshooting

- Negative absorbance values are nonphysical; if observed, re-zero with the blank.
- Baseline drift or excessive noise may indicate that the lamp has not fully warmed up, the flame is unstable, or there are air bubbles or blockages in the nebulizer line.
- If the burner slot becomes dirty, extinguish the flame, allow it to cool, and gently clean using a soft brush or compressed air.

Shutdown Procedure

- Rinse the system: Aspirate deionized water for several minutes to clean the nebulizer and burner.
- Extinguish the flame: Turn off the flame using the switch icon in the software.
- Depressurize gas lines: Close both gas tanks, then click “Bleed Lines” to release any residual pressure.
- Save data: Export results as a PDF file and save them to a USB drive or shared folder.
- Close software: Exit WinLab 32 completely before powering down.
- Power down: Switch off the instrument power, then turn off the ventilation system.
- Cool down: Allow the burner head and components to cool completely before cleaning or disassembly

6.2 Experiment #1

Determination of Potassium in Fruit Juices Using a Flame Atomic Emission Spectrometer

Background

Potassium is an essential nutrient required for normal muscle function, nerve transmission, and fluid balance in the human body. Fruit juices, particularly orange and apple juice, are major dietary sources of potassium. Monitoring potassium levels in these beverages is important for nutritional labelling accuracy, quality control, and consumer health.

In this experiment, the potassium content of commercial fruit juices is determined using flame atomic emission spectroscopy (AES), a technique that measures the light emitted by excited atoms returning to their ground state. This procedure is based on AOAC Official Methods 920.149 (sample preparation) and 977.29 (analysis), which outline standardized approaches for sample preparation and elemental analysis.

Plasticware is used throughout this experiment—including volumetric flasks, pipettes, and beakers—to prevent contamination from potassium leaching out of glassware, minimize ion exchange at glass surfaces, and ensure compatibility with mildly acidic solutions. This practice reduces the risk of false-positive results and improves accuracy in trace metal determination. Emphasis is placed on precise sample dilution, calibration curve generation, and verification of system performance as part of laboratory quality assurance and quality control.

Learning Objectives

After completing this experiment, students will be able to:

1. Prepare calibration standards and fruit juice samples for potassium analysis by flame atomic emission spectroscopy.
2. Explain the role of an ionization suppressant and its effect on signal stability and accuracy.
3. Evaluate calibration linearity, reproducibility, and system suitability as part of quality assurance.
4. Quantify potassium concentrations in orange and apple juices and compare them with labelled nutritional values using statistical analysis.

Safety Precautions

- Operate the flame AES instrument only under instructor supervision.
- Ensure ventilation is on before igniting the flame and during all measurements.
- Dispose of liquid waste and rinses in the inorganic waste container.

Procedure

Note: Use plastic volumetric flasks, beakers and pipettes only to avoid potassium contamination from glassware. (no glass)

A. Standard Preparation

Potassium and Cesium Stock Standards (1000 ppm) will be provided by the instructor. Prepare the following standards according to the table below. All solutions should be diluted to volume using deionized water.

Standard	Preparation	Std. conc. K (ppm)
A	Pipette 0.5 mL of the 1000 ppm K stock std. into a 100.0 mL volumetric flask. Add 2.0 mL of 1000 ppm Cs std. Dilute to volume with deionized water and mix well.	
B	Pipette 0.2 mL of the 1000 ppm K stock std. into a 100.0 mL volumetric flask. Add 2.0 mL of 1000 ppm Cs std. Dilute to volume with deionized water and mix well.	
C	Pipette 0.1 mL of the 1000 ppm K stock std. into a 100.0 mL volumetric flask. Add 2.0 mL of 1000 ppm Cs std. Dilute to volume with deionized water and mix well.	

B. Sample Preparation

Samples 1, 2, and 3 (Orange Juice) – Pipette 0.1 mL of orange juice into a 100.0 mL plastic volumetric flask. Add 2.0 mL of 1000 ppm Cs std. Dilute to volume with deionized water and mix well.

Sample 4 (Standard without Cs) – Pipette 0.2 mL of the 1000 ppm K stock std. into a 100.0 mL volumetric flask. Dilute to volume with deionized water and mix well. This solution has a K concentration equivalent to standard B.

Samples 5, 6, and 7 (Apple Juice) – Pipette 0.1 mL of apple juice into a 100.0 mL plastic volumetric flask. Add 2.0 mL of 1000 ppm Cs std. Dilute to volume with deionized water and mix well.

Sample 8 (Apple Juice Matrix Spike) – Pipette 0.1 mL of apple juice and 0.1 mL of 1000 ppm K stock into a 100.0 mL plastic volumetric flask. Add 2.0 mL of 1000 ppm Cs std. Dilute to volume with deionized water and mix well. This solution will be used to calculate % recovery for the apple juice matrix.

F. AES Instrument Setup, Operation, and Shutdown

1. **The instructor will indicate the location of the following components:**
   a. Acetylene gas tank and air gas tank
   b. Burner head assembly
   c. Nebulizer tube
   d. Lamps
2. **Instrument Operation** – ***only to be performed with instructor's supervision***
   a. Turn on the ventilation.
   b. Turn on gases – check levels in tanks.
   c. Check liquid level in waste container and water trap within lid of waste container.
   d. Turn on power switch and give 2–3 minutes for it to initialize.
   e. Open WinLab 32 software – takes a few minutes to completely load.
   f. Click the Wkspc icon and open the workspace for the element you wish to analyze (potassium in this case).
   g. Make sure that the nebulizer is only in air – not sitting in a solution.
   h. Click on the flame switch button and turn the flame on.
   i. Click on File → Open and open the appropriate method (Potassium in juice) and sample information file, which acts as a "job list."
   j. Aspirate the highest-concentration standard solution to initialize the spectrometer.
   k. In the Manual Analysis Control panel, click on the desired sample and take your measurements (blank, standard, or sample); results will appear in the Results pane.
   l. Use deionized water as the blank.
   m. System suitability – 3 parameters to be tested/calculated:
      - Std. B (first reading) must have intensity within 1% of the theoretical value predicted by the calibration curve.
      - Duplicate readings for sample 2 must be ≤1.4% difference

$$\%\ \text{difference} = \frac{|(\text{reading 1} - \text{reading 2})|}{\text{average of reading}} \times 100\%$$

      - Duplicate readings for Std. B must be ≤1.4% difference to ensure no significant signal drift.
   n. Order of sample readings:
      - Water blank
      - Calibration standards C, B, A
      - Sample 1
      - Sample 2
      - Sample 3
      - Sample 4
      - Sample 2 duplicate
      - Std. B duplicate
      - Sample 5
      - Sample 6
      - Sample 7
      - Sample 8
3. **Instrument Shutdown** – ***only to be performed with instructor's supervision***
   a. Aspirate deionized water for several minutes to rinse the nebulizer and burner system.
   b. Turn off the flame using the switch icon in the software.
   c. Turn off the gases at the tanks and then click on the Bleed Lines button.

d. Click on the results window and print the data to a PDF file, save on a memory stick.
e. Close the software completely and shut down the instrument using the main power switch.
f. Turn off ventilation.

Experiment-Specific Lab Report Guidelines and Evaluation Criteria (20 marks)

Introduction (3 marks)

- What is an ionization suppressant, and how does it work? (3 marks)

Data and Calculations (14 marks)

- **System suitability calculations:**

  a. Using the calibration curve equation obtained from your standard solutions, calculate the theoretical intensity for Standard B based on its known concentration. Compare this theoretical value with the first measured intensity for Standard B. Verify that the measured value is within ±1% of the theoretical value. (1 mark)

$$\%\ \text{difference} = \frac{|\text{Measured} - \text{Theoretical}|}{\text{Theoretical}} \times 100\%$$

  Is the result within ±1%?

  b. For Sample 2, calculate the percent difference between the two intensity readings. (1 mark)

$$\%\ \text{difference} = \frac{|\text{Reading 1} - \text{Reading 2}|}{\text{Average of Readings}} \times 100\%$$

  Is the result within ≤1.4%?

  c. At the end of the run, Standard B is remeasured. Calculate the percent difference between the initial and final readings for Standard B. (1 mark)

$$\%\ \text{difference} = \frac{|\text{Initial Reading} - \text{Final Reading}|}{\text{Average of Readings}} \times 100\%$$

  Is the result within ≤1.4%?

  d. Show your calculations to verify that the calibration curve is linear. Determine the coefficient of determination (R^2) for your calibration data and report the equation of the line. An R^2 value of 0.998 or higher indicates acceptable linearity. (1 mark)

  Did your calibration line meet this criterion?

- Calculate the amount of potassium in the undiluted sample of orange and apple juice (mg/bottle) for samples 1, 2, and 3, as well as samples 5, 6, and 7 and determine the mean and %RSD for both apple and orange juice. (4 marks)
- Compare the mean of the mg K/bottle results to the value listed on the bottle label by determining the % recovery and performing a one-sample t-test to assess if there is a statistically signifi-

cant difference for both orange juice and apple juice. (4 marks)

- Using the measured potassium concentrations for the spiked (sample 8) and unspiked apple juice samples (average of concentration of samples 5, 6, and 7), calculate the percent recovery of potassium using the equation below: (2 marks)

$$\%\text{Recovery} = \frac{C_{\text{spiked}} - C_{\text{unspiked}}}{C_{\text{added}}} \times 100\%$$

Results & Discussion (3 marks)

- Compare the intensity readings between the two standard B solutions: one with Cs and one without (sample 4). Which one is higher? Was this the result you expected – why or why not? (2 marks)
- Discuss possible sources of error for this experiment and the possible effect they would have had on the results. (1 mark)

CHAPTER 7: FOURIER TRANSFORM INFRARED SPECTROSCOPY

Chapter Outline

Image by Fanshawe College. © All Rights Reserved.

7.1 Theory: Fourier Transform Infrared Spectroscopy

FTIR spectroscopy is an analytical technique used to identify chemical substances and their functional groups by measuring how they absorb infrared (IR) radiation. When IR light passes through a sample, certain wavelengths are absorbed, causing vibrations in chemical bonds—such as stretching, bending, or twisting of chemical bonds—and generating a unique spectrum for each compound.

In an FTIR spectrometer, infrared radiation from the source passes through an interferometer, which modulates the light into an interferogram. This beam passes through the sample compartment, and the transmitted or reflected light is detected and converted into an electrical signal. A Fourier Transform converts the interferogram into a spectrum of intensity versus wavenumber (typically 4000–400 cm^{-1}).

Each molecule produces a distinct pattern of absorption bands—its infrared fingerprint—allowing identification of functional groups and overall molecular structure. Absorbance intensity can also be used semi-quantitatively, following Beer–Lambert's Law.

Typical absorption ranges include:

- C=O stretching: 1820–1660 cm^{-1}
- O–H stretching: 2500–3000 cm^{-1}
- C–H stretching: 2800–3000 cm^{-1}

Instrument Components

An FTIR spectrometer consists of the following key components:

Infrared Source

A broad-spectrum infrared source provides continuous radiation necessary for molecular vibration excitation.

- In our laboratory instrument (Thermo Scientific Nicolet iS5 FTIR), a globar source—a silicon carbide rod heated to approximately 1200°C—emits continuous mid-infrared radiation (4000–400 cm^{-1}), suitable for most organic and inorganic samples.

Interferometer

The core of the FTIR system, typically a Michelson interferometer. A beam splitter divides the IR beam into two paths: one reflected by a stationary mirror and the other by a moving mirror. When the beams recombine, they produce an interferogram containing information from all wavelengths simultaneously.

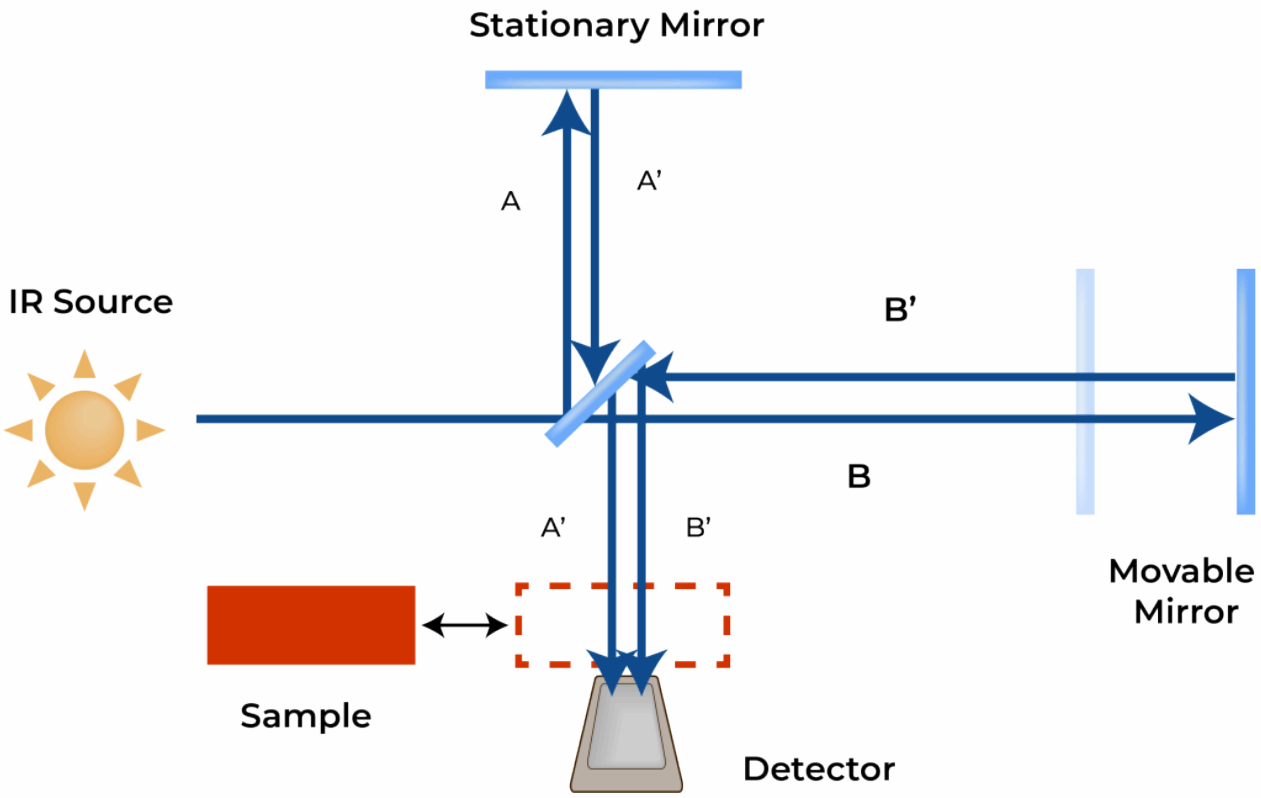

Figure 7.1.1. "Schematic Diagram of Fourier Transform Infrared Spectrometer" by Koen Liddiard, CC BY-NC-SA 4.0. Source: "Figure 1b" by Jing Zhao, in The Power of the Fournier Transform for Spectroscopists, CC BY 4.0.

Image Descriptions

Diagram of a Fourier transform infrared (FTIR) spectrometer based on a Michelson interferometer. On the left, an "IR Source" symbolized by a sun sends a horizontal infrared beam to the right, where it strikes a central beam splitter. From the splitter, one beam (A) is reflected upward to a "Stationary Mirror" and returns as A', while the other beam (B) continues to the right to a "Movable Mirror" and returns as B'. The returning beams recombine at the beam splitter and travel downward together toward a "Detector" at the bottom. A dashed red box in this downward path marks the location of the sample compartment, with a solid red rectangle labelled "Sample" shown to the left and arrows indicating that the sample can be inserted into the combined beam before it reaches the detector.

Sample Compartment

Holds the sample and allows IR radiation to interact with it using various sampling techniques:
Transmission cells

Uses NaCl or KBr discs for liquids or thin films; the IR beam passes through the entire thickness of the sample, and transmitted light is measured.

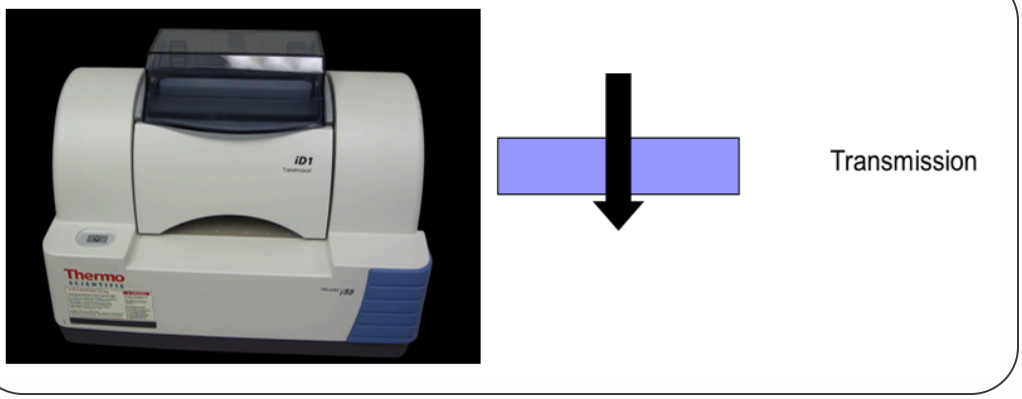

Figure 7.1.2. Thermos Scientific Nicolet iS5 FTIR Spectrometer with iD1 transmission accessory (left) and illustration of IR light passing through the full sample thickness in transmission mode (right). Image by T. Sarchami, CC BY-NC-ND 4.0.

ATR (Attenuated Total Reflectance)

A common modern accessory for solids and liquids that requires minimal sample preparation. The IR beam passes through an internal reflection crystal (commonly diamond or ZnSe), generating an evanescent wave that penetrates only a few micrometres into the sample surface.

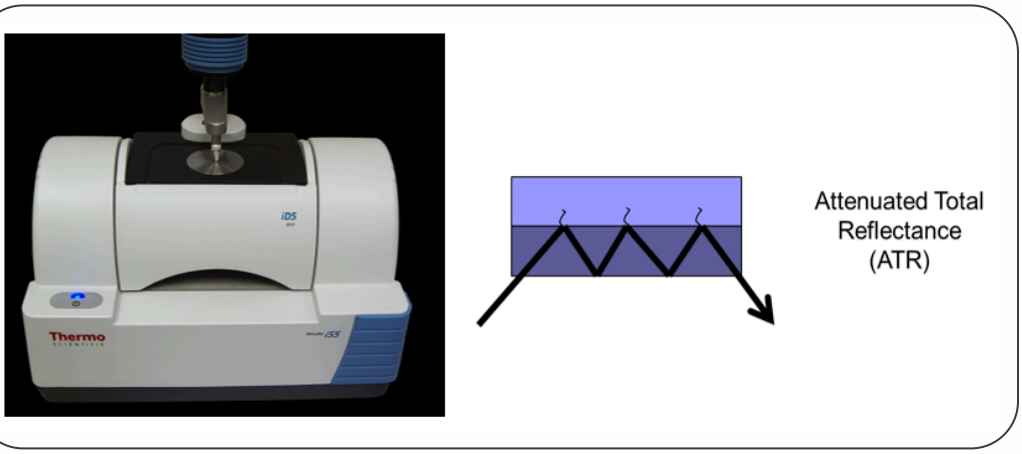

Figure 7.1.3. Thermo Scientific Nicolet iS5 FTIR Spectrometer with iD5 ATR accessory (left) and illustration of IR light internally reflecting within the ATR crystal to interact with the sample surface (right). Image by T. Sarchami, CC BY-NC-ND 4.0.

DRIFT (Diffuse Reflectance Infrared Fourier Transform)

Suitable for powdered or rough solid samples. In DRIFT, the IR light interacts only with the first few microns of the sample surface, making it useful for surface-sensitive analysis of heterogeneous materials.

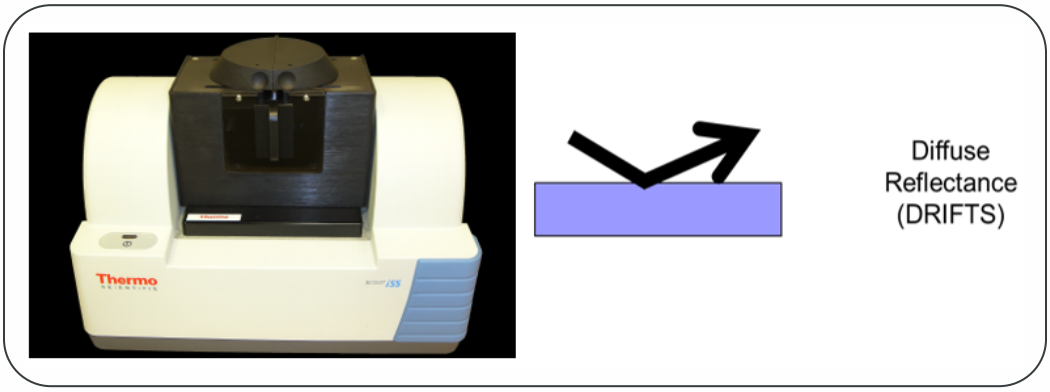

Figure 7.1.4. Thermo Scientific Nicolet iS5 FTIR Spectrometer with DRIFT accessory (left) and illustration of IR light diffusely reflected from the surface of a powdered sample (right). Image by T. Sarchami, CC BY-NC-ND 4.0.

Detector

Measures the intensity of transmitted or reflected IR light after interaction with the sample and converts it into an electrical signal.

- Our instrument uses a DTGS (deuterated triglycine sulfate) detector, a pyroelectric detector that operates at room temperature and provides stable, wide-range detection across 4000–400 cm^{-1}—ideal for routine laboratory analysis.

Computer and Fourier Transform Processor

Processes the detector output by applying a Fourier Transform, converting the interferogram (time-domain signal) into a spectrum (frequency-domain signal) that plots absorbance vs. wavenumber, revealing characteristic molecular vibrations.

Operating Tips for Accurate FTIR Measurements

Instrument Preparation

- Turn on the FTIR 15–30 minutes before use to allow the IR source, interferometer, and detector to stabilize.
- Always collect a background spectrum before analyzing samples (no sample in the beam path).

- Keep the sample compartment closed to prevent interference from atmospheric CO_2 and H_2O vapour.
- If available, use the purge system (dry air or N_2) to minimize moisture and CO_2 absorption.

Sample Preparation and Handling

A *Transmission Mode*

Used mainly for liquids, thin films, or transparent solids.

- Handle NaCl or KBr discs carefully — they are soft, fragile, and water-soluble. Avoid touching with bare fingers.
- Clean discs with a lint-free tissue lightly moistened with acetone or isopropanol before and after use.

For Liquids (Open-Disc Method):

- Place one small drop of the sample on one disc and gently place a second disc on top.
- The sample should form a thin, even film with no trapped air bubbles.
- Mount the holder in the FTIR so the IR beam passes through both discs evenly.

For Liquids (Sealed Cell Method):

- Alternatively, use a liquid transmission cell, which contains two parallel NaCl or KBr windows held inside a special cell assembly with inlet and outlet ports.
- Use a syringe to inject the sample gently through the inlet port until it fills the space between the discs.
- Ensure there are no air bubbles, then close the ports with plugs or plaques.
- This method prevents sample evaporation or contamination and is preferred for volatile, corrosive, or aqueous samples.

For Solids:

- Finely grind the sample with dry, spectroscopic-grade KBr and press it into a clear, thin pellet using a hydraulic press.
- Mount the pellet in the sample holder with the optical path aligned to the beam.

B *ATR (Attenuated Total Reflectance) Mode*

Used for solids, liquids, gels, or coatings — minimal preparation required.

- Clean the ATR crystal (diamond, ZnSe, or Ge) before and after each run with a lint-free tissue and acetone or isopropanol.
- Place a small amount of the sample on the crystal.

- Apply firm, even pressure using the built-in pressure arm to ensure good contact — weak contact gives low-intensity peaks.
- For liquids, place 1–2 drops directly on the crystal.
- For powders or rough solids, press the sample evenly against the crystal.
- Avoid scratching the crystal surface; do not use abrasive cleaning materials.
- After measurement, clean and verify the crystal by running a background scan.
- Note: The IR beam penetrates only a few micrometres into the sample (evanescent wave region), making it ideal for surface analysis.

C *DRIFT (Diffuse Reflectance Infrared Fourier Transform) Mode*

Used for powders, soils, or rough solid surfaces.

- In DRIFT, the IR beam is scattered by the sample surface, and the diffusely reflected radiation is analyzed to generate a spectrum.

Sample Preparation:

- Dry the sample to remove moisture (H_2O strongly absorbs in the IR).
- Grind the sample finely for uniform particle size — coarse grains cause uneven scattering.
- Mix a small portion of the sample (≈1–2%) with dry, spectroscopic-grade KBr as a diluent.
- Fill the DRIFT sample cup evenly, level the surface, and avoid compacting.
- Handle the cup carefully — do not touch optical surfaces.
- Collect a background scan using pure KBr before analyzing samples.
- DRIFT measures only the surface few micrometres, so ensure even mixing for reproducible results.

Measurement and Baseline

- Always collect a background scan before each sample run using identical parameters (e.g., 32 scans, 4 cm^{-1} resolution).
- Then, collect the sample scan; the instrument automatically subtracts the background to yield the absorbance spectrum.
- Ensure strong peaks are not saturated (absorbance < 2.0).
- Verify that the baseline is flat; re-run the background if distortion appears.
- Label and save spectra clearly with sample ID and mode (ATR, Transmission, or DRIFT).

Data Interpretation

- Identify functional groups by their characteristic absorption bands (e.g., O–H ≈ 3400 cm^{-1}, C=O ≈ 1700 cm^{-1}, C–H ≈ 2800–3000 cm^{-1}).
- Compare the obtained spectrum with reference spectra or spectral libraries.
- Note that ATR spectra often show slight wavelength shifts and altered intensities due to refractive index effects.
- DRIFT spectra may show baseline slopes due to scattering — this is normal for powdered samples.

Post-Measurement Care

- Remove all sample residues immediately after measurement.
- Clean NaCl/KBr discs and store them in a desiccator.
- Wipe ATR and DRIFT accessories thoroughly with solvent and dry them before storage.
- Cover optical components with protective caps when not in use.
- Record instrument parameters (scan number, resolution, date) in your lab notebook for traceability.

7.2 Experiment #1

FTIR Analysis of Liquid and Solid Samples Using Transmission Mode

Background

In this experiment, the transmission mode of the FTIR spectrometer is used. In transmission mode, the infrared beam passes through the entire thickness of the sample, and the detector measures the fraction of light transmitted at each wavelength. This setup provides high-quality spectra for thin films or liquid samples placed between NaCl discs, as well as for solid samples dispersed in a suitable medium such as Nujol.

In this experiment, students analyze liquid samples (benzonitrile and 2-pentanone) and a solid sample (vanillin) to explore how molecular structure influences infrared absorption. Each compound exhibits characteristic absorption bands:

- Benzonitrile contains a strong C≡N stretching vibration.
- 2-Pentanone shows a prominent C=O stretching band.
- Vanillin, with hydroxyl, aldehyde, and methoxy functional groups, provides a more complex spectrum ideal for studying overlapping vibrations.

Infrared absorption occurs when molecular vibrations cause a change in dipole moment. Polar functional groups (C=O, O–H, C≡N) thus produce strong diagnostic peaks in the mid-IR region (4000–600 cm^{-1}).

Characteristic Bands

Compound	Key Functional Group	Expected Band (cm^{-1})
Benzonitrile	C≡N stretch	~2220
2-Pentanone	C=O stretch	~1715
Vanillin	O–H, C=O, C–O, aromatic	3500–3200, 1670–1680, 1260–1020, 1600–1500

Learning Objectives

By the end of this experiment, students will be able to:

1. Operate and interpret data from an FTIR spectrometer.
2. Identify and assign functional group vibrations.
3. Compare sample preparation techniques for different physical states.

Safety Precautions

- Handle NaCl discs carefully—they are fragile and water-soluble.
- Perform all steps involving benzonitrile and 2-pentanone inside a fume hood.
- Avoid skin contact with Nujol, acetone, and organic solvents.
- Dispose of acetone wipes and chemical residues in designated waste containers.

Procedure

A. Software Familiarization

1. Click on the *OMNIC* icon to load the instrument software, then click on the “Collect” option from the menu bar at the top of the screen and select the “Experimental Set-Up” option. Make sure that you are in the “Collect” tab. This screen indicates the conditions for the optical setup of the instrument. Check to see that the instrument is set up with the following parameters:
   a. No. of scans = 16
   b. Resolution = 4
   c. Final format = %Transmittance
2. Go to the “Bench” tab. This screen indicates how the signal will be electronically amplified and gives the wavenumber range that will be used for data collection. It also gives specific information on the various parts of the instrument, such as sample compartment and sampling accessory, detector type, and light source. Make sure that the instrument is set up with the following parameters:
   a. Gain = 1.0
   b. Detector = DTGS KBr
   c. Source = IR
   d. Accessory – Transmission E.S.P
   e. Sample Compartment: Main
   f. Spectral Range 4000–600 cm^{-1}

Click on “OK” to exit this window.

B. Liquid Sample Analysis (Benzonitrile and 2-Pentanone)

1. Collect a background scan – the “image” the detector is receiving when there is no sample in the light path. Click on the “Col Bkg” icon and then click on OK at the next prompt.
2. Once the instrument has collected all 16 scans, the reading is complete. Print out a report by clicking on the “Window” icon and select print/preview.
3. You will be provided with two NaCl discs from your instructor. Ensure that they are clean by gently wiping them with a Kimwipe tissue lightly soaked with acetone. Avoid scratching the surface, as this can affect your measurements.
4. Place the cleaned discs into the liquid sample holder and insert the holder into the FTIR transmission accessory. Set the number of scans to 32 in the “Collect” tab. Click on “Collect Background (Col Bkg)”. Treat this measurement as a blank to obtain a baseline spectrum for the instrument. Print or save the background spectrum once the scan is complete.
5. Remove the sample holder from the instrument. Using a syringe with a Luer-lock tip, carefully inject benzonitrile into one of the cell’s fill ports. Fill the cell slowly to avoid trapping air bubbles, which can interfere with your spectrum. For volatile liquids, you can invert the cell while filling to minimize splashing. Once filled, seal both ports securely with the cell caps or plaques. Reinsert the liquid sample holder into the transmission attachment of the FTIR instrument for analysis.
6. This time, click on the “Col. Smp” icon to collect your sample spectra. Click OK at the collection prompt, and if necessary, click on a second collection prompt near the right-hand, top corner of the screen (Start Collection). In the bottom left-hand corner, you should see the instrument collecting 32 scans, and your sample spectrum should appear.
7. When the instrument is done scanning, click on the “Analyze” option from the top menu bar and select “Find Peaks”. A horizontal line will appear on the screen, and the instrument should label the bands below this line. You can raise or lower the line by clicking on various parts of the screen. Once all the desired peaks are labelled, click on the print icon on the left side of the screen. This will give you a printout with your spectrum, as well as a table listing the wavenumber and intensity of each band.
8. Click on the “Replace” button (right side of screen), and the spectrum will be sent to another window. Select “Analyze” from the top menu bar and then go to the “Library Set-up” option. Select several libraries from the left menu and use the arrow buttons to transfer them to the right side. Click on the search button to start the library search.
9. Print a copy of your search (print icon).
10. Clean the cells with acetone and then repeat steps 5–8 for 2-pentanone.

C. Solid Sample Analysis (Vanillin)

1. Clean the NaCl discs by gently wiping them with a Kimwipe tissue soaked with acetone.
2. Place the discs in the solid sample holder and put the holder in the sampling accessory. Click on “Col Bkg” again. This time, we are treating this “sample” as a “blank” and will get a baseline signal to start off our analysis with. Print the scan when completed.
3. Remove the discs from the instrument. Separate the NaCl plates and add a drop of Nujol to one of them. Place the other disc on top and gently twist the plates together so that the liquid will be spread out evenly between the plates, and all air bubbles should be removed.
4. Clamp the plates in the holder and then inside the instrument. This time, click on the “Col. Spl.” icon to collect your sample spectra. Click OK at the collection prompt, and if necessary, click on a second collection

prompt near the right-hand, top corner of the screen (Start Collection). In the bottom left-hand corner, you should see the instrument collecting 32 scans, and your sample spectrum should appear.

5. When the instrument is done scanning, click on the “Analyze” option from the top menu bar and select “Find Peaks”. A horizontal line will appear on the screen, and the instrument should label the bands below this line. You can raise or lower the line by clicking on various parts of the screen. Once all the desired peaks are labelled, click on the print icon on the left side of the screen. This will give you a printout with your spectrum as well as a table listing the wavenumber and intensity of each band.
6. Weigh out 5 mg of vanillin powder into a plastic weigh dish. Transfer to a marble mortar and grind to a fine powder using the pestle provided. Add one or two drops of Nujol (mineral oil) to the powder and mix well.
7. Take the discs out of the instrument and wipe off the excess Nujol with a Kimwipe. Use a rubber policeman to spread the vanillin/Nujol mixture evenly on one of the plates. Place the other disc on top and twist gently until the mixture is evenly spread and no air bubbles are visible. Collect this sample, find the peaks, and print out the corresponding report.

Experiment-Specific Lab Report Guidelines and Evaluation Criteria (20 marks)

Introduction (5 marks)

Briefly describe the major parts of the instrument and explain how a spectrum is generated for your sample.

- source
- interferometer
- sample compartment
- detector
- computer/data processor

Results and Discussion (9 marks)

- Draw the structure of benzonitrile on the spectrum from part B. Identify the major functional groups on this molecule, then identify at least FIVE major bands on the spectrum. (3 marks)
- Draw the structure of 2-pentanone on the spectrum from part B. Identify the major functional groups on this molecule, then identify at least FIVE major bands on the spectrum and indicate some of the bands on the spectra that correspond to these groups. (3 marks)
- Draw the structure of vanillin on the spectrum from part C. Identify the major functional groups on this molecule, then try to identify five major bands on the spectrum that are only due to the vanillin. Keep in mind that you will see several large bands, which are due to the Nujol and not vanillin itself. (3 marks)

Questions (6 marks)

Instructions:

- Open Windows File Explorer.
- Navigate to **Program Files (x86) → OMINC**.
- Locate and launch **OMTh-enu** (the icon appears as a coloured triangle).
- Answer the following questions.
- Please type your answers in **dark blue font colour**.
- **Marking**: 0.25 marks deducted for each incorrect blank answer, and 0.5 marks deducted for each incorrect short answer.

Downloads

Visit page 7.2 Experiment #1 in the online version of the textbook to access a PDF or Word version of these questions.

Questions:

1. Electromagnetic rays consist of _____ and _____ fields that vibrate at right angles to each other. (0.5 marks)
2. It is more convenient to report infrared radiation as wavenumbers because: (0.5 marks)
3. The intensity of vibrating atoms in a molecule _____ when IR radiation is absorbed. (0.25 marks)
4. Each chemical bond requires _____ to make it vibrate. (0.25 marks)
5. In the ethyl acetate spectrum: (1 mark)
    - A characteristic band occurs at 1750 cm^{-1} due to the _____ functional group
    - CH_2 and CH_3 stretches occur at _____ cm^{-1}
    - CH_2 and CH_3 bends occur at _____ cm^{-1}
    - Based on this data, which motion requires more energy – a stretch or bend?
6. The optical bench measures the _____ after it has passed through a sample. The resulting signal, called an _____, contains information from all frequencies present in the beam. The computer reads the interferogram and uses _____ to decode the intensity information for each frequency, and presents the _____. (1 mark)
7. The source emits _____. (0.25 marks)
8. Three steps involved in making a sample measurement are: (0.75 marks)
    a. collecting a _____
    b. collecting _____
    c. calculating the _____
9. Briefly summarize (one or two sentences maximum) what you learned about the importance of these four characteristics: (1.5 marks)
    - instrument sensitivity
    - precision and accuracy of peak locations
    - x-axis (wavenumber) resolution

7.3 Experiment #2

Identification of Recyclable Plastics by FTIR Using Attenuated Total Reflectance (ATR) Mode

Background

Attenuated Total Reflectance–Fourier Transform Infrared (ATR-FTIR) spectroscopy is a rapid, nondestructive technique for identifying materials based on their molecular vibrations. Unlike traditional transmission methods, ATR requires minimal sample preparation and allows direct analysis of solid surfaces such as plastic polymers.

In this experiment, students analyze six major categories of recyclable plastics (PET, HDPE, PVC, LDPE, PP, PS) using an ATR crystal that enables the infrared beam to penetrate only a few micrometres into the sample. Each plastic exhibits a distinct IR "fingerprint" corresponding to its chemical bonds and molecular structure, allowing identification of an unknown sample by spectral comparison.

Learning Objectives

After completing this experiment, students will be able to:

1. Explain the working principle of ATR-FTIR, including total internal reflection and the role of the evanescent wave in sampling surface layers.
2. Acquire and interpret ATR spectra of common recyclable plastics and use characteristic absorption bands to identify polymer types.
3. Compare and classify an unknown plastic sample based on spectral matching with known reference spectra.
4. Relate the functional groups observed in IR spectra to the chemical structure and composition of polymer materials.

Safety Precautions

- Handle the ATR crystal carefully—avoid scratching or touching it with bare fingers.
- Use only a small amount of acetone to clean the ATR window.
- Do not overtighten the pressure clamp when mounting samples.
- Dispose of acetone wipes in the designated waste container.

Procedure

In this experiment, spectra will be collected for the major categories of recyclable plastics. An unknown sample will then be analyzed and classified accordingly based on the comparison of its spectrum to those of the known samples.

A. Instrument Set-Up & Sample Analysis

1. Turn on power switch on the instrument (located near the bottom on the backside). The ATR attachment should already be installed (see instructor for details). Click on the OMNIC icon to load the software.
2. When you are ready for sample analysis, ensure that the Experiment line near the top of the screen is set for “iD5-ATR-Diamond”.
    - Click on the “Collect” option from the menubar at the top of the screen and select the “Experimental Set-Up” option.
    - Make sure that you are in the “Collect” tab. This screen indicates the conditions for the optical setup of the instrument.
    - Check to see that the instrument is set up with the following parameters:
        a. No. of scans = 16
        b. Resolution = 4
        c. Final format = %Transmittance
3. Go to the “Bench” tab. This screen indicates how the signal will be electronically amplified and gives the wavenumber range that will be used for data collection. It also gives specific information on the various parts of the instrument, such as sample compartment and sampling accessory, detector type, and light source.
    - Make sure that the instrument is set up with the following parameters:
        a. Gain = 1.0
        b. Detector = DTGS KBr
        c. Source = IR
        d. Accessory – iD5 ATR
        e. Spectral Range 4000 – 600 cm-1
    - Click on “OK” to exit this window.
4. The sample compartment should be empty. Clean the ATR window with a Kimwipe tissue that has been wetted with a small amount of acetone.
    - Collect a background scan – the “image” the detector is receiving when there is no sample in the light

path. Click on the “Col Bkg” icon and then click on OK at the next prompt. Click on the “Start Collection” button. Watch the counter on the bottom left corner of the screen.
    - Once the instrument has collected all 16 scans, the reading is complete. Click “Yes” in response to the prompt “Add to Window 1”.
    - Print out a report by clicking on the “Print” icon. Select “doPDF9” as the printer. Click Browse to make sure the file is going to the correct directory and enter the file name. Click Save, then when you are back in the doPDF9 window, you need to click on the OK button in the middle of the window to complete the process – otherwise the file will not save.
5. Using the recyclable plastic samples provided and an unknown sample, collect and print the spectrum for each one. See the instructor for details on how to correctly mount the sample in the holder so that there is sufficient contact between the sample and the ATR crystal.
    - From the Window option at the top of the screen, select “New Window” and create a window with the name of the sample you wish to run. Click on the “Col Smp” icon to start collection. Enter your sample name at the prompt (e.g. known1).
    - When the instrument is done scanning each sample, add each spectrum to its own window. Click on the “Analyze” option from the top menu bar and select “Find Peaks”. A horizontal line will appear on the screen, and the instruments should label the bands below this line. You can raise or lower the line by clicking on various parts of the screen. Once all the desired peaks are labelled, click on “Replace” near the top right of the screen to complete the analysis.
    - From the main top menu line, click on Report and then choose the Preview/Print Auto Report option. A report will appear on the screen with the spectrum and a table listing wavenumbers for the labelled peaks and their corresponding absorbance readings (intensity). Click on the print button for this window and save your file to the memory stick by using the doPDF9 printer option.
    - Repeat this process for all six of the known plastic samples, and for an unknown sample selected from the plastic bin.
6. After running the unknown sample, look through the windows for the known plastics until you find one that appears to be a match. Copy the known plastic spectrum over to the window of your unknown using the following procedure:
    a. While in the window for the known plastic, select Edit from the top menu, then Copy.
    b. Go to the window of the unknown sample, select Edit from the top menu, then Paste.
    c. From the top menu, select View, and then the Stack Spectra option.
    d. Send a copy of stacked spectra to the memory stick by hitting the Print icon and follow the prompts for the doPDF9 printer option.

Experiment-Specific Lab Report Guidelines and Evaluation Criteria (10 marks)

Introduction (3 marks)

- Give a brief description of the ATR sampling mode attachment and explain how it works. (2 marks)
- Perform an online search to discover the identity and structures of the plastics that make up recyclable categories 1 through 6. Include these in your report introduction and reference your sources. (1 mark)

Results and Discussion (7 marks)

- Prepare copies of your spectra with the peak labels on them. This can be done by taking snapshots of your spectra from the .pdf files and copying them over to PowerPoint, and then using textboxes to label the peaks. Alternatively, you can print a hard copy of your instrument report, label what's needed by hand for parts b) and c) below, and then take a picture or scan the document as a .pdf file to submit to the FOL submissions folder.
- Label each spectrum of the six known plastics with the appropriate chemical name based on your literature search.
- Include the polymer structure on each spectrum and identify four major bands. A graphics program can be used to draw the structure, or you can use an online image as long as you reference the source and there are no copyright restrictions governing its use for a student report. State the bond and whether or not it's a stretch or bend (e.g. O-H stretch) and include the structure of your analyte (can be hand-written on your spectrum). (6 marks)
- For your unknown plastic, label the spectrum with the identity of the plastic structure and circle four major bands that confirm it. You should base your identification on plot of the stacked known and unknown spectra for the best
match. (1 mark)

7.4 Experiment #3

Analysis of Paint Curing and Milk Composition by FTIR Using Attenuated Total Reflectance (ATR) Mode

Background

In this experiment, two practical industrial applications of ATR-FTIR are explored: polymer curing and food composition analysis.

In the polymer industry, FTIR is commonly used to monitor the curing reaction of acrylic paints, which involves solvent evaporation and polymer cross-linking. During the drying process, the disappearance of the broad O–H stretching band (around 3300 cm^{-1}) indicates water loss, while the increasing intensity of C–O stretching bands (around 1250 cm^{-1}) reflects cross-link formation. These spectral changes allow for estimation of the paint's drying time and assessment of its chemical stability, crucial parameters in quality control and product performance.

In the food industry, FTIR spectroscopy offers a rapid and non-destructive means of analyzing the major macronutrients in milk—fats, proteins, and carbohydrates (sugar). Each component exhibits characteristic absorption bands corresponding to ester carbonyl (fat), peptide (protein), and hydroxyl (sugar) functional groups. By comparing absorbance values between milk types (2%, skim, and chocolate) and reference standards (0% milk), it is possible to estimate composition and assess the accuracy of IR-based quantification against nutritional label values.

Together, these applications demonstrate how FTIR spectroscopy extends beyond basic molecular identification to quantitative and kinetic analysis, supporting both product development and regulatory compliance in industrial and food chemistry contexts.

Learning Objectives

After completing this experiment, students will be able to:

1. Explain the working principle of ATR-FTIR and its application for surface analysis of solids and liquids.
2. Monitor and interpret spectral changes during acrylic paint curing to assess drying and cross-linking.
3. Acquire and analyze ATR spectra of milk samples to estimate fat, protein, and sugar content.
4. Compare experimental results with nutritional label data to evaluate the analytical accuracy of ATR-FTIR.

Safety Precautions

- Handle the ATR crystal carefully—avoid scratching or touching it with bare fingers.
- Use only a small amount of acetone to clean the ATR window.
- Do not overtighten the pressure clamp when mounting samples.
- Dispose of acetone wipes in the designated waste container.
- Do not spill liquids on the instrument.

Procedure

This experiment will explore applications in the polymer and food industries. Students will estimate the drying time of acrylic paints based on chemical changes during the curing reaction over time, and compare the fat, protein, and lactose content of various milk samples.

Investigation of the Curing Reaction in Acrylic Paint Samples

Paints consist of pigments, binders to hold the pigments in place, extenders to improve adhesion and film strength, solvents, and additives for a variety of purposes, such as accelerating drying time and bactericides and fungicides. Acrylic paints contain water as the solvent and binders made from acrylic resin polymers based on monomers such as ethenyl ethanoate (vinyl acetate) and acrylic esters such as methyl 2-methylpropenoate, ethyl propenoate, butyl propenoate, methyl 2-methylpropenoate. As the paint dries or “cures”, the solvent evaporates, and cross-linkages begin to form between the polymer chains (The Essential Chemical Industry, 2023).

A. Instrument Set-Up & Sample Analysis

1. Click on the **OMNIC** icon to load the instrument software, then click on the "Collect" option from the menu bar at the top of the screen and select the "Experimental Set-Up" option.
    - Make sure that you are in the "Collect" tab. This screen indicates the conditions for the optical setup of the instrument. Check to see that the instrument is set up with the following parameters:
        - No. of scans = 8
        - Resolution = 4
        - Final format = Absorbance
2. Go to the "Bench" tab. This screen indicates how the signal will be electronically amplified and gives the wavenumber range that will be used for data collection. It also gives specific information on the various parts of the instrument, such as sample compartment and sampling accessory, detector type, and light source.
    - Make sure that the instrument is set up with the following parameters:
        - Gain = 1.0
        - Detector = DTGS KBr
        - Source = IR
        - Accessory – iD5 ATR
        - Sample Compartment: Main
        - Spectral Range 4000 – 600 cm^{-1}
    - Click "OK" to exit this window.
3. Collect a background scan – the "image" the detector is receiving when there is no sample in the light path. Click on the "Col Bkg" icon and then click on OK at the next prompt. Once the instrument has collected all 8 scans, the reading is complete.
    - Print out a report by clicking on the "Print" icon. Select "doPDF9" as the printer. Click Browse to make sure the file is going to the correct directory, and enter the file name. Click Save, then when you are back in the doPDF9 window, click OK to complete the process – otherwise the file will not save.
4. Pour out a few drops of paint onto a large watch glass and let it run down the edges toward the depression in the center. Using a glass rod with a rubber tip, gently transfer some of the paint to the surface of the crystal on the ATR attachment.
    - Go to Window on the top menu line and click on New Window to open a new window to collect your sample. Name the window "Wet Paint." Click on the "Col. Smp" icon to collect your sample spectrum. Name the sample (e.g., "wet sample"). Click OK at the collection prompt. After scanning, you will be prompted to add the sample to the window – click OK.
    - Immediately after collection, gently wipe the paint off the ATR crystal using Kimwipes wetted with water and also with acetone.
5. Click on the "Analyze" option from the top menu bar and select "Find Peaks". A horizontal line will appear on the screen, and the instrument will label the bands below this line. Adjust the line as needed. Once labelling is complete, click "Replace".
    - From the main menu, click Report → Preview/Print Auto Report. Save the file using the doPDF9 printer.
6. After 10, 20, 30, and 40 minutes, remove dried paint from the watch glass and transfer it to the ATR crystal. Collect each spectrum in its own window and label the peaks. Analyze the dry paint sample provided.
    - No new background is required between samples for paint or milk analysis.

Comparison of Fat, Protein, and Lactose Concentrations in Milk Samples

According to AOAC Method 972.16 (Fat, Lactose, Protein, and Solids in Milk), the following characteristic bands may be observed in milk samples:

- Carbonyl groups in ester linkages of fats — 5.723 µm
- Peptide linkages of amino acids in proteins — 6.465 µm
- OH-groups of lactose molecules — 9.61 µm

Instrument Set-Up & Sample Analysis

1. Collect IR spectra for 2%, skim, and chocolate milk using the conditions listed below, as well as the spectrum for a few drops of water.
    - Use plastic transfer pipettes to add one or two drops of each sample to the ATR crystal.
        - No. of scans = 8
        - Resolution = 4
        - Final format = Absorbance
        - Gain = 1.0
        - Spectral Range 4000 – 600 cm^{-1}
    - Immediately after collection, thoroughly but gently wipe the paint off the ATR crystal with a kimwipe and several wipes of a clean kimwipes wetted with water and also some wetted with acetone.
2. Each spectrum should be collected in its own window. Since milk has a high water content, the spectrum for water will need to be subtracted off each sample prior to analysis.
    - Click on the Window option from the main menu and open the window with the water sample. Click on Edit from the main menu and then Copy. Now open the window for the milk sample you are interested in, and then click on Edit, and then paste.
    - From the pull-down menu listing the available samples in this window (wide menu located just below the instrument control icons), select both the water and the milk sample by holding down the Shift key and using the mouse, then click on the Subtract icon. You should see small bands pointing upwards. If they are pointing down, click on a double-headed arrow near the right side of the screen.
3. On the water-subtracted spectra, click on the “Analyze” option from the top menu bar and select “Find Peaks”. A horizontal line will appear on the screen, and the instrument should label the bands below this line. You can raise or lower the line by clicking on various parts of the screen. Once all the desired peaks are labelled, click on “Replace” near the top right of the screen and print a copy of your report for each sample using the doPDF9 printer (see step 3 from the paint analysis instructions).

Experiment-Specific Lab Report Guidelines and Evaluation Criteria (15 marks)

Introduction (5 marks)

- Perform a brief literature search on the structure and use of acrylic paints. (2.5 marks)
- Using the nutritional labels on the milk cartons, prepare a table summarizing fat, protein, and sugar content (g/250 mL). Adjust values if carton volume is 237 mL. (2.5 marks)

Data and Calculations (10 marks)

1. Drying Time Predictions (5 marks)

From your spectra of the paint samples, make a chart of the absorbance readings for the bands at 3300 cm^{-1} and 1250 cm^{-1}. Use your data from the initial, 10, 20, 30 and 40 minute time points to plot two graphs in Excel. Each should have Absorbance on the y-axis and time (minutes) on the x-axis. One graph should plot the decrease in signal for the 3300 cm^{-1} band due to water, and the other for the 1250 cm^{-1} for the increase in signal due to the cross-linkage formation during the curing reactions. If desired, both lines can be plotted on the same graph but use different colours or line formats (e.g. solid or dashed) for each parameter.

Use Excel to calculate and display the equation of each line. Use a linear line fit. For each equation, enter in your absorbance values for the dried paint sample and calculate the expected drying time.

2. Milk Analysis (5 marks)

Part 1: Quantitative Accuracy Assessment

Convert the wavelength values from the AOAC method into wavenumber values. Using the absorbance values for the appropriate bands, calculate the amount of fat, protein, and lactose in skim and chocolate milk using the formula provided. The 2% milk sample is used as a reference. Present the calculated concentrations for fat, protein, and lactose for all three milk samples in Table 7.4.1 below.

$$\frac{\text{Absorbance of band in sample}}{\text{Absorbance of bank in 2\% milk}} \times \frac{\text{mass in 2\%}}{250mL}$$

For the 2% milk portion of the table, the g/250mL values should be based on the label; for the skim and chocolate milk g/250 mL values, use the above equation to calculate them based on the 2% milk as the reference

Table 7.4.1

2% Milk					
Fat		**Protein**		**Sugar**	
Fat Abs.	g/250 mL	Fat Abs.	g/250 mL	Fat Abs.	g/250 mL
Skim Milk					
Fat		**Protein**		**Sugar**	
Fat Abs.	g/250 mL	Fat Abs.	g/250 mL	Fat Abs.	g/250 mL
Chocolate Milk					
Fat		**Protein**		**Sugar**	
Fat Abs.	g/250 mL	Fat Abs.	g/250 mL	Fat Abs.	g/250 mL

Part 2: Statistical Comparison to Label Values

Calculate the accuracy of the IR method by comparing your results to the nutritional label information compiled in the introduction. For the skim and chocolate milk samples, compute the Percent Error for each component using the formula:

$$\text{Percent Error} = \frac{|\text{IR Calculated Value} - \text{Label Value}|}{\text{Label Value}} \times 100\%$$

Present these results in Table 7.4.2. Based on the percent error, discuss the accuracy of the FTIR method for each nutritional component. Provide possible reasons for any significant discrepancies observed (e.g., percent error > 10%).

Table 7.4.2

Milk Sample	Component	Label Value (g/250 mL)	IR Calculated (g/250 mL)	Percent Error (%)
2% Milk	Fat			
	Protein			
	Sugar			
Skim Milk	Fat			
	Protein			
	Sugar			
Chocolate Milk	Fat			
	Protein			
	Sugar			

CHAPTER 8: LAB PROJECT

Chapter Outline

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8.1 Lab Project: Project Overview

Development and Validation of a Standard Operating Procedure (SOP) for Allura Red AC Quantification in Sports Drinks

Project Overview

In this lab project, your group will function as an analytical chemistry team in a quality control laboratory. You are tasked with developing, validating, and executing a Standard Operating Procedure (SOP) for determining the concentration of the colourant Allura Red AC (FD&C Red No. 40) in commercial sports drinks using a double beam UV-Vis spectrophotometer.

The project is divided into three distinct phases:

1. Pre-Lab: SOP Development - Your group will research and write a complete SOP.
2. In-Lab: SOP Execution & Validation - You will perform the experiment exactly as outlined in your approved SOP.
3. Post-Lab: Reporting - You will analyze your data, assess your method's performance, and write a formal report.

Learning Objectives

Upon completion of this project, you will be able to:

- Design a detailed Standard Operating Procedure (SOP) for a quantitative analytical method.
- Justify the selection of sample preparation techniques and instrumental parameters based on scientific literature.
- Operate a UV-Vis spectrophotometer to generate high-quality analytical data.
- Implement a comprehensive method validation protocol to assess key performance characteristics: Linearity (LOL, LOD, LOQ), Precision, Accuracy (via % Recovery and Relative Error), Robustness, and stability.
- Incorporate system suitability checks to ensure data integrity during analysis.
- Quantitatively determine the concentration of an analyte in a complex real-world sample (sports drinks: Gatorade Fruit Punch, Powerade Red Fruit Punch, and Prime Strawberry Watermelon).
- Critically evaluate the effectiveness of a self-developed analytical method.

The Task: Your Mission

Your client has provided three sports drink samples and a pure Allura Red AC standard. Your deliverables are:

- *A pre-approved Standard Operating Procedure (SOP)* titled: “SOP for the Quantification of Allura Red AC in Sports Beverages via UV-Vis Spectrophotometry.”
- A *formal analytical report* presenting the findings, including the calculated concentration of dye in each sample and the results of your method validation.

8.2 Phase 1: Pre-Lab SOP Development

To be completed BEFORE the lab session

Your group must develop a comprehensive SOP. This document must be typed and submitted for review by the instructor ***before your scheduled lab session***. You will not be permitted to begin lab work without an approved SOP.

Your SOP must include the following sections with sufficient detail for another chemist to replicate your work exactly:

1. Scope and Purpose

- **Briefly state the goal:** to quantify Allura Red AC in sports drinks.

2. Principle

- Explain the scientific theory behind the method (e.g., Beer-Lambert Law, absorption of light by chromophores).

3. Reagents, Standards, and Samples

- **List all materials:** Include specific sports drink brands and flavours (Gatorade Fruit Punch, etc.).
- Detail the preparation of all solutions:
  - **Stock Standard Solution (e.g., 100 mg/L)**: Specify the exact mass of Allura Red and the volumetric flask size. Show the *calculation* for this preparation.
  - **Calibration Standards**: Propose a series of at least five standard concentrations (e.g., 2, 4, 6, 8, 10 mg/L). Detail the dilution scheme from the stock solution, specifying the volumes and flask sizes to be used. Show *example calculations*.
  - **Sample Preparation**: Describe your procedure for preparing the sports drink samples for analysis. Justify your chosen dilution factor based on expected dye concentrations. How will you address potential interferences (e.g., carbonation, turbidity)?

4. Instrumentation and Conditions

- Identify the instrument (Spectronic 200 UV-Vis Spectrophotometer).
- Specify the cuvette type and pathlength.
- Detail the procedure for determining the wavelength of maximum absorption (λ_{max}).

5. Analytical Procedure

- Provide a step-by-step, numbered list of instructions.
- System Suitability Checks: Your procedure MUST include:
  - **Blank Verification**: The blank (deionized water) will be measured after every three samples to monitor for baseline drift and contamination.
  - **Standard Precision Check**: A mid-level calibration standard (e.g., 6 mg/L) will be measured as an unknown at the beginning, middle, and end of the sample run. The precision of these measurements will be used to assess instrument stability.

6. Method Validation Protocol

Your SOP must outline how you will validate the following parameters during the lab session:

Linearity and Range

- **Construct a calibration curve**. Report the correlation coefficient (R^2), slope, and y-intercept.
- **LOD (Limit of Detection)**: $3.3\sigma/S$ (where σ is the standard deviation of the response of the blank or the y-intercept, and S is the slope of the calibration curve).
- **LOQ (Limit of Quantification)**: $10\sigma/S$
- **LOL (Upper Limit of Linearity)**: The highest standard concentration for which the response is demonstrably linear (e.g., where the deviation from the regression line is <5%).

Precision

- **Standard/Instrument Precision**: Calculate the Percent Relative Standard Deviation (%RSD) of the mid-level standard measured for the system suitability check.
- **Sample Precision (Repeatability)**: Specify which sports drink sample will be analyzed six times independently to calculate the RSD.

Accuracy

- **% Recovery**: Design a spike recovery experiment. Specify which sample will be spiked, the concentration of the spike solution, and the calculation (any formula).
- **Relative Error (% Error)**: If a reference value is known or can be approximated from literature for a typical concentration, calculate the relative error for your measured sample concentration

Robustness

- Design a simple test to evaluate the method's resilience to a small, deliberate change in procedure.
  - For example, prepare and measure one standard and one sample at a wavelength slightly different from the determined λ_{max} (e.g., $\lambda_{max} \pm 2$nm). Compare the results (absorbance and calculated concentration) to those obtained with the standard procedure.

7. Data Analysis and Calculations

- Explain how the sample concentration will be calculated from the calibration curve, including how to account for the dilution factor.

8. Safety and Waste Disposal

- List all required PPE.
- State the proper disposal procedures for all solutions.

8.3 Phase 2: In-Lab SOP Execution & Validation

During the lab session, you will:

1. Have your SOP reviewed and signed by the instructor.
2. Prepare your solutions and perform the analysis *exactly as written in your SOP*, including all system suitability and validation tests.
3. Record all raw data (weights, volumes, absorbance values) directly into your lab notebook in real-time. The instructor will sign your notebook pages at the end of the session.
4. Clean your work area and dispose of waste according to your SOP and college policy.

8.4 Phase 3: Post-Lab Reporting

20 Marks

The final group report must be a maximum of 15 pages, double-spaced, and submitted as a single PDF file.

Report Format:

Introduction (5 marks)

- **Objective**: Clearly state the project's aim.
- **Context**: Importance of testing for Allura red dye in food and drink and its concentration limits (regulatory limit), reason for various sample preparation steps, interference prevention measures, why use UV-Vis for this testing?
- **Theory**: Different components of the instrument and how they work, Sample container material, what happened to the sample in the instrument.

Materials and Methods (5 marks)

- Include the ***final, executed version of your SOP***. This should be the same document used in the lab, with any minor, instructor-approved deviations noted.

Data and Results (5 marks)

- **Summary of Raw Data**: Present all data in clear, well-labelled tables (e.g., calibration standard absorbances, sample absorbances, system suitability data).
- **Calibration Curve**: Include the graph with the linear regression equation and R^2 value.
- **Sample Analysis**: Table of calculated Allura Red concentrations for each sports drink.
- **Method Validation**: A comprehensive summary table of results for LOD, LOQ, LOL, Precision (Standard and Sample RSD%), Accuracy (% Recovery and/or % Error), and Robustness.
- **Calculations**: All calculations must be shown in a step-by-step manner; however, only show one representative set of calculations.

Discussion and Conclusion (5 marks)

Interpret your results. How do the dye concentrations in the three drinks compare?

- Critically evaluate your method's performance based on the full validation data. Was the method linear (up to the LOL), precise, accurate, and robust? What do the LOD/LOQ values imply about the method's sensitivity?
- Discuss the results of your system suitability checks. Did the instrument perform stably throughout the run?
- Discuss potential sources of error and the effect of the sports drink matrix on the analysis.
- Propose specific improvements to your SOP for future use.

8.5 Report Submission and Laboratory Conduct Guidelines

1

Report due date – five school days from the date of your lab session. Reports should be submitted to the FOL Dropbox. A folder has been set up for the lab project. ***PDF format ONLY!*** Late reports will not be accepted.

2

All department safety and waste disposal guidelines for laboratory procedures must be followed as per college policy; otherwise, marks will be deducted from the report.

3

Instrumental analysis often involves accurate work at very low concentrations. Good lab technique is critical, and housekeeping procedures need to be followed to reduce the possibility of contamination. Marks may be deducted if these procedures are not followed. Examples of these procedures include:

- Accurate pipetting and quantitative sample transfer.
- Labelling all beakers and solutions.
- Proper disposal of solutions at the end of the lab session.
- Leaving work area clean after use.

Steps for Developing an Instrument Test Procedure

1. Sample Information
2. Analysis Goals
3. Equipment Needed
4. Sample Preparation
5. Test Run
6. Optimization
7. Validation: verify the performance of the method parameters (eg. sensitivity, selectivity, reproducibility, robustness, linear range, accuracy/recovery, stability)

References

The Essential Chemical Industry. (2013, March 18). Paints. https://www.essentialchemicalindustry.org/materials-and-applications/paints.html

Version History

This page provides a record of changes made to the open textbook since its initial publication. If the change is minor, the version number increases by 0.1. If the change involves substantial updates, the version number increases to the next full number.

Version	Date	Change	Affected Web Rage
1.0	December 11, 2026	Publication	N/A