CH 426 – Instrumental Analysis Laboratory Experiments

Southern Oregon University
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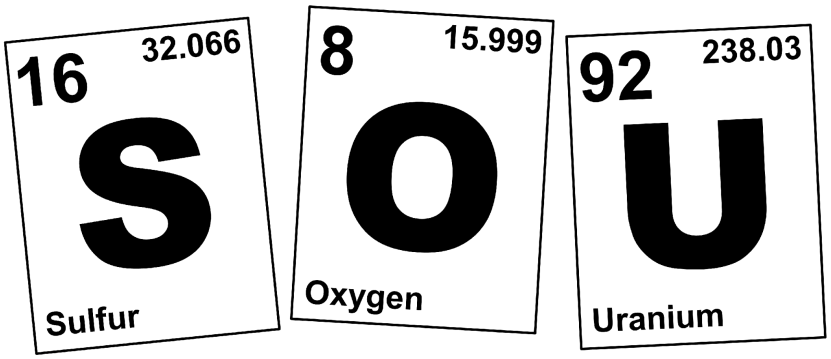

Instrumental Analysis Laboratory Experiments

Pre-lab Report Rubrics

Pre-laboratory reports are mandatory for all sessions. Each pre-lab report should include the following elements: date, your name, experiment title, background, objectives, hypotheses, materials and methods, and safety considerations. The purpose of the pre-lab is to ensure you are well-prepared and to give you an opportunity to demonstrate your understanding before the experiment.

In your report, briefly explain the lab's objective and hypotheses—what the experiment involves and what you aim to achieve. Discuss key concepts or techniques that will be applied, followed by an overview of the procedure, focusing on the main steps. Where applicable, simplify these steps into a diagram or schematic graph (this will also be evaluated in your notebook). Be sure to highlight any specific safety precautions required for the experiment. You are welcome to include any questions or concerns you may have about the experiment.

Students who fail to submit their pre-laboratory report on time will be considered late arrivals. Per the course's late arrival policy, late arrivals will not be permitted to participate in that session's experiment. For more details, refer to the late arrival policy outlined in the course guidelines. Over the term, you will complete four pre-laboratory reports, three of which will be combined reports due to multi-week labs. Each report will be evaluated using the provided rubric.

If you have any questions or encounter technical issues, feel free to reach out.

Table 1. Grading rubric and point allocation for the pre-lab report.

Section	**Points**
Date, name, experimental title	1
Background	2
Objective and/or hypothesis	3
Materials and Methods	3
Safety	1
Total	10

Laboratory Report Rubric

Over the course of the term, you will write four formal laboratory reports. The following rubric will be used to evaluate each report.

Table 2. Grading rubric and point allocation for the full laboratory report.

Section	Key Components	Points
Abstract	Provides a brief, one-paragraph summary of all laboratory report sections. The abstract should provide context for the study, the techniques used, and an overview of the findings.	5
Introduction	The introduction should provide context for the experiment that has been performed. What are you hoping to find through the experiment? What are the key chemical techniques used to address this topic? Where appropriate, peer-reviewed literature should be used to support the background and claims made in the introduction.	20
Materials & Methods	A concise summary of the experimental procedures used is provided, providing sufficient technical detail that the experiment could be repeated by a scientist familiar with experimental techniques. It is **not** appropriate to copy the laboratory manual verbatim as more detail is provided than would be appropriate.	15
Results and Discussion	The results and discussion section can either be a single unified section or broken up into a results section and a discussion section. However, the following areas should be addressed: ● The provided data summarizes the results from the experiment. Representative data is provided to support the analysis of the data provided. ● An explanation of the findings from the laboratory. ● A description of the importance of these findings. ● A discussion of the experimental limitations of this technique should be provided. Keep in mind that the results portion of this section describes the findings, while the discussion portion describes the impact/importance of these findings. It is not sufficient to tell your reader what you have found, you must also tell them what that means and why that might be important.	20
Figures and Tables	The figures (tables, graphs, and any pictures) included are clearly labeled and cleanly presented to improve the reader's understanding.	10
Writing Quality	The laboratory report is well written, using appropriate scientific English. Only primary sources are used to support the claims made in the report, and all sources include appropriate citations. Your laboratory report should not be more than three 1.5 spaced pages including figures and tables.	10
Pre-lab		10
Notebook		10
	Total	50

Laboratory 1: Analysis of Ions and Nutrients in Surface Water

Background

Water is one of the most essential resources on our planet, supporting all forms of life. Clean and safe water is crucial for human health, agricultural productivity, and the stability of ecosystems. Unfortunately, water quality can be compromised by pollution, leading to adverse effects on both human and environmental health. Monitoring water quality is a critical step in identifying pollution sources and developing effective strategies for prevention and remediation.

In this lab, we will measure key water-quality parameters to evaluate the health of different water systems and identify potential pollution sources. Water samples may include stormwater runoff, drinking water, and tap water, depending on student interest and availability. During the first two weeks, we will analyze trace micronutrients such as zinc (Zn) and copper (Cu), which are essential for biological function but may become toxic at elevated concentrations. These metals will be quantified following microwave digestion using UV–Vis spectrophotometry. In Week 3, we will measure additional water-quality indicators, such as ammonium, nitrate, and chloride, using Vernier ion-selective electrodes (ISEs). These parameters are important for understanding nutrient loading, contamination sources, and overall water chemistry.

Nitrate concentration is a key parameter in water quality studies, often elevated by acidic rainfall, fertilizer runoff, and waste decay. The Nitrate ISE will measure nitrate ions (NO_3^-), commonly introduced through sewage, agricultural runoff, and animal waste. Bacterial processes convert ammonia (NH_3) from these sources into nitrate.

Chloride ions (Cl^-) enter freshwater from minerals like sodium chloride (NaCl) or potassium chloride (KCl). The EPA sets a maximum drinking water limit of 250 mg/L for chloride, with seawater containing about 19,400 mg/L. Monitoring chloride helps detect salinity and contamination.

Ammonium ions (NH_4^+) are critical in areas near fertilized fields. Safe drinking water levels should not exceed 0.5 mg/L, but agricultural runoff can cause seasonal spikes. Nitrate-nitrogen (NO_3^- as N) levels above 10 mg/L are unsafe for drinking, while unpolluted waters are typically below 1 mg/L.

To understand the significance of these elements in water quality, students will research and document relevant regulatory standards and background information. They will identify EPA guidelines, such as Maximum Contaminant Levels (MCLs), Action Levels, or Secondary Maximum Contaminant Levels (SMCLs), for zinc, copper, calcium, sodium, lithium, and potassium in drinking water, and compare these standards with World Health Organization (WHO) or other international guidelines if applicable. For each element analyzed, students can include background information on its biological role (why it is essential for humans or the environment), potential toxicity (health or environmental risks at elevated levels), and natural and anthropogenic sources (how it enters water systems, such as through industrial waste, natural mineral deposits, or agricultural runoff).

Experimental Procedures

General Laboratory Equipment and Supplies

1. Quartz Cuvettes (4)
2. Zincon T-98

3. pH meters (2)
4. 500-mL polyethylene bottles (5)
5. 50-mL beakers (10)
6. 5-mL volumetric flasks (10)
7. 10-mL volumetric flasks (15)
8. 25-mL volumetric flasks (5)
9. 100-mL volumetric flasks (10)
10. 500-mL volumetric flasks (1)
11. 1000-µL adjustable Eppendorf pipets and tips (2)
12. 100-µL adjustable Eppendorf pipets and tips (2)
13. Vernier LabQuest 2 (3)
14. 10-mL graduated cylinders
15. Vernier chloride Ion-Selective electrode (1)
16. Vernier ammonium Ion-Selective electrode (1)
17. Vernier nitrate Ion-Selective electrode (1)
18. UV-Vis spectrophotometer
19. Anton Paar Microwave Digestion System

Chemicals

1. 1 M sodium hydroxide
2. 1 M hydrochloric acid
3. Boric acid
4. Potassium chloride
5. Copper (II) nitrate hemipentahydrate
6. Zinc (II) nitrate hexahydrate
7. Concentrated (15.7 M) nitric acid (trace metals grade)
8. Ammonium high standard (100 mg/L) (if the solution runs out, make from ammonium chloride)
9. Ammonium low standard (1 mg/L) (if the solution runs out, dilute the high standard)
10. Nitrate high standard (100 mg/L) (if the solution runs out, make from sodium nitrate)
11. Nitrate low standard (1 mg/L) (if the solution runs out, dilute the high standard)
12. Chloride low standard (1 mg/L) (if the solution runs out, make from sodium chloride)
13. Chloride high standard (100 mg/L) (if the solution runs out, dilute the high standard)

Water Sample Collection and Preparation

Each group will collect two water samples. Use a clean, acid-washed 500-mL polyethylene bottle for sampling. Rinse the bottle with the sample water before collection. For tap samples, allow the water to run briefly to remove stagnant water, then fill the bottle, cap it securely, and clearly label it with the sampling location, date, and time.

Each group should collect one sample expected to be relatively contaminated and one expected to be relatively clean. Possible sources may include drinking water, wastewater, tap water, and stormwater runoff.

Microwave Digestion

For total metals, an unfiltered original sample will be digested using EPA Method 3015A: Microwave-Assisted Acid Digestion of Aqueous Samples and Extracts on the microwave digestion system. A measured aliquot of the well-mixed sample (typically 22.5 mL, depending on expected concentration) is transferred into a clean microwave digestion vessel, and concentrated trace-metal–grade nitric acid (approximately 2.5 mL) is added. The vessels are then sealed and placed into the microwave rotor.

The digestion program follows EPA 3015A guidance, ramping the temperature to approximately 170–180 °C under controlled pressure and holding for ~10–15 minutes to ensure dissolution of particulate-bound metals. After cooling, the vessels are carefully vented, and the digested samples are quantitatively transferred to acid-washed 100-mL volumetric flasks and diluted to volume with milli-Q water. The final solution typically requires nitric acid to maintain appropriate sample solution acidity and stability of the elements. Commonly, a 2% (v/v) nitric acid concentration is desirable. The final digestate is now preserved at pH < 2 with nitric acid and stored until analysis. Appropriate reagent blanks will be processed alongside the samples to verify digestion efficiency and monitor contamination control.

Spectroscopic determination of copper and zinc binding

The concentration of free metal ions in aqueous solution is determined spectroscopically using a modified Zincon-based protocol adapted from previously reported methods. The borate buffer is prepared by adding 53.25 mL of 1 M sodium hydroxide to a 250 mL volumetric flask, followed by the addition of 9.33 g potassium chloride and 7.75 g boric acid. The solution is diluted to volume with Milli-Q water and mixed thoroughly.

The Zincon reagent solution is prepared by dissolving 0.013 g of Zincon in a 10 mL volumetric flask, followed by the addition of 0.20 mL of 1 M sodium hydroxide to facilitate dissolution, and dilution to volume with Milli-Q water. The Zincon solution is stored under refrigeration when not in use to maintain reagent stability.

To prepare the metal–Zincon complex, add 0.30 mL of Zincon solution, 6.0 mL of borate buffer (0.5 M boric acid, 0.5 M KCl, adjusted to pH 9.0), and 1.0 mL of the digested water sample or calibration standard to a 10.0 mL volumetric flask. Dilute to volume with Milli-Q water and mix thoroughly.

Maintaining a final pH of approximately 9.0 is critical for effective metal–ligand complex formation. Because the digestion step produces an acidic solution, the buffer volume may be adjusted as needed to ensure complete neutralization prior to analysis.

Calibration standards are prepared from zinc nitrate and copper nitrate hydrates (you may begin with making a 100 ppm stock solution for each metal). Calibration standards should be diluted using 2% nitric acid as the solvent to mimic the sample matrix.

Preliminary observations indicate linear absorbance responses in the 1–8 ppm range for both Zn^{2+} and Cu^{2+}, although some literature reports linearity only within the 1–4 ppm range. Therefore, the optimal linear working range will be determined experimentally using at least five calibration points (including a blank, if appropriate; otherwise, five calibration standards should contain the analyte).

For each metal, the dynamic range, sensitivity, linear range, limit of detection (LOD), and limit of quantification (LOQ) will be evaluated based on the calibration data.
For each calibration standard, full absorbance spectra (400–700 nm) are collected to confirm metal–Zincon complex formation by identifying the characteristic absorbance maxima. The absorbance at the maximum wavelength is then recorded as a function of concentration. The copper–Zincon complex typically exhibits a maximum absorbance near 600 nm, while the zinc–Zincon complex peaks near 620 nm.

If the absorbance is too high (the recommended range is generally 0.2–0.8 AU) and/or you believe the standard concentration can be reduced, feel free to prepare lower-concentration standards and evaluate the lower end of the calibration range. There is flexibility in this step, and you are encouraged to explore different concentration ranges, including values below 1 ppm, to determine the optimal linear working range.

In some cases, more than one metal may be present in a sample, requiring a multi-element calibration approach, even though only a single absorbance value can be measured at a given wavelength. Under these conditions, a linear relationship can still be established between absorbance and the total Zincon-complexed metal concentration (Zn + Cu) by measuring at an isosbestic point (defined as a wavelength at which two interconverting species have identical molar absorptivities).

For the Zincon system, the isosbestic point is observed at approximately 615 nm (typically between 600 and 620 nm). New calibration standards can therefore be prepared based on the total (Zn + Cu) Zincon-complexed metal concentration, and the resulting calibration curve will be evaluated. Calibration solutions should span a similar total concentration range to that determined previously, but with the total metal concentration split 50:50 between zinc and copper. Prepare at least five calibration points and evaluate the linearity and dynamic range of the response like how you prepare for separate metals. If this approach is successful, individual metal concentrations can subsequently be determined by selectively adding a competing chelating agent such as EDTA, which preferentially complexes Zn^{2+}. Because the Cu–Zincon complex is more stable, copper can be measured independently after zinc has been stripped from the Zincon complex.

Potentiometric determination of ammonium, nitrate, and chloride

To prepare the ISE for use, soak the electrode in the high-standard solution for approximately 30 minutes, ensuring it does not touch the bottom of the container and that the small white reference contacts are fully immersed without air bubbles trapped below. Calibrate each ISE with appropriate high- and low-standard solutions based on the ion being measured. For best results, use 1 ppm and 100 ppm standards for nitrate and ammonium ISEs, and 10 ppm and 1000 ppm standards for chloride. If the unknown ion concentration falls outside the linear range, dilute the sample with DI water. For lower ammonium concentrations (e.g., <0.5 mg/L NH_4^+-N), calibrate with 0.1 mg/L and 1.0 mg/L NH_4^+-N standards to improve accuracy, as sensitivity changes at these levels. Before measurement, turn on the Vernier ISEs and allow them to warm up. Select the parameters to measure and dip the probes into the water sample, letting the readings stabilize before recording them. The electrodes can be securely held in place using the clamp and stand. After each measurement, rinse the probes with DI water.

For long-term storage, moisten the sponge in the bottom of the storage bottle with DI water, rinse the ISE with DI water, blot it dry, and insert it into the storage bottle without allowing the tip to touch the sponge. Ensure the white reference mark is inside the bottle, then tighten the lid to maintain a humid environment and prevent the reference junction from drying out. For short-term wet storage (less than 24 hours), fill the soaking bottle ¾ full with the high-standard solution, insert the electrode, and tighten the cap. These steps ensure accurate measurements and proper maintenance of the ISE.

References

Säbel, C. E.; Neubert, R. H. H.; Voigt, A. A Spectrophotometric Method for the Determination of Zinc, Copper, and Cobalt Ions in Metalloproteins Using Zincon. *Anal. Biochem.* 2010, *397*, 218–226. https://doi.org/10.1016/j.ab.2009.10.036.

Kocyła, A.; Pomorski, A.; Krężel, A. Molar Absorption Coefficients and Stability Constants of Zincon Metal Complexes for Determination of Metal Ions and Bioinorganic Applications. *J. Inorg. Biochem.* 2017, *176*, 53–65. https://doi.org/10.1016/j.jinorgbio.2017.07.012.

Laboratory 2: Microbial Water Quality of Surface Water

Background

Antibiotic resistance is a growing public health concern around the world. Antibiotics are medicines used to treat bacterial infections, but overuse and misuse of antibiotics have led to the emergence of antibiotic-resistant bacteria. These bacteria are able to survive exposure to antibiotics that would normally kill or inhibit their growth, making it difficult or even impossible to treat infections caused by them with common antibiotics. Antibiotic-resistant bacteria can cause serious illnesses and even death. The development of new antibiotics is a slow and challenging process, making the prevention and control of antibiotic-resistant bacteria a critical public health priority.

The plate method is a widely used laboratory technique to measure antibiotic-resistant bacteria. This method involves growing bacterial cells on a solid medium, such as agar, that contains a specific concentration of an antibiotic. The bacteria that are able to grow on the antibiotic-containing medium are considered resistant to that antibiotic. To perform the plate method, a bacterial culture is first grown in a liquid medium to reach a certain density. Then, a small volume of the bacterial culture is spread evenly onto a plate that contains the antibiotic of interest at a specific concentration. The plate is then incubated for a period of time, allowing the bacteria to grow. After incubation, the bacterial colonies on the plate can be counted, and the number of resistant colonies can be compared to the total number of colonies to determine the percentage of resistant bacteria (resistance ratio).

The IDEXX method is also a commonly used laboratory technique for the detection of bacterial contamination in environmental samples. One of their products is the Colilert test, which is used for the detection of total coliforms and E. coli bacteria in water samples. Total coliforms are a group of bacteria that are commonly found in the environment. E. coli is a specific type of coliform bacteria that is commonly found in the intestines of warm-blooded animals, including humans. In some cases, antibiotics can be added to the culture media used in the IDEXX method to detect antibiotic-resistant bacteria. If bacterial growth is observed in the presence of the antibiotic, it indicates the presence of antibiotic-resistant bacteria. The addition of antibiotics to the IDEXX method can provide valuable information on the prevalence of antibiotic-resistant bacteria in different types of samples. This information can be used to develop strategies for the prevention and control of antibiotic-resistant infections, such as reducing the use of antibiotics in agriculture and healthcare settings.

Week 1 of this laboratory focuses on water sampling and microbial analysis using the IDEXX Colilert method. Students collect environmental water samples using sterile techniques and analyze them for total coliforms, *E. coli*, and antibiotic-resistant *E. coli* using IDEXX Quanti-Trays and Most Probable Number (MPN) analysis. Week 2 will focus on chemical water quality analysis using a Hach spectrophotometer. We will analyze the same water samples for total lead and total copper concentrations. Samples are acid-digested to ensure all metals are fully dissolved, and metal concentrations are measured using Hach TNT vial tests following manufacturer protocols.

Experimental Procedures

General Laboratory Equipment and Supplies

1. Lab Incubator
2. Single use sterilized pipettes (10)

3. UV-viewing cabinet
4. Milli-Q water
5. Lab electric pipette controller
6. 500-ml Nalgene bottles (10)
7. 15-mL Falcon tubes (5)
8. 125-ml Nalgene bottles (20)
9. IDEXX Quanti-Tray sealer
10. IDEXX Quanti-Trays
11. IDEXX Colilert-18 powder packets (20)
12. 5-mL syringe with 0.45 μm pore size cellulose ester membrane filter (5)
13. Hach TNT 850 Lead Vial Test (8)
14. Hach Copper TNTplus Vial Test (8)

Chemicals

1. Cefotaxime salt
2. Concentrated nitric acid (analytical grade)

Water Sampling

Each student is assigned two water samples, preferably from visibly impacted or "dirty" water sources (e.g., surface water, wetlands, runoff-affected areas). Samples are collected using 500 mL sterile Nalgene bottles. To collect a grab sample, remove the bottle cap immediately before sampling, taking care not to touch the inside of the cap or bottle. Submerge the bottle below the water surface with the opening facing upstream or away from disturbance, allowing it to fill completely. Cap the bottle securely while still submerged if possible, then remove it from the water. Label each bottle clearly with the sample ID, location, date, and time of collection. Samples should be kept cool and transported to the laboratory as soon as possible for analysis.

Cefotaxime Solution

Dissolve 10 mg (0.01 g) of cefotaxime (CTX) salt in 10 mL of milli-Q water to prepare the stock solution (1 mg/mL). Sterilize the stock solution by filter sterilization using a 0.45 μm filter and a 5 mL syringe. Transfer the filter-sterilized solution into a 15-mL falcon tube, and label the tube with the solution name, date, and concentration. Store the aliquots at −20 °C for later use. When needed, allow the CTX solution to thaw to room temperature before use. Each aliquot should be thawed and refrozen no more than twice; after this, the solution must be neutralized and disposed of in the appropriate chemical waste.

IDEXX

Two sterile bottles are prepared per sample and labeled to indicate *E. coli* and ESBL-producing *E. coli*, respectively. IDEXX Quanti-Trays are labeled with tape indicating the date, dilution factor, and test type. Sterile Milli-Q water is added to the bottles to prepare the required dilutions. Original sample bottles are shaken thoroughly, allowed to settle briefly, and the appropriate volume of sample is added to complete each dilution. IDEXX Colilert reagent packets are added to each diluted sample bottle, after which the bottles are sealed and shaken until the reagent is fully dissolved. For ESBL analysis, the diluted samples are shaken again and 100 μL of filtered cefotaxime (CTX) solution is added. Each prepared sample is poured into its designated, labeled Quanti-Tray, which is then carefully placed into the Quanti-Tray insert and sealed using the Quanti-Tray sealer, ensuring the tray is facing upward. The sealed trays are incubated at 35 °C for 18–22 hours in the Biotechnology Center on the first floor of the Science Building, not exceeding 22 hours. After

incubation, total coliforms are quantified by counting the number of yellow wells (49 large and 48 small wells per tray). To determine the presence of *E. coli*, incubated trays are examined under a UV-viewing cabinet and the numbers of fluorescent and yellow wells are recorded. ESBL-producing *E. coli* are recorded using the same approach. Most Probable Number (MPN) values are determined using the IDEXX MPN table, and the resistance ratio is calculated as the ratio of ESBL MPN to *E. coli* MPN. Calculations are repeated for each sample.

Hach Spectrophotometer

Total lead and total copper concentrations in water samples will be measured using Hach colorimetric test kits. Samples are first acid-digested as described in Laboratory 1 to ensure that all particulate-bound metals are fully dissolved. Following digestion, analyses are conducted using the Hach TNT 850 Lead Vial Test and the Copper TNTplus Vial Test, following the manufacturer's instructions. Results are reported as total dissolved lead and copper concentrations.

References

IDEXX Laboratories, Inc. Colilert-18® Product Insert; Colilert-18-06-02027-27; IDEXX Laboratories, Inc.: Westbrook, ME, USA.

Korir, N.; Kirby, A.; Murphy, J.; Berendes, D. Evaluation of Prevalence and Changes in Antimicrobial-Resistant Faecal Organisms in Faecal Sludge and Wastewater Treatment Plants, Naivasha, Kenya. Proc. 6th Int. Faecal Sludge Manag. Conf. 2021, Virtual Conference.

Jimenez, K.; Kong, Y.; Zhang, Y.; Ferketic, D.; Nagori, S. K.; Yang, J.; Yulo, A. A.; Kramer, B.; Prado, O. G.; Cason, T.; Chowdhry, R.; Kemsley, A.; Mendoza Espinosa, L.; Steele, J. A.; Griffith, J.; Jay, J. A. Evaluation of a Modified IDEXX Method for Antimicrobial Resistance Monitoring of Extended Beta-Lactamase–Producing Escherichia coli in Impacted Waters near the U.S.–Mexico Border. One Health **2025**, 20, 100997. https://doi.org/10.1016/j.onehlt.2025.100997

Laboratory 3: Identification of Metal Composition in Modern Pennies

Background

The composition of United States one-cent coins (pennies) has changed over time due to economic pressures and fluctuations in metal prices. Historically, pennies were produced using copper-rich alloys, consisting of approximately 95% copper with a small percentage of zinc added to improve mechanical strength. Copper was selected because of its durability, corrosion resistance, and ease of minting. However, as the market price of copper increased, manufacturing coins using primarily copper became economically inefficient.

To reduce production costs while maintaining the coin's familiar appearance, the U.S. Mint later adopted a different design using a zinc core coated with a thin outer layer of copper. This design preserves the traditional color and corrosion resistance associated with copper while significantly lowering material costs. Although coins produced using these two approaches appear visually similar, their internal compositions and overall metal content differ substantially.

In this experiment, students will use both handheld and bench-top X-ray fluorescence (XRF) spectroscopy to analyze the elemental composition of pennies from various years. XRF is a non-destructive analytical technique that identifies elements based on the characteristic X-rays emitted when atoms are excited by an external X-ray source. Students will compare elemental signals and relate composition to physical properties such as mass and density. Because pennies are manufactured with nearly identical dimensions, their volumes remain relatively constant across different years. As a result, any differences in mass primarily reflect changes in metal composition. Students will investigate how changes in coin design reflect economic decisions, manufacturing practices, and material properties while also evaluating differences between handheld and bench-top analytical instruments by comparing composition and mass.

Experimental Procedures

General Laboratory Equipment and Supplies

1. EDAX Orbis PC Micro-XRF
2. Bruker S1-Titan Handheld XRF Analyzer
3. Penny sets from multiple years (one set per group)
4. Analytical Balance

Chemicals

None

Mass Determination

For mass determination, each penny is weighed using an analytical balance, and the mass is recorded in grams. Because pennies are manufactured with nearly identical dimensions, their volumes are expected to remain relatively constant, while differences in mass and density primarily reflect changes in metal composition.

X-ray Fluorescence

Each group will analyze assigned pennies using both handheld and bench-top XRF instruments. Groups will rotate between instruments as directed by the instructor. Make sure pennies are clean and free of dirt, grease, or corrosion. If necessary, gently wipe coins using a lint-free tissue or paper

towel. Avoid scratching or damaging the coin surface, as surface changes may affect XRF measurements.

For bench-top XRF analysis, the EDAX Orbis PC Micro-XRF system is powered on and the operating software is launched. After verifying that the sample stage is clear and the X-ray source is properly initialized, the instrument shutter is closed and the penny is placed flat on the sample stage. The shutter is then reopened following instructor guidance, and the X, Y, and Z stage controls are adjusted to properly align and focus on the coin surface. A single scan is initiated to collect fluorescence spectra, and elemental signals, particularly copper (Cu) and zinc (Zn) are recorded. Record concentration results (both ppm and mass percentage) before preparing the stage for the next coin.

For handheld XRF analysis, use the Bruker S1-Titan analyzer in 30-second measurement mode. Confirm calibration and safety status with the instructor before beginning. Place the penny on the designated sample platform and position the analyzer so the measurement window is flush against the coin surface. Perform one measurement for each coin following instrument instructions. Once the scan is complete, record the elemental composition results, particularly copper (Cu) and zinc (Zn).

Data Analysis

For each penny analyzed, record the mint year, the elemental composition results obtained from both XRF instruments, and the measured mass. Using these data, compare composition and mass across the sample set to identify the year in which a significant change in metal composition occurs.

References

Department of Chemistry. *Fundamentals of Chemistry Laboratory Experiments: A Manual for Chemistry 100*; Southern Oregon University: Ashland, OR, 2009.

Laboratory 4: Assessment of Soil Heavy Metals in Lincoln School

Background

Soil is an essential component of Earth's ecosystem, serving as a habitat for diverse organisms and providing the foundation for agriculture and food production. However, soil can also contain metals that pose risks to human health and the environment. The primary exposure pathway for metals present in surface soils is ingestion, which can occur when individuals accidentally consume contaminated soil or dust. This exposure may happen through the consumption of fruits or vegetables grown in contaminated soil or through direct contact with soil during recreational or occupational activities. These metals may originate from a variety of sources, including mining operations, industrial activities, traffic emissions, and the historical or current use of pesticides and fertilizers.

In this laboratory experiment, we will investigate the presence of seven common heavy metals in soil: lead (Pb), copper (Cu), zinc (Zn), chromium (Cr), nickel (Ni), arsenic (As), and cadmium (Cd). These metals can cause adverse health effects when accumulated in the body and may also impact environmental quality by contaminating groundwater and surrounding ecosystems.

To evaluate the concentrations of these heavy metals, we will use a handheld X-ray fluorescence (XRF) analyzer, which allows for rapid and non-destructive measurement of elemental composition in soil samples. By analyzing soil samples collected from the Lincoln School playground in Ashland, we aim to assess the distribution and potential sources of these metals and evaluate whether they may pose a risk to human health or the environment. Preliminary screening results collected during the summer indicated that lead concentrations in some soil samples exceeded 1000 ppm.

The California Environmental Protection Agency (CalEPA) has established regulatory levels for several metals in soil. The regulatory levels are designed to protect human health and the environment from the potentially harmful effects of metal exposure. Here are the CalEPA regulatory levels for the metals:

a. Lead (Pb): The CalEPA regulatory level for lead in soil is 80 parts per million (ppm) for residential soil and 200 ppm for non-residential soil.
b. Arsenic (As): The regulatory level for arsenic in residential soil in California is 20 parts per million (ppm), while the regulatory level for non-residential soil is 75 ppm.
c. Copper (Cu): The CalEPA regulatory level for copper in soil is 140 ppm for residential soil and 690 ppm for non-residential soil.
d. Zinc (Zn): The CalEPA regulatory level for zinc in soil is 500 ppm for residential soil and 1,600 ppm for non-residential soil.
e. Chromium (Cr): The CalEPA regulatory level for total chromium in soil is 150 ppm for residential soil and 560 ppm for non-residential soil.
f. Nickel (Ni): The CalEPA regulatory level for nickel in soil is 50 ppm for residential soil and 220 ppm for non-residential soil.
g. Cadmium (Cd): The CalEPA regulatory level for cadmium in soil is 4.1 ppm for residential soil and 8.0 ppm for non-residential soil.

Experimental Procedures

General Laboratory Equipment and Supplies

1. Bruker S1-Titan Handheld XRF Analyzer
2. XRF sample cups (with XRF films) (10)
3. Laboratory Oven
4. 2-mm Sieves (2)
5. Ziploc bags (10)
6. Porcelain evaporating dishes (10)
7. Mortar and pestle (4)

Chemicals

1. Nitric acid, concentrated, analytical grade

Soil Sampling

Each student will collect two soil samples from Lincoln School as discussed during recitation. The sampling process will focus on approximately 1-square-meter areas of exposed surface soil to obtain representative samples. Using clean gloves, students should collect the top layer of soil (approximately the top 0–2 cm) by gently scraping the surface using a clean scoop or spatula. Avoid collecting rocks, plant material, mulch, or large debris. Each sample should be placed into a labeled Ziploc bag, ensuring the bag is sealed tightly to prevent contamination or moisture loss. Students should record the sampling location, date, and any notable site observations (e.g., soil color, moisture, nearby structures, or vegetation).

Sample Preparation

Soil samples will be dried to minimize moisture interference with XRF measurements. Spread each soil sample in a thin layer on a clean drying surface such as aluminum weigh boats, glass watch glasses, or porcelain evaporating dishes. Allow samples to air-dry if time permits. To ensure consistent moisture removal, samples will then be dried in a laboratory oven at approximately 105 °C for about 1–2 hours or until completely dry (this step may be completed during recitation the day prior to analysis). After drying, samples should be allowed to cool to room temperature before further handling.

Each dried sample will then be sieved through a 2 mm sieve into a clean container or labeled Ziploc bag to remove rocks, roots, and large debris. To prevent cross-contamination between samples, the sieve should be cleaned between uses by gently brushing or tapping out residual soil. If soil remains trapped in the mesh, lightly rinse the sieve with water and allow it to fully dry before processing the next sample. The fraction that passes through the 2 mm sieve represents the standard "fine earth" portion commonly used in soil screening and improves measurement consistency and repeatability. Alternatively, if sieving is not feasible, a mortar and pestle may be used to gently crush the dried sample, and forceps can be used to remove non-soil materials such as shells, sticks, or large organic debris.

Finally, the sieved soil should be thoroughly mixed within the bag by kneading or shaking to create a homogeneous sample. The homogenized soil will then be transferred into XRF sample cups for elemental analysis. Label each XRF sample cup clearly. Cover each sample cup with XRF film and secure the film using the locking ring to ensure the sample is properly sealed for measurement.

X-ray Fluorescence

Place the XRF sample cup containing the prepared soil sample into the sample holder of the XRF analyzer. Set the analyzer to 1-minute measurement mode. Start the XRF analysis. The XRF analyzer will emit X-rays onto the soil sample, causing the elements within the sample to fluoresce. The instrument will measure the intensity of the fluorescent X-rays and convert the signals into estimated concentrations of heavy metals in the soil sample. Perform three separate measurements for each sample, ensuring the sample cup remains properly positioned in the holder during each measurement. Record the measured concentrations for heavy metals including Pb, As, Cu, Zn, Cr, Ni, and Cd, and calculate the average concentration for each element based on the three measurements.

Laboratory 5: HPLC Method Development for the Quantification of Caffeine and Benzoic Acid in Commercial Beverages

Background

In this experiment, you will develop a High-Performance Liquid Chromatography (HPLC) method to quantify caffeine and benzoic acid in commercial beverages. These compounds are commonly found in diet sodas and other soft drinks, each serving a distinct function. Benzoic acid, typically found in its salt form as sodium benzoate, is a common preservative that inhibits microbial growth and extends shelf life. Caffeine, a natural stimulant, is added to enhance alertness and provide the characteristic energy boost associated with many soft drinks.

Due to the complex and often variable composition of commercial beverages, developing a precise and reproducible analytical method for quantifying these components is essential. HPLC is a powerful technique for separating and detecting compounds in complex mixtures. To enhance the precision and accuracy of your analysis, an internal standard will be incorporated. Theobromine and theophylline, which share structural similarities with caffeine, will be considered as potential internal standards to account for variations in sample preparation and instrument response. By the end of this experiment, you will have established a reliable HPLC method for quantifying caffeine and benzoic acid in various diet sodas, providing valuable insights into their composition and supporting quality control in beverage production.

Experimental Procedures

General Laboratory Equipment and Supplies

1. 0.45 μm syringe filters and syringes
2. Mobile phase filtration apparatus
3. Sonicator system
4. Centrifuge (capable of 1000 g)
5. 500 mm 5/16" vacuum hose
6. Autosampler vials
7. C18 column (150 mm, 4.6 mm, 3 μm)
8. 500 mL HPLC bottle × 2
9. 100-mL volumetric flasks × 2
10. 10-mL volumetric flasks × 10

Chemicals

1. Caffeine
2. Benzoic acid
3. Theobromine
4. Theophylline
5. Methanol (HPLC grade)
6. Acetonitrile (HPLC grade)
7. Formic acid
8. Triethylamine
9. Water (HPLC grade)
10. Snapples zero sugar peach tea
11. Mtn Dew soda diet

12. Rockstar pure zero energy drink
13. Pepsi soda diet
14. Other commercial beverages

Standard Solutions

Prepare individual stock solutions of caffeine and benzoic acid by dissolving 0.1 g of each analyte in a 100 mL volumetric flask and diluting to the mark with HPLC-grade water solution, yielding a concentration of 1000 ppm. Calibration standards are prepared by mixing and diluting aliquots of these stock solutions to achieve a final calibration range of 50 ng to 10 μg per injection (10 μL), corresponding to 5–1000 ppm. A recommended distribution of calibration points includes 10, 50, 100, 250, 500, and 600 ppm. Typical sample concentrations for caffeine and benzoic acid are expected to fall within the 1–100 ppm range with benzoic acid anticipated to be significantly lower.

Sample Preparation

Each beverage sample is degassed in an ultrasonic bath for 15–30 minutes or longer if necessary. Then, transfer 5 mL of the sample into a 10-mL volumetric flask, add 1 mL of the internal standard, and dilute to 10 mL with HPLC-grade methanol. If a sample contains high levels of dissolved solids (e.g., coffee or fruit juices), it should first be centrifuged at 12,500 rpm for 5 minutes, and the supernatant is then diluted as described. Before analysis, all prepared solutions are filtered using a 0.45 μm syringe filter.

Internal Standards

Prepare two separate internal standard solutions by dissolving 0.1 g of Theobromine and 0.1 g of Theophylline in individual 100 mL volumetric flasks, then diluting each to the mark with the HPLC-grade water to achieve a final concentration of 1000 ppm. Before dilution, 1 mL of the internal standard solution is added to each sample to ensure consistent quantification. Theophylline is more commonly used in caffeine-related studies and exhibits a distinct retention time with slightly different polarity. We will begin with Theophylline to assess its suitability as an internal standard, and adjustments will be made if necessary, based on its performance in the separation.

Chromatographic Conditions[1]

Separations are carried out using a C18 column (150 mm × 4.6 mm I.D., 3 μm) maintained at 25°C. The mobile phase consists of two separately prepared solutions: (A) 0.08% formic acid and 0.25% triethylamine in HPLC-grade water/methanol (99:1), and (B) HPLC-grade acetonitrile/methanol (1:1), delivered at a flow rate of 1.0 mL/min. Acetonitrile used for flushing should not be used for mobile phase preparation; a dedicated bottle should be used for each. Both mobile phases must be filtered using a mobile phase filtration apparatus before being placed in the HPLC solvent reservoirs. The instrument will operate under the following gradient: 0–4 minutes, 5–40% B; 4–6 minutes, 40–90% B; 6–7.5 minutes, 90% B; and 7.5–9.5 minutes, 5% B.

Detection is performed using a UV-Vis detector set at 285 nm for caffeine and 235 nm for benzoic acid. For diode array detection (DAD), use 235 nm with a 5 nm bandwidth and a reference at 380 nm (40 nm bandwidth), and 285 nm with a 5 nm bandwidth and a reference at 360 nm (100 nm bandwidth). These settings are optimized for detecting caffeine, benzoic acid, and related compounds including theobromine and theophylline. Samples are injected using a 10 μL loop (adjustable if needed), and a 10-minute initial elution time (adjustable if needed) is recommended to fine-tune. The required volume of mobile phase should be estimated based on the number of

samples, run time, flow rate, column equilibration, and expected waste. All solutions must be filtered and degassed before use, and the UV-Vis baseline should be checked for stability before injecting samples.

Experimental Considerations

- Your goal is to develop a method using either theobromine or theophylline as a possible internal standard. The materials above do not account for these compounds in the analysis, so you will need to decide the best method for moving forward. Before preparing your calibration standards, you may wish to consult your notes to refresh yourself on how internal standards are included in an experimental method. The inclusion of this analyte may also require you to adjust your chromatographic conditions to separate these compounds.
- HPLC is highly susceptible to particles and dissolved gases in samples. To avoid damaging the instrument, ensure that you have prepared all solutions in HPLC grade solvents and have thoroughly filtered all solutions. If you are not sure if you have filtered the sample enough, ask your instructor.
- You will be collecting UV-vis spectra for your samples using the HPLC. You will need to determine the best wavelength for the quantification of each species based on these data. Additionally, in your analysis of the commercial beverages, you will want to verify that the peak you are analyzing is relatively pure by comparing the UV-vis spectra collected to one of your standards.
- The HPLC should be reserved for two consecutive laboratory periods to ensure that you have sufficient time to complete the analysis. Even so, you will need to work efficiently during these two lab periods to analyze your samples and to determine if either of the proposed internal standards would be effective.

Before lab, briefly reviewing either of the following may be beneficial (both are available on the bookcase outside the computer lab)

- Chapters 21 and 23 in *Exploring Chemical Analysis* by Harris
- Chapters 26 and 28 in *Principles of Instrumental Analysis* by Skoog

References

(1) Delaney, M. F.; Pasko, K. M.; Mauro, D. M.; Gsell, D. S.; Korologos, P. C.; Morawski, J.; Krolikowski, L. J.; Warren, F. V. Determination of Aspartame, Caffeine, Saccharin, and Benzoic Acid in Beverages by High Performance Liquid Chromatography: An Undergraduate Analytical Chemistry Experiment. *J. Chem. Educ.* **1985**, *62* (7), 618. https://doi.org/10.1021/ed062p618.