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OREGON STATE UNIVERSITY DEPARTMENT OF CHEMISTRY

Experiment 6

Integrated Laboratory Experiment - CH 461 & CH 461H

TEAM SPECIAL PROJECT

Overview

During the last four weeks of the term, you and your team will work on a special project of your own design. The basic idea is to analyze a real sample for one or more components. Harris 8th Ed (Sections 0-1 - 0-3), recognizing that "chocolate has been the savior of many a student on the long night before a major assignment is due," describes a student project to answer the question, "how much caffeine is in a chocolate bar? how does that amount compare to the quantity in coffee or soft drinks?" These sections of Harris are an excellent introduction as you think about developing your team project.

We expect the project to be generally in the area of analytical chemistry although we entertain any reasonable variation. You may wish to acquire more experience with some instrumentation you learned about during this term or in a previous term or to explore other instrumentation available in the department that you have not had a chance to use. There are a number of factors to consider.

1. Choose a project that is appealing to you. Depending on your interests, you may wish to work with a certain instrument or technique or you may be more concerned with the type of sample, analyte, or problem.

2. Choose a challenging, but reasonable project. You do not want to do something trivial. However, you need to accomplish something in only 4 lab periods. You want to have enough time to run through the experiment once, reflect on your results, and then try it one more time.

3. You can work in your present teams or form new teams of 2-3 people. If your project requires 4 people, check with Dr. Pastorek first.

4. Define the project carefully. Clearly state the hypothesis. It should be clear to a reasonable chemist why or for what purpose you are doing your project, what type of sample or samples you will analyze, and what chemical species you wish to determine. In general, you will want to either compare values which you obtain to known or expected values or to prove or disprove a hypothesis you have proposed.

5. Spend some time in the library, and online using for example, scientific database search engines, e.g., SciFinder Scholar, Reaxsys, or Google Scholar, or vendor catalogs, or the web finding information related to your project. Your probability of success is going to be much higher if you

find a scientific source in the literature describing experiments similar to what you propose using instrumentation similar to what we have in our lab. Remember, postings on the internet at-large are not guaranteed to be tested, whereas, a published protocol in a scientific journal has usually been peer reviewed and is more likely to be a reliable source.

Sources of Ideas and Information

1. Your imagination.
2. Faculty or the TA's.
3. Newspapers or the news on the web. Often there are articles about some new concern about a food, water supply, your local environment, or other consumer product that may be contaminated.
4. The library. Yes they still have books and journals at Valley Library!
5. Journal of Chemical Education. This journal publishes many laboratory experiments. These experiments can be searched at thttps://pubs.acs.org/journal/jceda8
6. Supplemental Materials for CH 461 on the course WEB page. Under the heading "Special Project Background Material" is included information about "Instruments Available for Special Projects", "Ideas for Special Projects", and a list of titles of special projects from previous years. The actual special project reports for these titles are found in 3-ring binders in GBAD 313 and are organized according to topic area (see table of contents in each binder).
7. Journal articles. See "Examples of on-line journals in the scientific literature" which is a link on the course Supplemental Material page. You should use Sci-Finder Scholar and Reaxsys that OSU subscribes to, to search "research topics". This will point you to abstracts and journal articles for specific species or samples (i.e., look up for example, As determination by GFAA).
8. Books and documents of applications from manufacturers. A large collection is found in GBAD 311 & 313 which include:

   a. The applications book for the Varian AA-6, "Analytical Methods for Flame Spectroscopy", a good source for determining metals in many types of samples with flame atomic absorption (AA) spectrophotometry. Also on PC's on AA instruments in room 314.

   b. "Microwave Digestion Applications" - a notebook from the manufacturer (CEM) of the microwave oven.

   c. The Hach Chemical Company "Procedures, Chemical Lists and Glassware for Water and Wastewater Analysis" which contains the standard procedures for determining many metal ions and other inorganic ions with UV-visible molecular absorption spectrophotometry.

d. The book "Standard Methods for Examination of Water and Wastewater" has accepted procedures for many inorganic and organic species in water samples.

e. Several books on solid phase extraction (SPE) which are a good source for applications involving determination of organic species by HPLC and sometimes GC in environmental or food samples (e.g., Baker Application Notes).

f. The book "Official Methods of Analysis - Association of Official Analytical Chemists", volumes 1 and 2 and supplements. A comprehensive collection of accepted methods of analysis.

g. Catalogs from suppliers of chromatographic equipment such as (Agilent, Altech, Supleco) which contain many chromatograms for real samples.

Please don't remove documents or books from the laboratory except for a moment to make copies. Thank you!

Factors to Consider in Planning your Project

1. The method of sampling and preservation should be chosen to prevent contamination or loss of the analyte between the time of sampling and analysis.

2. At least two and preferably three equivalent samples of each type of real sample should be obtained so that the homogeneity, contamination, and loss problems can be evaluated. For instance, for a water sample, use two different bottles to obtain two "equivalent" water samples in the field rather than dividing up one sample from one bottle back in the lab.

3. Each of the equivalent samples would be carried through the complete sample preparation procedure (e.g., digestion) to evaluate the reproducibility of the sample preparation procedure.

4. Careful consideration should be given to the blank. A distilled water or other appropriate blank should also be carried through the complete sample preparation procedure and the blank should contain all reagents that are added to the samples at the same concentrations. For a water sample, it is a good idea to add distilled water to an equivalent bottle used for sampling with the same length of storage as samples to test for contamination from the sampling container.

5. Standards should be matrix-matched to the samples where reasonably feasible and should contain all reagents at the same concentrations as added to the samples. Where appropriate, a standard should be carried through the sample preparation procedure. This check is particularly important if a pre-concentration procedure is used since such procedures are not often 100% efficient and can cause some contamination or loss of analyte.

6. Usually three replicate measurements should be made on each sample and the blank to evaluate the precision of the measurement. Report the precision (associated with replicate measurements of equivalent samples) of measuring equivalent samples and the detection limit of the technique in each case.

7. There is a limited amount of time for the project. You do not have time to develop a totally new analytical procedures. It is highly recommended that you find a procedure in the literature that discusses the specific instrumental techniques, species, and sample type that you plan to investigate. Look for a specific protocol and discuss with your Consultant in advance.

8. **When you are ready to make measurements, first measure your standards to establish that the instrument is working and to optimize experimental conditions.** Measuring your sample first is not prudent because there may be little analyte in your sample or the analyte concentration may be much less than you expected.

9. After your first attempt at analysis, you might consider spiking a sample with a standard to test the recovery or use the standard addition method.

Special Project Proposal Form - posted on course web page and at the end of this document.

Once you have decided on an idea for your special project, and discussed with one of the Consultants, the next step is to write a short proposal. The Project Proposal will help you organize your own thoughts about what you will need to do to have a successful project, and it will serve as the basis of discussion between your team and the staff member who will be the Consultant on the project. Your Proposal must also have a list of all chemicals or special materials that you'll need for your project (some may need lead time to order).

Your team must submit a Special Project Proposal (one proposal per team) by the posted deadline that includes the following information:

A. A sheet with one or two paragraphs (with complete sentences) that provide an overview of your project and the overall objectives and the hypothesis you wish to test;
B. One copy of each reference article that you found in the literature and plan to use as a basis for your project.
C. The completed table of information which is found on the Proposal Form, plus any additional material requested on that sheet.

Your ***Special Project Proposal*** is due for review at the end of the lab period dedicated to the preparation of proposals (in 2019, due date is by noon, Friday, Nov 15). We will use your written Proposal to judge the feasibility of the project and make appropriate suggestions. On the basis of your Proposal, your team will be assigned a Consultant who will help you throughout the project. You should meet with your Consultant well before the actual start of lab work on your project.

Required Data Sheets

Once your proposal is accepted, **but before you start lab work**, you are required to design, prepare and submit your own "data sheet" (similar to those used in the other experiments) listing the samples and standards and data to be recorded. You are required to obtain the signature of your Consultant on your datasheet before you begin the actual lab work. All observations and data should also be recorded in each person's lab notebook. The notebook should be initialed by your Consultant at the end of each lab.

Class Presentation and Audience Participation

Each Team will make an class presentation of ~15 minutes during the last lab period, Thursday of dead week. Each member of the Team is expected to contribute. Team presentations tend to be more effective when each team member is given an entire topic to present (e.g., introduction, or methods, or analysis, or results, etc.) and the number of transitions between speakers is minimized.

In addition, each student in the class is required to ask at least one good question of another Team during the class presentations.

Characteristics of an exceptional presentation are listed on the CH 461 web page. You should give an overview of the project and include enough detail on sample prep, instrumentation used, data collection and data analysis so that a scientist can follow what you did. Use tables to summarize important data and results – give enough detail for inquiring scientists but don't overwhelm with details (e.g. we don't need to know all of the sample mass data, or the total volumes of standard solutions, etc., unless this is important – don't repeat unnecessary details from your notebooks just to fill up space and time).

The oral presentation should cover the following points:

Overall objectives and hypotheses

- Overall objectives and hypotheses clearly and succinctly stated
- Background or context clearly presented, clear why someone would be interested
- Idea shows originality, consistent with time and resources available

Experimental design

- Design appropriate to address hypotheses with enough replicates for meaningful test of hypothesis
- Design clearly presented and easy to follow
- All major steps are given with at least one example and some detail so the audience can understand what was done (e.g., how are standards prepared, how are samples prepared and tested, etc.)

Results

Present at least on one figure (e.g., calibration curve showing slope and intercept), and any other figures you think will add to the understanding and interest for the audience.

- Just the right amount of detail in tables and figures
- Easy to follow steps from calibration to analysis including showing a calibration curve including the calibration equation
- Estimation of precision of final results All numerical results should be reported to 3 or 4 significant figures, with scientific notation format for very large or very small values (again showing 3-4 sig figs) . Report averages and estimate errors using either % deviations (if only 2 values) or standard deviation or 95% confidence based on your experience, for example. *(Cont'd next page)*

- Estimation of accuracy of your final results. Compare your results with accepted/published results, or similar results, when these are available.
- Problems encountered and possible scientific solutions

Conclusions

- Conclusions clearly and succinctly stated
- Conclusions supported by data and appropriate statistical tests applied to data (see Harris)
- All assumptions, approximations and simplifications stated explicitly (i.e., come absolutely clean on any short-cuts)
- Any editorial comments explicitly separated from the data and scientific based conclusions.
- Next steps – what you would do if you had it to do over again or had another full month / term to work on the project

A data projector is available with access to the internet, but you are encouraged to bring a copy of your presentation on a USB drive in case the internet is not available..

Written Reports

A written report (one per *team*) is due by 1:00 PM on Tuesday (Dec 10, 2019) of finals week. Your written report should follow the following basic outline, but it can be somewhat modified to fit your particular investigation. Avoid using a bulleted format and avoid labeling sections. Follow an ACS professional format for an analytical paper. Pages must be numbered on the lower right side.

Abstract. A one or two paragraph summary with one or two sentences devoted to each of the following: a statement of the problem and your hypothesis, the methodology used, the results (especially numerical results including estimate of precision (i.e., standard deviations)) and the significance of the results, including was your hypothesis supported or not.

Introduction. Include any relevant background material about the type sample analyzed or analyte determined and analytical procedure chosen and the justification for the project. Pertinent literature should also be discussed.

Experimental.

a. A complete description of blank, standard, and sample preparation procedures.

b. A description of the instrumentation used and experimental conditions. There is no need to review the standard operation of the instrument. If you use an experimental setup equivalent to that used in class, just reference the experimental writeup. Any modifications to a standard procedure or instrumentation should be discussed in detail. **The explanation should be detailed enough** that another student in the class could reproduce your results by following only the material in your paper.

Results. All data should be summarized in tables along with mean and standard deviation data and the number of data points used in the calculations. The detection limit and precision of analysis should be reported. Exemplary calculations should be included. **All tables and figures should be numbered and have descriptive captions.** In the text, when you are referring to tables and figures do so by number**.** Proper calibration curves are required.

Significant Discussion. Summarize the results and discuss their significance. Discussion should include a comparison with literature values (accepted, reported, etc.) where possible. Estimate the uncertainty in your results and discuss using scientific reasoning probable sources of error that cause results to either be too high or too low. If the results are inconclusive, give a plausible reason why. Indicate any improvements that could be made in the future.

Supplemental Material. The raw data taken in your laboratory notebook should be attached in an appendix with a table of contents as a first page. This section should include hard copies or charts of spectra, chromatograms, etc., all important raw data.

Numbered List of References. All relevant literature used should be listed with a complete proper ACS reference citation. See the ACS Style Guide or Supplemental Materials course web page for format. List references in order you cite them in the text and number these references in order. When referring to a reference in the text, refer the reader to the number of the reference on your list.

ADDITIONAL INFORMATION ON SAMPLE PREPARATION AND ANALYSIS

Methods of Analysis

The general type of analyte that you choose to study determines to a large extent the method of analysis. First consider if your analyte is organic or inorganic. Inorganic analytes are further divided into metals, non-metals, and gases. For metals, such as Na, K, As, Ca, Mg, Fe, Cu, Ni, Pb, and Cd in samples, one usually uses atomic spectrometric techniques. These techniques include flame atomic absorption spectrometry, ICP emission spectrometry, electrothermal atomic absorption spectrometry, or ICP mass spectrometry. Within the chemistry department, only the first three techniques are available. For non-metal inorganic species such as nitrate and phosphate, common analysis techniques include spectrophotometric (colorimetric) methods and ion chromatography. Only the former is available within the department. Gases such as O_2 or N_2 are often determined by gas chromatography.

For determination of organic species, separations are often necessary and include GC and HPLC. In general, GC is used for volatile compounds while HPLC is used for compounds such as vitamins that would decompose if heated. Spectrophotometric or fluorometric methods are also common for some organic species.

Sample Collection and Preparation

Often the most difficult part of an analysis procedure is acquiring the unknown and preparing the sample for measurement rather than the actually measuring the analytical signal of the standard and unknown. First one has to be sure that the sample obtained is representative of the body of material you wish to sample. When sampling the ocean (except very near the coast), one will generally be able to assume that the body is homogeneous in that region, but for something like a garden, this will probably not be so, and therefore, more care must be taken.

The integrity of the sample must also be maintained so that contamination or unwanted changes do not take place. This is particularly critical for trace species (less than 0.01%) where both contamination of the sample (e.g., from storage containers) and loss of the sample (e.g., volatilization) may cause considerable error.

Before the sample can be placed in the instrument, the sample must sometimes be converted into the proper form, which usually means some form of digestion. The analytical technique used and the type of sample preparation depends on the species to be determined and the matrix of the sample. It is always a good idea to consult the literature for potential interferences and proven methods of sample preparation. If the interferences cannot be compensated for, then some separation step may be required before the final analytical measurement. Also one must insure that the analyte is adjusted to the proper concentration range for the analytical technique. If the final concentration is expected to be near or below the detection limit, some form of pre-concentration (e.g., solvent extraction, evaporation, or solid-phase extraction) is required.

Samples generally encountered can be roughly divided into four groups:

- solid (e.g., rocks, minerals and alloys),
- biological (plant or animal),
- water (e.g., river, drinking, and sea waters),
- and gaseous samples (e.g., atmospheric pollutants).

Gases will not be considered further. Different approaches are needed for organic versus inorganic compounds or particulate versus dissolved matter. Inorganic, and particularly metal determinations, are primarily addressed below.

For solid samples, it is often necessary to homogenize or pulverize it before the subsequent digestion procedures. Grinding of geological samples and soil can be carried out in a simple mortar and pestle for many samples. If the hardness of the sample is not too great, a regular ceramic one is adequate. For harder samples, an agate mortar and pestle may be used, and for very difficult samples, alumina mortar and pestles are available. Manual grinding can be tedious, and a dental amalgamator is often a real work saver. Only chunks on the order of a few mm in diameter can be inserted into the capsules used with the amalgamator, but in a matter of a minute or so the samples are quite adequately pulverized.

For biological material, such as leaves or roots, a food blender will often serve to adequately chop and blend a good quantity of sample for a subsequent analysis. Alternately one can freeze the sample with liquid nitrogen and then grind the hard, brittle sample in a mortar and pestle under liquid nitrogen. The nitrogen can then be evaporated away, leaving a pulverized homogenized sample. This technique is particularly useful for fibrous matter which will wrap around the blades of the blender.

For homogenizing two liquids or gases in liquids, surfactants (soaps) can be helpful. An ultrasonic mixer will greatly facilitate this mixing process as well.

Water Samples. Water samples are usually the easiest to handle since the sample is already dissolved in a solvent. After collection, the sample is refrigerated at 4EC as soon as possible, and 2-5 mL of HNO_3 or HCl to a pH of 2-3 are added per liter of water if metals are to be determined. HNO_3 has the disadvantage of providing NO_3^- as a nutrient for organisms.

Water always contains particulate matter so one must decide whether to analyze the whole sample or to separate the water from the particulate matter and analyze each one separately. Separation is normally achieved with a membrane filter with 0.45 Fm pores. Dissolved metals are arbitrarily considered to be those that pass through the filter, while suspended metals are considered to be retained by the filter.

A **total metals analysis** includes the concentration of metals in solution after vigorous digestion of an unfiltered sample or the sum of the concentrations of metals in both the dissolved and suspended fractions. Acid digestion is carried out with various combinations of concentrated nitric acid and hydrochloric acid.

If the metal concentration is too low, one of the following **pre-concentration techniques** may be used: evaporation, chelation and solvent extraction, ion exchange, or co-precipitation. The disadvantages of evaporation are that dissolved solids increase, which may be intolerable since the concentration may cause some species to precipitate out (e.g., $BaSO_4$, $PbSO_4$). The high salt content may also present other problems, depending on the analysis technique itself. Chelation involves adding an organic ligand to the sample to chelate the desired metal ions and extracting the complex into an organic phase. Ammonium pyrolidine dithiocarbamate (APDC) and methyl isobutyl ketone (MIBK) are commonly used for this purpose.

Biological Samples. Before metal analysis, the organic matter in samples such as leaves, roots, organics, blood, and urine must be destroyed. The two methods commonly used for the destruction of organic matter in biological samples for analysis are acid digestions with one or more mineral acids and dry ashing in a high temperature muffle furnace. Various methods of sample preparation have been reviewed in detail (2-16).

Open-vessel acid digestions are relatively simple. The disadvantages are contamination from

the acids and the difficulty of subsequent pH adjustment.

To dry ash a sample in a muffle furnace, the sample must first be oven dried and then placed in a crucible. The crucibles are transferred to a muffle furnace and heated to between 500EC and 600EC for 4 to 5 hours (10-12). After cooling, the samples must be put into solution which often takes a small amount of acid. Problems with dry ashing are volatilization of some elements, difficulty in removing the ashed sample from the crucible walls, contamination from the crucible, and the dissolution of the compounds formed.

Microwave digestions are now considered standard procedure in many circumstances. Samples are placed in a Teflon-lined bomb and heated in a special microwave oven. Digestion times are relatively short (1 hr or less) compared to open-vessel acid digestions.

Geological Samples. There are a variety of methods for dissolution of geological samples depending upon the composition of the sample. For simple salts, the sample may be dissolved in water or acidic solutions. Minerals and rocks present more of a problem and fusion techniques with different fluxes are employed. The ground sample and flux are mixed and place in a graphite or Pt crucible in a muffle furnace at 800 to 900EC. There are two major types of rocks and minerals -- basic and acidic. Silicate and phosphate rocks are examples of acidic rocks, and a basic flux should be used to dissolve them. Examples of basic fluxes are Na_2CO_3, NaOH and other alkali metal bases. Carbonates and oxides are examples of basic rocks, and they should be dissolved with an acidic flux such as boric acid (H_3BO_3). A good general purpose flux (as it combines the alkaline and acidic properties) is lithium metaborate ($LiBO_2$).

For Organic substances in the water, it is most common to use gas-liquid chromatography or high performance liquid chromatography to separate the compounds for detection. Some pre-concentration technique such as solvent extraction or purge and trap (P/T) may be used before chromatography (the department has a P/T apparatus in GBAD 318).

For Organic analysis of biological samples, solvent extraction is commonly employed, after suitable mulching of the sample. The solvent can then be freed of water by filtering the sample and solvent through a bed of anhydrous Na_2SO_4, evaporating to a suitable volume, and analyzing via chromatography, infrared spectrophotometry, or Raman Spectroscopy. Although the latter two

instrumental techniques are not generally applicable to trace constituents, HPLC and GC/MS are particularly well suited for trace analysis with detection limits in the ppb range.

Preparation of Solutions

If one weighs out a sample, care must be taken to insure that the resolution of the balance is not limiting. Since a typical analytical balance has a resolution of 0.1 mg, sample sizes of 10 and 100 mg should be weighed for 1 and 0.1% accuracy, respectively.

Also one must be concerned with the accuracy and precision of using volumetric flasks and pipets. Typical values are given in Harris and on the CH 461 web site. Clearly, transfer (volumetric) pipets are preferred over measuring pipets for the most accurate work.

In preparing standard metal solutions, one must use suitable chemicals (officially certified in some cases). Suggested compounds are given on the CH 461 web site.

Statistical Analysis

You should make repetitive measurements of all samples and report means, standard deviations, and confidence intervals ($\pm ts/n^{½}$) (see Harris, 8th edition, 68-90). If you can compare a value you obtained for a concentration to some literature or accepted value, use a statistical difference test based on the Student t to state whether your result agrees or disagrees with the accepted value.

References

Many other references in ACS journals of e.g.,, Analytical Chemistry and Journal of Chemical Education, and using scientific search engines , e.g., SciFinder Scholar and Reaxsys (through Valley Library), plus Google Scholar.

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