Analysis of Phosphorus Concentrations in a Natural Water System

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This project was developed for implementation in an honors level quantitative analysis course. The following set of notes and comments are meant to help instructors implement or adapt pieces of this project into their own courses.

Our overall learning objectives for students:

- Learn quantitative analysis in the context of a real-life environmental problem;
- Learn how to apply the scientific method as student develop hypotheses, design experiments, analyze data and develop conclusions;
- Understand some of the complexities and uncertainties associated with scientific measurement, especially when working with complex systems and the natural environment;
- Learn to effectively communicate scientific results, both in writing and by oral presentation.

The project described here revolves around Lake Mendota and the Yahara River system of south-central Wisconsin. You can easily adapt the methods described here to something local to your area by contacting your local DNR to ask about current data of lake characteristics such as what is reported in Table 2 of the Introduction document, Analysis of Phosphorus Concentrations in a Natural Water System. A Google search of the desired lake or natural water system may reveal similar results.

Course Organization at Wisconsin

Our quantitative analysis course spans a 15 week semester. We list this as a 4 credit course. Students meet for lecture twice per week for 50 minutes, twice a week for four hour lab sessions, and once a week for a 50 minute discussion. A faculty member or lecturer leads the lecture sections. Teach assistants (TAs) or faculty assistants manage the labs and teach the discussion section. The lecture section is divided into lab/discussion sections of about 20 students. One TA manages one section of students and is responsible for teaching those student good laboratory practice and safety in the lab, as well as leading the discussion.

The class size averages about 100 students. For the spring semester of our honors quantitative analysis course students are mostly second semester freshmen, many of them chemistry or engineering majors. Our course demographic changes for the fall semester and is made up mostly of upper classmen looking to finish up lab requirements for their majors.

Our chemistry faculty primarily fill the lecturer role. Chemistry graduate students entering in our analytical or materials science programs serve as our teaching assistants. A laboratory director is responsible for innovating and managing the laboratory curriculum, managing and training the teaching assistants, and facilitating connections between the lecture, laboratory and discussion components of the course. We also have two stockroom staff members that prepare and dispense reagents and solutions, set up the teaching space with appropriate instrumentation, glassware, and other equipment necessary for experimentation. And finally we utilize the help of hourly student employees to help with cleaning the lab space and managing the stockroom counter where students will check out extra glassware or special equipment or reagents for a specific laboratory period.

Project Organization

We consider the project primarily a laboratory activity. Out of a fifteen week semester we dedicate 5 weeks (a total of 40 contact hours) to implementing the project labs. A sixth week of lab time is used to assess student oral presentations and provide feedback to students on the written papers they have submitted. Students find it helpful to have some experience with the following concepts before beginning the project, which we cover in lecture and in problem sets:

- Uncertainty and statistics
- Spectrophotometry
- Equilibrium
- Ionic strength/activities
- Monoprotic acids and bases
- Systematic treatment of equilibrium.

Lecture topics progress as students work on the projects and complement many of the concepts they work with in the lab, including:

- Polyprotic acids and bases
- Acid and base titrations
- Complexation chemistry
- Atomic spectroscopy.

Our strategic plan for implementing the project in this course is outlined in Table 1. The project is divided into three sections:

1. Compare two spectrophotometric methods of analysis for total phosphorus.
    - Provide students with the ascorbic acid method as described in Standard Methods for the Analysis of Water and Wastewater (Eaton et. al., 2005) and the malachite green method proposed by Van Veldhoven and Manaerts (1986).
    - Collect absorption spectrum for each method and verify the wavelength of analysis proposed is appropriate.
    - Calculate the absorptivity coefficient for each method.

- Determine the linear and dynamic ranges for each method.
- Calculate the method detection limit for both methods using the U.S. EPA Method Detection Limit

2. Measure total phosphorus in a Lake Mendota water sample.
3. Design and carry out an experiment using one of the methods of analysis explored in part 1 to answer a "research type" question, which student groups define related to measuring phosphorus in a fresh water system (last four or five lab periods).

Students report their results in two ways: Group members write a formal report using the *ACS Style Guide (*Coghill and Garson, 2006). Group members also prepare a presentation for the rest of the class focused on results and conclusions of their research question. The formal report is typically due 7 to 10 days after the final laboratory period.

Table 1: Implementation strategy for the lake study project

Schedule	How Long	Activity	Related Documents
Before the first lab day	45 minutes	Introduce the project in lecture	• Project Introduction PowerPoint • Analysis of Phosphorus in a Natural Water System—Introduction • Standard Methods for the Examination of Waters and Wastewaters 4500-P • Inorganic/organic Phosphate Measurements (Van Veldhoven, P.P., Mannaerts, G.P. Analytical *Biochemistry* **161,** 45-48 (1987))
First lab period	4 hours	Planning Activity	Introductory assignment on getting started*
Three lab periods	12 hours	Students conduct experiments to compare methods	• Progress Report 1 • EPA Method Detection Limit Procedure
One or two lab periods	4-8 hours	Analyze lake sample with chosen method and perform Spike Recovery	• Progress Report 2/Proposal for Part 3 • The Art of Writing Science (Plaxco, K.W., *Protein Science* **Vol. 19:**2261-66 (2010))
Four to five lab periods	16-20 hours	Carry out experimental design for the group research question	Final Report Details/Rubric

**We have also developed detailed experiments for the Malachite Green and Ascorbic Acid Methods of Phosphorous Detection, as well as an explicit experiment illustrating techniques of*

Spike Recovery and Method Detection Limits. These experiments are included, and can be substituted for the first four lab periods.

Roles Staff Members Play

Lecturer: The lecturer in our scenario primarily manages organizing and teaching lecture content for the course. They write problem sets, exams, dictate the weight of components of the course towards a student's final grade, and provide guidance on how to grade homework and exams. The more support the lecturer gives to the lab project, the happier the students! Some lecturers become quite involved in the projects and write supplemental questions related to project material for homework and exam questions. Lecturers often visit the lab to interact with students and TAs, but most lab sections may only see the lecturer in the lab for 10 or 20 minutes of each 4 hour period.

Teaching Assistants: The teaching assistant serves as the primary lab instructor. For the projects, their role is as a "thought leader". Especially during the first few days of lab work, students feel overwhelmed with the body of work ahead of them, and uncomfortable with having to develop their own procedures for measuring P. Students come to the TAs wanting to know what to do and how to do it. We instruct TAs to NOT answer those types of questions directly unless it involves a safety issue. Instead, they are asked to share how they would go about finding the answer to the question, assuming they don't know the answer themselves. A TA's response might point students to the project documents available to them, or to the literature, or perhaps the lecturer or lab director. If a student asks if a particular procedure is going to "work" (my guess would be this question ranks at the top of the "Most Frequently Asked"), a typical TA response might be, "I don't know, but you should try it and see what happens." **TAs model problem-solving skills for their students, instead of solving the problems for them.** At the beginning of each lab period, TAs will give a 5 minute debriefing to their lab class. They cover what things they should be working on, safety issues with any chemicals or procedures coming up in the lab queue, and disposal practices. Next they open the floor to allow students to address general issues, concerns, or questions before starting experiments. Once students set to work on experiments, TAs oversee experiments, addressing lab technique issues, safety, and answering individual questions as they come up.

Stockroom staff: The stockroom staff manages the inventory of chemicals, instrumentation, and special glassware. They prepare stock solutions in large quantities (such as 50 L of 1 M HCl or H_2SO_4 or NaOH) and dispense in smaller quantities to our general lab space. Staff also manage a counter, where students can request to special chemicals or glassware not provided in their lab drawers in the lab. These items are "checked out" from the stockroom, and students must return them to the counter by the end of the lab period.

Laboratory Director: The lab director oversees the TAs, stockroom staff, student help, and serves as a secondary resource for students when the TAs are either very busy or stuck on a problem. The lab director grades all assignments, provides feedback and direction as students problem-solve experimental challenges, and gives guidance on developing interesting and relevant experimental questions that can be explored reasonably given the time limitations of the lab.

Apparatus

-Spectrophotometer capable of doing single and full-wavelength visible analysis
-Analytical balance
-Hot plate
-pH meter
-Filtering apparatus, such as a Buchner funnel, coarse filter paper with filter flask and sink aspirator
-variety of beakers, pipets, and volumetric flasks that should be acid-washed

We teach the students how do to this. They warm ~0.1 M HCl on a hot plate, using small aliquots they rinse out the inside of their glassware with this solution. Rinse well with DI water. This glassware should only be used for the P determination.

-1 L plastic bottle acid washed for the lake grab sample

Reagents for Parts 1 and 2

9 M H_2SO_4—This is a stock solution students can subsequently dilute for experiments
1 M NaOH—This is a stock solution students can use or dilute to use as needed for experiments
ammonium potassium tartrate solution--1.3715 g in 500 mL volumetric flask. Store in a glass-stoppered bottle
ammonium molybdate solution—20 g dissolved in 500 mL DI water
0.035% w/v malachite green solution
Prepared phenolphthalein indicator
0.35% w/v polyvinyl alcohol (PVA)
Ascorbic acid solid: *a group of 4 uses about 6 g for the project. It only stores for about a week so students often remake the solution.*
anhydrous potassium phosphate monobasic: *a group of 4 typically used about a gram over the project period.*
-ammonium persulfate: *a group uses 0.4 g per sample for a digestion. Plan on about 5 g per group for the entire project period. Note that we do not ask them to digest the standards, although this is a good lab practice.*

Reagents/Equipment for Part 3 depend on the research questions students will explore. Listed below are some common items students ask for:

Metal salts of calcium, iron, aluminum
Acetone (for extraction of chlorophyll from algae)
O_2 and or N_2 tanks with regulators and a means to control bubble flow to a sample (for enriching a lake sample with either O_2 or N_2)
Access to Inductively Coupled Plasma Atomic Emmission Spectrometer (ICP-AES)
Oxygen probe
Other ion selective electrodes to measure metal cation concentrations in solution

The instructor should emphasize the following important details when addressing safety and laboratory technique. The instructions are included as a handout provided in the "Getting Started" assignment.

- Do not use a metal spatula when handling the ammonium persulfate. If spilt in balance, clean immediately as it is a strong oxidant and will react with metal.
- PVA may produce bubbles that result in a soapy looking solution. This will make it hard for you to accurately dilute reagent C to 100 mL; however, an accurate dilution in this step is not imperative. As long as you use the same solution for all measurements, the exact concentrations will not really matter. Along these same lines, you need to be careful when pipetting from reagent C for this same 'bubble problem'. Taking aliquots at least 1 cm below the surface of this solution should circumvent any issues.
- It is OK to dilute the samples with undigested DI water; however, DO NOT add DI water to a hot digested flask. The heat causes any organic phosphorous that may be in the distilled water (and there is some) to be hydrolyzed causing the inorganic phosphate concentration of the solution to be higher.
- Perchloric acid is a pretty nasty acid to work with, and we will not have it available for you to use in this project. For all your digestions, make sure you follow the procedure describing the persulfate digestion method.

First Day of Lab

Prior to lab:

- Students should review the project overview, "Analysis of Phosphorus Concentration in a Natural Water System"
- Students should review the associated literature, including "Standard Methods for the Examination of Waters and Wastewaters 4500-P" and "Inorganic/organic Phosphate Measurements (Van Veldhoven, P.P., Mannaerts, G.P. Analytical *Biochemistry* **161,** 45-48 (1987))"
- Organize student groups

Handouts for first lab day activity:

- Lake Study_Getting Started ASDL.docx
- Lake Study_Reagent info for students ASDL.docx

Activity description:

The goal for the planning activity is for student groups to adapt the procedures stated in the research paper and standard methods description, such that they can perform the experiments in their own lab. The questions in the Getting Started document in part guide them through some of the literature details so they can make some educated decisions on what to change. It should also inspire them to look up related literature articles (most are listed in the Works Cited section of the Introduction handout), as a way to enrich their understanding on the topic. When reviewing the proposals students submit, the grader should check that the procedure is basically correct and safe. There may be small errors in the procedure, which can be commented on. We tend to let students try what they propose even with the mistakes, provided what they propose will be safe. We value the problem-solving experience just as much as getting things working really well!

Leading up to Progress Report 1

Relevant Handouts:

- Lake Study_Progress Report#1 ASDL.docx
- Lake Study_Modified MDL ASDL.pdf

Activity Description:

Student groups should have several periods to explore the chemistry of these methods. The goal is to achieve linear, reproducible, and accurate standard curve plots to eventually use to analyze a fresh lake water sample. They will need to modify the methods described in both papers and therefore must explore that those modifications will still lead to a reliable working curve.

They should discover that the malachite green method indeed has a lower detection limit than the ascorbic acid method. However, the malachite green method has limitations in how stable the complex is. The complex must age for about 20-30 minutes, for example. And while the paper states it is stable for quite some time, our experience is that the more concentrated samples sometimes gel after about an hour. Both methods will work for samples in the 40 – 200 μg/L P range. If students choose to use the malachite green method for the high end of concentrations, they will need to dilute samples.

There is no wrong or right answer as to which method works better. The important point to make is that whatever they decide should be defended with a sound body of data.

Instructors who would prefer to give more guidance to this portion of the project could consider using a more traditional undergraduate laboratory format. Groups could perform "Ascorbic Acid Method for Phosphorus Determination" followed by "Malachite Green Method for Phosphorus Determination". These experiments are included (where?) with relevant staff notes.

Leading up to Progress Report 2

Relevant Handouts:

- Lake Study_Progress Report#2 ASDL.docx

Activity Description:

Having gained proficiency and confidence in using one of the methods for measuring phosphorus, students will now use their procedures to measure a real sample. The sampling procedure is described in the Introduction document. Be sure the bottle they use is acid washed. The sample should be stored in a refrigerator. In evaluating the reported answer, students should reference what is already known about the system they are measuring. Is the value they discover within a reasonable range of the reported value?

The second progress report requires groups to develop and perform a spike recovery experiment the check for any matrix effects. The instructor should point students to the text book in deriving the procedure. The text written by Daniel Harris, **Quantitative Chemical Analysis, 8th Ed.,** covers this nicely in Chapter 5, page 98. Our experience is that students report recoveries in the 95-104% range and therefore do not observe matrix effects on the measurement. We have written a traditional experiment that explains and gives practice on performing both the spike recovery and MDL determination. This experiment along with staff notes is included (where?).

One important feature of progress report 2 is that groups will now formally submit their proposals for Part 3 of the project. In our arrangement, the TAs and lab director have interacted with students frequently, and therefore we already have a pretty good idea on what will be submitted. Each TA is asked to compile a list of what is likely planned for each group before the proposals are submitted. We also make available to the students what chemicals we have on hand that they can use to set up these experiments. If we don't have it, we usually won't order it unless a group has proposed a really interesting question we would like answered, or it is something we can get in easily without too much expense.

Look over the experimental details described in the proposal for part 3 carefully. The digestion of solid sample, for example, is risky and should be closely supervised by an experienced chemist. We have only allowed this experiment a few times, and only when we had the time to sit with students to monitor the digestion. In evaluating the proposal, make sure students have picked ONE variable to explore. Often times students enthusiastically propose a multidimensional approach to answering an experimental question. They should change ONE variable in their design, and then measure the impact of that change on the concentration of P in the system. The first experiment should have a maximum of 4 samples plus a control. We encourage them to run multiple trials either in series or in parallel, depending on the complexity of the experiment. If students are inspired do to more, we suggest they sign up in a subsequent semester for a credit of independent research.

Final Paper

Relevant Handouts:

- Lake Study_Final Paper and Oral Report ASDL.docx
- Lake Study_Final Paper rubric ASDL.docx
- Lake Study_Individual grade rubric ASDL.docx

Activity Description:

Groups will submit a formal paper describing their experimental work over the course of the project. We also ask groups to prepare a short presentation focused on the results and discussion portions of the paper. Before meeting with the students, the lecturer of the course and the lab director read the student papers carefully, adding notes and suggestions to the written document. At this point we propose a current grade for the paper.

The lecturer, lab director and the section TA will meet with the student groups to first hear their oral reports and then ask questions about what they did. We then discuss the paper with students and suggest informally, ways to revise the paper to improve how the science is presented and therefore improve the grade. After this meeting, groups have one week to revise the paper and resubmit for a final grade. If they choose not to revise the paper, they can simply hand it the original paper up to one week later, and we evaluate the paper using the rubric provided.

We evaluate group members individually as well. The individual grading rubric is also included. The points distribution for the entire project is suggested in Table 2.

Table 2: Points distribution for the Lake Study Project

Activity	Maximum Points
Getting Started	10
Progress Report 1	10
Progress Report 2	10
Final Paper	35
Individual Assessment (Oral presentation, peer evaluation and TA evaluation)	20
Total Points	90