

Lab Design Project Getting Started

The first day of the project lab will be devoted to planning. You will need to plan not only what you will do, but also how you will divide the work for maximum productivity and efficiency. Before your group begins experimental work, you will submit a design proposal. In your proposal, you will need to specify procedural details for carrying out Part 1 of the experimental objectives (p. 9 of the Project Introduction handout). You may also wish to identify which group members will take the lead role in various aspects of the project. Who is the best at experimental design? Who is the best communicator? Who is the best writer that will maintain the primary documentation of the group's work? These people will not have exclusive responsibility for a particular part of the project; rather they can act as the leader for that aspect. All group members are expected to be productive, contribute significantly, and understand all parts of the project.

Your design proposal is the starting point for your experiments. Once your experiments are underway, you will likely need to adjust and refine your procedures. Experiments rarely work the first time exactly as planned! Problems occur and new questions arise. You will modify your experiments to solve the problems and/or answer the questions that arise. This process will be repeated multiple times over the course of the project.

Design Proposal

The primary objective for Part 1 is to determine which method of analysis works best for the determination of total phosphorus (P) in lake water samples, which you'll later be collecting from the Madison area lakes. **Your group's experimental design proposal should summarize each method concisely and as completely as possible.** It will be necessary to modify some of the procedures described in the published methods in order to save time, reduce chemical waste, and to accommodate for limitations in available equipment and resources. Points to include in your design proposal follow:

- 1) Both procedures instruct you to prepare Normal (N) concentrations in some cases, rather than prepare solutions using units of Molarity. Describe the differences between N and M. What is the molar concentration of 1 N NaOH? 5 N H₂SO₄? Provide explicit calculations to explain your point.
- 2) Phosphorus analysis methods are highly sensitive to trace amounts of contamination commonly present in tap water, on fingers, in soap and some detergents, as well as from other reagents. What quality assurance measures will you use in your analysis to show that your samples and standards are not contaminated? Provide a procedure for how the glassware should be cleaned in order to minimize P contamination.
- 3) The end-goal for Part 1 is to validate a method for measuring P in Madison lakes. Justify a useful range of concentrations for your standard curve with this end goal in mind. Be very clear on which units your group will use in reporting the concentrations. Note that the methods described in these two papers use different units! Your group must use the same units when comparing the methods. Provide a sample calculation showing how to convert between the units presented in the two papers provided.
- 4) How will you prepare your standards for the experiment? What chemical will you use to make your P standards? Provide detail on the stock concentrations you'll begin with and how you will proceed in making other standards from the stock solution. The "Adventures with Buffers" lab might be of some help for this question.
- 5) Explain the reasoning for your P concentration range you choose in evaluating the linear and

dynamic ranges of the two methods under investigation. What criteria will you use to determine linearity?

- 6) Provide a procedure for how you will verify the best wavelength(s) for the analysis for both methods. What spectrometers will you use? Will you use different spectrometers to verify the best wavelengths for analysis versus what you'll use to actually measuring P for the bulk of the experiment?
- 7) Our spectrometers use sample chambers that accommodate 1 cm² cuvettes. What is the MINIMUM volume of sample you'll need in order to make a reliable measurement? Note that your sample should fill at least ½ of the volume of the cuvet. Explain what changes your group MUST make to the procedure for developing color using malachite green to accommodate for the volumes you need to make the measurement.
- 8) Your group should take care to minimize the excess preparation of chemicals and solutions you'll use in this lab. For example, in the Ascorbic Acid method, the procedure instructs you to make 500 mL of some of the bulk solutions, and later tells you to use 50 mL or less of these solutions to prepare the "combined reagent". Modify this procedure such that you'll make only 100 mL of bulk reagents. Will this impact your ability to make the combined reagent described in 3-e? Show your calculations and take care that your units remain consistent. Give one other example where you group could modify a procedure to reduce wasteful chemical preparation. (Note: Some of these bulk solutions will be prepared by the stockroom for you to use, such as the solutions described in 3-a through 3-e.)
- 9) Develop a list of glassware and reagents you'll need to carry out the experiments. Take care to note whether or not phosphorus exists as an impurity in the reagents your group proposes to use. Also, take care that you don't make solutions that you'll actually not use. For example, in the Malachite Green method description, the procedure calls for four solutions to be made, when you actually will use only two of the solutions.

Your initial design proposal should explain the procedure of each method in enough detail that the reader could do the experiment. Assume the reader has some background in chemistry. For example, you do not need to write out how to make a 0.1 M ascorbic acid solution. Write your proposal in paragraph format. Points will be deducted for unclear or missing procedural components. Your proposal should be terse (No more than 2 pages of text outlining the proposed procedures. Answers to questions may take additional pages.), yet with enough detail that someone else could set up your experiment without asking a lot of questions.

Once you begin your experiments, it will be important that your group keeps a research notebook documenting your lab work. You will be required to periodically submit notebook pages during the project course. As your group begins to pursue more intricate experimental details, keep in mind the following set of questions. Be ready to discuss your answers for the progress report, due in a few weeks, which will ask your group to comment on these and other issues:

- What quality assurance measures did your group perform to ensure your results remained sufficiently accurate and precise?
- Explain your phosphate concentration choice your group used to determine the method detection limit?
- How is your group evaluating any matrix effects in your lake water sample? If there are matrix effects present, how do you compensate for them?
- The first two parts of the project are the same for everyone. The third part of the project is

very open. You are asked to design and perform an experiment in which you use one of the methods of analysis to answer a question you ask about concentrations of phosphorus in freshwater systems. What variable will you investigate? How will you set up your experiments? What equipment will you need? Will you need to measure the concentrations of other substances besides phosphorus? If so, how will you do it?

List of Available Reagents and Solutions

The following solutions have been made for you and will be available in the lab:

- 9 M H₂SO₄ – This is a stock solution that you can subsequently dilute for your experiments.
- 1 M NaOH—This is a stock solution that you can use or dilute as needed for your experiments.
- Antimony potassium tartrate solution—1.3715 g dissolved in 500 mL DI water stored in glass.
- Ammonium heptamolybdate solution—20 g dissolved in 500 mL DI water stored in glass.
- Ammonium heptamolybdate solution—1.75% (w/v) in 6.3 N H₂SO₄ (Reagent A)
- .035% w/v malachite green and 0.35% w/v PVA solution (Reagent C)
- Phenolphthalein solution
- MilliQ water is stored in 50 L carboys in each lab. Be sure to conserve this and only take what you need.
- Solid ammonium persulfate
- Solid ascorbic acid
- Various phosphate salts such as those we make available for the buffer lab

Some other helpful tips to consider as you begin experiments:

- Do not use a metal spatula when handling the ammonium persulfate. If spilt in the 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 water; however DO NOT add distilled 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 described in the 4500-P Inorganic Nonmetals Standard Methods document.