EXPERIMENT 25
THE FERTILIZER PROJECT—ANALYSIS OF PHOSPHORUS IN FERTILIZER

INTRODUCTION

In this project we will investigate the phosphorus content of soluble fertilizers. The idea is to simulate the type of problem that might be solved in a real job situation. The scenario is that an analytical company has been hired to evaluate the phosphorus content of water-soluble plant fertilizers (phosphorus is generally present in the form of phosphate ion in fertilizers). A decision must be made about how to do the analysis. You are part of a team in this company who has been assigned the task of developing the method needed to perform this analysis.

Typically, the steps in developing a method for a specific type of analysis are as follows:

1) The scientific literature is researched to find similar types of analyses—ones that analyze for the phosphorus content of samples that aren't necessarily fertilizers. These are referred to as **standard procedures**. Two procedures that analyze the phosphorus content of the salt KH_2PO_4 are given in this handout.

2) The published procedures are modified to specifically analyze the material of interest—in this case, fertilizer. Modifications include determining how much of the fertilizer sample to use in the analysis, and whether any unique properties of the fertilizer (presence of insoluble impurities, color) will interfere with the published procedure.

3) After a number of analyses have been performed using the different published procedures, the data is analyzed, and a decision is made as to which procedure is most appropriate. Some considerations in the choice will be: the ability of inexperienced lab technicians to execute the procedures reliably in a reasonable amount of time, the reproducibility (precision) of the results obtained, the accuracy of the results obtained, the cost of the reagents and equipment required, and the ease of disposing of any waste generated in the analysis. One way to make such a decision would be to establish a team to make a recommendation, and that is what we shall do here.

PROCEDURE

Each team will consist of four members with assigned roles. The **team leader** does just what the name suggests—organizes the tasks, assigns equivalent work to each team member (including him/herself). The **recorder** keeps a systematic record of the results provided by each team member and the conclusions of discussions reached. The **assistant team leader** assembles the final group report to be submitted. The **information coordinator** is responsible for formulating questions to be asked of the instructor, obtaining the answers and conveying the information obtained to the other team members.

At the start of the project each team will develop two protocols—one for a **gravimetric** analysis of the fertilizer and one for a **spectrophotometric** analysis. A preliminary version of each protocol will be submitted and returned for revision after suggestions from the instructor. The final versions of the

protocols will be submitted one lab period after receiving the preliminary protocol back from the instructor. Each team member will receive a score *only for the protocol they worked on*, which will be twenty percent (20%) of each person's total grade for the project. At the end of the project, each team will submit a series of tables summarizing the results from all team members (25%), and an essay describing the environment impact of phosphates (13.33%). In addition, each team member will write an individual report using his/her own data and the shared data of team members to recommend one of the analytical methods; 31.33% of the grade will be based on this report. Ten percent (10%) of the grade will be based on an evaluation of each team member's contribution to the project, both by the TA and the other team members.

Fertilizers contain nutrients required by plants. Among these are the elements N, P (reported as P_2O_5) and K (reported as K_2O). The composition is reported on the container as three numbers such as 13-13-13, which means that the formulation contains 13% by weight N, 13% by weight P_2O_5 and 13% by weight K_2O. Even though fertilizers do not actually contain P_2O_5 (which would react violently with water) or K_2O, it is customary for historical reasons to report the content in this way. In fact, P is generally present in fertilizers as the phosphate ion PO_4^{3-}. Many products are over formulated; that is, they contain more of some ingredient than is promised on the label. Also, the content of a particular sample may vary as much as ±0.5% because of the problem of achieving complete homogeneity in the mixing of solids. Thus, in evaluating analytical procedures, the agreement among large numbers of results is more important than obtaining the exact percentage on the label (unless the result obtained is ridiculously different).

Two types of analytical methods will be used. In **spectrophotometric analysis** the substance of interest (in our case phosphate, PO_4^{3-}) is converted to a colored compound. The intensity of the color produced can be measured and is proportional to the amount of phosphate present. In **gravimetric analysis** phosphate is precipitated as an insoluble compound which can then be weighed. The standard methods for analysis are described in **APPENDICES 25B** and **25C.**

WHAT EACH TEAM AND TEAM MEMBER IS EXPECTED TO DO

The **team** is responsible for the following:

1. Writing an **essay** of about 500 words detailing the environmental significance of phosphate. **APPENDIX A** gives some suggestions for researching this essay via the Internet.
2. Developing a set of directions (a **protocol**) for analyzing the fertilizer sample. Two team members will develop a protocol for the gravimetric procedure and the other two for the colorimetric procedure; *only one protocol for each method should be submitted*. The intent of the protocol is to provide detailed information for someone to 1) **perform the procedure** and 2) **do the necessary calculations to determine the %P_2O_5 in the fertilizer sample** (typically, a lab technician with limited chemical knowledge is assigned to perform the analysis). The protocol should be typed (the calculations may be written by hand).

   Protocol procedure: You will be using the procedures given in **APPENDICES B** and **C** as a basis for your fertilizer procedure. Note that the procedures given are for the analysis of a known phosphate sample; much of your protocol procedure will be identical. However, when you get to the **Sample Analysis** portion of the procedure, **you will need to modify each procedure to analyze for fertilizer, rather than the known sample**. Some ideas on how to modify the procedure are given in the **Protocol Considerations** section after each procedure.

Sample calculations to be included in the protocol are given below:

Gravimetric method:

- The amount of fertilizer sample needed for the analysis
- The mass of phosphorus atoms present in the $Mg(NH_4)PO_4 \cdot 6H_2O$ product
- The percent of P_2O_5 in the fertilizer sample

Spectrophotometric method:

- the concentration of PO_4^{3-} in any one of the solutions used to generate the calibration curve;
- how the fertilizer sample is diluted to the desired concentration (the mass of fertilizer to be weighed out);
- the mass of P in the fertilizer sample solution;
- the % of P_2O_5 in the original fertilizer sample.

Note that these calculations will not include actual measured data, but simply show how the calculations are set up.

The preliminary versions of these protocols will be turned in; your TA will make comments and return them to the team for revision.

Each **team member** is expected to:

1. Execute tasks in designing protocols and researching the essay as assigned by the team leader.
2. Give legible copies of all results to the team recorder.
3. Make at least two determinations of the % phosphate in a pure sample of known phosphate content (KH_2PO_4) and in the sample of commercial fertilizer by the method for which he/she helped develop the protocol.
4. Teach this method to a team member who developed the other protocol and help him/her make measurements as in 3.
5. Learn the second analytical method from a team member and make measurements as in 3.
6. Share the results with all other team members. This will give a total of eight measurements for the fertilizer sample and four for the known phosphate sample for each procedure—two from each team member—as well as four for the known phosphate sample for each procedure. Ideally, the measurements in 3 and 5 should all agree with one another within 1.5% of the quantity being measured. If they do not, some decision as to why must be made. Does one person's data seem out of line? If so, what is the problem? Measurements may need to be repeated until each person is satisfied that accurate results are being obtained through improved lab technique as a result of practice. (This is important because one needs to choose procedures that will give the same results no matter which lab tech does the analysis.)
7. Assist in compiling the team data into a series of tables.
8. Write a report which
    a. incorporates a standard **INTRODUCTION;**

b. presents the published methods as well as your protocols as the **PROCEDURE** section.

c. Uses data obtained to perform the calculations necessary to determine the $\%P_2O_5$ in a representative sample of fertilizer for both methods;

d. evaluates the two analytical methods based on criteria mentioned above. If the results of the two methods are different from one another (for example, the gravimetric method consistently gives around 30.2% phosphate while the colorimetric method consistently gives around 31.8% phosphate) a discussion of the reasons why should be given.

9. Evaluate on a scale of 1 (worst) to 10 (best) the contribution by the other three team members using the form provided later in the quarter.

The Time Line

Lab Period prior to the start of the project—

Form teams and assign roles. Determine which team members will begin with the spectrophotometric method and which will begin with the gravimetric method (this will also determine which draft protocol they should work on. Begin research on the essay.

Second Lab Period—

Draft protocol for project due

Begin analysis of **KH_2PO_4**

a. Two or three members perform the colorimetric analysis (see **APPENDIX B).**
b. Two team members each do the gravimetric analysis (see **APPENDIX C).**

Third Lab Period—

Team members discuss progress with TA

Teams will go over last period's results; TA will return comment on protocols.

Begin analysis of fertilizer

Each team member does the same analysis as last lab period, except now analyzing for phosphate in **fertilizer** rather than the KH_2PO_4.

Fourth Lab Period—

Final protocol for project due

Team members discuss progress with TA

Switch Methods for analysis of **fertilizer**

Team members teach analysis methods to each other.

APPENDIX 25A: DOING RESEARCH VIA THE INTERNET

The Internet has available **search engines** such as Google. Each search engine offers directions for executing a search on given topics. In general, you supply key words; the more you supply, the more you narrow the search. Here, you might use at least three key words such as: phosphate, fertilizer, environment or phosphate, water, environment. The search engine brings up hits, which you can examine and read one by one to determine their usefulness.

APPENDIX 25B: SPECTROPHOTOMETRIC ANALYSIS

In a spectrophotometric analysis, the element of interest is incorporated into a colored compound which remains dissolved in solution. The intensity of the color (and consequently the amount of light absorbed) is related to the amount of the element present by the equation $A = \varepsilon bC$.

It is usually a good idea to experimentally determine the relation between *A* and *C*. The procedure for doing this consists of preparing several solutions having different known concentrations *C* of the colored compound and measuring *A* for each one. A plot of absorbance *A* versus *C* gives a so-called **calibration curve** relating *A* and *C*. If one now takes a solution of unknown concentration and measures *A*, its concentration can be read from the curve. Beer's Law suggests that the calibration curve should be a straight line which passes through the origin (*A* = 0 when the concentration of the colored compound *C* = 0) having slope εb. Actually preparing a calibration curve takes into account any minor deviations from Beer's Law.

Activity Chart for Spectrophotometric Analysis

Prepare Calibration Curve

- Obtain 5.00 x 10^{-3}M PO_4^{3-} **stock solution**
- Dilute 3mL of stock solution to 250mL to make **working solution**
- Prepare **colored solutions** containing 1.0, 2.0, 3.0, 4.0 and 5.0mL of the working solution and 4 mL of heteropoly blue solution diluted to 10 mL
- Obtain absorbance spectra for each colored solution
- Determine absorbance values at a selected wavelength using LabQuest analysis
- Calculate [PO_4^{3-}] for each colored solution and plot A vs. [PO_4^{3-}] using Excel program

Obtain Sample Spectra

- Weigh out sample (KH_2PO_4 1st lab period; fertilizer 2nd and 3rd periods)
- Dissolve sample in 250 mL water to make **sample solution**
- Dilute 3mL of sample solution to 250mL to make **working solution**
- Prepare **colored solutions** containing various aliquots of the working solution and 4 mL of heteropoly blue solution diluted to 10 mL
- Obtain absorbance spectra for each colored solution
- Determine absorbance values at a selected wavelength (same one as for the calibration curve)using LabQuest analysis

To prepare a calibration curve, you need to take a solution having a known percent of P and prepare samples by dilution containing concentrations of P close to the ones you expect to encounter with your fertilizer. You will be provided with a **stock solution** of known PO_4^{3-} concentration. Solutions of lesser molarity can be prepared by pipetting out portions of this solution into smaller volumetric flasks and diluting them to the mark to create a **working solution** (this step is done so that the final colored solutions will have an absorbance value between 0.1 and 1.0). Portions of the working solution are subjected to a procedure that develops a **colored solution** and *A* is read. A plot of *A* versus the molarity of the colored species establishes a relationship between a known amount of P and the solution Absorbance.

In the sample analysis, the solid sample weighed out and dissolved in water to make a **sample solution**, which is in turn diluted to make a **working solution** in the same manner as for the dilted solutions above**;** this solution is used to develop the colored compound and the quantity of P actually present in the fertilizer can be related to the concentration of P in the colored solution experimentally determined by *A.*

In this method, the phosphoric acid is converted into a blue colored "heteropoly blue" complex by adding it to a developing solution containing 2.5×10^{-3} M ammonium molybdate, 2.40×10^{-3} M ascorbic acid and 0.75 M H_2SO_4; the following reaction occurs:

$$7\ PO_4^{-} + 21\ H^{+} + 12\ (NH_4)_6Mo_7O_{24}{\cdot}4H_2O \rightarrow 7\ (NH_4)_3[PO_4(MoO_3)_{12}] + 51\ NH_4^{+} + 51\ OH^{-} + 33\ H_2O \qquad (25\text{-}1)$$

The molybdophosphate product (which contains Mo(VI)) is colorless. This compound is reduced by ascorbic acid to a blue compound containing Mo(V) and Mo(VI). The developing solution is made up so that there is an excess of both molybdate and ascorbic acid large enough to convert all of the phosphate ions in the solutions you will prepare to the reduced molybdophosphate product. (That is, PO_4^{3-} is the limiting reagent in all your solutions.)

Standard Procedures for Spectrophotometric Analysis

Heteropoly Blue Method (*Colorimetric Determination of Nonmetals* , D. F. Boltz, Ed., Interscience, New York, 1958. p. 32)

EQUIPMENT NEEDED

cuvets (2)

50 mL beaker

10 mL volumetric flask (1)

250 mL volumetric flask

5 mL Mohr (graduated) pipet

5 mL volumetric pipet

graduated disposable pipets

spectrometer

Medium test tubes

CHEMICALS NEEDED

5.00 x 10-3 M PO_4^{3-} solution

Heteropoly blue developing solution

KH_2PO_4 (1st lab period)

Fertilizer (2nd lab period)

Constructing the Calibration Curve

Note: Team members may work together to construct the calibration curve. You must construct a new calibration curve at the beginning of each laboratory period.

Rinse all glassware with distilled water before starting.

Before preparing your samples, fill a 800 mL beaker ~1/2 full with tap water, place it on a hot plate, and turn the dial to the highest heat setting.

1. A **standard stock solution** containing 5.00 x 10^{-3} M PO_4^{3-} will be provided. Obtain ~10 mL of the stock solution in a clean dry 50-mL beaker. (Why must the beaker be dry?). Obtain ~60 mL of the heteropoly blue developing solution in a clean dry 150 mL beaker. Obtain ~300 mL of distilled water in a clean 400 (doesn't have to be dry!) mL beaker
2. Use a Mohr (graduated) pipet to deliver 3.00 mL of the phosphate stock solution into a 250-mL volumetric flask and dilute to the mark with water. Mix the solution thoroughly. This is your **working solution**. **Before preparing any colored solutions, rinse the pipet with distilled water to remove any residual stock solution.**
3. Use a volumetric pipet to deliver 5.00 mL of your **working solution** into a 10-mL volumetric flask; use a disposable pipet to add ~4 mL developing solution (**CAUTION! The developing solution contains acid.)**, dilute to the mark with distilled water and mix. This is one of your **colored solutions.** Pour the solution into a labeled medium test tube. Rinse the volumetric flask thoroughly with distilled water.
4. Prepare four more **colored solutions** by repeating the procedure in step 3, starting with 4.00, 3.00, 2.00 and 1.00 mL of the working solution. Pour each solution into a separate medium test tube, making sure each is labeled carefully.
5. A so-called **reagent blank** must also be prepared in case any other constituents of the solution besides the blue one might also contribute to the absorbance at the selected wavelength, as well as correcting for any residual PO_4^{3-} present in the distilled water. This reagent blank can be used as the reference solution when obtaining the absorption spectra. To do this, add 4 mL of developing solution to the 10-mL volumetric flask and dilute to the mark with distilled water. Pour this solution into another labeled medium test tube.
6. Place the test tubes in a boiling water bath for 10 minutes to develop the blue color. Cool each test tube to room temperature by transferring them to a beaker of cold water.

Note: If the workstation is displaying a plot of data from a previous user's experiment, select **New** from the **File** menu and touch **Discard**.

7. Fill one cuvet ¾ full with the reagent blank solution (this will be the reference), and another with one of the colored solutions.
8. If the spectrometer is not plugged in yet, do so using the USB port on the side of the workstation.
9. Touch the green **Collect** arrow—this will start the lamp warmup process and will zero the spectrometer. When the warmup is complete, insert the reference cuvet into the spectrometer with one

of the clear sides pointing toward the white arrow to the left of the opening, and touch the **Finish Calibration** box. Once the calibration is completed (it will take a few seconds), touch **OK**.

10. Place your sample cuvet in the cell holder, clean, properly oriented, and fully inserted. In a few seconds the absorbance curve will appear on the display.
11. Once you are satisfied with your spectrum, touch the red **Stop** square.
12. Obtain and save absorption spectra for each of the remaining colored solutions (Runs 2-5) by touching the **File Cabinet** icon to change the run number, touching the **Collect** arrow, and repeating steps 10-11. **Note:** for best results, use the same cuvet for each of your colored solutions. Rinse the cuvet with a small amount of the next solution to be measured before filling it in preparation for the next spectrum. **Be sure to record which solution corresponds to each run number.**
13. Touch the **Run** box and select **All Runs**. Touch the spectrum near the absorbance maximum and adjust the line using the cursor controls to find the wavelength of maximum absorbance.
14. Calculate the concentration of PO_4^{3-} in each of your colored solutions (recall that the formula for calculating dilutions is $M_1V_1=M_2V_2$). Go to your TA's computer and consult with him or her to make sure the spectra look OK. If they do, you can email yourself (and your lab partner) a screen shot of the spectra from the computer.
15. Use the calibration curve program on your TA's computer to make a Beer's Law plot of *A* vs. concentration of "heteropoly blue" (in mol/L). Since **EQUATION 25-1** shows an equimolar relationship between phosphate ion and the "heteropoly blue" species, **your calibration curve also represents a plot of A vs. $[PO_4^{3-}]$**. Note that the linear regression line passes through the point (0,0). (That is, A=0 when $[PO_4^{3-}] = 0$.) If any of the points deviate greatly from the rest of the data, you should remeasure the spectrum for that point and repeat the analysis.

Sample Analysis

Note: Each team member must prepare and analyze their own sample solutions.

16. Using solid KH_2PO_4, prepare a known phosphate **sample solution** containing a PO_4^{3-} concentration similar to that of the standard stock solution. **Determine the amount of KH_2PO_4 needed to make 250 mL of this solution *before* you come to lab.** Add 3.00 mL of this solution to another 250 mL volumetric flask as in step 2 to make a **working solution**, then prepare three **colored solutions** as in step 3, using different aliquots (1,3 and 5 mL) of the working solution. Prepare another reagent blank solution to serve as a reference as in step 5. Develop the color of each solution by placing them in a boiling water bath as in step 6.
17. Record the absorption spectrum for each of these solutions using steps 7-13, being careful to keep an accurate record of the file names you use and the solutions to which they apply (you may skip the warmup step when prompted during the calibration). Go to your TA's computer and consult with him or her to make sure the spectra look OK. If they do, you can email yourself a screen shot of the spectra from the computer. **You do not need to generate a Beer's Law plot for the sample data.**
18. Dispose of all blue solutions in the waste container in the hood. If you have any leftover heteropoly blue developing solution, check with neighboring team to see if they need more. If not, disposed of this solution in the waste container as well. All other solutions can be poured down the sink.
19. Rinse all glassware thoroughly with distilled water before returning.

Protocol Considerations

When you develop the protocol for the fertilizer, you need to weigh out an amount of fertilizer that will provide a quantity of P comparable to what was used in determining the calibration curve. As a rough guess to determine the appropriate mass of sample, we will estimate that the sample will contain approximately 18% P by mass(meaning a 100 g sample of fertilizer will contain 18 g of P); you can use that approximation to **calculate the approximate mass of solid fertilizer needed** to make 250 mL of **sample solution** that has a concentration similar to that of the PO_4^{3-} stock solution; that is, ~5 x 10^{-3} M. **You should dissolve the solid in ~40 mL water and filter it by suction to remove insoluble substances**, then add the entire filtrate to a 250 mL volumetric flask and dilute to the final volume of 250 mL. Don't forget to dilute this sample solution by 3 mL to a second 250mL flask to prepare the **working sample solution** before developing the colored solutions**.**

For preparing the colored solution, select a three volumes of your working sample solution that will give an absorbance reading in the range of your calibration curve (usually 1,3 and 5 mL).

Also, since the fertilizer may itself be colored with dye and so contribute to the absorbance at the wavelength of measurement, **the reagent blank should be run using 3 mL of the working fertilizer sample solution, omitting the developing solution, so that the colored compound doesn't form**.

APPENDIX 25C: GRAVIMETRIC ANALYSIS

In a gravimetric analysis, the element of interest is incorporated into an insoluble compound which precipitates from the solution and can be weighed. Since the identity of the insoluble compound is known, the per cent composition can be calculated and the number of grams of the element calculated. For example, insoluble AgCl is composed of 24.75% Cl and 75.24% Ag; so for every 1.000 g of AgCl precipitated from a solution, the solution must have contained 0.2475 g Cl-. Knowing the weight of the element contained in the original sample, allows one to calculate what per cent of the original weight the element contributes. Obviously, the original sample must be dry since any unknown amount of water in the sample will contribute to the original weight taken and thus throw off the percent P_2O_5 determined. (***How?***)

Once the sample has been dissolved, you must take care that absolutely none of the solution is spilled or allowed to remain behind in a beaker, on filter paper, etc.; otherwise, an unknown amount of phosphate will be lost. Thus, it is important to rinse out flasks and to wash filter paper and to put the rinsings and washing back into the sample-containing solution.

Standard Procedure for Gravimetric Analysis

Magnesium Ammonium Phosphate Method (Solomon, S.; Lee, A. and Bates, D. *J. Chem. Ed.*, 1993, *5*, 410)

$$6\ H_2O + PO_4^{3-} + Mg^{2+} + NH_4^{+} \rightarrow Mg(NH_4)PO_4{\cdot}6H_2O_{(s)} \qquad (25\text{-}2)$$

EQUIPMENT NEEDED

50 mL beaker
10 mL graduated cylinder
250 mL suction flask
Büchner funnel
watch glass
red litmus paper
filter paper
graduated disposable pipet
vacuum tubing

CHEMICALS NEEDED

0.4 M $MgSO_4$ solution
6M NH_3
KH_2PO_4 (1st lab period)
Fertilizer (2nd lab period)

PROCEDURE

1. Weigh out enough KH_2PO_4 sample to contain about 0.1 ± 0.01 g of P. **Have this mass calculated *before* coming to lab**.
2. Dissolve the sample in about 10 mL of deionized water in a 50 mL beaker**. *(Does the exact volume make a difference? Why or why not?***)
3. Add 10 mL of the 0.4 M $MgSO_4$ solution. Using a disposable plastic pipet, ***slowly*** add 1 mL 6M NH_3 dropwise while stirring; after adding all of the NH_3, touch the stirring rod to a piece of red litmus paper. Repeat this, adding 1 mL of NH_3 at a time until the litmus turns blue when the solution contacts it. Then add a few drops more of NH_3. This will precipitate insoluble $Mg(NH_4)PO_4 \cdot 6H_2O$. Let the solution stand about 15 min to complete the precipitation.
4. Filter off the precipitate using suction filtration. Wash the precipitate with two 5-mL portions if *iso*-propyl alcohol. (This helps the precipitate dry faster; the alcohol dissolves any water remaining on the precipitate and evaporates very rapidly.) Suck air through the filter for about twenty minutes to help dry the precipitate and then carefully scrape it onto a pre-weighed watch glass. Be sure that you get all of the precipitate, and that it is completely dry.
5. While you are waiting for the product to dry, perform a second trial by the previous steps.
6. Measure the mass of the product for each trial. Discard the product in a wastebasket.

Protocol Considerations

As a rough guess to determine the appropriate mass of sample, we will estimate that the sample will contain approximately 18% P by mass (meaning a 100 g sample of fertilizer will contain 18 g of P); you can use that approximation to **calculate the approximate mass of solid fertilizer needed.** When you formulate the protocol for the fertilizer analysis, you should also filter the dissolved sample by gravity to remove any insoluble impurities. (***How would insoluble substances affect the result?)***