

# ASCORBIC ACID METHOD FOR PHOSPHORUS DETERMINATION

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*In this experiment you will determine the phosphorus (P) concentration of an aqueous sample using a standard method of analysis from Standard Methods for the Examination of Water and Wastewater.*

## BACKGROUND

Since 1905, Standard Methods for the Examination of Water and Wastewater (Eaton et al., 2005) has been the standard text for water analysis. It contains hundreds of the best available, generally accepted procedures for analyzing water, wastewater and related materials. Three techniques for the colorimetric analysis of phosphorus are described in Standard Methods: the vanadomolybdophosphoric method, the stannous chloride method, and the ascorbic acid method. The technique most commonly used is the ascorbic acid method. In this method, ammonium molybdate  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$  and antimony potassium tartrate  $(\text{K}(\text{SbO})\text{C}_4\text{H}_4\text{O}_6\cdot \frac{1}{2}\text{H}_2\text{O})$  react in an acid medium with dilute solutions of orthophosphate to form an intensely colored antimony-phospho-molybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid (Murphy and Riley, 1977) and the absorbance of the complex is measured at 880 nm. In this experiment, you will use the ascorbic acid method to determine the concentration of phosphorus in a water sample.

## PRELABORATORY EXERCISES:

1. Your group will need to prepare a stock solution of phosphorus (P) from which you will prepare standard phosphorus solutions. How many grams of  $\text{KH}_2\text{PO}_4$  (MW = 136.09) must be weighed out to make 100 mL of a 50 mg P/L stock solution? What is this concentration in mmol P/L?
2. After reading the experimental procedure below, create a table in your laboratory notebook that describes how you will use the stock P solution and subsequent dilutions to prepare a set of standard solutions of P ranging in concentration from 0.1 mg/L to 2.0 mg/L. Plan to prepare a minimum of 6 standards.

## BEFORE YOU BEGIN:

1. Understand how to determine the concentration of an unknown solution using Beer's Law.
2. Determine the concentration of standard phosphorus solutions needed to prepare a calibration curve suitable for determining your unknown concentration.
3. Know what a blank is and how to use it in spectrophotometric measurements.
4. Calculate the concentration of phosphorus in a solution prepared from potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ).
5. Understand the relationship between percent transmittance and absorbance.
6. Understand how a spectrophotometer works.

## EXPERIMENTAL

The procedure below is from Standard Methods for the Examination of Water and Wastewater. You will need to make some procedural changes based on the availability and type of equipment we have in the laboratory. You will also have to decide on what concentrations to make for your standards. Use the procedure from Standard Methods and the added comments to make your own detailed experimental procedure. Be specific in how you will make solutions, including how much  $\text{KH}_2\text{PO}_4$  you plan to weigh out and what the final concentrations of your solutions will be after dilution. Write your procedure in your

notebook and follow it carefully when performing the analysis.

## 1. General Discussion

*a. Principle:* Ammonium molybdate and antimony potassium tartrate react in acid medium with orthophosphate to form a heteropoly acid—phosphomolybdic acid—that is reduced to intensely colored molybdenum blue by ascorbic acid.

*b. Interference:* Arsenates react with the molybdate reagent to produce a blue color similar to that formed with phosphate.

Concentrations as low as 0.1 mg As/L interfere with the phosphate determination. Hexavalent chromium and  $\text{NO}_2^-$  interfere to give results about 3% low at concentrations of 1 mg/L and 10 to 15% low at 10 mg/L. Sulfide ( $\text{Na}_2\text{S}$ ) and silicate do not interfere at concentrations of 1.0 and 10 mg/L.

*c. Minimum detectable concentration:* Approximately 10- $\mu\text{g}$  P/L. P ranges are as follows:

Approximate P Range mg/L	Light Path cm
0.30–2.0	0.5
0.15–1.30	1.0
0.01–0.25	5.0

## 2. Apparatus

*a. Colorimetric equipment:* One of the following is required:

1) *Spectrophotometer*, with infrared phototube for use at 880 nm, providing a light path of 2.5 cm or longer.

2) *Filter photometer*, equipped with a red color filter and a light path of 0.5 cm or longer.

*b. Acid-washed glassware:* See Section 4500-P.C.2b.

## 3. Reagents

*a. Sulfuric acid,  $\text{H}_2\text{SO}_4$ , 5N:* Dilute 70 mL conc  $\text{H}_2\text{SO}_4$  to 500 mL with distilled water.

*b. Antimony potassium tartrate solution:* Dissolve 1.3715 g  $\text{K}(\text{SbO})\text{C}_4\text{H}_4\text{O}_6 \cdot \frac{1}{2}\text{H}_2\text{O}$  in 400 mL distilled water in a 500-mL volumetric flask and dilute to volume. Store in a glass-stoppered bottle.

*c. Ammonium molybdate solution:* Dissolve 20 g  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$  in 500 mL distilled water. Store in a glass-stoppered bottle.

*d. Ascorbic acid, 0.1M:* Dissolve 1.76 g ascorbic acid in 100 mL distilled water. The solution is stable for about 1 week at 4°C.

*e. Combined reagent:* Mix the above reagents in the following proportions for 100 mL of the combined reagent: 50 mL 5N  $\text{H}_2\text{SO}_4$ , 5 mL antimony potassium tartrate solution, 15 mL ammonium molybdate solution, and 30 mL ascorbic acid solution. *Mix after addition of each reagent.* Let all reagents reach room temperature before they are mixed and mix in the order given. If turbidity forms in the combined reagent, shake and let stand for a few minutes until turbidity disappears before proceeding. The reagent is stable for 4 h.

*f. Stock phosphate solution:* See Section 4500-P.C.3e.

*g. Standard phosphate solution:* Dilute 50.0 mL stock phosphate solution to 1000 mL with distilled water; 1.00 mL = 2.50  $\mu\text{g}$  P.

- The laboratory spectrophotometers use 1.0 cm path length cuvettes, which suggests a concentration range of 0.15–1.30 mg P/L is appropriate for your calibration curve; however, the spectrophotometers we use are better than those used to set these limits. You will be able to detect phosphorus concentrations below 0.15 mg P/L. Your calibration curve should range from 0.1 to 2.0 mg P/L
- Save your stock phosphate solution and standard phosphate solutions. Do not discard them.** These solutions may be used for later labs and the laboratory project.

**4500-P.C.2b. Acid-washed glassware:** Use acid-washed glassware for determining low concentrations of phosphorus. Phosphate contamination is common because of its adsorption on glass surfaces. Avoid using commercial detergents containing phosphate. Clean all glassware with hot dilute HCl and rinse well with distilled water. Preferably reserve the glassware only for phosphate determination, and after use, wash and keep filled with water until needed. If this is done, acid treatment is required only occasionally.

- 1 M HCl will be available in the laboratory for acid washing glassware.
- You will need to prepare Reagent *a* (5 N  $\text{H}_2\text{SO}_4$ ) using 9 M  $\text{H}_2\text{SO}_4$ , instead of concentration  $\text{H}_2\text{SO}_4$ . Normality, N, is moles of reactive unit per liter. For  $\text{H}_2\text{SO}_4$ ,  $\text{H}^+$  is the reactive unit, so 1 M  $\text{H}_2\text{SO}_4$  is 2 N  $\text{H}_2\text{SO}_4$ .
- Reagents *b*, *c*, and *d* will be prepared for you.
- When preparing Reagent *e*, the Combined reagent, the reagents must be added in the order listed and mixed after each reagent is added in order for the proper chemistry to occur. Make sure you have recorded this information correctly in your notebook.
- Reagent *f*. The directions ask you to prepare 1 L of this stock solution; you only need to prepare

**4500-P.C.3e.** Dissolve in distilled water 219.5 mg anhydrous  $\text{KH}_2\text{PO}_4$  and dilute to 1000 mL; 1.00 mL = 50.0  $\mu\text{g}$   $\text{PO}_4^{3-}\text{-P}$

- Reagent *g*. Prepare 1 L of this solution.

#### 4. Procedure

*a. Treatment of sample:* Pipet 50.0 mL sample into a clean, dry test-tube or 125-mL erlenmeyer flask. Add 0.05 mL (1 drop) phenolphthalein indicator. If a red color develops add 5N H<sub>2</sub>SO<sub>4</sub> solution dropwise to just discharge the color. Add 8.0 mL combined reagent and mix thoroughly. After at least 10 min but no more than 30 min, measure absorbance of each sample at 880 nm, using reagent blank as the reference solution.

*b. Correction for turbidity or interfering color:* Natural color of water generally does not interfere at the high wavelength used. For highly colored or turbid waters, prepare a blank by adding all reagents except ascorbic acid and antimony potassium tartrate to the sample. Subtract blank absorbance from absorbance of each sample.

*c. Preparation of calibration curve:* Prepare individual calibration curves from a series of six standards within the phosphate ranges indicated in ¶ 1c above. Use a distilled water blank with the combined reagent to make photometric readings for the calibration curve. Plot absorbance vs. phosphate concentration to give a straight line passing through the origin. Test at least one phosphate standard with each set of samples.

- While the standard method asks you to record absorbance, you should record percent transmittance (%T) at 880 nm and convert %T values to absorbance using the relationship  $A = \log T$ .
- It is important to measure the percent transmittance for all of your samples after the same time interval (i.e., consistently measure %T 15 minutes after addition of the combined reagent).
- Instructions on how to use the LabQuest spectrophotometer are on page 125 in the laboratory manual.
- Create a table similar to the one below in your laboratory notebook. Fill in the rows titled "Expected Concentration", "Standard P Solution (mL)", and "Distilled H<sub>2</sub>O", with the values you plan to use to make your standards. The sum of the volumes of Standard Phosphate Solution you use plus the amount of distilled H<sub>2</sub>O should add up to 50 mL. "Actual Concentration" is the concentration of your phosphorus solutions using the exact mass of KH<sub>2</sub>PO<sub>4</sub> that you weighed out on the balance in lab.
- Even though you dilute your solutions further by adding 8.0 mL of the combined reagent before analysis, since the standards and unknown are diluted by the same amount, it is not necessary to take this dilution into account, meaning you can directly determine your unknown concentration by plotting absorbance versus "Actual Concentration".
- Starting with the lowest standard phosphorus concentration, rinse the cuvet several times with the solution, then fill the cuvet 3/4 full and measure %T of the solution. Discard the solution in the cell, rinse the cuvet several times with small portions of the next solution, fill the cuvet 3/4 full and measure %T of the solution. Repeat this procedure for the standard solutions and sample solution, proceeding from lowest to highest concentrations.

- Although Table 1 doesn't show this, make multiple measurements of %T of each solution to increase the precision of your final reported values.

**Table 1:** Suitable data table for planning the preparation of solutions for the Ascorbic Method of analysis of P

	Blank	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Unknown
Expected Concentration (mg P/L)	0							--
Unknown (mL)	--	--	--	--	--	--	--	50.0
Standard P Solution (mL)	0							--
Distilled H <sub>2</sub> O (mL)	50.0							--
Actual Concentration (mg P/L)	0							--
Phenolphthalein	drop	drop	drop	drop	drop	drop	drop	drop
Combined Reagent (mL)	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Percent Transmittance								
Absorbance								

## RESULTS/CALCULATIONS

Hand in the completed answer sheet and carbonless copies of the notebook pages to you TA. Be sure your calculations include the following:

1. Calculate the absorbance from the measured percent transmittance values and determine an average absorbance value for each of the solutions measured.
2. Use EXCEL to prepare a calibration curve of average absorbance versus phosphorus concentration. Make sure to include the point from the blank you prepared. Turn in a copy of your calibration curve along with your notebook pages and answer sheet.
3. Using the calibration curve, determine the concentration of phosphorus in your unknown solution. Report the concentration of the unknown in units of mg P/L and mmol P/L.

## REFERENCES

Eaton, A.D.; Clesceri L.S.; Rice E.W.; Greenberg A.E.; Franson M.H., Eds., Standard Methods for the Examination of Water and Wastewater: 21<sup>st</sup> ed.; American Public Health Association: Washington, DC, Water Environment Federation: Alexandria, VA, and American Water Works Association: Denver, CO, 2005.

Murphy, J.; Riley, J.R. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chem.* **1977**, *27*, 31-36.

## Ascorbic Acid Method for Phosphorus Determination

Name \_\_\_\_\_ Course \_\_\_\_\_ Section \_\_\_\_\_ Date \_\_\_\_\_

Chemical formula of orthophosphate salt used for stock solution \_\_\_\_\_

Mass of phosphate salt weighed out \_\_\_\_\_

Concentration of stock phosphorus solution (in mg P/L) \_\_\_\_\_

	Concentration of P (mg P/L)	Average Absorbance
Standard 1		
Standard 2		
Standard 3		
Standard 4		
Standard 5		
Standard 6		
Unknown Solution	-----	

Concentration of P in unknown solution \_\_\_\_\_ mg P/L

\_\_\_\_\_ mmol P/L

## STAFF NOTES

### Overview

Students work in groups of two or three to prepare for and to carry out this experiment. There is no wrong way to form groups. You may either assign students or let them self-select group members. What is important is that group members equally plan, carry out, and participate in all aspects of the experiment. Notice students must develop the procedure for this experiment, so preplanning on their part will serve them well! Besides teaching the normal safety aspects and lab technique, your role in this experiment is to facilitate group cooperation and ensure all group members participate.

### What the Students are Supposed to Learn

- How to navigate a technical document, extract relevant information, and develop a modified procedure for analysis.
- Good laboratory technique in making absorbance and transmittance measurements.
- How to use Beer's Law to determine the concentration of an unknown chemical/compound that can be complexed with something to generate a color.
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### Equipment

Chemicals	Apparatus	Check out from stockroom
1 M HCl (for acid washing) 9 M H <sub>2</sub> SO <sub>4</sub> 1.3715 g antimony potassium tartrate in 500 mL DI 20 g ammonium molybdate in 500 mL DI FRESH 0.1 M ascorbic acid solution KH <sub>2</sub> PO <sub>4</sub> solid Orthophosphate unknowns stored in sample room	Acid-washed glassware VIS spectrometer	None

### Safety

- Concentrated sulfuric acid and 1 M HCl is caustic and must be handled with care. Rinse exposed skin immediately for several minutes with water. Students should avoid inhaling the fumes.
- Any spills should be cleaned up immediately.

#### Disposal

- All chemicals may be rinsed down the sink with water.

#### Things You Need To Tell Them

*In Discussion/prelab talk*

- Review Beer's law, including the relationship between A and %T.
- Work with them to navigate the technical document in the procedure. They may have trouble picking out the relevant text. The final procedure should be clear to you and to them! Check that the stock P concentration calculation makes sense.

*In the Prelab*

- Notify the TA of any chemical spills.

## The Experiment

There is a lot to do in this experiment. Monitor that the work is divided between the group and you follow the outline in the student notebook. Most students have experience with the LabQuests and the spectrometer, however do a quick review on how to use the equipment for single wavelength absorbance and transmittance measurements. Spend time on this on your own, before lab, if you do not know how to operate the equipment.

## Preparation – Students

It is recommended students acid wash their glassware the period BEFORE the lab is scheduled. The procedure is noted in the lab document. They should warm 1 M HCl, rinse and agitate the wash solution on the inside of volumetric flasks and beakers they will use for the experiment, rinse again with DI water, then store especially the volumetric flasks with a small amount of DI water. The flask should be capped. If they keep their flasks stored like this, they do not have to repeatedly acid wash their glassware for the project.

Review good laboratory technique for making spectrophotometric measurements.

- Always use the same spectrometer.
- Frequently blank the spectrometer.
- Always take your measurements for the standards and the sample in the same time period. (e.g. don't do the standards on one day and then think your samples only need to be measured on the next).
- The cuvetts should only be handled on the clouded sides.
- Measurements for the standards should begin with the lowest concentration and continue in sequence to the highest concentration.
- The cuvet should be rinsed three times with the new standard before taking a measurement.
- Record multiple measurements for the same standard by removing and reinserting the cuvet into the sample holder. Average your measurements for the final recorded value.

Students will have to make some of their own stock solutions. The prelab questions address making the stock P solution. They will have 9 M sulfuric acid available to make 1.4 M  $\text{H}_2\text{SO}_4$  solution. The technical document starts with 5 N  $\text{H}_2\text{SO}_4$ . You may need to introduce Normal units, and you may need to guide their thinking in making the adjustment. Avoid simply giving them the answer.

## Preparation - Staff

Collect a clean and DRY sample bottle from each group. Fill the sample bottle with the phosphorus unknowns, stored in plastic, labeled bottles in the sample room. Record which sample you give each group. Reference the answer key for the unknowns when grading the reports.

Check your labs that you have the listed chemicals and equipment. Contact stockroom staff if anything is missing.

Check the “made on” date of the 0.1 ascorbic acid solution. It should not be more than 1 week old.

## Probable Pitfalls

- Aging the complex for the same amount of time for the samples and the standards is important in obtaining a linear plot for the standards. I recommend they make their standards from low to high concentration and they keep track of the time each one is allowed to age before taking the measurement.

- If the mixed standard or sample immediately turns blue there is a problem. It could be students did not use DI water. (2012 the DI apparatus had actually failed and this is what we observed).
- Ascorbic acid can only be stored as a solution for a week. It oxidizes. If it seems the color is very slow to develop, check the “made on” date of the agent. It may be true the students will have to remake their solutions.