

Determination of Orthophosphate Ion

Introduction

Phosphorous, in the form of phosphate, is one of several important elements in the growth of plants. Excessive algae growth in water is stimulated by the availability of this element. Subsequent decay of the algae causes serious deterioration of water quality. The amount of phosphorous in an environmental sample is an important water quality parameter.

The analysis of the various forms of phosphorous is based on a determination of the orthophosphate (PO_4^{3-}) ion. A series of sample pre-treatments permit differentiation among the several forms. Orthophosphate ion is determined spectrophotometrically by reacting it with molybdate and reduction, forming an intensely blue phosphomolybdate complex. The intensity of this blue complex is proportional to the orthophosphate concentration.

Solutions needed for this experiment:

Solutions prepared by the student	Solutions provided by the instructor
Acid molybdate reagent	12M Hydrochloric acid
Phosphate stock and calibration standards	
Reducing agent	
6M Hydrochloric acid	

Specialized equipment needed:

In this experiment, you will be using the Unico Spectrophotometer. You are already familiar with this instrument from your penny experiment.

Experimental Procedure

Cleaning glassware.

1. Carefully read the section "Preparation of calibration standards and unknown" (below), and make a list of the number and kinds of glassware you will need in this experiment.
2. Wash the glassware with dish soap, rinse 3 times with tap water, and 3 times with small portions of deionized water.
3. Prepare a water bath by putting ~500 mL of tap water into a 1000 mL beaker. In the hood, set up a hot plate, and heat the 1000 mL beaker (with 500 mL tap water) almost to boiling.
4. For the volumetric flasks, and any other glassware that will conveniently hold liquid, fill the flasks with 6M HCl, and immerse the filled glassware into the water bath. Heat for about 30 minutes, remove the glassware, empty out the acid, and rinse 3 times with small portions of deionized water. The 6M HCl can be reused for several pieces of glassware, so don't throw it away until you are finished with all of your washing.
5. For glassware that can't conveniently hold liquids (stirring rods, pipets), put ~50 mL of 6M HCl into a 100 mL graduated cylinder. Put the stirring rods or pipets into the graduated cylinder, put the graduated cylinder into the water bath, and heat for about 30 minutes. At the end of 30 minutes, remove the pipets/stirring rods, turn them upside down, reimmerse and reheat for 30 minutes. At the end of heating, rinse the glassware 3 times with small portions of deionized water.

Solution preparation

1. Dry potassium phosphate monobasic (KH_2PO_4) in an oven at ~110 °C for at least two hours. Accurately weigh ~0.1360 grams of the dry potassium phosphate monobasic into a 100 mL beaker and dissolve it in deionized water. Analytically transfer this solution to a 1.000 L volumetric flask and QS with deionized water. If you have used the exact mass indicated above, then the solution contains 1.000 $\mu\text{mole/mL}$ phosphate.

You do NOT need to acid wash this flask; any phosphate in it will constitute a systematic error, which will cancel out as you perform the experiment.

2. Prepare the acid molybdate reagent by first adding 13.6 mL of reagent grade sulfuric acid to 36 mL of water, cooling the solution in an ice bath. Dissolve 2.5 grams of ammonium molybdate tetrahydrate in 50 mL of deionized

water, and add this solution to the cooled sulfuric acid solution. Dilute to 100 mL with deionized water. **You don't need to use volumetric glassware to prepare this solution.**

You do not need to acid wash equipment used to prepare this reagent; any phosphate present constitutes a systematic error, which will cancel out as you perform the experiment.

3. Prepare the reducing agent by dissolving 3 grams of sodium bisulfite and 1 gram of Elon (p-methylaminophenol) in 100 mL of deionized water. **You don't need to use volumetric glassware to prepare this solution.** The reducing agent is stable for ~ 1 week if stored in a brown bottle. However, it is safer and just as fast to prepare the solution immediately before use.

You do not need to acid wash equipment used to prepare this reagent; any phosphate present constitutes a systematic error, which will cancel out as you perform the experiment.

Preparation of calibration standards and unknown.

NOTE: The standards and unknowns are unstable; do NOT prepare them unless you are prepared to analyze them immediately!

1. Into separate 50.00 mL volumetric flasks, pipet 2.00, 4.00, 6.00, and 8.00 mL aliquots of your phosphate stock solution. Add 10-15 mL of deionized water.

In this specific order, add 10 mL of acid molybdate reagent, 10 mL of reducing agent, and QS with deionized water. Mix by inversion and allow 20 minutes for the color to fully develop. A blank (0.00 concentration) sample is made using DI water, acid molybdate, and reducing agent. The blank should be made in a 50.00 mL volumetric flask.

2. Pipet 5.00 mL of your unknown solution into a 50.00 mL volumetric flask. Add 10 mL deionized water, 10 mL of acid molybdate reagent, 10 mL of reducing agent, and QS with deionized water, mixing by inversion. Allow 20 minutes for the color to fully develop.
3. The analytical wavelength for this experiment is 660 nM. Follow the proper procedures for warming up the spectrophotometer, setting the dark current, and setting the wavelength.
4. Within one (1) hour of the initial mixing, measure the absorbance of all standards, the blank, and your unknown. NOTE: you must wait 20 minutes after mixing for the color to develop, and all measurements must be completed within 1 hour of mixing, so you only have 40 minutes to make all measurements!!

5. Plot absorbance versus concentration for the calibration standards (the blank, and the 2.00, 4.00, 6.00, and 8.00 standards). Perform linear regression analysis on the straight line to determine slope and intercept.
6. Using the absorbance of your unknown, and the slope and intercept values from your linear regression analysis, calculate the concentration of phosphate in your unknown.

Laboratory report.

A sample lab report is included at the end of the procedure. You must turn in your calibration curve with this experiment.

This experiment is based upon an experiment described by M. Ackerman, J. Chem. Ed., 55, 795 (1978).

WASTE DISPOSAL: All solutions containing ammonium molybdate must be disposed of in the aqueous metals container. All other waste can be disposed of down the sink.

SAMPLE REPORT – ALL NUMERICAL VALUES ARE FICTITIOUS!

Name: Mxyzptlk
Orthophosphate
Unknown # 666

Calibration data

Sample	Absorbances
Blank, 0.00 μmole	0.00218
2.00 μmole	0.143
4.00 μmole	0.287
6.00 μmole	0.431
8.00 μmole	0.572
Unknown	0.385

Slope = 0.0714

Intercept = 0.0014

Unknown:

Volume used = 5.00 mL

$$\text{Absorbance} = (\text{slope} \times \text{concentration}) + \text{intercept.}$$

$$(\text{Absorbance} - \text{intercept}) / \text{slope} = \text{concentration (in } \mu\text{moles)}$$

$$(0.385 - 0.0014) / 0.0714 = 5.37 \mu\text{moles}$$

$$5.37 \mu\text{moles} / 5.00 \text{ mL} = 1.07 \text{ mmol/L} = 102 \text{ mg/L}$$

Calibration curve must be attached to the report.