17 chapter

Phosphorus Determination by Murphy-Riley Method

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17.1 INTRODUCTION

17.1.1 Background

Phosphorus is one of the important minerals found in foods. Murphy-Riley method, a dry ashing colorimetric method, has been widely used to measure the phosphorus content in natural waters as well as in foods. In this procedure, it is necessary to ash the sample prior to analysis. The method described here is applicable to most foods following ashing. If the food sample has a low magnesium content, several milliliters of saturated $Mg(NO_3)_2 \cdot 6H_2O$ in ethanol should be added to the sample prior to ashing to prevent volatilization and loss of phosphorus at the high temperatures used in ashing.

17.1.2 Reading Assignment

Ward, R.E., and Legako, J.F. 2017. Traditional methods for mineral analysis. Ch. 21, in *Food Analysis*, 5th ed. S.S. Nielsen (Ed.), Springer, New York.

17.1.3 Objective

Determine the phosphorus content of milk using a colorimetric method, with the Murphy and Riley reagent.

17.1.4 Principle of Method

Ammonium molybdate reacts with phosphorus to form phosphomolybdate. This complex is then reduced by ascorbic acid with antimony serving as a catalyst. The reduced phosphomolybdate complex has an intense blue color. The maximum absorptivity of this complex is at approximately 880 nm. This wavelength is above the operating range of most spectrophotometers. The absorptivity of the molybdate complex is great enough in the region of 600–700 nm, however, to allow determination of the phosphorus content of most foods at any wavelength within this region. Most spectrophotometers have a maximum usable wavelength of 600–700 nm.

17.1.5 Chemicals

	CAS no.	*Hazard(s)*
Ammonium molybdate	13106-76-8	Irritant
Potassium antimonyl tartrate	28300-74-5	Harmful
Ascorbic acid	50-81-7	
Monopotassium phosphate	7778-77-0	
Sulfuric acid	7664-93-9	Corrosive

17.1.6 Reagents

(** It is recommended that these solutions be prepared by the laboratory assistant before class. Glassware cleaned by phosphate-free detergents and double-distilled or distilled deionized water must be used in all dilutions and preparation of all reagents.)

- Ammonium molybdate**
  Dissolve 48 g of ammonium molybdate to 1000 mL in a volumetric flask.
- Potassium antimonyl tartrate**
  Dissolve 1.10 g of potassium antimonyl tartrate to 1000 mL in a volumetric flask.
- Ascorbic acid**
  Prepare fresh daily. Dissolve 2.117 g to 50 mL in a volumetric flask.
- Phosphorus stock solution and working standard solution**
  Dissolved 0.6590 g of dried (105 °C, 2 h) monopotassium phosphate (KH_2PO_4) to 1000 mL in a volumetric flask. This solution contains 0.150 mg phosphorus/mL (i.e., 150 μg/mL). To prepare a working standard solution, dilute 10 mL of this solution to 100 mL in a volumetric flask. The solution now contains 15 μg phosphorus/mL.
- 2.88 *N* sulfuric acid (H_2SO_4)**
  Prepare 200 mL of 2.88 *N* sulfuric acid (H_2SO_4) using concentrated sulfuric acid (H_2SO_4) and double-distilled or distilled deionized water. Always add concentrated acid to water, not water to concentrated acid. Do not use a mechanical pipette to pipette concentrated sulfuric acid and 2.88 *N* sulfuric acid, since this would corrode the pipettor.
- Murphy and Riley reagent
  Combine 14 mL of 2.88 *N* H_2SO_4, 2 mL of ammonium molybdate solution, and 2 mL of potassium antimonyl tartrate solution in a 50 mL Erlenmeyer flask.

17.1.7 Hazards, Precautions, and Waste Disposal

Adhere to normal laboratory safety procedures. Wear gloves, lab coat, and safety glasses at all time. Care should be exercised in pipetting any solution that contains antimony since it is a toxic compound. Waste containing antimony, sulfuric acid, and molybdate must be discarded as hazardous waste. Other wastes likely may be put down the drain using a water rinse, but follow good laboratory practices outlined by environmental health and safety protocols at your attention.

17.1.8 Supplies

(Used by students)

- 1 Crucible (preheated at 550 °C for 24 h)
- Glass funnel
- Glass stirring rods
- Kimwipes

- Mechanical adjustable volume pipettes, 1000 µL with pipette tips
- Nonfat liquid milk, 5 g
- Repipettor (for fast delivery of 2 mL H_2SO_4)
- Test tubes (13 × 100 mm)
- 1 Volumetric flask, 250 mL
- Whatman No. 41 ashless filter paper

17.1.9 Equipment

- Analytical balance, 0.1 mg sensitivity
- Forced draft oven
- Hot plate
- Muffle furnace
- Spectrophotometer
- Vortex mixer

17.2 PROCEDURE

(Instructions are given for analysis in duplicate.)

17.2.1 Ashing

(Based on note in Chap. 10, Sect. 10.2.7.3.)

1. Predry crucible at 550 °C for 24 h and weigh accurately.
2. Accurately weigh ca. 5 g of sample in the crucible.
3. Heat on the hot place until a majority of water has been evaporated.
4. Dry in a forced draft oven at 100 °C for 3 h.
5. Ash in a muffle furnace at 550 °C for 18–24 h.

17.2.2 Phosphorus Measurement

1. Preparation of Murphy and Riley reagent: Add 2 mL of ascorbic acid solution to pre-prepared 18 mL of Murphy and Riley reagent. Swirl to mix.
2. Standards: Prepare phosphorus standards using working standard solution (15 µg phosphorus/mL) and water as indicated in the table below. Pipet aliquots of the phosphorus standard into clean test tubes (duplicated for each concentration) and add water so each test tube contains 4 mL. Add 1 mL of Murphy and Riley (M&R) reagent to each tube. The final volume of each tube should be 5 mL. Mix with a vortex mixer. Allow the color to develop at room temperature for 10–20 min. Read the absorbance at 700 nm.

µg P/5 mL	*15 µg P/mL*	*Vol. water (mL)*	*Vol. M&R (mL)*
0 (blank)	0	4.0	1.0
1.5	0.1	3.9	1.0
3.0	0.2	3.8	1.0
4.5	0.3	3.7	1.0
6.0	0.4	3.6	1.0

3. Sample analysis: (Note: *Do not* use an automatic pipettor to obtain 2 mL of the sulfuric acid. This would corrode the pipettor.) Analyze one ashed sample. Carefully moisten ash in crucible with H_2O and then add 2 mL of 2.88 N H_2SO_4. Filter through ashless filter paper into a 250 mL volumetric flask. Thoroughly rinse crucible, ash, and filter paper. Dilute to volume with distilled water and mix. Analyze in duplicate by combining 0.2 mL of sample, 3.8 mL of distilled water, and 1 mL of Murphy and Riley reagent. Mix and allow to react for 10–20 min. Absorbance is read at 700 nm as per the phosphorus standard solutions.

17.3 DATA AND CALCULATIONS

1. Report data obtained, giving A_{700} of the duplicate standards and sample and the average A_{700}.

	Absorbance (700 nm)		
µg P/tube (5 mL)	*1*	*2*	*Average*
1.5			
3.0			
4.5			
6.0			
Sample			

2. Construct a standard curve for your phosphorus determination, expressed in terms of phosphorus (A_{700} vs. µg phosphorus/5 mL). Determine the equation of the line for the phosphorus standard curve.
3. Calculate the concentration of phosphorus in your milk sample expressed in terms of mg phosphorus/100 g sample. Show all calculations.

Sample calculation for milk sample:

Weight of milk sample: 5.0150 g
A_{700}=0.394
Equation of the line:

$$y = 0.12x + 0.0066$$
$$y = 0.394$$
$$x = 3.3\ \mu g\ P/5\ mL$$

P content of milk sample

$$= \frac{3.2\,\mu g\,P}{5\,mL} \times \frac{5\,mL}{0.2\,mL} \times \frac{250\,mL}{5.0150\,g}$$
$$= \frac{820\,\mu g\,P}{g} = \frac{82\,mg\,P}{100\,g}$$

17.4 QUESTIONS

1. How does your value compare to literature value (US Department of Agriculture Nutrient Database for Standard Reference) for phosphorus content of milk?

2. If you had not been told to use a 250 mL volumetric flask to prepare your ashed milk sample, how could you have calculated the dilution scheme was appropriate if you wanted to use 0.2 mL ashed milk sample in the assay. US Department of Agriculture Nutrient Database for Standard Reference indicates ca. 101 mg phosphorus/100 g. Show all calculations.
3. What are the possible sources of error using this method to determine the phosphorus content of milk?
4. What was the function of ascorbic acid in the assay?

RESOURCE MATERIALS

Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27:31–36

Ward RE, Legako JF (2017) Traditional methods for mineral analysis. Ch. 21, In: Nielsen SS (ed) Food Analysis, 5th edn. Springer, New York