

## PHOSPHORUS

### PRINCIPLE

The sample is ignited in the presence of a fixative to destroy organic matter and convert phosphorus to inorganic phosphates which are not volatilized during ignition. Residual phosphates are taken up in acid, hydrolyzed to orthophosphate, and determined spectrophotometrically as a reduced phosphomolybdic acid complex (Note 1).

### SCOPE

The procedure is applicable to the determination of phosphorus in unmodified and modified starches, and syrups and sugars obtained from the corn wet milling process (Note 2).

### SPECIAL APPARATUS

1. VYCOR Dishes: 100 mL capacity
2. Muffle Furnace: Equipped with a pyrometer and capable of operating at controlled temperatures up to 650 °C
3. Spectrophotometer: An instrument capable of accurate absorbance measurements at 825 nm and equipped with a red-sensitive detector and matching 1.0 cm cuvettes.

### REAGENTS

1. Zinc Acetate Solution, 10%: Dissolve 120.0 g of reagent grade zinc acetate dihydrate [ $\text{Zn}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 2\text{H}_2\text{O}$ ] in 880 mL of purified water. If the solution is hazy, filter through Whatman No. 2V filter paper.
2. Nitric Acid Solution, 29%: Add 300 mL of concentrated nitric acid (70%  $\text{HNO}_3$ , sp g 1.42) to 600 mL of purified water and mix.

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3. Sulfuric Acid Solution, 26%: Cautiously add 167 mL of reagent grade concentrated sulfuric acid (96% H<sub>2</sub>SO<sub>4</sub>, sp g 1.84) to 833 mL of purified water and mix thoroughly.
4. Ammonium Molybdate Solution, 2%: Dissolve 10.6 g of reagent grade ammonium molybdate tetrahydrate [(NH<sub>4</sub>)<sub>6</sub> Mo<sub>7</sub>O<sub>24</sub>•4H<sub>2</sub>O] in 500 mL of purified water and mix thoroughly.
5. Ascorbic Acid Solution, 5%: Dissolve 5.0 g of ascorbic acid in 100 mL of purified water. Make fresh every 48 hrs.
6. Standard Phosphorus Solution, 2 µg Phosphorus per mL:

Stock Solution: Dissolve exactly 0.4395 g (Note 3) of reagent grade potassium dihydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>) in 500 mL of purified water in a 1 L volumetric flask; dilute to volume with purified water and mix thoroughly.

Standard Solution: Pipet 10.0 mL of the stock solution into a 500 mL volumetric flask; dilute to volume with purified water and mix thoroughly.

**PROCEDURE**

Standardization: Pipet 5.0, 10.0, 15.0, 20.0 and 25.0 mL of the standard phosphorus solution (respectively 10, 20, 30, 40 and 50 µg of phosphorus) into respective 50 mL volumetric flasks (Note 4); reserve another flask for a blank. To each flask add, in order, and mix after each addition, 10 mL of 26% sulfuric acid solution and 5 mL of 2% ammonium molybdate solution. Add purified water to make a total volume of about 45 mL; add 2 mL of 5% ascorbic acid solution and mix thoroughly. Place the flasks in a boiling water bath for 10 mins. (Note 5). Cool to 25 °C in an ice bath. Dilute to volume with purified water and mix thoroughly. Using the blank as a reference at absorbance (A), determine A of each standard in a 1 cm cuvette at 825 nm. Plot A against µg of phosphorus per 50 mL.

Analysis: Weigh 2 g to the nearest milligram of starch or an amount containing not more than 500 µg of phosphorus in a 100 mL VYCOR dish. Add 10 mL of

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10% zinc acetate solution; distribute the solution uniformly through the sample, adding purified water if necessary. Evaporate to dryness on a steam bath and char the sample on a hot plate. Ignite in a muffle furnace at 550 °C for 2 hrs. Cool to room temperature and wet all of the residue by cautious addition of 3 mL of 29% nitric acid solution (Note 6). Evaporate to dryness on a steam bath and dehydrate by brief heating on a hot plate. Return the dish to the muffle furnace for 20 mins.

Cool to room temperature; carefully wash down the sides of the dish with 3 mL of 26% sulfuric acid solution and 5 mL of purified water (Note 7). Heat to incipient boiling and hold at that temperature for 10 mins. (Note 8). With the aid of purified water, quantitatively transfer the contents of the dish to a 100 mL volumetric flask. Cool to room temperature, dilute to volume and mix thoroughly. Transfer 10.0 mL to a 50 mL volumetric flask, add 10 mL of 26% sulfuric acid solution and 5 mL of 2% ammonium molybdate solution mixing after each addition. Dilute to about 45 mL with purified water and add 2 mL of 5% ascorbic acid solution. Mix, heat, cool and dilute as directed under standardization (Note 9). Using the reagent blank as reference at O A (Note 10), determine A of the samples in 1 cm cuvettes at 825 nm. Determine  $\mu\text{g}$  of phosphorous in the sample from the standardization curve.

**CALCULATION**

$$\text{Phosphorus, } \mu\text{g} = \frac{(\mu\text{g Phosphorus From Graph})(10)}{\text{Sample Wt., g}}$$

**NOTES AND PRECAUTIONS**

1. Reduced phosphomolybdic acid ("moly blue") procedures have long suffered from errors arising from color instability and lack of reproducibility. The procedure described here is adapted from that of Fogg and Wilkinson [Analyst 83, 406 (1958)]; in sharp contrast to other procedures of its type, it shows excellent reproducibility and more than adequate color stability.
2. The method as written is limited to an analytical solution containing no more than 50  $\mu\text{g}$  of phosphorus per 50 mL. Sample size is adjusted to meet this limitation.

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3. Most samples of potassium dihydrogen phosphate assay slightly over 100%  $\text{KH}_2\text{PO}_4$ . Obtain the exact weight of standard to be taken by dividing 0.4395 by the decimal purity factor. (Example: Assay = 100.4%  $\text{KH}_2\text{PO}_4$ ; decimal purity factor = 1.004; weight of standard = 0.4377 g).
4. As is common in determination of trace constituents, most errors in this procedure stem from use of contaminated equipment. Invisible traces of detergent may contribute as much phosphorus as is contained in the sample. Dishes used for ignition of high phosphorus samples will often contain significant quantities (e.g., several micrograms) of residual phosphorus. For this analysis, volumetric flasks and pipets should be cleaned with warm chromic acid (or non-phosphorus containing equivalent solution) and rinsed thoroughly with purified water. VYCOR dishes should be treated in boiling concentrated hydrochloric acid and rinsed thoroughly before use. Ideally, glassware used in this procedure should never come in contact with detergents or high-phosphorus samples.
5. Heating time may be shortened to 5 mins. if bath capacity is such that bath temperature does not fall below 90 °C when samples are inserted. Longer heating times (up to 45 mins.) result in green colors but do not affect net absorbance at 825 nm.
6. The residue will seldom be carbon-free at this point; treatment with nitric acid and re-ignition assures complete combustion in a reasonable time. Residue from the evaporation contains hydrates which must be decomposed, by heating as directed, to avoid loss by spattering in the furnace.
7. Adhere closely to quantities of acid specified. Excess acid inhibits color development; low acidity results in complete reduction of the molybdate reagent.
8. Heating as described is necessary even if the residue dissolves immediately. Any pyrophosphate in the residue must be hydrolyzed to orthophosphate before color development.

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9. The method is somewhat sensitive to variation in experimental conditions; to avoid risk of serious error, include at least one phosphorus standard together with its reagent blank in each set of samples.
  
10. Zinc acetate dihydrate has not been observed to contribute apparent phosphorus to the system; however, it is advisable to check an ignited zinc acetate blank against a reagent blank whenever a new lot of zinc acetate dihydrate is used.