PHOSPHATE DETERMINATION BY ASCORBIC ACID METHOD (SPECTROPHOTOMETRY)

INTRODUCTION: Phosphorous occurs in waters and wastewaters almost solely as phosphates. These are classified as orthophosphates, condensed phosphates (pyro, meta, and polyphosphates) and organically bound phosphates. They occur in solution, in particles, or in the bodies of aquatic organisms. Domestic wastewater is relatively rich in phosphorous compounds. Most heavy duty synthetic detergent formulations designed for household market contain large amounts of polyphosphates. The organisms involved in the biological processes of wastewater treatment all require phosphorous for reproduction and synthesis of new cell tissue. Domestic wastewater contains amounts of phosphorous far in excess of the amount needed to stabilize the limited quantity of organic matter present. Many industrial wastes however do not contain sufficient quantities of phosphorous for optimum growth of the organisms used in treatment. In such cases, the deficiencies may be supplied by the addition of inorganic phosphates. Phosphate compounds are widely used in steam power plants to control scaling in boilers.

OBJECTIVE: The objective of the experiment is to determine PO₄ (phosphate) in water and wastewater.

REAGENTS:

Stock Solution:
This solution is only needed to make standard solution. Dissolve 219.5 mg of anhydrous KH₂PO₄ and dilute to 1,000 ml. This solution will be 50 ug P/ml.

Standard Solution:
Dilute 25 ml of stock solution to 500 ml. This will be 2.5 ug P/ml. The working standard is 2.5 ug/ml (2500 ppb) of phosphorus in phosphate (PO₄-P). Use it to make a series of standards.

<table>
<thead>
<tr>
<th>Volume (ul) used of the working standard, diluted to 25 ml.</th>
<th>Standard Conc. (ppb PO₄-P)</th>
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<tbody>
<tr>
<td>ZERO</td>
<td>BLANK</td>
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<tr>
<td>100</td>
<td>10.0</td>
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<tr>
<td>200</td>
<td>20.0</td>
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<td>400</td>
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<tr>
<td>600</td>
<td>60.0</td>
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<tr>
<td>800</td>
<td>80.0</td>
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5N Sulfuric Acid:
Dilute 70 ml of conc. sulfuric acid to 500 ml.

Potassium antimonyl tartarate - K(SbO)C₄H₂O₆.1/2 H₂O:
The bottle of the solid salt may be labeled as: Potassium antimoy(III) oxitartarate, or
Antimony Potassium Tartarate.
Dissolve 1.3715 g in 400 ml H₂O, Dilute to 500 ml. Store in glass bottle.

4% Ammonium Molybdate - (NH₄)₆Mo₇O₂₄.4H₂O:
Dissolve 20 g in 500 ml. Stir for sometime (HARD TO DISSOLVE !!)
Store in glass stoppered bottle.

Ascorbic Acid:
Dissolve 1.76 g in 100 ml H₂O.
This solution is stable for ONE WEEK ONLY at 4 degrees C.

Combined Reagent:
For 100 ml of the combined reagent add the following, IN ORDER:
- 50 ml of 5N sulfuric acid.
- 5 ml Potassium antimonyl tartarate. STIRR THOROUGHLY
- 15 ml Ammonium Molybdate. STIRR THOROUGHLY
- 30 ml Ascorbic acid. STIRR THOROUGHLY
- Let the reagent cool down to room temperature.

This combined reagent is stable for 4 HOURS ONLY !!

PROCEDURE:
- Take 25 ml sample in a 50-ml graduated tube
- Add 4 ml combined reagent.
- Cover the tube with parafilm and shake well.
- Wait for blue color to develop. It needs 10-30 minutes time.
- Measure absorbance on the Spectronic-21 at the wavelength 880 nm.