Spectrophotometric determination of P

INTRODUCTION

For measuring orthophosphate content of samples (e.g. soil extracts, plant digests)

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

SCOPE

Soil extracts and acid digests can be analysed with this method. In fact, almost any orthophosphate-containing sample can be measured (subject to satisfactory spike and recovery tests)

PRINCIPLE

Ammonium molybdate and potassium antimony tartrate react in an acid medium with orthophosphate to form a heteropoly acid – phosphomolybdic acid – that is reduced to intensely colored molybdenum blue by ascorbic acid. Absorbance of the (blue) solution is measured at 880 nm. The colour is stable for around 12 hours according to the literature, but should be reda within 1-2 hours.

REAGENTS

Reagent 'A':

- dissolve 12.0 g of ammonium paramolybdate in 250 mL of DI water.
- Dissolve 0.291 g of potassium antimony tartrate in 100 mL of DI water.
- Add both solutions to 1000 mL of 5 N sulphuric acid (= 138.8 mL of conc sulphuric in 100 mL DI water).
- Make up to 2000 mL and mix well.
- Store the solution in a brown glass bottle in a cool, dark area (usually it is stored in the fridge)

Reagent 'B:' (to be made up on the day required)

- Dissolve 1.32 g of ascorbic acid in 250 mL of reagent 'A'.
- This reagent should be prepared as required as it is only stable for 12 hours.

2,4-DNP:

- Make a saturated solution of 2,4-DNP in water (Solubility =2.5 mg/mL).
- Only very small volumes are required (1-2 drops per sample). Therefore, don't make up too much. It is best if it is in a container from which it is easy to dispense dropwise.

5 N HCl

- Add ~100mL of DI water to a 200.0 mL volumetric flask.
- Slowly add 82.6 mL of 37.2% HCl.
- Make up to 200.0 mL with DI water.

5 N NaOH

- In a 250 mL beaker dissolve 40.0 g of NaOH pellets in ~100 mL of water.
- Transfer to a 200 mL volumetric flask and make up to 200.0 mL with DI water

STANDARDS

Stock Solution: 100 ppm P

- Dissolve 0.4394 g of potassium dihydrogen orthophosphate in a 500 mL beaker.
- Transfer to a 1.00 L volumetric flask and make up to 1.00 L with DI water.

Intermediate Stock: 10 ppm P

- Dilute 2.00 mL of 100 ppm stock to 20.00 mL in a 20.00 mL volumetric flask

Calibration Standards:

- All standards are made up in the same way as samples in 25.00 mL volumetric flasks.
- If concentrations are very low, you should make the maximum standard about 0.2 –to 0.3 ppm, and include standards of 0.025, 0.05, 0.15 ppm.
- A 1-cm pathlength is OK for 0.15-1.0 ppm, a 5-cm pathlength can be used for 0.01 to 0.25 ppm P. Make standards as appropriate for the pathlength used.

METHOD

- Turn on the spectrophotometer and allow to warm up for 30 minutes
- Pipette an aliquot of sample into a 25 mL volumetric flask. The aliquot will vary but the amount of P in the flask should not exceed 12.5 µg.
- Add 3 drops 2,4-DNP. If yellow, adjust pH to 3 by adding 5 N HCl dropwise until it goes clear. If clear, add 5 N NaOH until solution is yellow, then adjust pH to 3 by adding 5 N HCl.
- Add 4.0 mL of reagent ‘B’, make to volume with DI water and mix well
- Allow colour to develop for 15 minutes before reading absorbance (against a blank)