Determination of phosphate in clinical samples

Introduction

More than 80% of the body's phosphorus is present in bones as calcium phosphate. The remainder is found intracellularly as organic phosphates such as phospholipids, nucleic acids and ATP or extracellularly as inorganic phosphorus. There is generally a reciprocal relationship between serum calcium and inorganic phosphorus levels. Increased levels of serum phosphorus are seen in renal disease, hypoparathyroidism and excessive Vitamin D intake. Decreased levels are seen in rickets, osteomalacia (adult rickets), hyperparathyroidism and diabetic coma.

The analysis is also important for water and effluent monitoring in the environmental market.

Principle

Phosphate and molybdate ions combine in acidic solution to form 12-molybdophosphoric acid, which can be measured at 340nm. Alternatively upon treatment with a suitable reducing agent such as hydrazine sulphate, the reaction yields a highly coloured blue product called heteropoly blue. Acidic molybdate can also be reduced to a blue substance, but only in less acidic solutions. Although the blue heterpoly compound has not been characterized completely, it appears to have a molecular composition similar to that of the unreduced species, differing only in that some of the covalently bound molybdenum atoms are in a +5 rather than +6 oxidation state.

The absorbance can be measured at 650 or 830 nm, but the method is more sensitive and subject to fewer interferences when the longer wavelength is used. Formation of unreduced phosphomolybdate is reported to overcome problems of variable colour formation and reagent instability and this reaction is the basis of the example shown here.

$H_3PO_4 + 12H_2MoO_4 \rightarrow H_3P(Mo_3O_{10})_4 + 12H_2O$

$H_3P(Mo_3O_{10})4 + N_2H_2SO_4 \rightarrow$ Blue product

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Method

Reagents

NaH_2PO_4 (2g) [KH_2PO_4 is a suitable alternative]
Cl_3CCO_2H, 0.60 M (660 ml)
H_2SO_4, 0.42 M
$(NH_4)2MoO_4$, 1.0 X 10^{-3} M in water

$N_2H_4SO_4$, 6.0 X 10^{-3} M (10 ml)

Preparation of standard phosphate solution:

For optimum accuracy the standard should be prepared as below but this stage can be omitted for demonstration purposes.

1. Dry 1g of reagent-grade NaH_2PO_4at 100oC for 1 hour and cool in a desiccator.
2. Weigh and dissolve 0.0125g of the dry reagent in deionised water and dilute to volume in a 200ml volumetric flask. Store in a plastic bottle.

Preparation of the calibration graph

1. For analyses on materials requiring de-proteinisation, add 90 ml of the trichloroacetic acid reagent to the phosphate standard before dilution to volume with water.
2. Pipette 20-200 microlitres of the solution into separate cuvettes, adding deionised water to a total volume of 200 micolitres. Add 1.5 ml of the molybdate and 1.5 ml of the sulphuric acid solution. Mix each thoroughly.
3. Allow to stand for 3 mins at 25 oC.
4. Measure the absorbance of each solution at 340 nm in a 1-cm cell against a reagent blank (i.e. with no phosphate).

Analysis of unknowns

Pipette 1.0 ml of blood serum and 9.0 ml of the trichloroacetic acid reagent into a small centrifuge tube. Mix and centrifuge for 5 minutes. Proceed as for the calibration standard.

Results

A standard curve measured at 340nm should be plotted. More concentrated samples may need appropriate dilution before measurement.

Discussion and Conclusions

This protocol can be applied to group surveys for the purpose of collecting typical population data. Expected values are 2.5-4.5 mg/100ml in serum and 0.4-1.3 mg/100ml in 24hr urine.

Ordering Details

Libra S5	80-2115-00
Libra S11	80-2115-15
Libra S12	80-2115-10
Libra S21	80-2115-25
Libra S22	80-2115-20
Libra S32	80-2115-30

The reaction can be accelerated for increased sensitivity if warmed. For this purpose the Libra S21/S22 have the following accessories:

- 8 position water heated cell changer (80-2109-70) used with an external heating bath
- 6 position Peltier heated cell changer (80-2106-04) and Temperature Control Unit (80-2112-49)
- Single position water heated cell holder (80-2106-08) used with an external heating bath
- Single position electrical cell holder (80-2106-12), temperatures selectable from 25, 30 and 37°C
- Single position Peltier cell holder, temperatures selectable over the whole range from 20-49°C (80-2106-13).

The Sipper (80-2112-25) enables some automation of the analyses, and can be used together with a heated (not water heated) or non-heated single cell holder.