CCLII. A METHOD FOR THE DETERMINATION OF SMALL AMOUNTS OF PHOSPHATE BY THE USE OF 8-HYDROXYQUINOLINE

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With phosphomolybdic acid 8-hydroxyquinoline forms a salt $(C_9H_7ON)_3 \cdot H_7[P(Mo_3O_7)_6] \cdot 2H_2O$, which is highly insoluble at a mildly acid reaction [Berg, 1935]. The salt is formed as a bulky precipitate which is easily washed and isolated. It is readily soluble in alkali. The 8-hydroxyquinoline is easily determined colorimetrically by the phenol method of Folin & Ciocalteu [1927]. This method furnishes a very convenient and accurate procedure for the estimation of substances having a phenolic hydroxyl group. Three molecules of 8-hydroxyquinoline are precipitated for each atom of phosphorus. The quinoline gives a very intense blue colour with the phenol reagent. Approximately 5–10 times as much blue colour is obtained with the quinoline in the precipitate from a given amount of phosphate by the phenol method, as is got with the same amount of phosphate in the phosphate methods currently in use.

SOLUTIONS REQUIRED

Stock standard phosphate. A stock solution is made by dissolving 2.194 g. of pure KH_2PO_4 in 500 ml. in water. This solution contains 1 mg. P per ml.

Dilute standard phosphate. A standard solution containing 2 γ P per ml. is made by diluting 2 ml. of the stock solution to 1 litre with water. Both solutions should be kept saturated with chloroform to prevent bacterial growth.

8-Hydroxyquinoline molybdate solution. Berg's [1935] "oxin-molybdate" solution is made by dissolving 0.8 g. 8-hydroxyquinoline and 4.2 g. ammonium molybdate separately in 5 N HCl, mixing and making to 100 ml.

N/10 sodium hydroxide.

*Folin and Ciocalteu [1927] phenol reagent.*¹ 50 g. sodium tungstate and 12.5 g. sodium molybdate are dissolved in 350 ml. of water together with 25 ml. of 85% phosphoric acid and 50 ml. of concentrated HCl. The mixture is boiled under a reflux condenser for 10 hr. 75 g. lithium sulphate and 25 ml. of water are added and a few drops of liquid bromine. The mixture is boiled gently without the reflux for 15 min. to remove excess bromine. It is then cooled, diluted to 500 ml. and filtered if not perfectly clear. The solution should be preserved in a glass-stoppered brown bottle.

10% sodium carbonate (Na_2CO_3) solution.

20% trichloroacetic acid.

THE METHOD

An amount of the phosphate-containing solution is carefully measured into a conical 15 ml. centrifuge tube and distilled water added to 5 ml. 1 ml. of oxin-molybdate solution is added and the mixture is shaken. The tube is then placed

¹ This reagent can be obtained from British Drug Houses.

in a beaker of warm water at about 60° and left for 30 min., when the precipitate of 8-hydroxyquinolinephosphomolybdate is well flocculated. The tube is then spun in the centrifuge for 5 min. so that the precipitate is well packed in the bottom of the tube, and by careful decantation the supernatant fluid can be completely separated from the precipitate. The tube is left inverted on filter-paper to drain for 5 min. The lip of the tube is then wiped clean and the precipitate stirred up with 5 ml. of ice-cold water blown into the tube from a fine-tipped pipette. The final portion of the distilled water is allowed to run down the sides of the tube. The tube is immediately centrifuged and the supernatant carefully poured off as before. After a few minutes' drainage on filter-paper the tube is wiped at the rim and the precipitate dissolved in 5 ml. *N*/10 sodium hydroxide. 0.5 ml. of Folin-Ciocalteu phenol reagent is added and 2 ml. of the 10% sodium carbonate. After mixing, the coloured solution is placed in a beaker of warm water (about 40°) for 10 min. for the blue colour to develop.

EXPERIMENTAL

Proportionality of colour. Measured quantities of dilute phosphate standard, corresponding to amounts from 2.5 to 10 γ P, were treated in the above fashion. The coloured solution containing 5 γ was used as a standard against which the other solutions were read. The results were as follows:

Taken	2.5	5.0	7.5	10.0
Found	2.6	4.9	7.6	10.0

(Figures represent γ inorganic P.)

Recovery of phosphate added to a trichloroacetic acid filtrate. Different amounts of phosphate standard were recoverable when added to trichloroacetic acid filtrates prepared from blood and other protein-containing liquids. A representative experiment gave the following results:

Taken	Filtrate	Filtrate + 5 γ	Filtrate + 10 γ
Found	2.3	7.5	12.1
Recovery		5.2	9.8

Recovery of phosphate added to blood plasma. To 0.2 ml. of plasma were added different amounts of phosphate together with water to 2 ml. Trichloroacetic acid (2 ml.) was added to each tube and the mixtures shaken. They were filtered and 2 ml. of filtrate diluted to 5 ml. with water used for each test. Two such experiments gave the following results:

Taken	Plasma	Plasma + 2.5 γ	Plasma + 5 γ
Found	4.8	7.3	9.9
Recovery		2.5	5.1
Taken	Plasma	Plasma + 7.5 γ	Plasma + 10 γ
Found	2.5	9.9	12.4
Recovery		7.4	9.9

Estimation of organic phosphate. A measured amount of a solution of an ester of phosphoric acid was placed in a conical 15 ml. centrifuge tube and digested with 0.2 ml. of 60% perchloric acid over a micro-burner. The tube was clamped in a slanting position and the flame of the burner applied a short distance above the bottom of the tube, so as to minimize the possibility of loss by bumping. The introduction of a fine glass rod with a small indentation at the lower end assured that even boiling took place. Destruction of the organic material was indicated by the mixture turning brown as it became more concentrated and then turning colourless after oxidation was complete. The tube was cooled and the stirring

rod washed off with about 3 ml. of water. The acid solution was neutralized by the addition of 2 ml. of *N* sodium hydroxide. The oxin-molybdate was added and the estimation carried through as described above.

The results obtained corresponded very closely with the theoretical percentage of phosphorus.

Sodium glycerophosphate solution.

P present	P found
10.0γ	10.0γ
	10.05γ

Barium phenylphosphate ($C_6H_5PO_4Ba \cdot 2H_2O$)

P theoretical	P found
8.98 %	8.90 %
	8.98 %

Barium ethylphosphate ($C_2H_5PO_4Ba \cdot H_2O$)

P theoretical	P found
11.11 %	11.1 %
	11.4 %

APPLICATION OF METHOD TO BLOOD ANALYSIS

Inorganic phosphate. 0.1 ml. of blood obtained by finger or ear prick is washed into 5.4 ml. of distilled water. 2 ml. of trichloroacetic acid are added; the mixture is shaken and filtered on a fine paper. 5 ml. of the filtrate and 1 or 2 ml. of the phosphate standard are placed in conical centrifuge tubes and the estimation carried out as above.

Organic phosphate (from total acid-soluble phosphate). 1.0 ml. of the above filtrate is digested with 0.2 ml. of perchloric acid until colourless.

Results obtained for inorganic and for total acid-soluble phosphorus corresponded closely with those given by King's [1932] modification of the Fiske & Subbarow [1925] method.

SUMMARY

A method for the determination of minute amounts of phosphate is described. Phospho-molybdic acid is precipitated as the salt of 8-hydroxyquinoline. Three molecules of the quinoline are precipitated for each atom of phosphorus. The quinoline in the precipitate gives a very intense blue colour with the phenol reagent.

REFERENCES

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