LXIV. THE GRAVIMETRIC ESTIMATION OF MINUTE QUANTITIES OF PHOSPHORUS.

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In the course of an investigation concerning certain of the phenomena of immunity, a method was required for the estimation of the ether-soluble phosphorus compounds of blood serum. The amount of serum available for the estimation was not more than 10 cc. and it was anticipated that only a few milligrams of the phosphorus compounds in question would be present. The isolation of this small amount and its direct weighing was therefore rejected as a possible method of estimation. An estimation of the phosphorus present appeared to be much more promising, since Neumann's method was known to be accurate for quantities of P_2O_5 as low as 10 to 15 milligrams. It was thought that by suitable modification (the use of decinormal instead of half normal solutions) this process could be used for smaller quantities of phosphorus. For the purpose of working out the method a standard solution of potassium dihydrogen phosphate solution was made such that 5 cc. contained 1 milligram P_2O_5 . Addition to this of 10 cc. of the acid mixture (equal parts of nitric and sulphuric acids), and subsequent dilution to about 100 cc. made a solution comparable to one which would be obtained by the Neumann method after oxidation had been completed and the acid residue diluted. A solution prepared in this way was therefore used in all experiments when it was desired to test the applicability of the method to actual working conditions with an unknown substance. In order to obtain rapid and complete precipitation of the phosphorus as ammonium phosphomolybdate it has been found most satisfactory to neutralise a portion of the acid present with ammonia, to add ammonium nitrate and then precipitate with ammonium molybdate at 80° . The titration of this precipitate by the Neumann method using decinormal solutions was found to

be unsatisfactory. The results were from 10 to 20 per cent. too high, when 1 milligram of P_2O_5 was being estimated. On this account the method was rejected as unsuitable, a method with an error of less than 5 per cent. being required.

The next procedure tried was the lead molybdate method of Brearley and Ibbotson [1902], which was devised by them for the estimation of phosphorus in steel. The principle of the method is very ingenious. The ammonium phosphomolybdate precipitate is dissolved in ammonia and the molybdic acid in it is precipitated as lead molybdate, which is capable of being ignited and weighed. This lead molybdate precipitate weighs 142 times as much as the phosphorus originally taken, so that even a tenth of a milligram of phosphorus gives rise to a weighable amount of precipitate. On applying this method to the precipitate of phosphomolybdate obtained in the Neumann method, the results were constantly between 10 and 12 per cent. too high when 1 milligram of P_2O_5 was being estimated. It was found, however, that if the phosphorus was precipitated by ammonium molybdate from a solution containing only ammonium nitrate and nitric acid, no sulphuric acid being present, the lead molybdate method gave very accurate results. The presence of sulphuric acid thus appears to cause precipitation of a small but appreciable quantity of molybdic oxide along with the ammonium phosphomolybdate, and although this causes no marked error when upwards of 20 milligrams of P_2O_5 are being estimated by the Neumann method, the error is magnified when only 1 milligram of P_2O_5 is in question. To overcome this difficulty the ammonium phosphomolybdate precipitate was dissolved in ammonia and reprecipitated in the presence of nitric acid and ammonium nitrate. This proved quite satisfactory and yielded results in which the average error with 1 milligram of P_2O_5 was only about 2 per cent. It was discovered later that the above influence of sulphuric acid in the estimation of phosphorus by the phosphomolybdate method had been observed by Hibbard [1913]. This author also recommends reprecipitation to overcome the difficulty.

Certain other interesting facts have been observed in working out the method finally adopted.

In determining the best amounts of ammonium nitrate and nitric acid to use in reprecipitating the ammonium phosphomolybdate, it was found that although ammonium nitrate in considerable amounts favoured rapid precipitation, the results were always from 6 to 10 per cent. too high unless the amount of nitric acid was largely increased. This in its turn rendered

precipitation less rapid and incomplete. The effect of the ammonium nitrate is due to the ease with which it causes precipitation of molybdic oxide when insufficient nitric acid is present. A determination of the ideal amounts of ammonium nitrate and nitric acid required to produce complete precipitation of 1 milligram of P_2O_5 in 100 cc. of liquid within 20 minutes without concurrent precipitation of molybdic oxide was therefore made; these concentrations were then adhered to in the reprecipitation of the phosphomolybdate.

In addition, it was observed that the use of old ammonium molybdate solution in which a white crystalline deposit had appeared, gave rise to high results. On examination it was shown that such solutions gave rise to precipitation of molybdic oxide in the presence of amounts of nitric acid and ammonium nitrate which did not cause precipitation of molybdic oxide when fresh molybdate solutions were used.

Whilst this work was in progress two methods for the estimation of small quantities of phosphorus were published by Taylor and Miller [1914]. The first method is a modification of the Neumann titration method, and the second is a colorimetric method. In both cases the substance analysed is ashed in the dry condition and the phosphorus subsequently precipitated as ammonium phosphomolybdate. The first method is stated to be accurate to 2 per cent. with amounts of P_2O_5 as low as 1 milligram. The second method is less accurate but can be used for smaller amounts than the first. Both appear to be more cumbersome than the one described in the present paper. Further, it has been found possible by the dry ashing method (which is always preferable to the wet method if it can be adopted), followed by precipitation as ammonium phosphomolybdate and then as lead molybdate, to estimate 0.1 milligram of phosphorus with an error of only 3 per cent. In the following description of the method, the process of precipitating the lead molybdate is given almost exactly as described by Brearley and Ibbotson. An alternative method suggested by them, in which the precipitate contains the phosphorus as lead phosphate in addition to the lead molybdate, has not been found as accurate.

Solutions required.

1. Ammonium Nitrate 50 per cent.
2. Ammonium Acetate 50 per cent.
3. Ammonium Chloride 20 per cent.
4. Lead Acetate 4 per cent.
5. Ammonium Molybdate 10 per cent. (To be made with cold water.)
6. Standard solution of KH_2PO_4 ; 0.3833 grams per litre; 5 cc. = 1 milligram P_2O_5 .

The substance is oxidised in the usual fashion, with nitric and sulphuric acids in a Kjeldahl flask. With the small amounts of substance used in the present investigation, rarely more than 10 cc. of the acid mixture were required. The volume of acid left after the oxidation is estimated by pouring water from a measuring cylinder into a similar Kjeldahl flask. The contents of the flask are rinsed into a 500 cc. conical flask which has been marked at the 100 cc. level. 15 cc. of ammonium nitrate are added, and for every cc. of acid left after the oxidation, 0.75 cc. of strong ammonia (S.G. 0.880) in addition. The solution is diluted till the volume is about 100 cc. and is then heated to 80°. 10 cc. of ammonium molybdate are added and the contents of the flask are constantly agitated for about a minute and a half. If precipitation of the phosphomolybdate has not begun, less than one milligram of P_2O_5 is present and 5 cc. of the standard phosphate solution should be added, or precipitation will not be completed quickly enough. The contents of the flask are again shaken, then maintained at 80° for 20 minutes, shaking twice during this period, each time for a minute. The precipitated ammonium phosphomolybdate is filtered off by means of suction on a pulp filter made by shaking a 9 cm. Swedish filter paper with very dilute nitric acid (about 2 per cent.), and pouring the pulp a little at a time on to a 2 cm. perforated porcelain plate in a funnel of suitable size. A 3 inch funnel has been found most convenient. The pulp is well packed down, employing suction by means of a Buchner flask, and is finally pressed down and at the sides with the finger. Prepared in this way the filter always retains the phosphomolybdate precipitate. The flask is rinsed out twice with cold water and the precipitate washed twice on the pulp with cold water using a wash bottle with the jet removed. The precipitate is now dissolved in 6 cc. of dilute ammonia (10 per cent.), which is allowed to filter through into the rinsed-out precipitation flask. The pulp is washed about half a dozen times with water, as much as possible being removed from the pulp each time by suction. For this purpose the stem of the funnel is passed through a rubber stopper bored with two holes. Through the second hole a bent tube passes which can be connected to the pump. The filtrate and washings, which should measure rather less than 100 cc., are now acidified by adding 11 cc. of concentrated nitric acid, and 8 cc. ammonium nitrate added. The liquid is heated to 80° and the phosphorus again precipitated as phosphomolybdate by adding 7 to 8 cc. of ammonium molybdate. The flask is well shaken for from one to two minutes and then allowed to stand at 80° for 20 minutes, with occasional shaking. The precipitate is filtered off on

a pulp filter, prepared as before, washed thoroughly with water and dissolved in 6 cc. of dilute ammonia, the pulp being then washed several times with water. The filtrate and washings are best collected in the washed-out precipitation flask. They are transferred to a beaker, 11 cc. concentrated hydrochloric acid added followed by 10 cc. of lead acetate, and the contents then heated almost to boiling. Meanwhile in the precipitation flask, a mixture of 50 cc. of ammonium chloride and 50 cc. of ammonium acetate is heated to boiling point. When both solutions are hot the solution in the beaker is poured into that in the flask, the beaker is rinsed out with a little hot water, and the precipitation of the lead molybdate facilitated by shaking. The precipitate is filtered off on a Gooch crucible, well washed with hot water, dried, ignited and weighed. The weight of the precipitate in milligrams multiplied by 0.0069 gives the weight of phosphorus in milligrams. The factor 0.0159 gives the weight of P_2O_5 . The factors theoretically are 0.00703 and 0.0161. Since the results are on an average 2 per cent. too high, the amended factors have been used when reprecipitation was necessary. When this is not the case the theoretical factors may be used. If 5 cc. of the standard phosphate solution have been added in the first precipitation, then 1 milligram P_2O_5 or 0.437 milligram of phosphorus must be subtracted from the weight found.

The method just outlined has been used for the estimation of the ether-soluble phosphorus compounds of serum. The dry ashing method for the preliminary oxidation was not convenient, because of the large volume of the ether solution of the phosphorus compounds, and the difficulty of evaporating this in a small platinum dish without loss. In cases where the dry ashing method can be used, however, the reprecipitation of the ammonium phosphomolybdate is not necessary. The method of ashing adopted by Taylor and Miller [1914] may be used with advantage. The substance, to which a few drops of saturated sodium carbonate solution have been added, is dried in a platinum dish and ignited. The mass is dissolved in a solution of 8.5 per cent. nitric acid containing 6.5 per cent. ammonium nitrate and after precipitation with ammonium molybdate the phosphorus is determined by the lead molybdate method. It is necessary to know approximately the amount of phosphorus present. For each tenth of a milligram of phosphorus about 25 cc. of the ammonium nitrate-nitric acid mixture must be used for dissolving the mass after ignition and rinsing out the platinum dish. The solutions used in the precipitation of the lead molybdate must be taken in about the same relative proportion, *i.e.* 25 cc. of the ammonium chloride

and ammonium acetate mixture, 2.5 cc. of concentrated hydrochloric acid and 2.5 cc. of 4 per cent. lead acetate solution. When about one to two tenths of a milligram of phosphorus is being estimated it is preferable to use a 100 cc. conical flask, a 1 cm. filter plate and a smaller amount of pulp in the filtration. Usually about a quarter of a 9 cm. Swedish filter paper has been found to be sufficient.

Special emphasis must be laid on the necessity for adhering to the relative concentrations of ammonium nitrate and nitric acid given above, when the phosphoric acid is being precipitated as ammonium phosphomolybdate. It is only in this way that a concurrent precipitation of molybdic oxide is avoided, and much time was lost in the early stages of working out the method through this fact not being appreciated. When Neumann's method of oxidation is used and reprecipitation of the phosphomolybdate is necessary, a trace of molybdic acid in the precipitate appears to be unavoidable, probably due to traces of colloidal matter from the filter pulp facilitating its precipitation. This error has been corrected by a slight reduction in the factor finally used for converting the weight of lead molybdate obtained into the corresponding weight of phosphorus.

The method has so far been used only for the purpose for which it was devised, but there is little doubt that it would serve equally well for the quantitative estimation of phosphorus in most organic substances.

Appended are some of the results obtained by the method both with known and unknown amounts of phosphorus:

Estimation of Known Amounts of Phosphorus.

<i>Single Precipitation Method.</i>			<i>Reprecipitation Method.</i>		
Phosphorus taken in milligrams	Phosphorus found	Mean	Phosphorus taken in milligrams	Phosphorus found	Mean
0.437	0.436	0.439	0.437	0.451	0.445
	0.443			0.435	
	0.443			0.453	
	0.434			0.454	
0.175	0.179	0.431			
0.089	0.094	0.448			
	0.091	Using the factor 0.0069 instead of the theoretical factor 0.007 the mean value becomes 0.437.			

*Estimation of Unknown Amounts of Phosphorus
(Reprecipitation Method).*

	Phosphorus found, milligrams		Phosphorus found, milligrams
Solution of crude lecithin in ether	0.66	Ether-soluble phosphorus compounds from 10 cc.	0.68
10 cc.	0.66	ox serum	0.68
	0.66		
5 cc.	0.33		
2 cc.	0.14		
	0.15		

SUMMARY.

A method is described for the gravimetric estimation of fractions of a milligram of phosphorus as low as 0.1 milligram. It is a modification of Brearley and Ibbotson's method for the estimation of phosphorus in steel.

After preliminary oxidation to phosphoric acid, the phosphorus is precipitated under special conditions as ammonium phosphomolybdate. The molybdenum in this precipitate is then estimated as lead molybdate.

The method is rapid and accurate and requires no expensive or special apparatus.

REFERENCES.

- Brearley and Ibbotson (1902). *Analysis of Steel Works Materials*, p. 55 (Longmans).
 Hibbard (1913). *J. Ind. Eng. Chem.*, **5**, 998.
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