CCXLV. THE MICRO-DETERMINATION OF PHOSPHORUS AS PHOSPHOMOLYBDATE.

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The oxidation of organic matter with sulphuric and nitric acids and the estimation of ammonium phosphomolybdate volumetrically was introduced into biochemical work by Neumann [1902]. Plimmer and Bayliss [1906] found that the precipitation was not certain and the filtration was slow and liable to error. They defined the conditions for precipitation, introduced a new way of rapid filtration and proved the accuracy of the method for quantities of phosphorus ranging from 10 to 20 mg. Gregersen [1907] also determined the conditions of precipitation and found the method accurate for 1 to 30 mg. of phosphorus. Woerner [1908] confirmed the accuracy of the method. Other workers, Raper [1914], Heubner [1914], Jodidi and Kellog [1915], Kleinmann [1919], Iversen [1920], Euler and Svanberg [1921], Kuhn [1923], Sørensen [1925], Macheboeuf [1926] and Flatter [1933] have maintained either that the method is inaccurate for micro-quantities, or of no value for quantities below 0·1 mg. Greenwald [1913] and Taylor and Miller [1914] however obtained satisfactory results with quantities of 0·015 to 0·12 mg. Pregl [1930], who weighed the precipitate, regarded the process as accurate and quotes Lieb as proving the method applicable volumetrically to 0·03 mg.

Since excellent results are generally agreed to be given with from 0·1 to 10 mg., there seemed no reason for the failure with 0·01 to 0·1 mg. Having used the method for estimating 1 to 30 mg. since 1906, we were interested to ascertain the reason for the general failure with the micro-quantities.

EXPERIMENTAL.

It was shown by Plimmer and Bayliss [1906, p. 441] that the condition for complete precipitation of ammonium phosphomolybdate was the presence of 30 cc. of 50 % ammonium nitrate (17 g.) solution for 10 cc. of conc. sulphuric acid in a volume of about 200 cc. The same conditions were applied to the precipitation of micro-quantities of phosphorus from 0·01 to 1 mg., but with smaller quantities of reagents and in a smaller flask. In all experiments there was complete precipitation.

Filtration and washing of the macro-precipitate was accomplished on a filter-paper on a perforated platinum plate fused into a glass funnel. This filter has been found unsuitable for micro-quantities, even with two papers of barium sulphate quality. The use of asbestos on this filter was rejected as a large amount of inert material would be present during the subsequent titration with the necessity of testing the filter before each estimation. Trial of an asbestos filter of the form described by Bertrand [1913] for glucose estimation showed that it retained the fine yellow precipitate, and that the latter could be dissolved on the filter in standard alkali and titrated in the usual way. Titration of the micro-quantities was carried out with $N/10$ or $N/20$ alkali and acid. The results with the micro-quantities of 0·1 mg. and less were generally from 5 to 10 % too high, just as had been found by other workers.

Numerous trials were made altering the amount of acid in the solution during precipitation of the ammonium phosphomolybdate. It was found that complete precipitation took place in concentrations of nitric acid from N to $5N$, and in concentrations of sulphuric acid from N to $3N$ in presence of the corresponding amount of ammonium nitrate. The results were still too high. Above $3N$ sulphuric acid, precipitation did not occur even on the addition of more ammonium nitrate.

Trials with alteration of the amount of ammonium molybdate in the solution gave irregular results, generally higher with more ammonium molybdate. Impurity in the ammonium molybdate was suspected as the cause of the high results. At this time Prof. J. L. Rosedale was testing Pregl's gravimetric method in this laboratory with which he obtained satisfactory results. Pregl used a special acid ammonium molybdate for precipitation. In its preparation it was noticed that a small yellow deposit was present in a larger bulk of white precipitate, which indicated the presence of small amounts of phosphorus in the reagent. On testing this reagent in place of the usual ammonium molybdate, the results were satisfactory. The cause of the high results was an impurity of phosphate in the molybdate reagent.

One other change was made in the procedure of Plimmer and Bayliss. The precipitate of ammonium phosphomolybdate was washed with 50 % alcohol, which was shown by Taylor and Miller not to dissolve it. The compound is slightly soluble in water.

The procedure of micro-estimation is as follows. To the measured volume of unknown solution in a 100 cc. flask are added 10 or 20 cc. of 10 % ammonium nitrate solution and 0·5 or 1 cc. of conc. H_2SO_4. The mixture is raised to the boiling-point and from 1 to 10 cc. of Pregl's ammonium molybdate solution are added[1]. Precipitation begins immediately and filtration can be carried out after 15 minutes. The acid solution is filtered through a Bertrand filter into a filter-flask, and the flask and filter are washed 6 times with 3–5 cc. of 50 % alcohol. It is not necessary that the whole of the precipitate be washed on to the filter. The filter-tube is removed, washed on the outside with water, and placed in one opening of a two-holed cork fitting the 100 cc. flask; the other opening carries a small bent tube for connection to a filter-pump. A slight excess of $N/10$ or $N/20$ alkali is run on to the filter from a burette; on stirring the precipitate dissolves and the solution is drawn into the flask, and the filter is washed 6 times with 3 to 5 cc. of water. The total volume is about 25 cc. A piece of porous plate is put in and the mixture is boiled for about 5 minutes to remove ammonia; one drop of phenolphthalein solution is added and the solution titrated with standard acid. A slight excess of the acid is added, the solution is again boiled to remove CO_2 and back-titrated with $N/10$ or $N/20$ alkali. The same factor, 1 cc. $N/20$ NaOH $= 0{\cdot}1268$ mg. P_2O_5 or $= 0{\cdot}05536$ mg. P,

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[1] Pregl's solution is made as follows. 150 g. of powdered ammonium molybdate are treated with 400 cc. of boiling water and shaken until dissolved. When cool, the solution is added slowly and with constant shaking to 50 g. ammonium sulphate in 500 cc. of nitric acid of sp. gr. 1·36 in a litre flask. After standing for 2 days the mixture is filtered and stored in a brown glass bottle. It has been found that 61 g. of ammonium nitrate can be used instead of the ammonium sulphate. The reagent slowly gives a deposit, and must be filtered before an estimation of phosphorus.

is used. Quantities from 0·01 to 0·1 and 1·0 mg. P have been accurately estimated as shown by the following figures:

mg. P taken	Titration cc. *N*/20	mg. P found	mg. P taken	Titration cc. *N*/20	mg. P found	mg. P taken	Titration cc. *N*/20	mg. P found
0·1	1·9	0·1052	0·09	1·75	0·0969	0·08	1·5	0·0830
	1·9	0·1052		1·55	0·0858		1·45	0·0803
	1·9	0·1052		1·6	0·0886		1·4	0·0775
	1·75	0·0969		1·5	0·0830		1·45	0·0803
	1·75	0·0969		1·6	0·0886		1·45	0·0803
0·07	1·3	0·0720	0·06	1·1	0·0609	0·05	1·0	0·0554
	1·3	0·0720		1·1	0·0609		1·0	0·0554
	1·2	0·0664		1·2	0·0664		1·0	0·0554
	1·2	0·0664		1·05	0·0581		1·0	0·0554
	1·25	0·0692		1·15	0·0637		1·0	0·0554
0·04	0·85	0·0471	0·03	0·5	0·0277	0·02	0·45	0·0249
	0·8	0·0443		0·6	0·0332		0·35	0·0194
	0·7	0·0388		0·6	0·0332		0·45	0·0249
	0·8	0·0443		0·6	0·0332		0·45	0·0249
	0·8	0·0443		0·55	0·0305		0·45	0·0249
0·01	0·2	0·0111	0·1	1·75	0·097			
	0·25	0·0138	0·2	3·5	0·194		cc. *N*/10	
	0·25	0·0138	0·3	5·1	0·282	0·7	6·0	0·664
	0·25	0·0138	0·4	7·05	0·390	0·8	7·0	0·775
	0·3	0·0156	0·5	8·75	0·484	0·9	7·7	0·852
			0·6	10·75	0·595	1·0	8·6	0·952

All the titrations were made with an ordinary burette. Better results would have been obtained with a micro-burette, and *N*/25 or *N*/50 alkali and acid could have been used.

Filtration, washing and titration can be effected in 15 to 20 minutes, so that four estimations can be made in 1 hour. For quantities above 1 mg. P, the perforated platinum plate is preferable.

The sensitivity of the precipitation of ammonium phosphomolybdate under the above conditions has been tested qualitatively. Quantities of P from 0·009 to 0·006 mg. began to come down in 2 to 3 minutes: from 0·005 to 0·003 mg. in 6 to 12 minutes; 0·002 mg. showed on standing 1 hour and 0·001 mg., which was just visible but quite evident on filtering on to the asbestos, in 1½ hours.

The method is applicable to solutions of inorganic phosphate as above described. It is applicable to all forms of organic phosphorus for estimation of total phosphorus. In this case, 1 cc. of conc. H_2SO_4 is added, oxidation is effected with nitric acid and to the cool residue 20 cc. of 10 % ammonium nitrate are added and then 5 to 20 cc. of Pregl's ammonium molybdate. Inorganic phosphate in the presence of phosphoric esters not rapidly hydrolysed by acid, such as are present in blood-filtrates, can be estimated, especially if filtration is carried out after 15 minutes, or if the precipitation is allowed to proceed at room temperature overnight, which was shown by Plimmer and Page [1913] to be quantitative. The conc. H_2SO_4 can be omitted if 10 cc. ammonium molybdate be added. The following determinations were made with 5 cc. of a trichloroacetic acid blood-filtrate:

(1) 7·74 mg. per 100 cc. (hot) as against 7·44 mg. by Fiske and Subbarow's colorimetric method
(2) 3·88 „ (hot) „ 3·34 „ kindly performed by Dr Griffiths
3·34 „ (cold)

It has not been possible to test the method for a compound like phosphagen which is very easily hydrolysed by acid. For this purpose it would be necessary to precipitate the inorganic phosphate as ammonium magnesium phosphate, to add acid to the filtrate to hydrolyse the compound and then estimate the inorganic phosphate. It would also be possible to make this estimation by dividing the solution into two parts, to add acid to one part and then estimate the inorganic phosphate in both portions as ammonium magnesium phosphate. The difference would give the phosphagen-phosphorus. The phosphorus in the ammonium magnesium phosphate can be estimated by the molybdate method after dissolution in dilute nitric acid.

SUMMARY.

The accurate estimation of micro-quantities of 0·01 to 0·1 mg. of phosphorus by the molybdate method depends upon (1) the conditions of precipitation; 20 cc. of 10 % ammonium nitrate solution must be present for every cc. of conc. H_2SO_4; (2) the use of a purified solution of ammonium molybdate such as that of Pregl, (3) the filtration of the precipitate on an asbestos filter such as that of Bertrand.

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