# INFLUENCE OF CERTAIN ANIONS ON THE ACCURACY OF THE TITRAMETRIC METHOD OF ESTIMATING PHOSPHORIC ACID IN SOLUTION.

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Several methods have been proposed for the determination of phosphoric acid in biological fluids. In most of these ammonium molybdate has been employed as the reagent for estimation. Among the methods based on its use may be mentioned the following: (1) Estimation of molybdenum in the precipitate colorimetrically; (2) Estimation of ammonia in the precipitate by the Folin-Nessler colorimetric method; (3) Conversion of the precipitate to magnesium phosphate and weighing as such or as pyrophosphate; (4) Conversion to barium, lead or strychnine phosphomolybdate; (5) Sedimetric method; (6) Nephelometric method; (7) Method of direct titration; (8) Method of weighing the precipitate and (9) The Titrametric method. Of these, the methods of direct weighing of the precipitate and of titration have found most favour among workers. Although the former method, as standardised by Lorenz, (*Landsw.-Versuchs-Stat.*, 1901, **55**, 183) has yielded good results in the hands of several workers, its use is restricted, owing firstly to the fluctuation in the composition of the precipitate (Hehner, *Analyst*, 1879, **4**, 23; Hundeshagen, *Zeit. Anal. Chemie.*, 1889, **28**, 141; Hibbard, *Jour. Ind. Eng. Chem.*, 1913, **5**, 998 and others) and secondly, because the precipitate cannot be dried without, disintegration. The titrametric method, on the other hand, has been most popular among workers both for its ease and speed in determination and for giving accurate and reliable results.

Originally developed by Pemberton (*Jour. Amer. Chem. Soc.*, 1893, **15**, 382; *Ibid.*, 1894, **16**, 278; *Ibid.*, 1895, **17**, 178) and perfected by Kilgore (*U. S. Dept. of Agric.*, *Div. of Chem.*, 1894, *Bull.* **43**, 68), it has been adopted as the Official method of the Association of Agricultural Chemists (*U. S. Dept. of Agric.*, *Div. of Chem.*, 1912, *Bull*, **107**). The method was applied to soil extracts by Prescott

(*Jour. Agric. Sci.*, 1914, **6**, 111). The sensitiveness of the method has, however, been found by a number of workers to depend on certain well defined conditions which must be strictly adhered to (Hundeshagen, *Chem. Zeit.*, 1894, **18**, 505, 547; *Zeit. anal. Chem.*, *loc. cit.*; Veitch, *J. A. C. S.*, 1896, **18**, 389; Hissink and van der Waerden, *Chem. Centr.*, 1905, **1**, 1188; Hissink, *Chem. Weekblad*, 1905, **2**, 115; *Ibid.*, 1909, **6**, 181; Schultze, *Chem. Zeit.*, 1905, **29**, 508; van Kampen, *Chem. Centr.*, 1906, **2**, 1357; Shuey, *J. Ind. Eng. Chem.*, 1917, **9**, 367; Kleinmann, *Biochem. Zeit.*, 1919, **99**, 95; Ross, *Jour. Asso. Off. Agric. Chem.*, 1929, **12**, 170; Caldwell, *Chem. News.*, 1900, **48**, 61; and, Kitajima, *Sci. Papers, Inst. Phys. Chem.*, Tokyo, 1931, **16**, 285). Indeed, as has been emphasised by Carpenter (*Jour. Ind. Eng. Chem.*, 1910, **2**, 157) 'the best way to obtain good results is for each analyst to take some standard samples of known composition and adjust the working conditions in such a manner that correct results may be obtained'.

In the course of an investigation on the mode of action of colloidal silica in increasing phosphorus availability of soils to cereal crops (Sreenivasan, *Proc. Ind. Acad. Sci.*, 1935, **1B**, 607; *Ibid.*, 1935, **2B**, 201; *Ibid.*, 1936 **3B** 283), some difficulties were encountered in the application of the titrametic method for estimating phosphates in soil extracts. In particular, it was found that the presence of chlorides, sulphates, silicates and citrates or their corresponding free acids in solution interfered with the method of determination. It was therefore thought necessary to study and, if possible, overcome the influence of these factors on the accuracy of the method. Similar studies on the interfering action of free acids and of neutral salts in solution have been carried out by other workers (*vide infra*) but, as their findings are, in most cases, contradictory and therefore inconclusive, the present investigation along similar lines was undertaken. The results have been discussed in the text together with the corresponding observations of earlier investigators.

## EXPERIMENTAL.

The reagents employed for determining phosphorus in solution were the same as those detailed by the U. S. Bureau of Soils (1912, *loc cit.*). The molybdate reagent was prepared afresh from time to time and was always filtered before being used. A standard solution of potassium dihydrogen phosphate (A. R.) was used for the estimations. 10 c.c. of this solution corresponded to 42.8c.c. of standard (0.0426N) alkali. In all the following experiments, 10 c.c. each of the phosphate solution (=about 5.5 mg. $P_2O_5$) was employed unless otherwise stated. Results are expressed in terms of c.c. of standard alkali.

***Conditions of precipitation*** :—At the outset it was found that the time and temperature of precipitation, the time of standing after precipitation and the total volume of solution affected the accuracy of the estimate. In the following experiments, 10 c.c. of the phosphate solution was mixed with 25 c.c. of saturated ammonium nitrate aq. and the volume raised to 100 c.c. with water. Precipitation was carried out as detailed in Table I with 20 c.c. of the molybdate reagent.

TABLE I

*Volume of solution = 100 c.c.*

| Precipitation | | Time of standing | Titre c.c. |
|---|---|---|---|
| at 0°C. (temp.) | for (mins.) | | |
| 28° | .... | 24 hrs.* | 40.7 |
| 28° | .... | 48 hrs. | 41.5 |
| 40° | 15 | 2 hrs.* | 41.5 |
| 40° | 15 | 24 hrs. | 42.5 |
| 50° | 10 | 2 hrs. | 43.0 |
| 50° | 15 | 2 hrs. | 42.6 |
| 50° | 15 | 24 hrs. | 43.5 |
| 50° | 30 | 2 hrs. | 46.4 |
| 60° | 15 | 2 hrs. | 44.5 |
| 60° | 30 | 2 hrs. | 52.0 |
| 70° | 15 | 2 hrs. | 45.2 |
| 70° | 15 | 24 hrs. | 45.6 |

* Filtration slow in these cases.

It may be observed that high temperature of precipitation together with longer periods of standing gives high results due obviously to separation of molybdic oxide. Low results are obtained by precipitating at ordinary temperatures. Pemberton (*loc. cit*). carried out his estimations at 70°C. while Prescott (*loc. cit*). recommends a temperature of 55°-60° and 2 hours' time to let stand before filtering. The above observations would however suggest that a temperature of 40°-45° together with overnight standing is always more desirable in practice.

In another set of experiments, the phosphate solution was mixed with 25 c. c. of saturated ammonium nitrate *aq*, and raised to different volumes before precipitation of the phosphate with the molybdate reagent. (Table II).

TABLE II

*Precipitation carried out at 45°C. together with overnight standing.*

| Volume of phosphate solution c. c. | Molybdate reagent added c. c. | Phosphate estimated c.c. of standard alkali |
|---|---|---|
| 50 | 10 | 42.8 |
| 50 | 20 | 43.5 |
| 100 | 20 | 42.8 |
| 150 | 20 | 42.4 |
| 200 | 20 | 41.6 |
| 200 | 30 | 42.0 |
| 300 | 20 | 39.8 |
| 300 | 30 | 39.8 |
| 300 | 40 | 41.0 |

It may be seen (Table II) that with too high a concentration or too large an amount of molybdate, high results are obtained, while with dilute solutions and with small amounts of the molybdate reagent, the values are low. It was however observed that even with dilute solutions, correct values were often indicated by suitable increase in the amounts of ammonium nitrate and of molybdate reagent employed together with longer periods of standing. Finkener (*Deut. Chem. Ges. Ber.*,) 1879, **10**, 1638) and Hibbard (*loc. cit.*) have stated that nitric acid delays the formation of the yellow precipitate or of molybdic acid while ammonium nitrate hastens their formation. At the same time, in the absence of sufficient amounts of ammonium nitrate, the yellow precipitate is liable to be less flocculent and run through during filtration (Tauber, *Landw. Versuchs-Stat.*, 1883, **28**, 333).

In the following experiments, all precipitations were carried out at 45° for 15 minutes together with overnight standing, unless otherwise stated.

*Effect of neutral salts in solution*:—That the presence of more than nominal amounts of certain neutral salts in solution (particularly chlorides and sulphates) distinctly affects the accuracy of the method, has been observed by a number of workers (Neubauer, *Landw. Versuchs-Stat*, 1905—06, **63**, 141; Hibbard, *loc. cit.*; Taylor and Miller, *Jour. Biol. Chem.*, 1914, **18**, 215; Ross, 1929, *loc. cit.*; and others).

*Chlorides*:—The phosphate solution (10 c.c.) together with 25 c.c. of saturated ammonium nitrate solution was made up to 100 c.c. and treated with different known amounts of ammonium chloride, sodium chloride and ferric chloride (chlorides of cations likely to occur in soil or soil extracts) respectively. The phosphates estimated are given in terms of c.c of standard alkali in Table III.

TABLE III

*Volume 100 c.c.*

| Chloride in solution per cent. | Titre in c.c. of standard alkali | | | | | |
|---|---|---|---|---|---|---|
| | $NH_4$ | Na | K | Mg | Ca | Fe |
| 0.5 | 42.5 | 42.2 | .... | .... | .... | .... |
| 1.0 | 42.5 | 41.5 | 41.9 | .... | .... | 40.2 |
| 2.0 | 43.0 | 42.0 | 42.0 | 42.2 | 42.5 | 39.6 |
| 5.0 | 42.1 | 40.5 | 41.8 | 41.6 | 40.8 | .... |
| 10.0 | 42.4 | 38.6 | .... | .... | 41.9 | .... |

It may be seen that the chloride ion in general retards the formation of ammonium phosphomolybdate; the effect is more pronounced with increasing concentration of chlorides and with the chlorides of the more highly electropositive metals. Ammonium chloride, however, does not have any adverse effect (*cf.* Meineke, *Chem. Zeit.*, 1896, **20**, 108).

*Sulphates.*—In the following experiments (Table IV) the procedure was the same as above, but instead of the chlorides, the corresponding sulphates were used.

TABLE IV

*Volume 100 c. c.*

| Sulphate in solution per cent. | Titre in c. c. of standard alkali | | | | |
|---|---|---|---|---|---|
| | $NH_4$ | Na | K | Mg | Fe |
| 0.5 | .... | 42.5 | 43.0 | 44.0 | .... |
| 1.0 | .... | 44.0 | 43.8 | 42.5 | 41.5 |
| 2.0 | 42.5 | 44.5 | .... | 43.2 | 42.0 |
| 3.0 | .... | 42.0 | 42.4 | .... | .... |
| 5.0 | 42.0 | 40.2 | 39.5 | 40.8 | .... |
| 10.0 | 42.0 | 37.5 | 38.0 | .... | .... |

Ammonium sulphate has no interfering action on the estimate of phosphate. In presence of the other sulphates, incorrect values are obtained. But, as distinct from chlorides, it may be noted that high values are often obtained upto about 2 or 3 per cent. concentration of added sulphates, while above that, low results are indicated. This would appear to be due in the first instance to the sulphate radical entering into the phosphomolybdate complex, thereby changing the composition of the yellow precipitate (Falk and Sugiura, *Jour. Amer. Chem. Soc.*, 1915. **37**. 1507: shuey, *loc. cit*) while excess of it has a solvent action on the latter.

It was observed that the filtrate and washings from the sulphate-treated series gave on standing, small amount of the yellow precipitate. This suggested that on dilution, part of the precipitate held in solution by the sulphate was reprecipitated. In the following experiments, the precipitate from the filtrate was separated, washed and titrated (Table V.)

TABLE V

*Volume 100 c. c.*

| Sodium sulphate added grams | Phosphomolybdate as c.c. of standard alkali in | |
|---|---|---|
| | Precipitate | Filtrate |
| 5 | 39.8 | 2.0 |
| 8 | 38.0 | 3.9 |
| 10 | 36.5 | 5.2 |

It would appear from the above that dilution is an important factor in determining the influence of sulphates on the estimation of phosphorus in solution. Accordingly in the following experiments the concentration of sodium sulphate was altered by suitable change in total volume before the precipitation was carried out.

TABLE VI

| Sodium sulphate (grams) | Total volume of solution | Titre c. c. |
|---|---|---|
| 5 | 100 | 40.0 |
| 5 | 150 | 41.1 |
| 8 | 100 | 38.2 |
| 8 | 150 | 40.3 |
| 10 | 100 | 36.5 |
| 10 | 200 | 37.2 |

It is thus seen that the solvent action of sulphates can, to some extent, be minimised by diluting the medium in which the precipitation is taking place. But, as has been observed before (Table II), with increasing dilution the precipitation becomes less liable to be complete. Since however the presence of larger quantities of ammonium nitrate or of molybdate reagent gives better values in such cases, some experiments were carried out increasing the quantities of these reagents in the presence of varying amounts of sulphate (Tables VII and VIII).

TABLE VII

*Total volume*: *100 c.c.* ; ***Molybdate reagent added***: *20 c. c.*

| Sodium sulphate grams | Ammonium nitrate added grams | Titre c. c. |
|---|---|---|
| 5 | 12 | 40.0 |
| 5 | 20 | 41.5 |
| 5 | 30 | 42.5 |
| 10 | 12 | 39.0 |
| 10 | 20 | 38.5 |
| 10 | 40 | 41.0 |
| 10 | 50 | 43.5 |

TABLE VIII

*Total volume: 100 c. c.; Ammonium nitrate added: 20 grams.*

| Sodium sulphate added grams | Molybdate reagent employed c. c. | Titre c. c. |
|---|---|---|
| 5 | 20 | 40.0 |
| 5 | 30 | 41.5 |
| 5 | 40 | 43.0 |
| 10 | 20 | 38.0 |
| 10 | 30 | 39.2 |
| 10 | 50 | 42.0 |

It may be seen (Table VII and VIII) that although larger quantities of molybdate reagent or of ammonium nitrate aid in the separation of the yellow precipitate, yet seldom correct values are obtained. Hence, the titrametric method would appear to be unreliable in presence of high concentrations of soluble sulphates.

*Effect of free hydrochloric acid*:—Varying amounts of hydrochloric acid were added to the phosphate solution before precipitation was made with the molybdate reagent. The phosphorus estimated (in c.c. of standard alkali) are given in Table IX.

TABLE IX

| Hydrochloric acid (1.20 d) added c. c. | Total vol. of solution c. c. | Ammonium nitrate added gms. | Titre c. c | Titre c. c. (after neutralisation of mix.) |
|---|---|---|---|---|
| 1 | 100 | 30 | 41.5 | 42.0 |
| 1 | 100 | 12 | 39.8 | .... |
| 1 | 200 | 30 | 42.0 | .... |
| 2 | 100 | 30 | 37.2 | 42.2 |
| 3 | 100 | 40 | 29.4 | 41.8 |
| 5 | 100 | 50 | Nil | 42.0 |
| 5 | 200 | 50 | Nil | .... |
| 10 | 100 | 50 | Nil | 41.6 |

Definite solvent action by the hydrochloric acid is indicated by the results although this is minimised by dilution or increase in the quantities of ammonium nitrate or both. Since ammonium chloride does not interfere, it was thought that the solvent action of $HCl$ can be overcome by neutralising the acid present in solution by means of ammonia before addition of the molybdate reagent. There salts obtained after neutralisation are given in the last column (Table IX). The precipitation is seen to be much more complete, although in some cases, results are yet slightly lower.

*Influence of sulphuric acid* :—The interfering action of free sulphuric acid has been pointed out by Richters (*Jour. Chem. Soc.*, 1871, **24**, 157), Finkener (*loc. cit.*), Hundeshagen (*loc. cit.*), Falk and Sugiura (*loc. cit.*) Shuey (*loc. cit.*), and others. In the following experiments (Table X), known quantities of sulphuric acid were added to the phosphate solution before precipitation of the phosphomolybdate.

TABLE X

| Vol. of $H_2SO_4$ (d. 1.82) added c. c. | Total vol. of soln. | Titre c. c. | Titre with 50g. extra of ammonium nitrate c.c. | Titre after neutralisation with | |
|---|---|---|---|---|---|
| | | | | $Na_2CO_3$ | $NH_4OH$ |
| 2 | 50 | 27.2 (14.9) | .... | 43.9 | 42.5 |
| 2 | 100 | 29.3 (13.0) | 36.8 | 43.0 | 43.6 |
| 1 | 50 | 35.6 ( 6.4) | 39.4 | 43.1 | 44.5 |
| 1 | 100 | 40.1 ( — ) | .... | .... | 43.2 |
| 4 | 50 | 24.0 (15.7) | 30.0 | 39.5 | 42.5 |
| 6 | 50 | Nil | .... | 40.8 | 41.5 |
| 6 | 100 | 19.6 (19.5) | .... | 41.2 | 42.0 |
| 10 | 100 | .... | .... | 36.0 | 41.5 |

As with sulphates, it was observed (Table X) that the filtrate and washings gave a precipitate on standing, which was filtered, washed and titrated. Results are given in bracket in the table above (column 3). In one set of experiments, the precipitation was done with a larger quantity (50 gms.) of ammonium nitrate, while in some others, the sulphuric acid was neutralised by addition of sodium carbonate or ammonia before estimation. Results are included in Table X above.

From the results, it may be seen that even small quantities of sulphuric acid have a solvent action on the phosphomolybdate and consequently very low values are obtained. Some investigators have stated that in small amounts (*cf.* Prescott, *loc. cit.*) the presence of sulphuric acid does not interfere with the method of estimation. Some others (Falk and Sugiura, *loc cit.*; Shuey, *loc. cit.*) have recorded that in small amounts $H_2SO_4$ gives higher figures for phosphorus, while with a larger amount, low values are obtained. But, from the above results it would appear that at all concentrations, sulphuric acid gives low values, the solvent action increasing with increased concentration, until at a concentration of about 20 per cent. precipitation is completely prevented. Larger amounts of ammonium nitrate in the medium give slightly better values while neutralisation with ammonia gives correct results in almost all cases.

According to Hibbard (*loc. cit*) the interefering action of soluble sulphates and sulphuric acid can be avoided by precipitating sulphate ion by barium chloride solution (*cf.* Richardson, *Jour. Amer. Chem. Soc.*, 1907, **29**, 1314). This should always be done in presence of considerable free acid to avoid precipitation of barium phosphate. Besides, such a procedure is tedious and takes much time.

*Influence of citric acid*:—Koenig (*Landw. Versuchs—Stat.*, 1851, **26**, 360), Ross (*Jour. Amer. Chem. Soc.*, 1894, **16**, 304), Wuyts (*Ann. Chim. anal.*, 1911, **16**, 134) and others have stated that citric acid, when present in appreciable amounts, entirely or partially prevents the precipitation of phosphoric acid by means of molybdate reagent. Others (Max Schmoeger, *Chem. Zeit.*, 1896, **20**, 497) find that phosphoric acid can be completely precipitated in presence of citric acid. With a view to finding the extent to which citric acid interferes with the estimation of citric soluble phosphorus in soils, some trials were carried out in which the phosphate solution was treated with varying amounts of citric acid. In a few cases larger amounts of ammonium nitrate were added, while in others, precipitation was carried out after neutralisation of the citric acid with ammonia. The details of the experiments together with the results are given in Table XI.

TABLE XI.

*Volume of solution = 100 c. c.*

| Citric acid added grams | Ammonium nitrate added grams | Titre c. c. | Neutralised with $NH_3$ Titre c. c. |
|---|---|---|---|
| 1 | .... | 40.4 | 43.2 |
| 1 | 10 | 41.8 | .... |
| 2 | .... | slight ppte. | 42.4 |
| 2 | 20 | do. | .... |
| 3 | 20 | Nil | 39.8 |
| 5 | 40 | Nil | slight |

It may be seen that citric acid has a pronounced solvent effect on the precipitate. The solvent action can be minimised by neutralisation of the acid with ammonia prior to precipitation, so long however as the concentration of citric acid does not exceed about 2 per cent. Beyond that, precipitation is prevented suggesting thereby that ammonium citrate also, in large quantities, has a solvent action on the precipitate. The only way then would appear to be to carry out the precipitation in a dilute solution and in presence of larger quantities of ammonium nitrate.

***Influence of silicic acid and soluble silicates*** :—Some previous workers (Pemberton, *Chem. News.*, 1882, **46**, 4 ; Preis, cited from *Brit. Chem. Abstr.*, 1890, **58A**, 825 ; Jenkins, *Jour. prakt. Chemie*, 1876,

**13**, 237 ; *Chem. News.*, 1877, **35**, pp. 60, 168, 177, 188, 201, 215 ; and others) have stated that the presence of soluble silica does not interfere with the method of estimation, while others (Richters, *loc. cit.* ; Atkinson, *Chem. News.*, 1877, **35**, 127 ; Melikoff and Becaia, *Compt. rend.*, 1912, **154**, 1478 ; and Prescott, *loc. cit.*) have found interference due to silica. Since it was proposed to study the action of soluble silica on the unavailable phosphates in the soil, it was thought necessary to study the interfering action, if any due to soluble silica. Accordingly the phosphate solution was treated with known amounts of a solution of sodium silicate and the phosphate estimated as before. Results are given in Table XII.

TABLE XII

*Volume of solution = 100 c c.*

| Sodium silicate aq. added as mg. of $SiO_2$ | Titre c c. | | |
|---|---|---|---|
| 25 | 42.6 | 43.5 | 41.9 |
| 50 | 41.8 | 44.0 | 39.2 |
| 75 | 43.4 | 44.0 | .... |
| 125 | 46.6* | .... | .... |
| 250 | 44.9* | .... | .... |
| 500 | ....* | .... | .... |

* Filtration very slow

It may be observed that with small concentrations of silicate, the values for phosphorus in solution were not very much affected, but the precipitate was greenish yellow in colour, flocculent, difficult to filter and very slow to dissolve in the alkali. Larger amounts also caused very slow filtration and difficulty in washing and always gave higher results due probably to formation of silicomolybdic acid ( *cf.* Melikoff, *Compt. rend.*, 1912, **154**, 1478; Prescott, *loc. cit.*; and others). The filtrate was also often coloured yellow, suggesting incomplete precipitation of phosphoric acid. To obtain correct values, it is therefore necessary to remove silica completely.

## SUMMARY.

1. In the estimation of phosphoric acid by the titrametric method, a high temperature of precipitation of the phosphomolybdate or longer periods of standing give higher results. Satisfactory results are obtained by carrying the precipitation at 40°–45°C. and allowing to let stand overnight.

2. In very dilute solutions, precipitation of ammonium phosphomolybdate is incomplete. Increasing the amounts of ammonium nitrate and of the molybdate in reagent in such cases give better results.

3. Chlorides have a solvent action on the yellow precipitate, the alkali chlorides being most pronounced in their solvent action. Ammonium chloride, however, has no adverse effect.

4. Sulphates - excepting ammonium sulphate—in small concentrations (upto about 2 per cent.) give higher values while with high concentrations low values are obtained. Increasing the amounts of ammonium nitrate or of molybdate reagent minimises the solvent action of sulphates.

5. Free hydrochloric acid or sulphuric acid if present in more than traces, has pronounced solvent action on the precipitate. Neutralising the acid prior to precipitation with the molybdate reagent often give correct values especially if the concentration of the acid present is low.

6. Citric acid has also a strong solvent action on the precipitate. Neutralisation with ammonia gives satisfactory results only if the citric acid present is small (less than 2 per cent.)

7. Silica, if present has to be completely removed from solution before estimating, as otherwise high values are obtained.

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