2. The presence of manganese sulfate and phosphoric acid in the ferrous solution allows the removal of the excess of permanganate and manganese dioxide without liberation of bromine or chlorine from the halides. Likewise the manganese and phosphoric acid insure a correct permanganate titration of the ferrous iron excess.

3. Iodide can also be determined in the presence of bromide and chloride by addition of potassium iodide to the final solution obtained as in 2 and titration of the liberated iodine with thiosulfate.

4. Both titrations can be made on a single sample, using the residual solution from the permanganate titration for the iodimetric determination.

Very small amounts of iodine are best determined by the iodimetric method. With moderate quantities of iodine both methods outlined are easy of manipulation, rapid in execution and accurate.

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THE PRECIPITATION OF PHOSPHORUS AS AMMONIUM PHOSPHOMOLYBDATE IN THE PRESENCE OF SULFURIC ACID.

BY K. GEORGE FALK AND KANEMATSU SUGIURA.

Received March 20, 1915.

The Neumann method for the determination of phosphorus in organic matter is based upon the oxidation of the material by a mixture of concentrated sulfuric and nitric acids, precipitation of the phosphorus as ammonium phosphomolybdate, and titration of the latter, after removal of ammonia, with standard sodium hydroxide solution. The formula of the phosphomolybdate precipitate has been taken to be essentially $(NH_4)_3PO_4.12MoO_3.2HNO_3$ under the conditions described by Neumann,[1] Gregerson,[2] and others. A substance possessing this formula, after the removal of ammonia either by boiling in alkaline solution or by combination with formaldehyde,[3] would require 56 equivalents of sodium hydroxide per gram molecule of P_2O_5 to be neutral toward phenolphthalein. If the precipitate is titrated with 0.5 N NaOH solution, one cc. would be equivalent to 1.268 mg. P_2O_5 or 0.553 mg. P; on the other hand, if it is dried at 110–120° before titrating, the nitric acid would be driven off and then one cc. would be equivalent to 1.365 mg. P_2O_5.

The possibility of the composition of the precipitate varying under different conditions has been indicated a number of times,[4] but the good

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[1] *Z. physiol. Chem.*, **37**, 115 (1902); **43**, 35 (1904).

[2] *Ibid.*, **53**, 453 (1907).

[3] Bang, *Biochem. Z.*, **32**, 443 (1911).

[4] Recently by Hibbard, *J. Ind. Eng. Chem.*, **5**, 998 (1913), who also gives a very complete bibliography. Cf. also A. E. Taylor and C. W. Miller, *J. Biol. Chem.*, **18**, 215 (1914).

results obtained in practice with the factor given by Neumann appeared to substantiate the formula ascribed. Recently, however, Heubner[1] found this factor to be too small and adopted the value 0.57 mg. in place of 0.553 mg. for the quantity of phosphorus equivalent to one cc. 0.5 *N* NaOH solution.

The work described in this paper developed from the study of the phosphorus determinations made in connection with enzyme investigations in this laboratory, and deals especially with the part played by sulfuric acid in the precipitation of the phosphomolybdate.

A number of different standard phosphate solutions were used. The experiments to be described were made with sodium ammonium phosphate 10.88 g. of $NaNH_4HPO_4.4H_2O$ (Kahlbaum's "zur Analyse") were dissolved in water and diluted to two liters. Two different solutions, designated I and II were made up at different times. The methods of analysis used will first be given.

A. Molybdate Method, no Sulfuric Acid Present.[2]—To the phosphate solution (generally 25 cc.) 12 cc. ammonium nitrate solution (750 g. NH_4NO_3 dissolved and diluted to 1 liter) and 50 cc. molybdate solution (60 g. MoO_3, 440 cc. H_2O, 60 cc. NH_4OH, sp. gr. 0.90, poured into a mixture of 250 cc. H_2O and 250 cc. HNO_3, sp. gr. 1.40, filtered after standing several days) were added, the mixture heated at 80° on a water bath for 10 minutes, cooled, and allowed to stand over night. It was then filtered on asbestos in a Gooch crucible, washed 5–6 times with an NH_4NO_3 solution (containing 50 g. of the salt and 10 cc. conc. HNO_3 in 1 liter), once with 1% HNO_3 solution, dried in an air oven at 120° and weighed as $(NH_4)_3PO_4.12MoO_3$.

B. Magnesium Ammonium Phosphate.—To 25 cc. of the phosphate solution, 10 cc. magnesia mixture (containing 55 g. cryst. magnesium chloride, 70 g. NH_4Cl, and 300 cc. NH_4OH, sp. gr. 0.96, diluted to 1 liter and filtered) were added, the mixture heated to boiling and 5 cc. 3 *N* NH_4OH solution added drop by drop; stirred, allowed to stand over night and filtered through a Gooch crucible with asbestos (or through an ashless paper). The precipitate was washed with 2.5% ammonia solution, dried, ignited to constant weight (after addition of conc. HNO_3) over a Meker burner, and weighed as $Mg_2P_2O_7$.

C. Ignition Method.—50 cc. of the solution were evaporated to dryness in a platinum dish at 100° in an air oven; the residue was then ignited to constant weight over a Meker burner and weighed as $NaPO_3$.

D. Neumann Method.—To the phosphate solution were added the stated amounts of conc. H_2SO_4, sp. gr. 1.84, water, and 50% NH_4NO_3 solution, the mixture heated to 75° and the stated quantity of a 10%

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[1] *Biochem. Z.*, **64**, 393 (1914).

[2] Cf. for instance, Olsen, "Quantitative Chemical Analysis" **1910**, p. 119.

ammonium molybdate solution (from Merck's product, highest purity) added with stirring. After 15 minutes, the mixture was cooled and either filtered at once or allowed to stand over night and then filtered.

DI. Weighing the Precipitate.—The precipitate was filtered on a Gooch crucible (asbestos), washed 7 times (unless otherwise stated) with 5–10 cc. ice-cold water each time, and dried at 120° in an air oven.

DII. Titrating the Precipitate.—The phosphomolybdate precipitate obtained as described in Method D was filtered on a Gooch crucible or on paper, washed with ice-cold water as described in DI, then the paper or the Gooch crucible containing the wet precipitate was returned to the beaker in which the precipitation had been made. If the phosphomolybdate precipitate had been dried and weighed as in Method DI, the Gooch crucible with the precipitate was placed in a beaker. The subsequent treatment of the wet or dry precipitate was the same, namely, 30 cc. water were added, then 45 cc. 0.5 *N* NaOH solution with stirring until all had dissolved; the solution was then either boiled for at least 30 minutes until litmus paper showed no further evolution of ammonia, cooled, made acid with 0.5 *N* H_2SO_4 solution, and titrated with 0.5 *N* NaOH solution using phenolphthalein as indicator; or, 50 cc. 30% formaldehyde solution, neutralized toward phenolphthalein, were added, the mixture allowed to stand 5 minutes, 7 cc. (or other suitable amount) 0.5 *N* H_2SO_4 solution added, and the solution titrated to alkaline reaction with 0.5 *N* NaOH solution.

E. Reprecipitation as Phosphomolybdate.—The weighed precipitate (from A or D, DI) was treated in a beaker with 50 cc. H_2O and 3 cc. conc. ammonia solution, warmed, filtered through paper, and washed successively with 2.5% ammonia solution and water. To the filtrate were added 20 cc. 50% NH_4NO_3 solution, and 1 cc. 10% ammonium molybdate solution. The mixture was heated on an electric stove until bubbles began to rise, and 20 cc. warm nitric acid (3 parts conc. to 1 part water) added drop by drop with stirring. It was then cooled, and after standing over night treated as in Method A.

F. Reprecipitation as Magnesium-Ammonium Phosphate.—The phosphomolybdate precipitate was transferred to a beaker, dissolved in ammonia (Method E), acidified with conc. HCl, and made slightly alkaline with ammonia; 10 cc. of the magnesia mixture (Method B) were then added, the mixture heated to boiling, cooled, and 20 cc. conc. ammonia solution added. After standing over night, the precipitate was filtered, etc., as in Method B.

G. Lead Molybdate.[1]—The phosphomolybdate precipitate was dissolved in ammonia (Method E), diluted to 100 cc. and 25 cc. portions taken for analysis. Each portion was diluted with 50 cc. H_2O, and 22 cc.

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[1] Raper, *Biochem. J.*, **8**, 649 (1914).

conc. HCl, followed by 20 cc. of 4% lead acetate solution and 200 cc. of a mixture of equal parts of 50% ammonium chloride and 50% ammonium acetate solutions added. After standing over night, the precipitate was filtered on a Gooch crucible (asbestos), washed with hot water 10 times (the wash water was somewhat turbid up to the sixth washing), then dried, ignited over a Meker burner, and weighed as $PbMoO_4$.

The results will be given as mg. P_2O_5 in 25 cc. solution, since this was the quantity used in the analyses. The phosphate contents of the two solutions were first determined by the standard methods. Four analyses of 25 cc. portions of Solution I by Method A gave 1.2338–1.2394 g. phosphomolybdate when dried at 120°, or a mean content of 46.8 mg. P_2O_5. Two of these precipitates, titrated by Method DII (formaldehyde), gave the same value (factor 1.365). Four analyses by Method B, filtered on a Gooch crucible, gave 0.0730–0.0742 g. $Mg_2P_2O_7$, or 46.8 mg. P_2O_5 as mean, and two analyses, filtered on paper, 0.0715–0.0728 g. $Mg_2P_2O_7$, or 46.0 mg. P_2O_5. By Method C, 50 cc. portions gave 0.1336 and 0.1337 g. $NaPO_3$, or 46.5 mg. P_2O_5 per 25 cc. Solution I contained, therefore, as the mean, very nearly 46.8 mg. P_2O_5 per 25 cc., taking into account the number of the determinations by each method, and the separate deviations. Four 25 cc. portions of Solution II by Method A gave 1.2350–1.2401 g. phosphomolybdate, mean P_2O_5 content 46.8 mg.; two portions precipitated by Method A, then dissolved without drying and reprecipitated by Method E, gave 1.2455 and 1.2469 g. phosphomolybdate, mean 47.1 mg. P_2O_5. These last two precipitates titrated by Method DII (formaldehyde) required 34.91 and 34.92 cc. 0.5 *N* NaOH solution, or 47.7 mg. P_2O_5 as mean. Solution II may, therefore, be taken as containing very nearly 47.1 mg. P_2O_5 per 25 cc.

A number of experiments which will not be given in detail showed that the titration of the phosphomolybdate precipitate by Method DII gave the same results whether the ammonia was removed by boiling or was combined with formaldehyde. The same results were also obtained whether the precipitates were filtered on paper or on asbestos in a Gooch crucible.

Four 25 cc. portions of Solution I by Methods D and DII, with 5 cc. conc. sulfuric acid, 8 cc. NH_4NO_3 solution, and 40 cc. molybdate solution required 37.33–37.51 cc. 0.5 *N* NaOH solution. Assuming the formula $(NH_4)_3PO_4.12MoO_3.2HNO_3$ and the factor 1.268, this corresponds to an average of 47.6 mg. P_2O_5. One portion of the same mixture by Methods D and DI gave 1.3565 g. phosphomolybdate, which, when titrated by Method DII required 37.36 cc. 0.5 *N* NaOH solution. Assuming no HNO_3 in the dried precipitate, these values correspond to 51.3 and 51.0 mg. P_2O_5, respectively. It will be noted, however, that the titration values are the same, whether or not the phosphomolybdate precipitate was dried before titrating. This was observed in every case. With mixtures containing 5 cc. sulfuric acid, 20 cc. ammonium nitrate solution, and 40 cc. molybdate solution, Methods D and DII, direct titration of the undried precipitate, gave 36.12 and 36.14 cc. 0.5 *N* NaOH solution, corresponding to 45.9 mg. P_2O_5 (1.268 factor). The same mixture by Methods D and DI, after drying and weighing the phosphomolybdate precipitate, gave 49.5 mg. P_2O_5, and titrating this dried precipitate 36.16 cc. With 50 cc. NH_4NO_3 solution, 70 cc. H_2O and the rest of the mixture the same, 35.80–35.88 cc. 0.5 *N* NaOH solution were required for the undried precipitates, or 45.5 mg. P_2O_5, assuming HNO_3 to be present. This value is considerably lower than the standard value (46.8), but, assuming no nitric acid to be present in the precipitate, would give 48.9 mg. P_2O_5, a value much too large. The filtrates from these last precipitates, on being neutralized with ammonia, acidified with nitric acid, and 15 cc. conc. nitric acid in excess added, gave no further precipitate. The results with 5 cc. conc. sulfuric acid showed that drying the precipitate had no effect on the titration, and that the results were either too large or too small, depending upon the amount of ammonium nitrate added. In the ordinary Neumann wet combustion method, often more than 5 cc. conc. sulfuric acid are present. A number of experiments were, therefore, made with the following mixture: 25 cc. Solution I, 10 cc. conc. sulfuric acid, 65 cc. H_2O, 50 cc. ammonium nitrate solution, and 40 cc. molybdate solution. Eight determinations by Methods D and DII required 35.55 to 36.02 cc. 0.5 *N* NaOH solution, giving a mean value 45.5 mg. P_2O_5 (factor 1.268). Assuming no nitric acid present in the precipitate, the value 48.9 mg. P_2O_5 (factor 1.365) is obtained. Four portions of the same mixture by Methods D and DI gave 1.2985 to 1.3047 g. phosphomolybdate precipitate, or 49.2 mg. P_2O_5 (for the formula $(NH_4)_3PO_4.12MoO_3$). These precipitates when titrated by Method DII required 35.83 to 36.03 cc. 0.5 *N* NaOH solution, or very nearly the amounts required by the undried precipitates. With the factor 1.268 this corresponds to 45.5 mg. P_2O_5; with the factor 1.365 to 49.0 mg. P_2O_5. The filtrates from these precipitates, neutralized with ammonia, acidified with nitric acid and 15 cc. conc. nitric acid in excess added, gave no further precipitate on standing. A few experiments in which more sulfuric acid was present, were made. Using 12.5 cc. conc. sulfuric acid in place of 10 cc., determinations by Methods D and DII required 34.81 and 35.29 cc. 0.5 *N* NaOH solution, while the filtrates, treated as described above, gave additional precipitates after standing over night, requiring 0.89 and 0.34 cc. The average total titration was 35.67 cc. With 15 cc. conc. sulfuric acid in place of 10 cc. the first precipitates required 28.83 and 30.13 cc., while from the filtrates were obtained precipitates requiring 7.70 and 6.53 cc. The average total titration was 36.60 cc., of which 15–20%

was obtained with the second precipitates. The total titrations with different amounts of sulfuric acid present were not in accord. This would appear to indicate a varying composition of the precipitates. With more than 10 cc. conc. sulfuric acid present, the precipitation was not complete. 50 cc. of the 50% NH_4NO_3 solution present in 190–195 cc. total solution is slightly more than the quantity recommended by Neumann, and somewhat less, 13% instead of 15%, than that recommended by Gregerson.

The experiments with Solution II were carried out with different ends in view, and the results obtained indicate some of the reasons for the irregularities observed by different workers. 25 cc. of this solution contained 47.1 mg. P_2O_5 as indicated before. The mixture used in all of the following work with this solution was made up of 25 cc. Solution II, 10 cc. conc. sulfuric acid, 50 cc. NH_4NO_3 solution, 65 cc. water, and 40 cc. molybdate solution.

The phosphomolybdate precipitate in the Neumann method must be washed with ice-cold water until the wash water is no longer acid. Two sets of two portions each, precipitated by Method D, then treated by Method DI, the first set washed four times with ice-cold water, the second seven times, gave 1.3079–1.3089 g. and 1.3042–1.3047 g. phosphomolybdate precipitate, respectively, corresponding to 49.5 and 49.3 mg. P_2O_5. Two other sets of three portions each by Methods D and DII, washed four times and seven times, respectively, required 36.43–36.72 cc. and 36.11–36.27 cc. 0.5 *N* NaOH solution, respectively, corresponding to 46.3 and 46.0 mg. P_2O_5 with the factor 1.268, or 49.9 and 49.4 mg. P_2O_5 with the factor 1.365. The fourth washings required 2.44 cc. 0.5 *N* NaOH, the 7th 0.12 cc. 0.5 *N* NaOH. The washings tested for sulfuric acid gave, in a series of similar experiments, for the sixth washing a test equivalent to 0.2–0.3 cc. 0.1 *N* H_2SO_4, and for the eighth washing a test equivalent to 0.02–0.05 cc. 0.1 *N* H_2SO_4. After the sixth washing the phosphomolybdate precipitate began to run through the filter, as shown by the turbidity. Practically all the free acid was, therefore, removed by seven washings, but not by four. No volatile substance, such as nitric acid, is present in the precipitates, as shown by the agreement between the dried and undried precipitates.

A number of experiments involving solution of the phosphomolybdate and reprecipitation were made. Three portions by Methods D and DI gave 1.3087–1.3116 g. phosphomolybdate precipitate, corresponding to a mean P_2O_5 content of 49.5 mg. These precipitates dissolved and treated by Method F gave 0.0728–0.0732 g. $Mg_2P_2O_7$, corresponding to 46.6 mg. P_2O_5. Another set of three by Methods D and DI gave 1.3137–1.3138 g. precipitate, corresponding to 49.7 mg. P_2O_5; these precipitates dissolved and reprecipitated by Method E gave 1.2561–1.2564 phosphomolybdate precipitate, corresponding to 47.5 mg. P_2O_5; these dissolved and reprecipitated by Method F gave 0.0728–0.0732 g. $Mg_2P_2O_7$ or 46.5 mg. P_2O_5.

These experiments show that the first precipitate of the phosphorus as phosphomolybdate from the solution containing sulfuric acid possesses a different composition from that of the precipitate obtained if little or no sulfuric acid is present. That no nitric acid is contained in the precipitate formed with sulfuric acid present in the solution is shown also by the fact that the same results were obtained whether or not the precipitates were dried at 120°. An attempt was, therefore, made to study the composition of these precipitates. The amount of sulfate in the precipitates was accordingly determined. Four portions of Solution II, with the same mixture as before, were treated by Methods D and DI. The precipitates were dissolved and reprecipitated by Method E; the mother liquors from these reprecipitations were evaporated to dryness on the water bath after the addition of one cc. conc. hydrochloric acid, 25 cc. water added, and the mixture filtered, the filtrates diluted to 100 cc. and 5 cc. concentrated hydrochloric acid and 5 cc. 10% $BaCl_2$ solution added while boiling. After the customary treatment, 0.0179–0.0214 g. $BaSO_4$ were obtained, equivalent to a mean value of 0.0080 g. H_2SO_4. Similar treatment of Solution II without added sulfuric acid, by Methods A and E, gave 0.0001 and 0.0004 g. $BaSO_4$. These results appear to show that sulfuric acid is an essential part of the precipitate formed under the given conditions.

Based upon the amounts of phosphate and sulfate present, and upon a number of nitrogen and molybdenum determinations, the composition of the precipitate was calculated to be $4[(NH_4)_3PO_4.12MoO_3] + (NH_4)_2SO_4.5MoO_3$. The mean of four closely agreeing nitrogen determinations by the Kjeldahl method[1] of the precipitates obtained from Solution II by Methods D and DI was 2.23%; calculated for the above formula, 2.35% N. Several determinations of the molybdenum in these precipitates by Method G gave, for 25 cc. portions of the phosphomolybdate precipitate dissolved in 100 cc., 0.7627 and 0.7496 g. $PbMoO_4$, corresponding to an average of 90.6% MoO_3. The calculated MoO_3 content for a substance of the above formula is 91.3%; for a substance of the formula $(NH_4)_3PO_4.12MoO_3$, 92.1%. To check up the method, similar portions of the phosphomolybdate precipitate from Solution II by Method A without the addition of sulfuric acid, gave 0.7216 and 0.7326 g. $PbMoO_4$, or a mean MoO_3 content of 92.6%.

The precipitate is probably present as a complex compound and not as a mixture, since similar solutions containing no phosphoric acid gave no precipitate of sulfomolybdate. The formula found, as given above,

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[1] Sherman, "Methods of Organic Analysis," 1912, p. 294.

naturally holds only for the precipitate formed under the given conditions. Under different conditions different proportions of sulfuric acid and molybdic oxide might be carried down, but the general relations would probably be similar. Thus, the carrying down of molybdic acid in excess of the 24 MoO_3 to one P_2O_5 has been observed at different times, recently by H. S. Halcro Wardlaw in a paper on the "Accuracy of Neumann's Method for the Estimation of Phosphorus."[1] That sulfate may be present in the precipitate has also been indicated at various times, but it was considered to replace the nitric acid which ordinarily forms part of the precipitate. The composition of the precipitates described in this paper have been given in the above form, because when no sulfuric acid was present, each P_2O_5 was combined with 24 MoO_3. The sulfuric acid which was precipitated was, therefore, assumed to carry down the further portion of MoO_3 which was found.

If the precipitate, whose complete formula may be written $(NH_4)_{14}(PO_4)_4SO_4.53MoO_3$ is titrated with alkali after removal of the ammonia, each gram molecule of P_2O_5 would require 58 equivalents of NaOH, instead of the 56 required by two molecules of the precipitate $(NH_4)_3PO_4.12MoO_3.2HNO_3$. From a consideration of the phosphosulfomolybdate precipitate, it is evident that the proportion of sulfate, phosphate, and molybdenum in the complex may well be subject to considerable variation. If the same ratio for MoO_3 to sulfate precipitated with the phosphomolybdate holds, a smaller concentration of sulfuric acid in the solution would be expected to result in titration values six times smaller than if the amount of sulfate in the precipitate alone varied. This may account for the entirely different factor, corresponding to less than 56 equivalents NaOH per gram molecule P_2O_5, found by Heubner, who worked with smaller amounts of phosphorus and sulfuric acid. Between these two sets of results lie those obtained by Neumann, Gregerson, and more recently by Greenwald.[2] These workers found the relation, 56 equivalents NaOH per gram molecule P_2O_5, to hold for the P_2O_5 content of the phosphomolybdate precipitate for the conditions of precipitation chosen by them.

In the course of this investigation a number of other phosphate solutions, and also solutions containing widely different amounts of phosphate, were used. The results obtained bear out, in general, the conclusions given, but not enough work was done to determine the variation of the composition of the precipitate with different amounts of phosphate and sulfuric acid. Finally, it may be stated that the titration of the phosphomolybdate precipitate gives satisfactory results only if the conditions of precipitation have been worked out with known amounts of phosphate

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[1] *J. Roy. Soc. New South Wales*, **48**, 73 (1914).

[2] *J. Biol. Chem.*, 14, 369 (1913).

and an empirical factor determined. Otherwise, solution and reprecipitation of the phosphomolybdate precipitate are essential.[1]

Conclusions.

The precipitate of ammonium phosphomolybdate formed in the presence of sulfuric acid (as in Neumann's method) contains sulfate apparently as an essential part of the molecule, together with an excess of molybdic oxide, and no nitric acid. The composition of this phosphosulfomolybdate precipitate may vary with the concentration of the different constituents in the solution. These variations explain the different factors found for the titration of the precipitate with alkali. The composition of the precipitate for a certain set of conditions was found to be $4[(NH_4)_3PO_4.12MoO_3] + (NH_4)_2SO_4.5MoO_3$.

FLUOBORIC AND FLUOSILICIC ACIDS IN THE QUALITATIVE ANALYSIS OF SODIUM.

By F. C. Mathers, C. O. Stewart, H. V. Housemann and I. E. Lee.

Received April 10, 1915.

The scheme for the detection of sodium is the only unsatisfactory test in the qualitative analysis of the soluble or alkali group of bases. The flame or spectrum test is so delicate that the quantity of sodium normally present in all reagents always distinctly gives it. This is confusing to inexperienced students. The pyroantimonate method has never given accurate results in this laboratory in the hands of beginning students. The following method is the final result of experiments which have been conducted by the authors for a number of years in an effort to devise a quick and accurate test for sodium.

Method of Analysis.—The filtrate containing the potassium, sodium, ammonium and magnesium (after the other metals have been removed in the ordinary way) is evaporated to dryness and ignited to remove the ammonium. The residue is dissolved in 10 cc. of water[2] by warming. A small portion of the solution (a few drops) is removed and tested for potassium with sodium cobaltic nitrite. The remainder of the solution is treated with an equal volume of alcohol and an excess of fluoboric acid in 50% alcohol. The potassium is completely precipitated as potassium fluoborate, a flocculent, easily filtered precipitate. Sodium and magnesium remain in solution. The sodium in the filtrate may then be precipitated with fluosilicic acid as sodium fluosilicate, a gelatinous, semi-transparent precipitate which settles slowly, consequently careful observation is necessary to detect it when only small quantities of sodium are present.

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[1] Cf. Hibbard, Raper, and others.

[2] Ten cc. of water should be used for each gram of sodium and potassium chloride present.