

On the Analysis of Calcium Phosphate.

By

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I. SIMULTANEOUS DETERMINATION OF CALCIUM OXIDE AND PHOSPHORIC ACID.

The usual method of determination of phosphoric acid consists of two procedures: (*a*) the preliminary precipitation of phosphoric acid from an acid solution by means of the ammonium molybdate reagent and (*b*) its final reprecipitation from the ammoniacal solution by means of the magnesia mixture. The new method here proposed for the analysis of calcium phosphate obviates the use of the ammonium molybdate reagent and possesses the advantage of enabling the simultaneous determination of calcium to be carried out.

Calcium is precipitated quantitatively from an acetic acid solution as oxalate in the presence of a measured excess of a standard solution of potassium bioxalate. Calcium oxalate is either titrated with potassium permanganate or ignited and weighed as oxide. The excess of oxalic acid in the filtrate is also titrated with potassium permanganate and the phosphoric acid in the reaction liquid thus obtained is determined by precipitating it as ammonium manganese phosphate.

PRECIPITATION OF CALCIUM OXALATE. 0.5000 gr. of the sample is dissolved in about 10 cc. of water containing 2 cc. of 6 *N* HNO₃. 10 cc. of 6 *N* acetic acid are added and the solution diluted to 200 cc. and heated to near boiling. 30 cc. of 0.2 molar solution of potassium bioxalate¹ are cautiously added drop by drop to the boiling

¹ Y. Osaka and K. Ando, Mem. Coll. Sci., Kyoto, 4, 371 (1921).

solution with a constant stirring, preferably from a burette or a pipette. 5 cc. of saturated ammonium acetate solution (nearly 6 *N* strong) are then added a few drops at a time with a constant stirring. The mixture is heated 5–10 minutes longer and then neutralized by slowly adding 12–14 cc. of 6 *N* ammonia and then allowed to stand for an hour on a warm plate: the precipitate becomes coarsely crystalline and settles to the bottom of the beaker. The clear liquid is then poured through a filter by decantation. The precipitate is covered with exactly 1 cc. of 2 molar KHC_2O_4 solution and about 10 cc. of boiling water with 1 cc. of 6 *N* ammonia. It is filtered by decantation again and the operation is repeated three times. Finally the whole precipitate is transferred to the filter and washed with hot water. (The total volume of the 2 molar KHC_2O_4 solution used in washing the precipitate is put down in the note-book and all the filtrates and washings are combined and reserved for the determination of phosphoric acid.)

The precipitate is now kept in an air bath until nearly dry, when it is placed together with the filter in a platinum crucible and ignited. The ignition of calcium oxalate is carried out in the ordinary manner, such as described in Treadwell-Hall, *Analytical Chemistry*, Vol. II. (1915), p. 70.

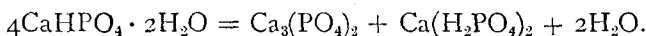
TITRATION OF THE EXCESS OF POTASSIUM BIOXALATE. The filtrates and washings from calcium oxalate are combined and treated with 1 cc. of a nearly 1 *N* manganous sulphate solution, containing 11.2 per cent. of $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$. It is warmed to 70° C. and, after addition of 5 cc. of 6 *N* H_2SO_4 , titrated with potassium permanganate solution. There will be needed in the present case about 30–32 cc. of 0.1 *N* solution to attain the end reaction.

PRECIPITATION OF AMMONIUM MANGANESE PHOSPHATE. The titrated liquid is concentrated on the water bath to about 200 cc. and then treated with 5 gr. of solid ammonium chloride. It is brought to boiling and there are added to it drop by drop about 5–6 cc. of 6 *N* ammonia with a constant stirring till the liquid smells of it very distinctly. This operation is carried out very slowly because the ammonium manganese phosphate that comes down assumes the crystalline form very much more easily while the liquid is acid with the liberated acetic acid than after it has become alkaline with ammonia. The mixture is heated 5–10 minutes longer and then allowed to stand

for an hour on the warm iron plate with a watch glass over the beaker to prevent the contact of the air as much as possible. The crystalline precipitate is filtered and washed with water containing 5 per cent. of ammonium chloride and 0.2 per cent. of ammonia. It is dried and ignited and weighed in the usual manner.

EXPERIMENTAL RESULTS. The calcium phosphoricum praecipitatum of the Japanese Pharmacopœia was used as the sample for analysis. Heated in the air bath at 160° C. for 5 hours, it lost 13.44 per cent. of water, while theoretically it should have lost 20.91 per cent. of water of crystallisation. Ignited at the red heat, it lost 24.76 per cent. in average, while the theory requires 26.14 per cent. for the total water of $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$. The deficit of water was thus 1.38 per cent.

In addition to the fact that the content of water in samples of calcium phosphate is rather irregular, it is also well known that secondary calcium phosphate left in contact with water gives up more or less of its PO_4 ions and becomes richer in calcium in the following sense :



It will be, therefore, very reasonable to assume that the known deficit of water in the sample is due to the above change and that it is compensated by the corresponding amount of tertiary calcium phosphate. Making a calculation based upon this assumption, it is found that the deficit of 1.38 per cent. of water in the sample means that 5.28 per cent. of $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ in it are replaced by the same percentage of $\text{Ca}_3(\text{PO}_4)_2$. This, furthermore, ultimately amounts to saying that the sample contains, in place of 1.38 per cent. of water in deficit, an excess of CaO to the extent of 1.14 per cent. over the theoretical requirement for pure sample of $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$.

There were made altogether thirty seven complete analyses of calcium phosphate for CaO and P_2O_5 with the method here proposed, and most of the results were closely concordant. Many of them were made by Mr. Tamaichi Kawamoto and various other students. The results of four analyses recently made by the author are given here as example :

Substance taken each time :	0.5000 gr.					
					average	theory
Percentage of CaO,	33.53,	33.55,	33.48,	33.53,	33.52,	32.59
Percentage of P_2O_5 ,	41.74,	41.77,	41.84,	41.67,	41.76,	41.27
Percentage of H_2O ,	24.75,	24.95,	24.88,	24.70,	<u>24.82,</u>	<u>26.14</u>
					100.10	100.00

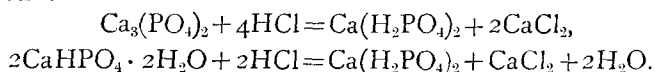
The quantity of CaO found is a little in excess of the theoretical value calculated for pure $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$, i.e. to an extent of 0.93 per cent. It is worthy of notice that this latter figure is a close approach to the value—1.14—calculated from the deficit of water upon the above hypothesis that the loss of water is compensated by an increase of CaO to form $\text{Ca}_3(\text{PO}_4)_2$.

REMARK. 1. All the filtrates and washings from the calcium oxalate precipitate may be combined and evaporated to near dryness. In this case, the oxalic acid is expelled by heating the residue with concentrated sulphuric acid and the phosphoric acid determined by precipitation as ammonium magnesium phosphate.

2. In order to be assured that the separation of phosphoric acid is complete, the weighed CaO may be dissolved in HNO_3 and tested with the molybdate reagent in a centrifuge tube. After repeated centrifugation and decantation, the precipitate is dried and weighed and the weight of P_2O_5 calculated out. This weight is subtracted from that of CaO and added to that of P_2O_5 . The residual P_2O_5 thus reclaimed was found in five experiments to amount to 0.03, 0.08, 0.04, 0.09, and 0.08 per cent. respectively.

II. ACIDIMETRY OF CALCIUM PHOSPHATE.

The primary phosphates are neutral toward methyl orange, while the secondary and tertiary phosphates of alkali metals react alkaline toward it. If a weighed sample of a phosphate is dissolved in a measured excess of 0.1 *N* HCl and titrated back with 0.1 *N* NaOH with methyl orange as indicator, the proportion of the secondary and tertiary salts will be easily calculated out on the basis of the following equations:



0.5000 gr. of $\text{Ca}_3(\text{PO}_4)_2$ contains 1.6115 mg. mols of it and will require $4 \times 16.115 = 64.46$ cc. of 0.1 *N* acid to form the primary salt, while 0.5000 gr. of $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ contains 2.9045 mg. mols and will require 29.045 cc. of the 0.1 *N* acid. If, therefore, 0.5 gr. of the commercial calcium phosphate was taken for analysis and if one per cent. of it

consisted of the tertiary salt and the remaining 99 per cent. consisted of the secondary salt, then there would be required $29.045 + (0.6446 - 0.2905) = 29.40$ cc. of 0.1 *N* acid to bring it to solution as the primary salt.

If, therefore, a 0.5000 gr. portion of a sample is thus analysed and *V* cc. of 0.1 *N* acid found to be consumed to form the primary salt, then the percentage of the tertiary salt in the sample will be indicated by the formula :

$$(V - 29.05) \div 0.354.^1$$

In the following experiments, 0.5000 gr. portions of a fresh sample of calcium praecipitatum were taken for analysis according to the principle just indicated. The numbers of cc. of 0.1 *N* acid consumed were :

29.44, 29.80, 29.49, 29.60, 29.80 ; average 29.63 cc.

Applying here the formula given above, the amount of tertiary calcium phosphate in the sample were found to be $(29.63 - 29.05) \div 0.354 = 1.7$ per cent. As the difference of percentages of CaO in the tertiary and secondary calcium phosphates is $54.21 - 32.57 = 21.64$, the above result ultimately amounts to saying that the actual excess of CaO in the analysed sample over that of pure secondary calcium phosphate is $1.7 \times 21.64 / 100 \equiv 0.37$ per cent. According to the hypothesis put forward in the first part of this paper, this quantity of CaO has entered the sample as the result of replacing a part of the secondary salt by an equal percentage of the tertiary salt.

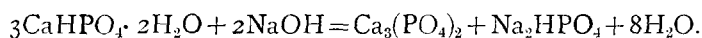
III. ALKALIMETRY OF CALCIUM PHOSPHATE.

As I have found by several experiments that secondary calcium phosphate has power to neutralize caustic soda toward phenolphthalein, an investigation was made regarding the possibility of applying this reaction to the determination of the secondary salt in the commercial sample. It may be noted at the outset that there is a marked difference in this respect between an alkali phosphate and an alkaline earth phosphate and that pure disodium phosphate reacts alkaline toward

¹ Here it must be borne in mind that in titrating phosphoric acid with methyl orange an excess of about 0.3 cc. of the tenth-normal alkali is necessary to cause the change from red to yellow. Treadwell-Hall, Analytical Chemistry, Vol. II. (1915), 542.

phenolphthalein while secondary calcium phosphate reacts acid toward it.

When secondary calcium phosphate is left in contact with caustic soda solution for 14 hours, or boiled with it for a few minutes, the latter disappears chiefly according to the following equation :



In the following experiments, 0.5000 gr. portions of the same sample that was used in the acidimetry above described were mixed with a measured quantity of 0.1 *N* NaOH, and, after standing for 24 hours with occasional shaking or after boiling for a few minutes, the mixture was filtered and well washed. The filtrate and washings were combined and titrated back with 0.1 *N* HCl with phenolphthalein as indicator. The difference between the two quantities is the quantity of 0.1 *N* NaOH absorbed. The results of four such determinations were :

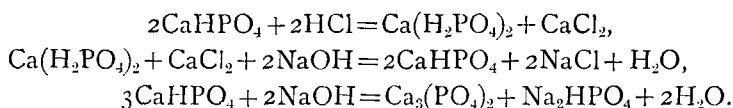
22.75, 22.80, 22.10, 21.00; average 22.16 cc.

If the reaction had run as required by the above equation so that two molecules of NaOH were consumed by every three molecules of secondary calcium phosphate, then the quantity of NaOH consumed should have been only about 19.5 cc. Thus the above results are by far too discordant to justify any use of this reaction for a quantitative purpose.

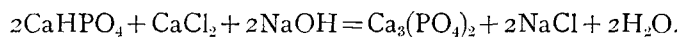
Experiments were then started to find out whether a more definite result could be obtained by first dissolving the sample in a measured excess of the deci-normal acid and neutralizing it with deci-normal NaOH toward methyl orange—i.e. bringing it to the stage of the primary salt—and then further titrating it back with phenolphthalein as indicator. It was ascertained beyond any doubt by the results that there is no well defined stage for either the secondary or the tertiary salt during the course of neutralization with NaOH. There was, indeed, a point reached sooner or later when a distinct phenolphthalein color showed up, but this color would invariably fade away in a few minutes to reappear by addition of a few more drops of NaOH and to fade away again, and so on indefinitely for several hours. By taking as criterion the first transient appearance of the pink color that remained about five minutes, the quantities of deci-normal NaOH required to attain that point were for 0.5000 gr. portions of the sample :

50.80, 48.88, 42.40, 42.72, 48.68; average 46.70 cc.

If the end-point were to be sharply at the stage indicated by the last of the following equations, then the corresponding figure should have been about 49.2 cc. instead of 46.70 cc.:



There still remained a possibility to be tested, that in the presense of an excess of calcium chloride the titration of the primary phosphate with NaOH toward phenolphthalein might give a definite end-point in the following sense:



Two experiments were made each with 0.5000 gr. of the sample dissolved in a measured excess of deci-normal acid. After bringing the solution to neutrality with deci-normal NaOH toward methyl orange, it was mixed with 20 cc. of 0.6 molar solution of neutral $\text{Ca}(\text{NO}_3)_2$. On titrating it toward phenolphthalein with 0.1 *N* NaOH at a nearly boiling temperatur until the permanent pink color was attained, the number of cc. of the alkali solution required for the above reaction was found to be:

69.70, 70.50, average 70.10 cc.; theoretical 59.00 cc.

The above given series of the alkalimetric titrations clearly shows the impracticability of applying this method to the determination of either secondary or tertiary calcium phosphate in the commercial sample. It also shows clearly the power of tertiary calcium phosphote to absorb, to a more or less extent, calcium oxide as well as caustic soda, probably in the form of solid solution.¹ The fact is well known, on the other hand, that tertiary calcium phosphate left in contact with water loses more PO_4 ions than Ca ions, the resulting solution becoming acid in reaction and the solid phase richer in lime than before.

In view of these results, the present author cannot but help entertaining some grave doubts as to the success of a similar alkalimetric method recently proposed by A. J. Jones² for analyzing calcium phosphate of the British Pharmacopœia.

¹ Moissan, *Traité de Chimie Minérale*, Tom. 3, 564-566; Seidell, *Solubilities*, (1916), p. 95; Cameron and Hurst, *J. Amer. Chem. Soc.*, **26**, 903-911 (1904); Cameron and Bell, *Ibid.*, **27**, 1512-1514 (1905); K. K. Jarvinen, *Zeits. Anal. Chem.*, **43**, 559-562 (1904).

² A. J. Jones, *Phar. J.*, cviii, 62-63 (1922); quoted in *The Analyst*, xlvii, 127 (1922).

SUMMARY.

(1) A method of simultaneous determination of calcium and phosphoric acid in calcium phosphate is described. Calcium is precipitated as oxalate from the acetic acid solution and the phosphoric acid in the filtrate is precipitated, after destroying the excess of oxalic acid in it with potassium permanganate, as ammonium manganese phosphate.

(2) The proportion of the secondary and tertiary salts in a sample can also be determined by ascertaining the number of cc. of the deci-normal acid solution consumed in converting a 0.5000 gr. portion of the sample to the stage of the primary phosphate.

(3) An attempt was made to base a method of determination on the results of titrating the solution of primary calcium phosphate, made neutral toward methyl orange, with a standard solution of caustic soda and with phenolphthalein as indicator. This attempt came out futile on account of the absence of any definite end-point for tertiary phosphate due to the fact that the latter absorbs more or less quantity of calcium oxide as a solid solution.
