

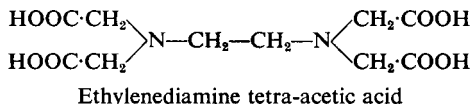
# THE VOLUMETRIC DETERMINATION OF CALCIUM AND MAGNESIUM

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THE classical methods of determining calcium and magnesium are lengthy and not well adapted to the routine determination of these elements in batches of preparations. The British Pharmaceutical Codex does not give limits for magnesium carbonate and calcium carbonate in compound powder of magnesium carbonate probably because of the lengthy nature of these determinations. A rapid volumetric method has been worked out which enables both these elements to be determined in two titrations.

The use of the disodium salt of ethylenediamine tetra-acetic acid as a volumetric reagent for the determination of hardness in water was first described by Schwarzenbach<sup>1</sup> and his co-workers in 1946. The same method was applied to the determination of calcium in pharmaceutical chemicals in America by Matock and Hernandez,<sup>2</sup> who reported that the method was more accurate and more rapid than the official method of the United States Pharmacopeia as well as being applicable to calcium phosphate for which, they stated, the official oxalate method cannot be used. Banks<sup>3</sup> has applied the same method to the determination of calcium and magnesium to coal ash and ceramic materials. Banks made the suggestion that stronger solutions might be worth investigating, and in the present work this suggestion has been adopted. Knight<sup>4</sup> suggested the use of a screened murexide indicator to make the end-point easy to recognise.



The strengths of solutions of ethylenediamine tetra-acetic acid used by previous workers have been low probably because of the small quantities of materials being tested. Banks used 0.02N solutions and direct titration, Matock and Hernandez used 0.03N solutions and back titration with magnesium chloride, which avoids interference from phosphate. The present method employs 0.5N solutions and back titration with calcium or magnesium solutions as applicable. These stronger solutions give sharper end-points and permit the direct titration of weighed amounts of material without the need for preparing dilutions. The method is of general application; the only important interfering substance is iron; this must be eliminated in the ordinary way if present. Other divalent salts, such as zinc or strontium, will titrate in a similar way if present, but the absence of such salts in a pharmaceutical preparation will normally be established. One molecule of the disodium salt of ethylenediamine tetra-acetic acid combines with one atom of calcium or magnesium to form a chelate compound in which the calcium or

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magnesium ions are completely sequestered. Eriochrome Black T is used as an indicator in the presence of ammonia, changing from blue to magenta on the addition of divalent ions. This gives an end-point with both calcium and magnesium. Calcium is estimated in the presence of magnesium by using murexide (ammonium purpurate) as an indicator in the presence of sodium hydroxide. Magnesium does not interfere because magnesium hydroxide is precipitated under these conditions. The colour change is from indigo to pink.

### EXPERIMENTAL

*Reagents.*—0.5N Disodium ethylenediamine tetra-acetate: 146 g. of the acid was dissolved in a slight excess of 5N sodium hydroxide (220 ml.) and diluted to 2 l. This was stored in a rubber stoppered bottle to exclude carbon dioxide which precipitates the acid.

0.5N Calcium chloride: 219.1 g. of hydrated calcium chloride was dissolved in water and made up to 4 l.

0.5N Magnesium sulphate: 246.5 g. of magnesium sulphate was dissolved in water and made up to 4 l.

Calcium indicator: 0.5 g. Naphthol green, 0.2 g. murexide ground together with 100 g. sodium chloride.

Combined calcium and magnesium indicator: 0.2 g. Eriochrome Black T (Solochrome) ground with 100 g. of sodium chloride.

The solutions were standardised against pure calcium carbonate, the equivalent weight being taken as 50.04 by analogy with the acidimetric titration.

*General Method.*—The calcium and magnesium salts are brought into solution with the addition of the minimum amount of hydrochloric acid, in about 150 ml. of water and excess of 0.5N sodium ethylenediamine tetra-acetate added. The solution is then titrated with 0.5N calcium chloride in the following two ways to give the calcium alone and combined calcium and magnesium. Magnesium is obtained by difference.

*Calcium titration.*—To the solution containing excess of reagent, 4 ml. of 5N sodium hydroxide is added (more if the solution is already acid), 0.2 g. of calcium indicator is added and 0.5N calcium chloride run in slowly. The end-point is reached when a tinge of red persists in the indigo blue solution.

*Calcium and magnesium titration.*—To the solution containing excess of reagent 10 ml. of 5N ammonia is added and 0.2 g. of Eriochrome Black T indicator. 0.5N calcium chloride or magnesium sulphate is added until the blue of the indicator turns through violet to magenta. There is some tendency for the magenta colour first produced to fade back to violet owing to the slow sequestration; the titration must be carried to a permanent colour.

*Estimation of calcium and magnesium in compound powder of magnesium carbonate.*

The B.P.C. gives limits for soluble alkali and total alkali, but the proportions of calcium carbonate and magnesium carbonate can vary

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widely without the powder falling outside these limits. In order to test the accuracy of the method, calcium carbonate and magnesium carbonate were assayed separately, then accurately weighed amounts were mixed together and the whole quantity dissolved in the minimum of hydrochloric acid and assayed by the general method. Table I shows the results obtained.

### *Estimation of calcium carbonate in compound powder of magnesium trisilicate.*

It was found that the whole of the calcium carbonate dissolved when compound powder of magnesium trisilicate was boiled with dilute hydrochloric acid but that the amount of magnesium present in the solution corresponded to all the magnesium carbonate and a variable

TABLE I  
DETERMINATION OF CALCIUM AND MAGNESIUM IN A MIXTURE OF CARBONATES

	By B.P. method	By titration
Calcium as CaCO <sub>3</sub> in mixture ..	0.322 g.	0.322 g.
Magnesium as MgO in mixture ..	0.108 g.	0.109 g.
CaCO <sub>3</sub> in calcium carbonate ..	98.2 per cent.	98.5 per cent.
MgO in magnesium carbonate ..	42.5 per cent.	42.1 per cent.

proportion of the magnesium present as magnesium trisilicate. It was decided therefore to adopt a method for the determination of calcium carbonate in the routine examination of batches of this preparation. Titration in the presence of the magnesium trisilicate was found unsatisfactory owing to the removal of calcium ions by the magnesium trisilicate by a base exchange mechanism at the endpoint. Filtration of the solution gave good results, the method adopted being as follows.

About 1.0 g. of compound powder of magnesium trisilicate is digested on a water bath for 20 minutes with 50 ml. of water and 5 ml. of 5N hydrochloric acid and filtered, the filter is washed with a further 50 ml. of hot water. The solution is cooled, 20 ml. of 0.5N disodium ethylenediamine tetra-acetate and 10 ml. of 5N sodium hydroxide are added and the solution titrated with 0.5N calcium chloride using the murexide indicator.

### *Calcium in calcium gluconate.*

Gluconic acid does not interfere with this titration and since magnesium salts are not present the combined titration is suitable and either 0.5N magnesium sulphate or 0.5N calcium chloride can be used for the back titration. While either method of titration may be used for calcium in the absence of magnesium, the less specific titration in the presence of ammonia and Eriochrome Black T was usually employed since either reagent may be employed for the back titration.

About 2.0 g. of calcium gluconate (or 10 ml. of 20 per cent. solution) is dissolved in 125 ml. of water, 25 ml. of 0.5N disodium ethylenediamine tetra-acetate, 10 ml. of 5N ammonia and 0.2 g. of Eriochrome Black T indicator are added and the titration completed with 0.5N calcium

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chloride or magnesium sulphate. For ampoules it is more convenient to use 2 ml. of 10 per cent. solution and titrate using 0.1N solutions.

TABLE II  
CALCIUM GLUCONATE BY TITRATION

	B.P. assay per cent.	Titration per cent.
Calcium gluconate .. ..	101.2	101.1
20 per cent. solution .. ..	21.1	20.9
10 per cent. solution .. ..	9.9	9.9

*Calcium or magnesium in the presence of phosphate.*

Estimation of calcium in the presence of phosphate using murexide indicator was not found to be satisfactory. This was due to the rapid precipitation of calcium phosphate which effectively removed calcium ions from solution and prevented the colour change of the indicator.

In the presence of ammonia and Eriochrome Black T a good end-point was obtained if magnesium sulphate was used for the back titration instead of calcium chloride. The following method has given good results for syrup of calcium lactophosphate.

10 ml. of syrup is measured in a pipette calibrated to contain 10 ml. and transferred by washing into a titration flask. 20 ml. of 0.5N disodium ethylenediamine tetra-acetate, 10 ml. of 5N ammonia, 100 ml. of water and 0.2 g. of Eriochrome Black T indicator\* are added. The solution is titrated with 0.5N magnesium sulphate solution.

It was found that this method did not give a satisfactory end-point when used for calcium phosphate. It then became apparent that the sugar in the syrup of calcium lactophosphate was preventing the rapid precipitation of calcium phosphate and so producing a satisfactory end-point. Addition of syrup to the titration of calcium phosphate gave satisfactory results.

About 0.5 g. of calcium phosphate is dissolved in 100 ml. of water with the addition of 2 ml. of 5N hydrochloric acid, then 20 ml. of syrup, 20 ml. of 0.5N disodium ethylenediamine tetra-acetate, 10 ml. of 5N ammonia and 0.2 g. Eriochrome Black T indicator are added and the excess reagent titrated with 0.5N magnesium sulphate. The same method is used for calcium hypophosphite and calcium glycerophosphate.

*Calcium in exsiccated calcium sulphate.*

About 0.5 g. of finely ground plaster of paris with 25 ml. of 0.5N of disodium ethylenediamine tetra-acetate and 10 ml. of 5N ammonia are shaken at 40° C. for 1 hour. The whole of the calcium sulphate dissolves and the excess of reagent is titrated with 0.5N calcium chloride using 0.2 g of Eriochrome Black T indicator and about 150 ml. of water.

*Influence of pH.*

The general titration of calcium and magnesium using Eriochrome Black T indicator was found satisfactory between pH 9.6 and 10.4 corresponding to a wide variation in the quantity of ammonia added.

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Below  $pH$  9.4 the end-point became less sharp. The titration of calcium alone was satisfactory above  $pH$  11.2, but at this  $pH$  it was not specific for calcium as magnesium ions could still bring about the colour change of the murexide. Above  $pH$  12.0 the titration proceeded normally, the ionisation of magnesium hydroxide being inhibited. The addition of extra sodium hydroxide up to a final concentration of 0.5N did not affect the titration.

### *Effect of temperature.*

Both titrations were carried out between  $10^{\circ}C.$  and  $40^{\circ}C.$  without influence on the results.

### *Accuracy of method.*

The end-point in these titrations could be judged to within 0.05 ml. giving an accuracy on a titration of 20 ml. of  $\pm 0.25$  per cent. Satisfactory replicate titrations could be obtained with this accuracy.

### *Effect of traces of iron.*

The presence of 0.5 mg. of iron did not interfere with the titration using Eriochrome Black T beyond changing the colour of the indicator to indigo just before the end-point. The addition of hydroxylamine hydrochloride improved the colour of the indicator only if the temperature was raised to  $60^{\circ}C.$  1.25 mg. of iron interfered with the titration even in the presence of hydroxylamine at a raised temperature.

The titration using murexide as the indicator is somewhat more sensitive to iron. The end-point was still visible if 0.25 mg. of iron was present or 0.50 mg. in the presence of hydroxylamine. Owing to the instability of murexide this titration cannot be carried out above  $50^{\circ}C.$

TABLE III  
COMPARISON OF RESULTS WITH THOSE OBTAINED BY OTHER METHODS

Compound assayed	Found by ethylenediamine tetra-acetate titration per cent.	Comparative result per cent.	Comparative method
CaCO <sub>3</sub> in compound powder of magnesium trisilicate.	24.8	24.9	Permanganate titration of the precipitated oxalate.
Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub> in calcium hypophosphite ..	101.5	101.7	do. do.
CaC <sub>2</sub> H <sub>2</sub> (OH) <sub>2</sub> PO <sub>4</sub> ·2H <sub>2</sub> O in calcium glycerophosphate.	100.2	100.4	do. do.
Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> in calcium phosphate ..	90.2	90.2	do. do.
CaSO <sub>4</sub> in plaster of paris ..	92.2	92.4	Residue on ignition.
MgSO <sub>4</sub> ·7H <sub>2</sub> O in magnesium sulphate ..	100.1	—	
MgO in magnesium oxide ..	99.2	99.3	By ignition.
Mg(OH) <sub>2</sub> in mixture of magnesium hydroxide.	8.62	8.62	By titration with sulphuric acid.

### *Stability of solutions.*

The solution of disodium ethylenediamine tetra-acetate has been stored for 12 months without change in ordinary glass bottles. The amount of calcium dissolved from the glass is not enough to affect these relatively strong solutions.

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0.5N magnesium sulphate is stable, 0.5N calcium chloride tends to form a slight deposit after a few months.

### SUMMARY

1. A rapid method of determining calcium and magnesium by titration has been described.

2. Factors influencing the accuracy of the method have been investigated.

I wish to thank the directors of Wright Layman and Umney, Limited, for permission to publish these results.

### REFERENCES

1. Schwarzenbach, Biedermann and Bangerter, *Helv. Chim. Acta.*, 1946, **29**, 811.
2. Matocks and Hernandez, *J. Amer. pharm. Ass., Sci. Ed.*, 1950, **39**, 519.
3. Banks, *Analyst*, 1952, **77**, 484-489.
4. Knight, *Chem. Ind.*, 1951, **51**, 1141.

### DISCUSSION

The paper was presented by THE AUTHOR.

DR. R. RUYSEN (Belgium) asked whether the method could be applied to the determination of calcium and magnesium in blood.

DR. G. E. FOSTER (Dartford) mentioned a paper published recently in *Die Pharmazie*, in which were listed a number of compounds which were determined by the use of ethylenediamine tetraacetic acid. He also said that this substance was added in the United States to certain pharmaceutical preparations where traces of calcium might cause opalescence.

MR. R. L. STEPHENS, in reply, said there would be difficulties in using the method for the determination of calcium and magnesium in blood; the colour changes would be masked and the iron present would interfere. The blood would have to be ashed and its iron content removed by precipitation in alkaline solution before the calcium and magnesium content could be determined.