Simultaneous Determination of Calcium and Magnesium of Serum by a Single Chelometric Titration

Sanford H. Jackson and Florence Brown

Titration of a trichloracetic acid filtrate of serum with EDTA is carried out at pH 9.8 using an excess of Calmagite indicator. Successive photometric readings of percentage transmission, made with an EEL titrator, are plotted against the volume of EDTA added. There is very little change at first while calcium is being complexed; the reading then decreases rapidly while magnesium is being titrated, followed by a flattening of the curve at completion of the titration. Hence, two sharp "breaks" are obtained in the titration curve, the first corresponding to the amount of calcium and the second to the amount of magnesium.

It is evident from the variety of procedures that has been introduced for the determination of magnesium in serum that difficulties are encountered in this estimation. These difficulties have also hindered the inclusion of the determination in the repertoire of the average clinical chemistry laboratory.

The phosphate precipitation procedures, e.g., that of Simonsen et al. (1), are slow, taking at least 6 hr., but appear to give accurate results.

Titan yellow (2, 3) procedures are difficult to control, both because the color is colloidal and, therefore, dependent on particle size, and because the spectral absorption of the blank may constitute 75% or more of the total absorption.

Flame photometric methods, particularly that of Alcock et al. (4) may be highly accurate, but require the use of a monochromator capable of separating the 285.2 m\(\mu\) line of magnesium from the 285.3 m\(\mu\) line.
of sodium. Such a flame photometer is not generally available in clinical chemistry laboratories. The flame photometer of Wacker and Vallee (5), using the 371 m\(\mu\) oxide line, appears to give high results since they report a mean normal value of 2.05 mEq./L., compared to the mean value of 1.67 mEq./L. found by a variety of other investigators (4).

The 8-hydroxyquinoline fluorometric method of Schacter (6) is convenient, but also appears to give a high mean normal value (2.0 mEq./L.), although a recent automated version (7) is reported to give more acceptable normal values.

Chelometric procedures, by which calcium and calcium plus magnesium are titrated separately and the difference determined, are subject to the large errors inherent in methods dependent on determining small differences. Procedures designed to limit this error by combining the separate titrations into one operation have been published. Flaschka and Ganchoff (8) titrated calcium at pH 10 in ammonia-ammonium chloride buffer using 2:2′ bis (carboxymethyl) amino diethyl ether (BAETA) with murexide indicator, after which the magnesium in the same solution was titrated with ethylene diamine tetra acetic acid (EDTA) using Eriochrome Black. End-points were determined photometrically. The procedure was not applied to serum. Kovacs and Tarnoky (9), using “plasma-corinth B” or Eriochrome Blue SE, titrated calcium in NaOH to a visual end-point with EDTA. They then reduced the pH by the addition of hydrochloric acid and an ammonia-ammonium chloride buffer and titrated the magnesium to a second visual end-point. Calcium and magnesium values for serum agreed with accepted normals.

This report concerns a procedure whereby both calcium and magnesium are titrated with EDTA at pH 9.8 using Calmagite* indicator. The changes in color of the indicator are followed photometrically. A graph of optical transmission against volume of titrant added exhibits two “breaks”; the first corresponds to the end of the calcium titration alone, while the second completes the titration of both calcium and magnesium.

Underwood (10) listed the following conditions necessary for successful simultaneous titration of two cations: (1) the stability constants of their complexes with EDTA must be sufficiently large; (2) the constants must differ sufficiently; and (3) the spectra of the com-

---

*G. Frederick Smith Company, Columbus, Ohio.
plexes must permit selection of suitable wave lengths. Underwood applied this type of titration to the determination of copper and iron.

Recently, Flaschka and Sawyer (11) reported a procedure for the simultaneous titration of calcium and magnesium in submicrogram amounts which is similar to that to be described here. They suggested its possible application to blood serum.

Method and Materials

All glassware is soaked in 50% nitric acid overnight and rinsed well with distilled water and finally with de-ionized water. De-ionized water is used throughout.

All reagents and standards are stored in polythene bottles.

Reagents

*Calcium stock standard, 50 mEq./L.* Dissolve 1.250 gm. CaCO₃ (Mallinckrodt primary standard) in about 50 ml. water by the careful addition of hydrochloric acid. Dilute to 500 ml.

*Magnesium stock standard, 20 mEq./L.* Weigh out 0.2432 gm. of metallic magnesium ribbon. Cover with water in a beaker and dissolve by the careful addition of hydrochloric acid. Dilute to 1 L.

*Dilute low standards, 2.5 mEq./L. of Ca and 1 mEq./L. of Mg.* Dilute 5 ml. stock Ca standard and 5 ml. stock Mg standard to 100 ml.

*Dilute high standard, 7.5 mEq./L. of Ca and 3 mEq./L. of Mg.* Dilute 15 ml. stock Ca standard and 15 ml. stock Mg standard to 100 ml.

*EDTA solution* Dissolve 5 gm. EDTA (disodium salt) in 1 L. water.

*Trichloracetic acid* Dissolve 10 gm. trichloracetic acid in water and dilute to 200 ml.

*Calmagite* Dissolve 10 mg. in 10 ml. water. Prepare fresh daily.

*Ammonium borate buffer, pH 9.8* Dissolve 7.0 gm. boric acid, 6.0 gm. sodium cyanide, and 0.5 gm. of hydroxylamine HCl in water. Add 24 ml. of concentrated ammonium hydroxide. Dilute to 1000 ml. Adjust the pH to 9.8, using a glass electrode pH meter. Add a further 6.5 ml. of concentrated ammonium hydroxide. This will neutralize the trichloracetic acid and return the pH of the titration mixture to 9.8

Apparatus

1. A micrometer syringe or the equivalent.* One rotation of the micrometer screw delivers 0.01 ml.

*Agla model, Burroughs-Wellcome & Co., Tuckahoe, N. Y.
2. An EEL titrator with filter,* consisting of a photometric unit into which a magnetic stirrer is incorporated, and a separate galvanometer.

**Procedure**

Add 4 ml. of trichloracetic acid to 1 ml. of serum in a small tube. Cap with parafilm and mix by shaking. Let stand for 10 min. or more, and centrifuge.

Place 0.1 ml. of Calmagite in a titration cuvet. Add 0.5 ml. of clear supernatant to the cuvet, followed by 2.5 ml. of borate buffer. Introduce a magnetic stirring bar and place the cuvet in position on the titrator. Set the sensitivity so that the galvanometer reads 100% transmission. Add EDTA from the syringe, taking the galvanometer reading after each addition of EDTA. The end-point is reached when two or three consecutive readings are the same.

The readings of %T are plotted against the titration "units," each unit being 10 divisions, or .002 ml. The best straight line is drawn through the points on the sloping part of the curve and extrapolated to intercept the 100%T line on one end and the best horizontal line through the last titration points on the other end (Fig. 1). The intercept on the 100%T line is the calcium titration while the intercept on the final titration line is the total titration of calcium plus magnesium. The titration from the calcium intercept to the final intercept is the magnesium value. These titration values are then converted to mEq./L. by reference to standard curves (Fig. 2).

To prepare the standard curves, dilute 1 ml. of each standard solution with 4 ml. trichloracetic acid. Titrate 0.5 ml. of this solution similarly to the serum titration. Plot the standard curves by joining the points with a straight line. The intercept on the abscissa is equivalent to the blank.

**Experimental and Discussion**

The use of the intercept of the magnesium part of the titration curve with the 100%T line (instead of with the slightly sloping initial part

---

Fig. 1. Titration curves of various concentrations of calcium and magnesium.

Fig. 2. Standard curves for calcium and magnesium.
of the curve) as the end-point of the calcium titration (Fig. 1) requires some explanation. There are factors other than the calcium concentration that influence this initial slope. Since the calcium and indicator have a low affinity the initial titration mixture will consist of an equilibrium mixture of calcium uncombined with indicator, free indicator, and calcium-indicator complex, as well as the firmly bonded magnesium indicator complex. The amount of free indicator, and hence the amount of calcium-indicator complex is related to the amount of magnesium. The more magnesium present, the less free indicator, and hence the less calcium-indicator complex there will be. Hence the initial slope is influenced by the magnesium as well as the calcium. This is illustrated by Curves B and D in Fig. 1. The initial drop (b) of Curve B is greater than the drop (a) of Curve D although there was three times the calcium in Titration D.

The concentration of EDTA, and hence the volume of titrant required, also affects the slope. More dilute solutions, requiring larger volumes, tend to flatten the curve by dilution of the indicator. Therefore it is advisable to use small volumes of concentrated EDTA solution.

In practice it has been observed that the use of the intercept with the initial sloping curve leads to high calcium and low magnesium results, and that the concentration of calcium will affect the magnesium determination and vice versa. However, if the intercept with the 100% T line is used, and the titrant volume is kept low, so that dilution can be neglected, the correct titration values for both calcium and magnesium are obtained.

An examination of the spectral absorbance curves (Fig. 3) of the free Calmagite indicator and its calcium and magnesium complexes reveals that the maximum spectral shift is in the 600–640 m\(\mu\) region. Accordingly EEL filter No. 607, which has the transmission characteristics shown in the same figure, was selected. The transmission in the region of 700 m\(\mu\) by this filter is of little consequence since the photo cell in the EEL titrator has little sensitivity at this wave length.

There is a marked effect of pH on the shape of the titration curve (Fig. 4). The optimum pH, giving the maximum spectral shift and sharpest breaks is 9.8–9.9. Departures from this pH range result in a loss of sensitivity. These curves were obtained using an ammonium borate-ammonia buffer at 0.1M concentration. Other buffers investigated were sodium borate, ammonia-ammonium chloride, monoethanolamine, and triethanolamine. Except with respect to stability there
was little to choose between them when they were used at the same pH and molar concentration. The ammonia-ammonium borate buffer was stable for at least 6 weeks. In all cases cyanide and hydroxylamine were added to complex copper and iron, the presence of which, in the

**Fig. 3.** Spectral absorbance curves of free indicator and in presence of excess calcium or magnesium. Also shown is spectral transmission curve of EEL filter 607.

**Fig. 4.** Effect of pH on titration curve.

free form in minute traces, would result in a loss of definition of the breaks.

The buffer concentration has an effect on the shape of the curve, as shown in Fig. 5. The more dilute buffers lead to a greater spectral shift. The apparent increase of the calcium and magnesium titration is an artifact resulting from the increased blank values with increased reagent concentration.

The effect of indicator concentration on the titration curve is critical
(Fig. 6). The affinity of Calmagite for calcium is very weak, so that the indicator is largely in the free form unless calcium is present in considerable excess. The affinity of the indicator for magnesium is strong, so that it is the changes in the magnesium-indicator complex that are reflected in the titration curve. If indicator added is insufficient to

![Figure 5](image1.png)

**Fig. 5.** Effect of borate concentration in buffer on titration curve. Borate solution adjusted to pH 9.8 with concentrated ammonia. (Ca, 5 mEq./L.; Mg, 3 mEq./L.)

![Figure 6](image2.png)

**Fig. 6.** Effect of indicator concentration on titration curve. (Ca, 5 mEq.; Mg, 3 mEq.)

complex all the magnesium, then some of the magnesium as well as all the calcium will be titrated before there is a spectral change, leading to an erroneously high calcium and low magnesium result. Hence the first consideration is to add sufficient indicator to combine with all the magnesium. On the other hand, excess indicator will be present largely in the free form so that the magnesium-indicator color will be diluted by the color of the indicator itself, leading to a lower proportional spectral shift. Ideally one should regulate the indicator to the actual
magnesium present. Some excess is necessary to obtain a satisfactory calcium break. We have chosen an amount of indicator sufficient to cope with a concentration of 3 mEq./L. in the serum. If a sample should be encountered containing more than this it would be necessary to repeat the titration on a smaller aliquot.

The effect of age of the Calmagite indicator on the titration curve is depicted in Fig. 7. There is some tendency for the calcium break to shift to the left, giving lower calcium recoveries and proportionally higher magnesium results.

Difficulties were encountered in the titration of calcium and magnesium in sera. Magnesium values were low and the slope of the curve was considerably flattened. Calcium results were satisfactory. This magnesium effect was not true of all sera, as horse serum gave a titration curve and magnesium recovery fully as satisfactory as the standards. It was suspected that the trouble arose from an effect of protein on the indicator. Figure 8 shows the effect of adding human albumin (Connaught laboratories 25% human albumin solution) to a standard titration. The flattening effect of the albumin was evident in the lowest concentration used. It was apparent that removal of the protein by precipitation with trichloracetic acid was essential. Sufficient extra ammonia was added to the buffer to neutralize the extra acid and return the pH of the titration mixture to 9.8. Standard solutions were also diluted in trichloracetic acid to maintain the same conditions of titration for both standard and tests.

A series of titrations were made in which the calcium and magnesium concentrations of the solutions were varied independently. The results are shown in Fig. 9. There was no indication that variations in
the calcium concentration affect the magnesium result or that vari-
ations in magnesium concentration affect the calcium titration.

Determinations on a series of 20 normal adults gave calcium values
ranging from 4.6 to 5.4 mEq./L., with an average of 4.98 mEq./L.
Magnesium values ranged from 1.40 to 1.88 mEq./L., with a mean of

1.64 mEq./L. The magnesium values agree closely with those of Al-
cock et al. (4), who reported a mean normal value of 1.66 mEq./L., with
a range of 1.45 to 1.85 mEq./L.

Careful replicate determinations showed a standard error for the
calcium titration of ± 0.08 mEq./L., and a standard error for the
magnesium determination of ± 0.12 mEq./L., when done with dupli-
cate titrations of the trichloracetic acid centrifugate.
References