A DIRECT ACIDIMETRIC MICROTITRATION METHOD
FOR CALCIUM

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The theoretical advantages of the acidimetric1 methods for
the microtitration of calcium have been pointed out by several
authors (1–5). In actual practise, however, oxidimetric methods
have gained more favor owing to their relative simplicity. Where-
as in the acidimetric methods the titrimetric equivalent is ob-
tained indirectly, in the oxidimetric methods this equivalent is
obtained directly. Difficulties are encountered that are common
to both the acidimetric and oxidimetric methods, owing to the
instability of the standard titrating solutions. This is caused by
the changing titer of the standard alkali2 as well as the standard
oxidimetric solutions at 0.01 N strength. In contrast, the titer
of standard acids of this strength is extremely stable. An acidi-
metric method which would eliminate the use of standard
alkali by employing a direct titration would therefore eliminate
the undesirable features of the present methods and yet retain

1 The term acidimetric is employed in this paper as a convention, when
a base is titrated with a standard acid. The term oxidimetric is used when a
reductant is titrated with a standard oxidizing agent. In both cases the
titration may be direct or indirect, depending upon the particular method.
In the direct titration an equivalent amount of standard solution is used.
In the indirect titration an excess of standard solution is employed and this
excess is titrated back with a standard alkali in the acid-base systems and
with a standard reductant in the oxidation-reduction systems.

2 With special precautions against absorption of carbon dioxide from the
air and by carefully lining the container with pure paraffin 0.01 N standard
alkali is fairly stable. In actual practise, however, it is not always possible
to observe these precautions. Thus, in the every-day experience of many
microanalysts, who are often pressed for time, a change in the titer of 0.01
N alkali is often encountered.
the theoretical advantages of the acidimetric and the simplicity of the oxidimetric procedures.

In order to obtain the desirable features of the present titrimetric methods, and eliminate the use of unstable standard solutions, the possibility of employing boric acid for the microestimation of calcium was investigated. Boric acid is used as the acid to trap ammonia in the microestimation of ammonia (6, 7). It is also used to trap the bases in the microestimation of total base (8). The trapped ammonia or base is titrated to the pH of a pure boric acid solution with standard acid. This titration represents the titrimetric equivalent of the ammonia or base. Thus, a direct titration is used instead of the customary indirect titration and the use of standard alkali is completely eliminated. After examination of various experimental conditions it was established that a hot solution of 10 per cent boric acid will dissolve calcium carbonate in a few seconds and that the titrimetric equivalent of the dissolved calcium may be obtained by adding a standard acid until the pH of the boric acid solution is reached. The quantitative precipitation of calcium as the oxalate and its conversion to the carbonate were carried out essentially the same way as previously described by one of the authors and his coworkers (9).

The principle of the method as finally developed is presented below.

$$\text{Ca}^{2+} + \text{C}_4\text{O}_6^\text{v} \xrightarrow{\text{pH 5.5-6.0}} \text{CaC}_2\text{O}_4$$

$$\text{CaC}_2\text{O}_4 \xrightarrow{\text{heat} \ 475-525^\circ} \text{CaCO}_3$$

$$\text{CaCO}_3 + \text{H}_2\text{BO}_3 \xrightarrow{\text{hot 10\% solution}} \text{Ca}_3(\text{BO}_2)_2$$

$$\text{Ca}_3(\text{BO}_2)_2 + 6 \text{HCl} \rightarrow 3\text{CaCl}_2 + 2\text{H}_3\text{BO}_3$$

Calcium ion is precipitated as the oxalate, which is then converted to the carbonate at a temperature of 475-525°. The carbonate is dissolved in a hot solution of 10 per cent boric acid. The solution is diluted and titrated directly with a 0.01 N standard acid to the pH of a pure boric acid solution of similar strength. This titration represents the amount of calcium in equivalents.

This method has a combination of desirable features, all of which are not present in any of the hitherto available micro-methods for calcium. The procedure is simple and accurate. A
direct titration is employed with a sharp end-point. Oxalate ion is employed in the washing fluid and thus loss of the calcium oxalate precipitate through solution is minimized. Only one washing is necessary, as adsorbed oxalate ions are volatilized during the conversion of the oxalate to the carbonate.

Method

Reagents—

0.01 N acid. 100 cc. of a carefully prepared solution of 0.1 N hydrochloric or sulfuric acid are diluted with double distilled water to 1000 cc. This solution is kept in an automatic burette calibrated to 0.01 cc. The 0.01 N acid is stable for years if carefully handled.

Indicators. 0.04 per cent bromocresol purple prepared according to Clark (10) and a mixture of methyl red and methylene blue prepared according to Patterson (11) as follows: 100 cc. of 0.02 per cent methyl red and 30 cc. of 0.1 per cent methylene blue. This mixture is diluted to 500 cc. The proportion of methyl red and methylene blue may be changed to suit the operator.

Ammonium oxalate reagent. A saturated solution of C.P. ammonium oxalate is prepared at 40-50° and allowed to cool to room temperature. The clear supernatant solution is used.

Boric acid solution. 10 gm. of boric acid are dissolved in 100 cc. of distilled water, by heating. This solution is supersaturated at room temperature and consequently it is necessary to dissolve any boric acid that precipitates on standing by heating just before use. This solution is used while hot.

0.5 per cent ammonium oxalate. 0.5 gm. of C.P. ammonium oxalate is made up to 100 cc. with distilled water.

Procedure

To 2.0 cc. of solution (containing about 0.1 to 0.4 mg. of calcium ions) in a 10 or 15 cc. Pyrex tube, 1 cc. of ammonium oxalate and 1 drop of bromocresol purple are added. This is washed down by 2 cc. of water and mixed. The pH is adjusted to the gray color which is between the yellow and purple of the indicator. The contents of the tube are allowed to stand for 1 hour or longer, after which it is centrifuged at about 2000 revolutions per minute for 10 minutes. The supernatant liquid is decanted or carefully
aspirated with a drawn out tube and rubber bulb without disturbing the precipitate. The precipitate is suspended in 3 cc. of 0.5 per cent ammonium oxalate and centrifuged for 5 to 10 minutes. The supernatant liquid is then carefully aspirated or decanted. The washed precipitate is dried at 100–110° and heated in a muffle furnace or sand bath at 475–525° for 20 to 30 minutes. After this the tube is placed in a boiling water bath and 0.5 cc. of the hot boric acid solution is added. The precipitate dissolves in about 1 to 2 minutes completely. The solution is diluted to 3 cc. with distilled water and 1 to 2 drops of Patterson's indicator added. This solution is then titrated with 0.01 N acid to the pH of a pure solution of boric acid of such concentration. (The titration back to the original pH of boric acid is most easily carried out by adding indicator to a control tube in which 0.5 cc. of boric acid is diluted to approximately 4.0 cc.) This represents the titrimetric equivalent of calcium.

Calculation—

\[ 1.0 \text{ cc} \times 0.01 \text{ N acid used} = 0.2 \text{ mg. Ca} \]

\[
\text{Mg. per 100 cc.} = \frac{\text{cc. acid used} \times \text{equivalent weight} \times 100}{\text{cc. sample}}
\]

Procedure for Blood Serum—The method is directly applicable to fresh serum except that at least 4 hours should be allowed for precipitation of the calcium oxalate.

Discussion

The optimum temperature for the conversion of calcium oxalate to the carbonate is between 475–525° (12). In order to heat the calcium oxalate to this temperature range, in a short time, it is necessary to keep the temperature of the muffle furnace higher. The actual temperature of the muffle furnace in most of these experiments was between 560–600°. With such a temperature the conversion of the oxalate to the carbonate is completed in 10 to

\[ \text{The muffle furnace is essentially an air bath where temperature exchange is relatively slow. This takes place in part by radiation from the sides of the furnace and partially by direct heat transfer from the heated air. Both of these processes are much slower than direct contact with a liquid or finely powdered solid.} \]
15 minutes. With a muffle furnace temperature of 490–530° this conversion takes at least 3 hours. One may substitute ordinary glass tube for Pyrex with careful temperature control provided the softening temperature of the glass is not reached. Aside from the undesirable effect of softened glass the calcium oxalate precipitate may be heated to above 525° and thus form some calcium oxide. This, however, does not influence the results, as the acidimetric equivalent of calcium oxide is identical with calcium carbonate. An insufficient heating period or a temperature much below 475° will prevent complete conversion of the calcium oxalate to the carbonate and thus give rise to results that are too low. In cases of incomplete conversion the precipitate will not dissolve completely in the hot boric acid.

The temperature as well as the concentration of boric acid influences its degree of dissociation. It is advisable, therefore, when a precision of a high order is required to standardize both of these factors carefully. In the present study, however, no special effort was made to standardize these conditions, as the precision of the method is satisfactory for our present purposes under the conditions described. As a rule 5 to 10 minutes elapsed between the dilution of the dissolved calcium carbonate and its titration. Thus, at the end of the titration the titrated solution is practically at room temperature. The difference between the volume of the pure boric acid solution (used for color matching) and the titrated solution was usually between ±0.5 to ±1.0 cc.

It is important to use an approximately 10 per cent solution of boric acid, as in more dilute solutions difficulties will be encountered in dissolving the calcium carbonate. Once the carbonate has dissolved, however, the solution may be diluted and better conditions for the titration thus obtained. In measuring out the boric acid it is advisable to preheat the pipette which is used for measuring and thus prevent precipitation of boric acid in the pipette.

Results

Representative results of known solutions are presented in Table I. It may be observed that the maximum error of estimation is within 1 drop (0.04 cc. ≈ 0.008 mg. of Ca) of the theoretically expected titration. The average error of 71 determinations
Acidimetric Microtitration of Ca

was $-0.0006$ mg. The average deviation of 71 determinations is $\pm 0.0030$ mg. Comparative results on blood serum by the Kramer-Tisdall (13) oxidimetric and the boric acid procedure are given in Table II. It is seen that excellent agreement is obtained by the two methods. The average difference between nineteen comparative estimations was $+0.25$ per cent of the Kramer-

**Table I**

**Determination of Calcium in Inorganic Solutions**

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<thead>
<tr>
<th>Ca present</th>
<th>Ca found</th>
<th>Ca present</th>
<th>Ca found</th>
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<tbody>
<tr>
<td>mg.</td>
<td>mg.</td>
<td>mg.</td>
<td>mg.</td>
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<tr>
<td>0.100</td>
<td>0.098</td>
<td>0.300</td>
<td>0.301</td>
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<tr>
<td>0.100</td>
<td>0.100</td>
<td>0.300</td>
<td>0.306</td>
</tr>
<tr>
<td>0.100</td>
<td>0.104</td>
<td>0.300</td>
<td>0.300</td>
</tr>
<tr>
<td>0.200</td>
<td>0.202</td>
<td>0.400</td>
<td>0.396</td>
</tr>
<tr>
<td>0.200</td>
<td>0.200</td>
<td>0.400</td>
<td>0.402</td>
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<tr>
<td>0.200</td>
<td>0.204</td>
<td>0.400</td>
<td>0.408</td>
</tr>
<tr>
<td>0.200</td>
<td>0.196</td>
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</tbody>
</table>

**Table II**

**Determination of Calcium in Serum**

The values are expressed in mg. per 100 cc.

<table>
<thead>
<tr>
<th>Permanganate method</th>
<th>Boric acid method</th>
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<tbody>
<tr>
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<td>9.3</td>
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<td>10.3</td>
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Tisdall values, while the average agreement between nineteen pairs (one titrated oxidimetrically, the other acidimetrically) was $\pm 1.57$ per cent of the Kramer-Tisdall values.

**SUMMARY**

1. A method is described for the acidimetric microestimation of calcium by a direct titration.
2. This is accomplished by precipitating calcium as the oxalate, which is then converted to the carbonate at 475–525°. The carbonate is dissolved in a hot solution of 10 per cent boric acid. The solution is diluted and titrated directly with a 0.01 n standard acid to the pH of a pure boric acid solution of similar strength. The titration represents the amount of calcium in equivalents.

3. This procedure retains the theoretical advantages of the acidimetric methods, employs only standard acid (which is stable) in a direct titration, and eliminates the use of standard alkali whose titer is difficult to keep at 0.01 n strength.

4. This method is directly applicable to blood serum.

BIBLIOGRAPHY

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