The determination of calcium in natural waters by atomic absorption spectrophotometry (AAS) is highly pH dependent. The response of the AAS for a constant calcium concentration in natural water increased with decreasing pH in the range of pH 7.0 to 3.8. It was constant in the pH range of 3.8 to 1.8 and decreased with decreasing pH below 1.8. Correct analyses were obtained when samples were adjusted to pH 1.8 to 3.8. The pH effect on calcium response above pH 3.8 is due to bicarbonate. Calcium carbonate precipitation occurs in the burner and causes the low response. In addition to pH adjustment, the addition of 50% of the EDTA required to titrate the hardness of the water or the addition of 1% lanthanum will eliminate the pH dependence of calcium response.

Since water samples are routinely analyzed for calcium, a quick, accurate, and precise analytical procedure is desirable. Analytical procedures currently employed for the determination of calcium are EDTA titration, precipitation, and weighing of calcium oxalate, redissolving precipitated calcium oxalate and titrating with permanganate, flame photometry, and atomic absorption spectrophotometry. The atomic absorption spectrophotometric method of analysis for calcium compares favorably with other methods (West and Herrin, 1965). Some of the problems encountered with other methods of analysis, such as difficulty in determining colorimetric end points, the large sample volumes required, and interferences from other elements, are not encountered in the atomic absorption spectrophotometric procedure (Butler and Drink, 1963). The literature on the use of atomic absorption spectrophotometry for water analysis has been reviewed (Bentley, 1967).

An atomic absorption spectrophotometer is currently utilized for calcium analysis of lake, stream, and river water samples in this laboratory. The procedure is quick, accurate, and precise; however, some problems have been encountered. The apparent calcium concentration obtained by this procedure is pH dependent—i.e., as the pH is varied, the apparent calcium concentration varies. This paper presents the results of a study to determine why the apparent calcium concentration obtained by this procedure is pH dependent.

Experimenta

Calcium analyses were performed using a Perkin-Elmer atomic absorption spectrophotometer, Model 303. Measurements were made at a wavelength of 4227 A, and a slit setting of 4. The visible range was used and the source current was set at 14 ma. A scale setting of 1 and an air flow rate of 5 flowmeter units was employed. Before the start of each series of analyses, the gas (acetylene) flow rate was adjusted to give maximum absorbance while aspirating a standard solution. This value was usually 9.5 flowmeter units. The aspiration rate was checked by using a stopwatch and graduated cylinder. Plugging of the aspirator was not excessive and, when it occurred, was rectified by aspirating 1 to 2 drops of hydrochloric acid for 1 minute.

A Beckman pH meter, Model GS, was used to make pH measurements on each sample before determination of calcium. Each sample was kept in a covered volumetric flask between the time of preparation and the time of analysis.

Sodium, potassium, and magnesium were determined with the atomic absorption spectrophotometer utilizing the manufacturer's recommended instrument settings (Perkin-Elmer Corp., 1966).

Reagent grade chemicals were used to prepare all solutions utilized in the experimental work.

Unless otherwise specified, sample refers to Lake Mendota water samples which were obtained from the Water Chemistry Laboratory lake water tap. This system provides untreated lake water from approximately 600 feet offshore from a depth of about 10 feet below the surface. Samples from other lakes were obtained on field trips and stored in polyethylene bottles at 5°C. All samples were filtered with a Whatman No. 1 paper, and glass-distilled water was used to prepare dilutions, as well as to zero the atomic absorption spectrophotometer.

Synthetic lake water was prepared by weighing and diluting with glass-distilled water, chemicals in sufficient quantities to give values of elements comparable to those found in Lake Mendota (Lee, 1966). This solution was used in the deter-
Table I. Apparent Calcium Concentration at Normal and Low pH

<table>
<thead>
<tr>
<th>Sample Source</th>
<th>Calcium Mg./Liter</th>
<th>pH adjusted to 2.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black Earth Creek</td>
<td>49.0</td>
<td>52.0</td>
</tr>
<tr>
<td>Comstock Lake</td>
<td>19.1</td>
<td>23.5</td>
</tr>
<tr>
<td>Big Twin Lake</td>
<td>21.5</td>
<td>23.0</td>
</tr>
<tr>
<td>Fox Lake</td>
<td>31.3</td>
<td>38.8</td>
</tr>
<tr>
<td>Lake Katrine</td>
<td>6.0</td>
<td>5.3</td>
</tr>
</tbody>
</table>

mination of the effect of organic material on the pH-calcium relationship.

Organic material was obtained by evaporation concentration of Lake Mendota water and placement of the concentrate on a Sephadex G-75 column. Separation of the sample, by molecular weight, was achieved and the high molecular weight, low conductance fraction was used in this study (Gjessing and Lee, 1967). The total hardness and alkalinity analyses were performed by Standard Methods (APHA 1965) procedures.

Results

A linear relationship was found for standard calcium solutions in the 1 to 10 mg. per liter range. All samples were diluted to obtain calcium concentrations in this range. The standard deviation of this analysis was ±0.5 mg. per liter, at the 95% confidence level.

Figure 1 is typical of the results obtained when samples are acidified and diluted 5 to 1, pH is measured, and calcium analyses are performed on a sample of Lake Mendota water. The apparent calcium concentration is constant in the pH range 1.8 to 3.8. Above pH 3.8, it decreases with increasing pH and, below pH 1.8, decreases with decreasing pH. These results show the pH range, in which the apparent calcium concentration is constant, to be approximately 2.0 pH units. A similar variation in apparent calcium concentration with pH was observed for other lake, stream, and river samples. Table I gives the results of determining apparent calcium at the original pH of the samples and at pH 2.4. The error that may be involved in the determination of calcium with atomic absorption, without pH adjustment, can be seen. For example, a Fox Lake sample had 38.8 mg. per liter of calcium; however, only 31.3 mg. per liter was found by atomic absorption analysis without acidification.

Experiments were run to determine if the apparent concentrations of sodium, potassium, and magnesium, as determined by atomic absorption, were pH dependent. Figure 2 shows that the apparent concentration of these elements is not affected by changes in pH above 1.8, but decreasing pH below this value caused a decrease in apparent concentration. These results indicated that the cause of variation in apparent calcium above pH 3.8 was not due to some instrumental parameter, as it would have been found in the determination of magnesium, sodium, and potassium. Below pH 1.8, the variation could be the result of a change in an instrumental parameter since all four elements showed a similar pH dependence.

In an effort to determine the cause of the pH dependence on calcium analyses above pH 3.8, studies were made on the
effect of organic matter and alkalinity on calcium response in atomic absorption analyses.

Series of experiments were run to determine the effect of organic material on the pH-calcium relationship. In the first series, dextrose was used as the source of organic material in preparing synthetic lake water. Material separated on a Sephadex column, after evaporation concentration, was used as the source of organic material in the next series of analyses. Figure 3 is typical of the results obtained for synthetic water devoid of bicarbonate alkalinity and containing dextrose or organic material. This material had no effect on the apparent calcium concentration from pH 1.8 to 6.5.

A sample of Lake Mendota water was used to determine the effect of the type of alkalinity on the calcium-pH relationship. The alkalinity of Lake Mendota is 140 to 149 mg. per liter as CaCO₃. As can be seen, the original Lake Mendota water, in which essentially all of the alkalinity is bicarbonate, showed a similar pH-calcium relationship (Figure 1). A sample of this water was treated with concentrated hydrochloric acid to remove all carbonic species. Concentrated ammonium hydroxide was added to restore the alkalinity and pH to their original values. Calcium measurements were made at various pH's on this water (hydroxide alkalinity curve, Figure 4). The apparent calcium is independent of pH in this sample. The sample was then treated with concentrated hydrochloric acid to remove the hydroxide alkalinity. Sodium bicarbonate was added to this water to increase the alkalinity to its original value, and calcium analyses, as a function of pH, were made on the solution (bicarbonate alkalinity curve, Figure 4). The addition of bicarbonate to this sample resulted in a similar calcium-pH relationship, as was found in the original untreated water. The amount of calcium present in the sample was different after each treatment because of the dilution of the sample by the addition of various reagents. These results indicate that the bicarbonate system is, in some manner, responsible for the low results obtained when the pH is above 3.8. Calculations based on solubility data do not indicate the possibility of calcium carbonate precipitation at pH 4.0 and that a sample containing 21.0 mg. per liter calcium must have its pH raised above 8.3 before carbonate precipitation will occur. These calculations, and the experienced lowering of apparent calcium content at pH 4.0, make it appear that the pH of the sample is being raised to a higher value between the time of aspiration and the time it reaches the flame.

To determine if the pH of the sample was being raised in the aspiration system, a 20% excess of the volume of EDTA required to titrate total hardness was added to the samples. Figure 5 shows apparent calcium to be constant in the pH range 1.8 to 7.9, and that essentially all of the calcium in a sample, with a measured injection pH of 4.0, is evidently complexed with EDTA when aspirated into the flame. As little as 50%, and as much as 200%, of the amount of EDTA needed to titrate total hardness of samples, allows the determination of the same apparent calcium concentration. Table II compares the results of the analysis of water from four different lakes on which 50% of the EDTA required to titrate their hardness was used or the pH of the sample was
Table II. Comparison of Apparent Calcium Concentrations in Lake Waters

<table>
<thead>
<tr>
<th>Sample Source</th>
<th>Calcium, Mg./Liter With 50% EDTA</th>
<th>Calcium, Mg./Liter Without EDTA</th>
<th>pH 2.4</th>
<th>1% Lanthanum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fox Lake</td>
<td>39.5</td>
<td>33.5</td>
<td>40.0</td>
<td></td>
</tr>
<tr>
<td>Cox Hollow Lake</td>
<td>26.0</td>
<td>22.0</td>
<td>26.5</td>
<td></td>
</tr>
<tr>
<td>Parker Lake</td>
<td>19.0</td>
<td>16.0</td>
<td>19.5</td>
<td></td>
</tr>
<tr>
<td>Lake Mendota</td>
<td>26.0</td>
<td>23.5</td>
<td>26.0</td>
<td></td>
</tr>
</tbody>
</table>

adjusted to 2.4. Both pretreatments yielded essentially the same result.

The decreased apparent concentration of calcium, magnesium, sodium, and potassium, at pH less than 1.8, must be due to properties of the instrument or aspiration system, since approximately equal effects were noted with each element. An increase in viscosity might have caused the observed results. However, measured changes in viscosity, as a function of pH on these samples, could not account for the marked apparent concentration-pH relationships observed based on the results of Winefordner and Latz (1961) and Zaugg and Knox (1966). Therefore, at this time, the effect of pH on apparent concentrations of pH below 1.8 cannot be explained.

Lanthanum chloride is routinely used in the determination of calcium by atomic absorption spectrophotometry (Perkin-Elmer Corp., 1966) and will eliminate interferences due to phosphate, sulfate, and bicarbonate (Fishman, 1966). Results obtained by the addition of 1% lanthanum and by pH adjustment are shown in Table III. The results agree within the experimental error of this procedure (±0.5 mg. per liter) on all of the samples, except Lake Mendota. To determine if sulfate or phosphate was causing the difference observed on Lake Mendota water, each compound was added separately to calcium standard solutions and their absorbances were determined. Phosphate, when added in excess of that found in Lake Mendota, caused no change in absorbance by the calcium solution, but sulfate at 20 mg. per liter caused a decrease in absorbance of approximately 4%. Sulfate could be responsible for the difference observed in Lake Mendota water because a 4% change would cause a difference of approximately 1.0 mg. per liter in a sample containing 20.0 mg. per liter calcium. Further study is needed to elucidate this difference.

Discussion

The apparent calcium concentration of water samples, containing bicarbonate alkalinity, is pH dependent. It is constant in the pH range 1.8 to 3.8 and samples must have their pH adjusted to this range before analysis. This is a more stringent pH requirement for calcium analysis than reported by Fishman (1966), who states that pH must be less than 7.0 to obtain good results.

Results indicate precipitation of calcium carbonate may be responsible for the low values obtained when a sample, with a measured pH greater than 4, is aspirated. The pH of the sample would have to be raised within the sampling system to a value greater than 8.3. The experiments with EDTA tend to support that this is happening, because at pH 7, approximately one-half the calcium in a sample is complexed with EDTA (Welcher, 1958). Results show that an appreciable amount of calcium is complexed when a sample, with a measured pH of 4.0, is aspirated into the flame. The increased temperature in the burner head and reduced pressure in the aspiration system could cause carbon dioxide to pass out of solution, increasing the pH. Ramakrishna, Robinson, et al. (1966) found that CO$_3^{2-}$ at 100 p.p.m. caused an interference in Ca$^{2+}$ determination. They did not report any pH effect on analysis. They found that the addition of EDTA and SrCl$_2$ tended to remove this interference.

The decrease in apparent concentration obtained below pH 1.8 is due, in part, to increasing viscosity with decreasing pH. A decrease in viscosity causes less sample to be aspirated per unit time, thereby causing a decrease in observed apparent concentration.

**Table III. Calcium Values Obtained at pH 2.4 and with 1% Lanthanum**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Calcium, Mg./Liter pH 2.4</th>
<th>Calcium, Mg./Liter 1% Lanthanum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Little Cedar Lake</td>
<td>30.0</td>
<td>30.0</td>
</tr>
<tr>
<td>Cox Hollow Lake</td>
<td>23.5</td>
<td>24.0</td>
</tr>
<tr>
<td>Lake Redstone</td>
<td>26.0</td>
<td>25.5</td>
</tr>
<tr>
<td>Lake Mendota</td>
<td>27.0</td>
<td>28.0</td>
</tr>
</tbody>
</table>

**Literature Cited**


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