Automated, Simultaneous Microdetermination of Calcium and Magnesium by Atomic Absorption

Nathan Gochman and Harry Givelber

A procedure is described for the simultaneous, automatic determination of serum calcium and magnesium with the AutoAnalyzer, and a dual-channel, double-beam atomic absorption spectrophotometer, the IL Model 153. Advantages over existing procedures include a high rate of analysis (90 samples or 180 tests per hour) and low sample consumption (a total of 30 μl for both determinations). We have compared the new procedure with a manual one in which the Perkin-Elmer Model 303 atomic absorption spectrophotometer is used.

Availability of an atomic absorption spectrophotometer (AAS) with two channels (one monochromator and one filter position), the Instrumentation Laboratory Model 153, provided the starting point for investigation of a simultaneous, automated procedure for serum calcium and magnesium.

The work of Klein et al. (1–3) indicated the feasibility of the AutoAnalyzer with an atomic absorption detection system for determining serum cations. These authors investigated the use of a Techtron Model AA-3 single-beam, single-channel AAS combined with a double-dialysis manifold, which provided a protein-free diluted sample. Both serum calcium and magnesium could be determined with their system using two sample runs at a net rate of analysis of 20 to 30 samples per hour. Sample consumption would be approximately 1.2 ml, which limits the procedure's usefulness for pediatric specimens, on which electrolyte determinations are frequently required.

Zettner and Seligson (4), Pruden et al. (5), Trudeau and Frier (6), and Johnson and Riechmann (7), have demonstrated that accurate determinations can be made directly by atomic absorption in the presence of serum proteins. Rodger son and Moran (8) reported a manual atomic absorption micromethod for calcium that requires 20 μl of serum and is based upon sample dilution and measurement without deproteinization. Their procedure was more reliable than a microfluorometric method. If deproteinization were unnecessary, an automated procedure of considerably higher sensitivity (lower sample consumption) would result, if recordings could also be made noise-free and the effects of serious burner clogging by protein could be eliminated.

This report describes the details of a combined AutoAnalyzer and IL 153 system that satisfies the requirements indicated above and correlates well with a manual atomic absorption procedure. Sample consumption at 90 samples per hour (sample to wash ratio, 1:1) is 30 μl, which fits the scope of automated microchemical determinations suitable for pediatric specimens as described by Mabry et al. (9).

Materials and Methods

Reagents and Standards

Lanthanum–butanol diluent (0.5 g of lanthanum per 100 ml of n-butanol, 40 ml/liter). Lanthanum oxide (La₂O₃), 5.85 g, (Alfa Inorganics, Inc., Beverly, Mass.), is dissolved in 25 ml of concd hydrochloric acid, then diluted with about 200 ml of de-ionized water; 40 ml of n-butanol is added and the solution is diluted to one liter with de-ionized water.

Stock calcium standard (100 mEq/liter). Dried calcium carbonate (CaCO₃), 5.0045 g, is dissolved in 4 ml of concd hydrochloric acid and diluted to one liter with de-ionized water.

Stock magnesium standard (50 mEq/liter). Mag-
nesium metal, 0.6080 g, is dissolved in 2 ml of coned hydrochloric acid and diluted to one liter with de-ionized water.

Stock saline (17 g/100 ml). Seventeen grams of sodium chloride is diluted to 100 ml with de-ionized water.

**Combined working standards**

<table>
<thead>
<tr>
<th>Ca, mEq/l</th>
<th>Mg, mEq/l</th>
<th>Ca stock, ml</th>
<th>Mg stock, ml</th>
<th>NaCl stock, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>0.5</td>
<td>2.0</td>
<td>1.0</td>
<td>5.0</td>
</tr>
<tr>
<td>4.0</td>
<td>1.0</td>
<td>4.0</td>
<td>2.0</td>
<td>5.0</td>
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<tr>
<td>5.0</td>
<td>1.5</td>
<td>5.0</td>
<td>3.0</td>
<td>5.0</td>
</tr>
<tr>
<td>6.0</td>
<td>2.0</td>
<td>6.0</td>
<td>4.0</td>
<td>5.0</td>
</tr>
<tr>
<td>7.0</td>
<td>2.5</td>
<td>7.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>8.0</td>
<td>3.0</td>
<td>8.0</td>
<td>6.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Aliquots of the stock solutions, as indicated, are combined and diluted to 100 ml with de-ionized water.

**Instrumentation**

*Atomic absorption spectrophotometer.* The Instrumentation Laboratory (Watertown, Mass.) Model 153 AAS used for this investigation was equipped with a magnesium hollow-cathode lamp in the turret position (Monochromator optical path), and a calcium hollow-cathode lamp in the fixed position (422.7 nm interference filter optical path). The burner used was a Boling-type with a three-slot head and 10-cm light path. This AAS has a linear recorder output, and when operated in the 2.5-scale position reads directly in absorbance units.

*Recorders.* Two SR recorders (E. H. Sargent Co., Chicago, Ill.) with variable range selector switches (up to 125 mV) were used. Chart speed was set at medium, 1-in./min. The IL 153 servo potentiometers required grounding for zero adjustment of these recorders.

*Automatic sample feed and dilution.* Technicon AutoAnalyzer modules were assembled as shown in the flow diagram (Figure 1). The Sampler II is operated with the 90-per-h cam, and a sample-to-wash ratio of 1:1. A check of the actual aspiration time of each cam lobe should be made as discussed by Young et al. (10).

**Operating Procedure**

Preliminary adjustment of the IL-153 AAS requires the following fixed settings:

- Test Mode—A, B; Channel A milliamp setting—4; Channel B—5; Scale—2.5; Curve correct—0; Calibration—full counterclockwise.

After "Power" and "Hollow Cathode" switches are turned on, the Channel A monochromator is peaked for maximum energy by rotation of the wavelength dial (nominal magnesium absorption peak at 285.2 nm), and energy-balance meters are adjusted. Monochromator slit setting is 160 μ.

The flame is ignited and the AutoAnalyzer manifold connected to the atomizer. Fuel (acetylene) pressure is adjusted to 4 lb/in.² and oxidant (compressed air) pressure to 12 lb/in². The AAS zero controls are adjusted so that the absorbance reads zero on the digital displays. The recorder zero controls are then adjusted to 5 divisions on the 100-division linear chart paper.

With the instrument sampling the highest combined working standard, the recorders are each set to about 90 lines while the steady state is plotted.

Samples are placed in microcups of 0.5-ml
capacity. As little as 100 µl of serum (first marked line) may be used if the sample pickup probe is carefully positioned.

Standards and samples are analyzed, and standard curves are prepared on linear graph paper for calculation of results for serum.

**Results**

Figure 2 shows a recording of the calcium-monitoring channel, including steady state of 8 mEq/liter, aqueous standards (including lowest following highest to note interaction level), and a number of random serum specimens. The concurrent magnesium recording is shown in Figure 3.

Occasionally, a serum magnesium value is extremely supranormal if a patient has been treated with magnesium salts, as illustrated by the off-scale specimen shown in this recording. Such samples are diluted with an equal volume of saline and reanalyzed.

The calibration curve for calcium is linear to about 7 mEq/liter, beyond which it deviates slightly downward (Figure 4). Magnesium deviates from linearity by about 10% at 3 mEq/liter (Figure 5).

The within-run precision of the automated procedure was tested by analyzing a serum pool 30 times consecutively in a single run; the results are listed in Table 1.

Frozen aliquots of the serum pool were analyzed on 20 consecutive days during routine operation of the method. The results, which give a measure of the day-to-day reproducibility of the procedure, are shown in Table 2.

| Table 1. Within-Run Precision of Automated Calcium and Magnesium Determination on a Serum Pool Sampled 30 Times |
|---------------------------------|----------|----------|
| Mean, mEq/liter | Calcium | Magnesium |
| SD       | 0.052   | 0.022    |
| CV, %    | 1.1     | 1.4      |
| Range   | 4.40–4.70 | 1.48–1.59 |
Table 2. Day-to-Day Precision of Calcium and Magnesium Determination on Aliquots of Frozen Serum Pool (20 Daily Analyses)

<table>
<thead>
<tr>
<th></th>
<th>Calcium</th>
<th>Magnesium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean, mEq/liter</td>
<td>4.53</td>
<td>1.57</td>
</tr>
<tr>
<td>SD</td>
<td>0.083</td>
<td>0.042</td>
</tr>
<tr>
<td>CV, %</td>
<td>1.8</td>
<td>2.7</td>
</tr>
<tr>
<td>Range</td>
<td>4.40-4.75</td>
<td>1.52-1.67</td>
</tr>
</tbody>
</table>

The automated procedure described here was compared with the routine laboratory procedure (4) that uses a Perkin-Elmer Model 303 AAS and a semiautomatic diluter for 50-fold dilution of serum. Diluting reagent for the Perkin-Elmer method is a mixture of 0.5 g of lanthanum, 6 ml of butanol, and 94 ml of water. Results of the correlation are shown in Table 3. The calcium results agree very well, while a small but significant difference in means for magnesium is evident. This difference is related to standardization of the two procedures and will be discussed later.

Table 4 summarizes analytical data obtained from the analysis of two types of commercial lyophilized control serum by the two procedures.

Discussion

The first consideration in setting up the dual-channel Instrumentation Laboratory Model 153 Atomic Absorption Spectrophotometer was the selection of either calcium or magnesium analysis with the grating monochromator. The numerous spectral lines in the 285.2 nm vicinity that could cause interference with the determination of magnesium demanded that magnesium be used with the higher resolution grating. A high-quality interference filter, of 222.7 nm nominal peak wavelength with a 6 nm band-pass, was used for the calcium analytical channel.

Factors influencing the determination of calcium on this instrument were reported by Bowers et al. [CLIN. CHEM. 14, 846 (1968), abstract]. These authors used strontium as an internal standard and measured the calcium-strontium ratio. Because of the many unsuspected abnormalities of magnesium metabolism observed in our patients, we thought it more desirable to use the second channel to provide a simultaneous magnesium determination. The results of the precision studies indicated that adequate stability could be obtained without resorting to an internal standard technique. The major instability factor that might be introduced is variation in hollow-cathode lamp intensity, but this is satisfactorily compensated by the double-beam operation (flame path vs. reference air path) of each analytical channel.

To satisfy the requirements for a high rate of analysis and low sample consumption, a nondialysis AutoAnalyzer manifold was investigated. The use of an aqueous lanthanum diluent with the manifold shown in Figure 1 resulted in rapid-forming deposits in the slot of the burner during aspiration of serum samples. This caused a ragged flame and a drop in sensitivity within 30 min.

The presence of butanol in the lanthanum diluent greatly reduced the rate of deposit accumulation, but also caused a slight decrease in washout characteristics of the manifold vs. the aqueous reagent. Butanol, 4 ml/100 ml, was chosen for

Table 3. Comparison of Results for Serum Calcium and Magnesium Obtained by Automated System with IL-153/AA and by Manual Atomic Absorption with PE-303

<table>
<thead>
<tr>
<th></th>
<th>Calcium, mEq/liter</th>
<th>Magnesium, mEq/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IL-153/AutoAnalyzer</td>
<td>PE-303</td>
</tr>
<tr>
<td>No. samples</td>
<td>160</td>
<td>160</td>
</tr>
<tr>
<td>Mean, mEq/liter</td>
<td>4.69</td>
<td>4.71</td>
</tr>
<tr>
<td>SD</td>
<td>0.51</td>
<td>0.49</td>
</tr>
<tr>
<td>Range</td>
<td>2.85-6.85</td>
<td>2.85-6.60</td>
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<tr>
<td></td>
<td>124</td>
<td>124</td>
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<tr>
<td></td>
<td>1.64</td>
<td>1.55</td>
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<tr>
<td></td>
<td>0.26</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>0.65-2.64</td>
<td>0.61-2.57</td>
</tr>
</tbody>
</table>

Table 4. Comparative Analysis for Ca and Mg in Commercial Lyophilized Control Sera by Automated Procedure (IL-153/AA) and Manual Procedure (PE-303)*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Calcium, mEq/liter</th>
<th>Magnesium, mEq/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IL-153/AutoAnalyzer</td>
<td>PE-303</td>
</tr>
<tr>
<td>Calibrate* 1</td>
<td>3.75</td>
<td>3.68</td>
</tr>
<tr>
<td>Calibrate 2</td>
<td>5.25</td>
<td>5.08</td>
</tr>
<tr>
<td>Calibrate 3</td>
<td>6.75</td>
<td>6.68</td>
</tr>
<tr>
<td>Monitrol*</td>
<td>4.84</td>
<td>4.77</td>
</tr>
<tr>
<td>Calibrate* 1</td>
<td>1.32</td>
<td>1.31</td>
</tr>
<tr>
<td>Calibrate 2</td>
<td>1.73</td>
<td>1.65</td>
</tr>
<tr>
<td>Calibrate 3</td>
<td>2.14</td>
<td>2.06</td>
</tr>
<tr>
<td>Monitrol*</td>
<td>2.5</td>
<td>2.32</td>
</tr>
</tbody>
</table>

* Values are av. of duplicate or triplicate determinations.
* "Calibrate" is a product of Warner-Chilcott Laboratories, Morris Plains, N. J. Lot No. 677108.
* "Monitrol" is a product of Dade Reagents, Miami, Florida. Lot No. 95-D.
the procedure since this concentration permitted 96–97% steady state to be attained for both aqueous standards and serum at 90 samples per hour and a sample-to-wash ratio of 1.

Over 200 serum specimens have been analyzed in a single run with this reagent without significant deterioration of the flame characteristics. The burner head is cleaned every day by immersing it in 10% nitric acid for 15 min or overnight.

Butanol also enhances magnesium absorbance and, at the 4% concentration used, produced a 30% increase in sensitivity compared to the reagent without butanol. There was no measurable effect on calcium absorbance; therefore, the effect of butanol on magnesium is apparently a direct enhancement rather than merely an increase in atomization efficiency, which would have affected both.

The AutoAnalyzer manifold was set up to provide a differential input of diluted sample to the AAS atomizer. The AAS is equipped with a variable aspiration-rate control capable of drawing between 0 and 10 ml/min of a solution open to the atmosphere. This was adjusted to draw from 5 to 6 ml/min, for optimum recordings when the AAS was connected to the AutoAnalyzer manifold delivering approximately 4 ml/min.

Absorbance values were about 0.700 for 3 mEq of magnesium per liter and about 0.150 for 8 mEq of calcium per liter with the manifold as described. These values were obtained with an actual manifold dilution factor of 42 compared to a nominal dilution of 50. This was calculated by comparing readings obtained by direct aspiration into the AAS of (a) an accurate 1-to-50 dilution of the standard and (b) the diluted effluent collected from the AutoAnalyzer during sampling of the combined standard.

When the correlation study reported in Table 3 was completed, the aqueous standards used for the respective procedures were compared with particular reference to the magnesium contents. Lithium was included in the aqueous standards for the Perkin-Elmer procedure because of occasional determinations of lithium in serum. However, lithium produced no interfering effects on the determination of either calcium or magnesium. New standards using magnesium metal, and high-purity magnesium sulfate were prepared. These agreed well with the automated system standards, but were 5% lower in concentration than the standards used for the data obtained on the Perkin-Elmer apparatus. This accounts for the lower magnesium values obtained on the Perkin-Elmer apparatus for the commercial control sera and individual serum samples when compared with the IL/AutoAnalyzer system.

After several weeks of routine operation of the automated procedure, we observed that occasional
lyophilized serum controls were producing results for calcium 5 to 10% lower than usual. When this problem became more consistent, we investigated and found the effect to be related to a change in transmission characteristics of the interference filter.

Samples were analyzed with the calcium filter currently used and a newly received filter, using standards prepared as described and another series of standards in which the 0.85% sodium chloride was omitted. The old calcium filter gave absorbance readings for the sodium-containing standards that were considerably higher than those prepared without sodium. As can be seen from Table 5, results for control serum were low when calculated against the sodium-free standards. Apparently, the sodium background effect is modified in serum, since the sodium level is approximately the same as in the standards but compensation is not achieved.

With the new filter, the two sets of standards were indistinguishable, as was the case with the old filter initially, and when we used the Channel A monochromator. Calculated data for the control sera agreed well with label values. We suspect that delamination of the initial filter caused the abnormal sensitivity to sodium, but we are not sure why this occurred.1

1 Subsequent examination of the old filter revealed missing epoxy sealer between the glass and holder, which could have admitted stray light. The new filter has been used for nearly a year without any difficulty.

References


Table 5. Comparison of Sodium Background Effects with Two Calcium Interference Filters (Calcium, mEq/liter)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Old calcium filter</th>
<th>New filtera</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Calc. v. regular standards</td>
<td>Calc. v. standards without NaCl</td>
</tr>
<tr>
<td></td>
<td>Found % label Found % label</td>
<td>Found % label</td>
</tr>
<tr>
<td>HSPb</td>
<td>6.35</td>
<td>5.65</td>
</tr>
<tr>
<td>LSPb</td>
<td>3.00</td>
<td>2.30</td>
</tr>
<tr>
<td>Calibrate 1</td>
<td>3.75</td>
<td>3.40</td>
</tr>
<tr>
<td>Calibrate 2</td>
<td>5.25</td>
<td>4.90</td>
</tr>
<tr>
<td>Calibrate 3</td>
<td>6.75</td>
<td>6.50</td>
</tr>
<tr>
<td>Monitrol</td>
<td>4.84</td>
<td>4.45</td>
</tr>
</tbody>
</table>

* Standard curves with and without NaCl indistinguishable, therefore only one set of calculated data.

HSP and LSP are aqueous calcium solutions prepared without NaCl and used as controls in the Clinical Chemistry Laboratory. Other samples identified in legend to Fig. 4.