Micellar Improvement of the Calmagite Compleximetric Measurement of Magnesium In Plasma

Maxwell H. Abernethy and Richard T. Fowler

Magnesium in plasma is determined by diluting a 50-μL sample with 5.0 mL of stable calmagite reagent containing the amphoteric detergent Empigen BB and buffered with 2-amino-2-methyl-1-propanol at pH 11.5. The calcium response is masked with strontium-buffered [ethylenebis(oxyethylenenitrilo)]tetraacetate (EGTA), while iron is masked with triethanolamine. The detergent causes an increased separation of the test and blank spectral absorbance bands. The test absorbance peaks, which may be measured immediately, are at 520 nm, while the blank peak shifts from 610 to 655 nm on addition of detergent. The blank absorbance at 520 nm is relatively decreased by the detergent. This allows the use of a more concentrated calmagite reagent, which in turn extends the linear relation of absorbance to magnesium concentration to 5 mmol/L in plasma. This method (y) has been compared with the atomic absorption procedure (x) of McDonald and Watson (Clin. Chim. Acta 14: 233, 1966) and gives favorable regression statistics (y = 1.001x + 0.057 mmol/L).

Calmagite [3-hydroxy-4-[(6-hydroxy-m-tolyl)azo]-1-naphthalenesulfonic acid] was proposed by Lindstrom and Diehl (1) in 1960 as an indicator in the titration of magnesium with EDTA. Ingman and Ringbom (2) took advantage of its ready solubility in water and high chelate molar absorptivity to use it directly as a compleximetric spectrophotometric reagent. It was used with biological materials by Chauhan and Sarkar (3), and in human plasma for a practicable determination of magnesium by several workers (4, 5).

The calmagite reagent has a blank absorbance peak at 610 nm, somewhat overlapping the magnesium-chelate peak at 520 nm. Use of the amphoteric betaine detergent Empigen BB shifts the blank peak to 655 nm, significantly decreasing the blank absorbance at 520 nm. Spectral changes result from association of light-absorbing molecules with the hydrophobic interior or with the highly charged surface of detergent micelles. Analysts may take advantage of favorable changes to design micellar-improved analytical methods, a field likely to find further applications in clinical chemistry (see 6 for a review).

In this paper we describe a manual plasma magnesium assay, and in the accompanying paper (7) extend its application to the Technicon SMAC analyzer.

Materials and Methods

Apparatus

All spectrophotometric measurements were made with a SP8-100 recording spectrophotometer (Pye-Unicam, Cambridge CB1 2PX, U.K.) set at a bandpass of 2 nm, and 10-mm glass cuvettes.

Reagents

The following reagent system is recommended for routine use. We used distilled, de-ionized water and polythene reagent bottles. The stock solutions are stable at room temperature for at least three months.

Stock reagent A: Dissolve 1.2 g of calmagite (55% pure, Sigma Chemical Co., St Louis, MO 63178) in 2 L of water. Store in the dark.

Stock reagent B: Dissolve 19 g of 2-amino-2-methyl-1-propanol (AMP; Fluka AG, Buchs, Switzerland) in 1500 mL of water. Add and dissolve 3.04 g of [ethylenebis(oxyethylenenitrilo)]tetraacetate (EGTA; Sigma). Add 67 mL of 300 g/L Empigen BB (Marchon Products, Whitehaven, U.K.), and 1 mL of triethanolamine (SLR grade; Fisons, Loughborough, U.K.). Adjust the pH to 12.5 ± 0.2 with potassium hydroxide, and dilute to 2 L.

Stock reagent C: Dissolve 1.28 g of strontium chloride hexahydrate, “AnalaR” grade (BDH Chemicals Ltd., Poole, U.K.) in 100 mL of water.

Magnesium reagent: To 800 mL of water add 100 mL of stock reagent A and mix completely; add 100 mL of stock reagent B and mix; add 10 mL of stock reagent C and mix. The pH should be 11.5 ± 0.2 (adjust with potassium hydroxide or hydrochloric acid if necessary) and its absorbance at 520 nm should be 0.50 ± 0.07. Magnesium contamination may be monitored by adding two to three drops of 0.1 mol/L ethylenediaminetetraacetic acid (EDTA) to 5 mL of magnesium reagent and noting the decrease in absorbance at 520 nm. If the absorbance change is equivalent to plasma magnesium of 0.5 mmol/L, or greater, as judged by comparison with the calibration standards, prepare fresh reagent, using magnesium-free reagents and containers.

Magnesium standards. Dissolve 44.61 g of magnesium iodate tetrahydrate (Sigma) in 1 L of water to prepare a stock solution of 0.1 mol of Mg per liter. Dilute with water to give solutions containing 1, 2, 3, 4, and 5 mmol of Mg per liter.

Method

Place 50 μL of plasma in a magnesium-free polyethylene test tube containing 5.0 mL of magnesium reagent. Stopper with a polythene cap or Parafilm, and mix by inversion. The colored chelate, produced immediately, is stable for at least 30 min. Measure the absorbance at 520 nm vs a blank of 5.0 mL reagent and 50 μL of water. Measure standards similarly with each batch of tests. Hemolyzed and lipemic plasma samples should be avoided.

Accuracy and precision studies. We assessed accuracy by duplicate measurements of 29 selected plasma samples ranging in concentration from 0.2 to 3.6 mmol/L. The atomic absorption method of McDonald and Watson (8) was used as the reference technique. To assess between-batch precision, we made repeated analyses over three weeks of two patients' plasma pools at 0.6 and 1.2 mmol/L and a commercial lyophilized control material (Dade Interlab QAP Level 1) at 1.9 mmol/L. Within-batch precision was assessed from the du-
Fig. 1. Spectral absorbance curves of calmagite reagent, showing the effect of added Empigen BB detergent on calmagite and its magnesium chelate

(1) Blank reagent, calmagite reagent without detergent; (2) blank reagent, calmagite reagent with detergent; (3) calmagite reagent with excess magnesium, without detergent; (4) calmagite reagent with excess magnesium, with detergent

plicate calmagite method results obtained in the accuracy study.

Results

The spectral absorbance curves (Figure 1) were obtained by using the reagent system as described but with excess EDTA (curve 2) or magnesium (curve 4), and by use of a reagent without Empigen BB, again with excess EDTA (curve 1) or magnesium (curve 3). The shift of the blank peak from 610 (curve 1) to 655 nm (curve 2) associated with micellar incorporation is noteworthy, as is the blank absorbance decrease at 520 nm. The chelate peak is not greatly altered. The Empigen BB was above its critical micellar concentration. (Below this concentration the detergent exists as individual molecules, not as micelles.) This was checked by measuring the shift in the blank peak absorbance with detergent concentrations increasing from zero. Above the micellar concentration, no further shift is noted.

The chelate absorbance was maximum at pH 12, in agreement with Chauhan and Sarkar (3). However, to use AMP buffering, we changed to pH 11.5, decreasing the chelate absorbance by 3%. At 0.01 mol/L, this buffer maintains the pH within 0.1 pH unit for plasma samples.

The concentration of EGTA is controlled by strontium, as recommended by Gitelman et al. (9), except that there possibly are changes in the pK values for EGTA and its metal chelates in the presence of detergent micelles (6). The interference of calcium was studied over the range 0–20 mmol/L in aqueous samples, both with and without magnesium at 1.0 mmol/L (Table 1). Throughout the range of ordinarily encountered plasma calcium concentrations (1.0–5.0 mmol/L) there was no interference; only when calcium exceeded 10 mmol/L was there a significant increase in analytical recoveries of magnesium.

Without triethanolamine, a slowly increasing turbidity developed, owing to iron interference. With the recommended reagent system, iron does not interfere significantly at physiological concentrations; however, 100 µmol of iron per liter increases the apparent magnesium concentration by 0.1 mmol/L. Similarly, copper and zinc react stoichiometrically, but at the maximum likely concentrations of 40 µmol/L, this interference is of no clinical significance.

Of other substances tested, there was no interference from bilirubin at 180 µmol/L; glucose at 80 mmol/L; phosphate at 14 mmol/L; or sodium, potassium, or chloride at 10-fold the normal concentrations in plasma.

The absorbance was linear with concentration over the range 0–5 mmol/L magnesium, to within 1%

The precision study is summarized in Table 2. Within-run precision (CV) was excellent (~1%). However, this is less important than the between-run precision, which indicates the ability of a laboratory to replicate results from day to day; 3% CV at normal plasma concentrations. The accuracy study, in which 29 patients' samples were analyzed by this rapid colorimetric method (y) and by the atomic absorption method (x), yielded a linear regression equation with a slope of 1.001, a y = intercept of 0.057 mmol/L, and a small scatter of points about the line (S_x = 0.045, x = 1.31, y = 1.37 mmol/L, r = 0.999).

Discussion

Detergents commonly are added to reagents in clinical biochemistry, as solubilizers or as wetting agents. They are usually regarded as inert additives. However, advantage can be taken of the microenvironment of the detergent micelle (6), especially when relatively hydrophobic reagents such as organic dyes are used. The spectral shift noted for calmagite when the betaine detergent Empigen BB is added is evidence that the dye is associated with the micelles (6), although the location remains a matter for speculation. Other combinations of reagents and detergents should also be explored for other clinical chemistry methods to yield similar analytical improvements elsewhere.

Neither the calmagite nor the Empigen BB detergent was purified. The method was assessed with reagents used as supplied, to allow direct applicability to the routine clinical chemistry laboratory. The calmagite reagent is claimed to be only 55% pure, and the Empigen BB contains sodium chloride (manufacturer's data). This latter is advantageous, however, because the added salt causes micelle formation at a lower concentration of ionic detergents (10). The spectral changes are dependent on micelles.

Table 1. Study of Calcium Interference

<table>
<thead>
<tr>
<th>Added calcium, mmol/L</th>
<th>In blank</th>
<th>In 1.0 mmol/L standard</th>
</tr>
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<tbody>
<tr>
<td>2</td>
<td>-0.018</td>
<td>1.006</td>
</tr>
<tr>
<td>5</td>
<td>-0.006</td>
<td>1.012</td>
</tr>
<tr>
<td>10</td>
<td>-0.006</td>
<td>1.006</td>
</tr>
<tr>
<td>20</td>
<td>-0.006</td>
<td>1.042</td>
</tr>
</tbody>
</table>

* Means of triplicate analysis.

Table 2. Precision Study

<table>
<thead>
<tr>
<th></th>
<th>Mg, mmol/L</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td><strong>Between-batch</strong></td>
<td></td>
</tr>
<tr>
<td>Plasma pool 1</td>
<td>0.62</td>
</tr>
<tr>
<td>Plasma pool 2</td>
<td>1.06</td>
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<tr>
<td>Dade Control 1</td>
<td>1.89</td>
</tr>
<tr>
<td><strong>Within-batch</strong></td>
<td></td>
</tr>
<tr>
<td>Group 1 (n = 10)</td>
<td>0.50</td>
</tr>
<tr>
<td>Group 2 (n = 9)</td>
<td>1.16</td>
</tr>
<tr>
<td>Group 3 (n = 10)</td>
<td>2.36</td>
</tr>
</tbody>
</table>

* n = 20 each, over a three-week period.  
* Mean of duplicate analyses of 29 patients' specimens distributed into three groups according to low, medium, and high Mg concentration.
The use of magnesium iodate as a standard is based on the rigorous examination of the properties of the tetrahydrate by Lindstrom and Stephens (11), who proposed its use as a primary standard because of its stability to both high temperatures and humidity.

Instead of measuring the increase in absorbance at 520 nm, one could measure decrease in the blank absorbance at 655 nm, a potential alternative that has not been extensively tested. Also, the test wavelength is not restricted to 520 nm; because the test absorbance peak is reasonably flat to 540 nm, the method may be expected to yield good results when used with a wide-bandpass spectrophotometer.

The method proposed by Gindler and Heth1 is available as a commercial kit. Our preliminary investigation showed that their method gives results that are linear with concentration only to 2 mmol/L. The addition of EDTA significantly decreases the blank absorbance, indicating the likely contamination of the reagents with magnesium. Clearly, linearity would be extended if contamination could be avoided.

We have chosen to avoid the use of cyanide as a general masking agent (12), and have substituted triethanolamine. Possibly cyanide would be necessary if the current method is extended to the measurement of magnesium in urine samples, where concentrations of interfering metals are likely to be higher than in plasma. Another modification for urinalysis would be a 10-fold increase in the concentration of the AMP buffer to prevent pH changes in the reagent system, especially if acidified urines were analyzed.

The method we have reported is proposed for use in emergency and routine laboratories that do not have ready access to an atomic absorption spectrophotometer. The accuracy and precision of the method are well within the requirements of routine laboratory requests for plasma magnesium analysis. The use of a moderately priced spectrophotometer and the freedom from timing constraints are attractive features of this technique.

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References