Ultrafiltrable Calcium and Magnesium in Ultrafiltrates of Serum Prepared with the Amicon MPS-1 System

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We evaluated the Amicon micropartition system (MPS-1) for preparing ultrafiltrates of serum for use in evaluating ultrafiltrable Ca and Mg. We found no adsorption of either to the filter and 99.6% retention of serum proteins on the membrane. Ultrafiltrate volumes recovered (100–450 μL) varied with centrifugation time (10–30 min) and temperature. Centrifugation time did not affect the measured concentration of ultrafiltrable calcium and magnesium, and pH change in the 1-mL serum specimen during a 30-min centrifugation at room temperature was negligible. There was an inverse relationship between temperature and ultrafiltrable Ca and Mg concentrations. The precision (CV) between filters ranged from 1.2 to 5.1% for ultrafiltrable Ca and 1.5 to 2.7% for ultrafiltrable Mg. The correlation between ultrafiltrable Ca (y) and ionized Ca (x) in samples from 115 patients with calcium-related metabolic disorders was good (y = 1.04x + 0.18; r = 0.9128). We find the MPS-1 to be a simple and convenient tool for the rapid production of serum ultrafiltrates.

Additional Keyphrases: reference interval • relation among forms of Ca, Mg in serum

There are three distinct forms of serum calcium and magnesium: a free or ionized fraction, a complexed form associated with a variety of anions such as phosphate and citrate, and a protein-bound nondialyzable fraction. The ionized form, the important physiological regulator of calcium and magnesium homeostasis, normally accounts for 46% of the total serum calcium and 55% of the total serum magnesium (1). Thus, measurement of ionized calcium and magnesium should provide a more accurate assessment of the metabolic status of these elements than would data on total concentrations. Several instruments equipped with ion-selective electrodes for the direct determination of ionized calcium are now available commercially. However, these dedicated instruments are generally expensive and often vary in performance (2, 3). No ion-selective electrode for determination of ionized magnesium is commercially available.

Measurement of ultrafiltrable calcium (protein-free ionized plus complexed calcium) may offer an alternative approach (4, 5) and may even provide a better index of calcium balance in certain clinical conditions.1 Traditional ultrafiltration methods have been tedious and time consuming and often involved intricate apparatus (6, 7). Recently, simpler devices have become available (8, 9). One such device is the Amicon Micropartition system (MPS-1; Amicon Corp., Danvers, MA 01923), which is designed for the rapid preparation of ultrafiltrates based on low-speed centrifugation through a nonabsorptive filter with a high degree of protein retention. In this paper we describe the detailed evaluation of the MPS-1 system for use in the determination of ultrafiltrable calcium and magnesium. A preliminary evaluation of this device was reported previously.2

Materials and Methods

Specimen: Blood for routine ultrafiltration studies were collected in plain red-top Vacutainer Tubes (Becton Dickinson and Co., Rutherford, NJ 07070) and maintained anaerobic by leaving stoppered during clotting and centrifugation. After centrifugation the stoppers were removed and the serum was promptly transferred into 1-mL plastic tuberculin syringes. To minimize introduction of air into the specimens, we made this transfer without using a needle and with a gentle draw on the plunger. The syringes were then capped with tight-fitting rubber seals. Routine ultrafiltration was performed without delay. However, in comparisons between ionized and ultrafiltrable calcium, the capped syringes were stored at 4°C and ultrafiltrable calcium and

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magnesium were determined within three to four days of collection. Stability studies indicated no significant change in the concentration of either the ionized or ultrafiltrable calcium for one week under these conditions. Ionized calcium was determined with the Nova 2 ionized calcium analyzer (Nova Biomedical, Newton, MA 02164) within 3 h of collection. The specimens were obtained from patients who were undergoing investigations for a wide variety of calcium-related metabolic disorders. In precision studies, freshly collected patients' specimens, collected as described above, were pooled and ultrafiltered in replicate filters. The pH of the serum pool was within the physiological range.

Ultrafiltration: The MPS-1 system is an all-plastic device, consisting of a narrow reservoir capped to prevent gas exchange with the atmosphere and mounted over a membrane support and receiving cup, which are all held together by means of two plastic clips (Figure 1). Except for the membrane, the entire device is reusable. In these studies we used the YMB membrane (Amicon cat. no. 40410). One milliliter of serum, obtained as described above, was placed in the reservoir and centrifuged in an angle-head rotor at 1800 × g for various intervals between 5 and 30 min. Unless otherwise stated, the centrifugation was done routinely at 4 °C. The ultrafiltrates were analyzed for calcium and magnesium without delay. Initially, we used mineral oil (Sera-Seed; Abbott Laboratories, North Chicago, IL 60064), layered over the serum surface in the reservoir, to minimize loss of CO₂ during ultrafiltration. However, we found that the pH of the serum in the reservoir with and without oil did not differ by more than 0.04 pH units during a 30-min centrifugation at room temperature.

Analytical: Calcium and magnesium were determined by atomic absorption spectroscopy (Model 151; Instrument Laboratory, Lexington, MA 02173). Ultrafiltrable calcium was determined in 100 μL of ultrafiltrate diluted with 2.5 mL of a 10 mmol/L solution of LaCl₃. Ultrafiltrable magnesium was analyzed in 50-μL samples of ultrafiltrates, each diluted with 2.5 mL of 10 mmol/L LaCl₃ solution. The overall between-run CV for the atomic absorption method, based on routine monthly quality-control data, was 1.5% at 2.0 mmol/L calcium and 2.0% at 1.0 mmol/L magnesium.

Protein in the ultrafiltrates was determined by the method of Lowry et al. (10).

Results

Protein retention. We tested protein retention by the YMB membrane with standard solutions of bovine serum albumin (50–100 g/L) as well as 20 serum specimens analyzed for total protein before and after ultrafiltration. Less than 0.4% of the total protein appeared in the ultrafiltrates, in accordance with the manufacturer's claim. In our experience with more than 500 membranes, fewer than 1% were found to leak protein. A leaky membrane is easily detected by the straw color of the ultrafiltrate and by examination of the membrane, which appears clearly pitted.

Analytical recovery. Standard mixtures of CaCl₂ and MgCl₂ (0.5 to 3 mmol/L in 200 mmol/L sodium citrate adjusted to pH 7.4) were filtered, and the analytical recovery of calcium and magnesium was in the range 97–103%. However, recent batches of YMB filters had unacceptably high calcium contamination (recovery up to 112%). These membranes are unsuitable for ultrafiltrable calcium determinations and it is important to test every batch before use.

Effect of centrifugation speed and duration. The volume of ultrafiltrate depends on centrifugation speed, duration, and temperature. Filtration pressure was tested in the range of 500 to 4000 × g at 25 °C for 15 min and at 1800 × g from 10 min to 90 min. A maximum of 450 μL of ultrafiltrate was obtained from 0.8 to 1 mL of serum centrifuged at 1800 × g for 30 min and 25 °C. At speeds exceeding 4000 × g the membranes tended to rupture. The concentrations of calcium and magnesium were not significantly altered in ultrafiltrates obtained at various speeds.

Because prolonged centrifugation causes protein to accumulate on the filter, it was pertinent to determine the effect of centrifugation time on the binding equilibrium for calcium and magnesium. We examined this effect by centrifuging a single serum pool at various times and analyzing for calcium and magnesium in the ultrafiltrates (Table 1). There was no significant difference in ultrafiltrable calcium and magnesium at 1800 × g and various intervals, even up to 90 min. Because the volume recovered was not linearly related to time beyond 30 min, we routinely centrifuged for 30 min. Although a 10-min spin at 4 °C would yield enough ultrafiltrate (175 μL) for both a calcium and magnesium determination, the precision was slightly better at 30 min, perhaps because consistently larger volumes were recovered.

Effect of temperature. The effect of temperature on ultrafiltrable calcium and magnesium (Table 2) was investigated

![Fig. 1. The MPS-1](image)

**Table 1. Effect of Centrifugation Interval on Ultrafiltrable Serum Ca and Mg**

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Ca</th>
<th>Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Range</td>
</tr>
<tr>
<td>10</td>
<td>1.54</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>1.42–1.86</td>
<td>0.71–0.79</td>
</tr>
<tr>
<td>20</td>
<td>1.56</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>1.48–1.88</td>
<td>0.67–0.71</td>
</tr>
<tr>
<td>30</td>
<td>1.51</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>1.47–1.55</td>
<td>0.69–0.73</td>
</tr>
</tbody>
</table>

A single serum pool was ultrafiltered at 4 °C, with various centrifugation intervals. n = 4 for each time period.
Table 2. Effect of Temperature on Ultrafiltrable Ca and Mg

<table>
<thead>
<tr>
<th>Temp range, °C</th>
<th>Ca</th>
<th>Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SD</td>
<td>SD</td>
</tr>
<tr>
<td>2-4</td>
<td>1.39</td>
<td>0.03</td>
</tr>
<tr>
<td>23-25</td>
<td>1.34</td>
<td>0.03</td>
</tr>
<tr>
<td>29-35</td>
<td>1.19</td>
<td>0.02</td>
</tr>
</tbody>
</table>

A single serum pool was ultrafiltered at 1800 × g for 30 min. n = 5 filters for each temperature.

by filtering serum pools at 4 °C, room temperature, and a higher temperature, which was achieved by preincubating the rotor head and filters overnight in a 37 °C incubator. Syringes containing serum specimens were incubated for 1 h at 37 °C before ultrafiltration. The temperature of the reservoir was measured by means of a Teflon-covered precision thermometer (YSI, Yellow Springs, OH 45387), mounted on a reservoir cap. Temperature was measured from resistance readings before and after centrifugation by means of a digital resistance meter. The thermistor was calibrated against a reference thermometer, which itself was calibrated against a reference National Bureau of Standards thermometer. At higher temperatures the degree of control was relatively poor and the lower temperature reading at the end of the run indicated cooling during ultrafiltration. With increasing temperatures serum ultrafiltrable calcium and magnesium declined significantly (p < 0.01). Compared with 4 °C values the magnitude of this change is a decrease of approximately 5% at 24 °C and 12% at 29-35 °C.

Precision. The between-filter precision of the MPS-1 system was investigated at 24 °C. For five different serum pools the CV for ultrafiltrable calcium in the mean range of 1.32 to 1.42 mmol/L was 1.2% to 5.1% and for magnesium (mean range 0.57 to 0.68 mmol/L) 1.5% to 2.7%. An analysis of the combined data (n = 56 for calcium and n = 60 for magnesium) resulted in mean and CV values for calcium and magnesium of 1.35 mmol/L (2.4%) and 0.61 mmol/L (2.4%), respectively. The corresponding values for total calcium and magnesium as determined by atomic absorption spectroscopy in these pools were 2.42 mmol/L (3.0%) and 0.86 mmol/L (1.7%), respectively. Expressed as the percentage of the total, ultrafiltrable calcium and magnesium were 56% and 71%, respectively, in the five pools studied.

Reference range for ultrafiltrable calcium and magnesium. Ultrafiltrable calcium and magnesium were determined in serum from 69 healthy volunteers (40 men and 29 women) ages 20 to 58 years, who attended a blood-donor clinic. The mean values for calcium and magnesium were 1.46 and 0.65 mmol/L, respectively. The reference interval (2.5th and 97.5th percentile) for calcium was 1.35 to 1.61 mmol/L and for magnesium 0.52 to 0.75 mmol/L. In a separate study we found the normal reference interval for serum ionized calcium to be 1.15 to 1.30 mmol/L.

Ultrafiltrable calcium compared with ionized calcium. Figure 2 shows the relationship (r = 0.9128) between ionized calcium and ultrafiltrable calcium in 115 specimens from patients who had ionized calcium analyses requested because of various calcium-related disorders. No outliers were excluded.

Discussion

The Amicon MPS-1 is a simple, convenient tool for the rapid production of serum ultrafiltrates. The only additional piece of equipment required is a bench-top centrifuge and an angle-head rotor—standard equipment in most clinical laboratories. Because the entire plastic device is reusable, the cost of routine operation is low, essentially only the cost of the membrane. An advantage of the MPS-1 device is that it can produce various amounts of ultrafiltrate in a single operation without having to reload the system, by simply prolonging the centrifugation. Thus, several analytes can be measured in a single ultrafiltrate. Centrifugation interval appears not to be critical, with yields of up to 450 μL for a 30-min spin at room temperature; however, this yield depends on a starting volume of serum of at least 0.8 mL. We recommend that 1 mL routinely be used, to minimize the dead space in the reservoir. This is of course a disadvantage when sample volume is critical, as in pediatric work. Currently, one needs to test batches of YMB membranes for calcium (but not magnesium) contamination. Apart from these limitations, the MPS-1 appears to be reliable for the determination of ultrafiltrable calcium and magnesium.

The reservoir chamber maintains adequate control of specimen pH, obviating the need for mineral oil for pH correction factors (11). There is little information concerning temperature effects on free ligand concentration and ultrafiltrable ligand. Temperature per se is known to affect the concentrations of ultrafiltrable calcium, independent of its effect on the pH of the specimen (11). We were surprised to see a decrease in ultrafiltrable calcium and magnesium with increasing temperature, because ligand complexes should be more stable at lower temperatures. Possibly, complexed calcium and magnesium precipitate at higher temperatures, but we have no evidence of this in our ultrafiltrates. Such an inverse relationship between ultrafiltrable calcium and temperature has also been reported by others using different techniques (7, 9). Because the room temperature may differ among laboratories, this may account for some differences in values reported for ultrafiltrable calcium at room temperature. Consequently, we chose to perform most of our experiments at 4 °C.

The precision of the MPS-1 method for ultrafiltrable calcium and magnesium was comparable with the precision noted in other methods (8, 5, 12). The reference intervals reported in this study were also similar to other reference values for calcium (8) and magnesium (12).
Ionized calcium and ultrafiltrable calcium correlated well, but it is evident from Figure 2 that the scatter of data is fairly large and that some samples would be normal by one technique, abnormal by the other. Therefore the measurement of ultrafiltrable calcium cannot be simply substituted for ionized calcium. The clinical usefulness of ultrafiltrable calcium has to be clearly established. As a substitute for assay of ionized calcium it is assumed that the complexed fraction remains constant in disease states. That this is not so is borne out by our findings. In fact the complexed, non-protein bound fraction may be altered independently in various disease states (13). It may itself play a critical physiological role (14) and function as an independent marker of calcium-related metabolic disorders. Measurements of ultrafiltrable calcium, in conjunction with total and ionized calcium determinations, may thus provide a measure of the complexed fraction. Certainly in some clinical situations—such as after multiple blood transfusions when blood citrate concentrations are high or during treatment with EDTA for the removal of lead stores—measurement of ultrafiltrable calcium would not be expected to correlate with the physiologically active calcium component.

In the absence of a magnesium-selective electrode, measurement of ultrafiltrable magnesium is now the only practicable alternative to measurement of total magnesium as a physiological index of its metabolism. Clinically important reasons for serum magnesium measurements are numerous (15), and the ultrafiltrable fraction may yet prove to be more important than the total (12).

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References