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Analytical Evaluation of Kone Microlyte Determination of Ionized Magnesium

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We performed an analytical evaluation of a commercially available instrument for determining ionized magnesium through use of a neutral carrier, liquid-membrane-based ion-selective electrode. Reproducibility (CV 2–4%), linearity (0.30–2.50 mmol/L), lower limit of detection (0.30 mmol/L), and absence of interference from Ca^{2+} indicate adequate performance for measuring ionized magnesium in plasma or serum samples in the normal to high-concentration range. Sodium in excess of 150 mmol/L caused a negative bias, which can be explained by ionic strength-induced changes in activity coefficients. The use of heparin as an anticoagulant must be restricted to concentrations <15 units/mL because of the binding of magnesium to heparin. The mean ± SD concentration of ionized magnesium and its fraction of total magnesium in 76 healthy volunteers were 0.56 ± 0.05 mmol/L and 0.65 ± 0.04, respectively.

Indexing Terms: *reference values/ion-selective electrode*

Ion-selective electrodes (ISE) have been developed for determining H^+, Na^+, K^+, Cl^-, ionized Ca^{2+} (iCa^{2+}), and Li^+ in clinical samples (*1–3*).[5] Until recently, no ISE was available that reliably measured ionized Mg^{2+} (iMg^{2+}) in plasma, serum, or blood. Efforts have been concentrated on the use of neutral ionophore-based membranes, composed of ionophore/poly(vinylchloride) (PVC)/plasticizer (1/33/66, by wt). To improve selectivity, researchers inserted into the membranes an organic anion in an equimolar ratio to the neutral ionophore (*4, 5*). Problems with such systems have mainly been caused by insufficient selectivity against Na^+, Ca^{2+}, and H^+, as well as the limited lifetime of the electrode.

Two experimental systems to measure iMg^{2+} in serum have been described. Rouilly et al. (*6*) used a cell assembly of the kind Hg:Hg_2Cl_2, KCl (saturated)|KCl (3 mol/L)|sample‖membrane‖$MgCl_2$ 0.7 mmol/L, $CaCl_2$ 1.2 mmol/L, KCl 4 mmol/L, NaCl 140 mmol/L, AgCl:Ag at 21°C. The membrane consisted of ionophore ETH 5282 [(*N'*,*N''*,*N'''*-imino-di-6,1-hexandiyl)-tris(*N*-heptyl-*N*-methylmalonamide)]/PVC/*o*-nitrophenyloctyl ether plasticizer (by wt, 1/33/65), and potassium tetrakis(*p*-chlorophenyl)borate (KTpClB) in a molar ratio of 1.5 relative to the ionophore. This system had two major drawbacks: Interference from calcium necessitated calibration of the electrode with solutions containing an iCa^{2+} concentration identical to that in the sample to be measured, and the measurements were performed in an open system, which led to rather large pH shifts by evaporation of CO_2.

Maj-Zurawska and Lewenstam (*7*) used a more elegant approach by incorporating a Mg^{2+}-selective electrode in the Microlyte 6 analyzer (Kone Instruments, Espoo, Finland), making possible the simultaneous measurement of Na^+, K^+, iCa^{2+}, and pH. The membrane composition of the Mg^{2+} ISE was ionophore ETH 5220 [*N*,*N'*-octamethylenebis(*N'*,*N'*-dioctylmalondiamide)]/PVC/chloroparaffin/*o*-nitrophenylphenyl ether (1/33/32.5/32.5, by wt) and KTpClB in a molar ratio of 0.70 relative to the ionophore. Measurement conditions were considerably improved over the aforementioned method by increasing the temperature to 37°C and using a closed system of flow-through electrodes. However, the authors still reported considerable interference from Ca^{2+}, for which no clear solution has been presented.

Recently, Kone introduced an iMg^{2+} determination on their Microlyte 6 ion analyzer that allows measurement of iMg^{2+} without interference from Ca^{2+} in the setting of a routine clinical chemistry laboratory. We have performed an extensive analytical evaluation of this instrument, including the determination of a limited set of reference values.

Materials and Methods

The Microlyte 6 ion analyzer was used with the following electrodes installed: Ag/AgCl reference electrode, Ca^{2+}, Na^+, K^+, Cl^-, pH, and Mg^{2+}. The Mg^{2+}-selective membrane was composed of ETH 5220 dispersed in a PVC matrix, with chloroparaffin and *o*-nitrophenylphenyl ether as plasticizers and KTpClB as additive.

To calibrate the instrument for all ions except Mg^{2+}, we used two aqueous standard solutions, followed by measurement of a third aqueous standard with an intermediate value as a linearity check. The Mg^{2+} ISE is calibrated with standard solutions 1 (Ca^{2+} 1.25 mmol/L, Mg^{2+} 0.60 mmol/L) and 2 (Ca^{2+} 0.75 mmol/L, Mg^{2+} 0.30 mmol/L). The third calibration solution has a Mg^{2+} concentration identical to calibration solution 2 (0.30 mmol/L) but Ca^{2+} at 1.75 mmol/L. This procedure enables calculation of the selectivity coefficient $K^{Pot}_{Mg,Ca}$, which is stored subsequently in the instrument and used for correction of iMg^{2+} values for iCa^{2+}, and neces-

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[5] Nonstandard abbreviations: ISE, ion-selective electrode; iCa^{2+}, ionized calcium; iMg^{2+}, ionized magnesium; PVC, poly(vinylchloride); KTpClB, potassium tetrakis(*p*-chlorophenyl)borate; AAS, atomic absorption spectrometry; and TES, 2-[(tris(hydroxymethyl)methyl]amino)ethanesulfonic acid.
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sitates simultaneous implementation of the Mg^{2+} and Ca^{2+} electrodes. Each measurement takes 2 min, followed by a one-point calibration, which also takes 2 min. Thus 15 samples can be measured in an hour. The instrument uses 150 μL of sample; measurement temperature is 37°C.

Total Mg^{2+} was measured by atomic absorption spectrometry (AAS) (PE 2100; Perkin-Elmer, Gouda, The Netherlands).

Selectivity coefficients were determined by the fixed interference method (*8*). Electromotive force values needed for this purpose were measured directly by disconnecting the electrodes from the instrument and connecting them with a Century SS-1 pH/mV meter (Beckman Instruments, Fullerton, CA). Interference from Ca^{2+}, Na^+, and Li^+ was also evaluated by addition of these ions as their chloride salts to a solution containing NaCl 140 mmol/L, KCl 4.5 mmol/L, $MgCl_2$ 0.6 mmol/L, and 2-{[tris(hydroxymethyl)methyl]amino}ethanesulfonic acid (TES) 5.0 mmol/L, pH 7.4.

Response times are defined as the length of time elapsing between the instant the concentration of iMg^{2+} changes and the first instant at which the electrode potential becomes equal to its steady-state value within 1 mV (*8*). We measured these times with the same experimental system as was used for the determination of the selectivity coefficients. The electrode was equilibrated with an electrolyte solution (composition identical to the solution used for the interference studies), after which serum samples with different iMg^{2+} concentrations were introduced.

The lower limit of detection was determined as the iMg^{2+} concentration at the intersection of the extrapolated two linear segments of the calibration curve (*8*). This curve was determined by addition of $MgCl_2$ to a solution containing NaCl 140 mmol/L, KCl 4.5 mmol/L, $CaCl_2$ 1.25 mmol/L, and TES 5.0 mmol/L, pH 7.4.

Intraassay reproducibility was determined with both commercial control materials (from human origin) and patients' samples. Commercial control samples were used for calculating day-to-day reproducibility.

Linearity was determined either by mixing standard solutions or by mixing pooled patients' samples to which $MgCl_2$ had been added.

Blood samples were drawn into either plain or heparin-containing tubes with an evacuated blood-collecting system (Venoject; Terumo Europe N.V., Leuven, Belgium). All procedures followed were in accordance with the rules laid down in the Helsinki Declaration of 1975, as revised in 1983.

All chemicals used were of analytical-reagent grade from E. Merck B.V. (Amsterdam, The Netherlands). Sodium heparin (Thromboliquine®, sodium salt) was obtained from Organon Teknika B.V. (Boxtel, The Netherlands). Lithium heparin for addition experiments was pooled from Venoject tubes.

Results

The response times of the Mg^{2+}-selective electrode were always <30 s. Thus, a measurement time of 120 s allows reading of the potential practically at steady state.

The selectivity coefficient $K^{Pot}_{Mg,Ca}$ was determined with three concentrations of the interfering ion. Both a relatively new electrode and one at the end of its lifetime were used. Results are shown in Table 1. The selectivity coefficient $K^{Pot}_{Mg,Na}$ was determined at two sodium concentrations (125 and 150 mmol/L); $\log K^{Pot}_{Mg,Na}$ was −2.9 and −3.1, respectively. These selectivity coefficients are equal to or smaller than earlier reported values (*7*).

Interference from Ca^{2+}, Na^+, and Li^+ was determined by the addition of standards to buffered solutions (Fig. 1). The addition of Na^+ resulted in a negative bias, whereas added Ca^{2+} and Li^+ produced no significant interference.

Within-calibration CVs, measured with assays of control serum, were 3.9% (iMg^{2+} 0.48 mmol/L, n = 10) and 1.3% (iMg^{2+} 0.92 mmol/L, n = 10). Assays of patients' samples gave within-calibration CVs of 2.2% (iMg^{2+} 0.50 mmol/L, n = 10) and 2.6% (iMg^{2+} 0.81 mmol/L, n = 10). Day-to-day reproducibility, calculated as CV, was 3.5% (iMg^{2+} 0.57 mmol/L, n = 36) and 2.9% (iMg^{2+} 1.05 mmol/L, n = 35).

The lower limit of detection was 0.30 mmol/L iMg^{2+}.

The linear range for assay of serum samples and dilute electrolyte solutions extended from 0.30 to 2.50 mmol/L iMg^{2+}. The comparison between Mg^{2+} in dilute electrolyte solutions determined by AAS and by ISE was analyzed by the regression method of Passing and Bablok, which resulted in ISE = 0.966AAS + 0.029 mmol/L (range: 0.30–2.50 mmol/L). Slope and intercept were not significantly different from 1 and 0, respectively.

The lifetime of the electrode was limited by a gradual decrease of the calibration slope, resulting in decreasing sensitivity. Determination of $K^{Pot}_{Mg,Ca}$ after measurement of 1000 serum samples showed a slight change in Ca^{2+} selectivity (Table 1). However, stable values of control samples proved that the instrument still applied an appropriate correction factor. After measurement of ~1000 serum samples, the electrode should be replaced because of the decrease in sensitivity.

The influence of heparin on iMg^{2+} and iCa^{2+} measurements was determined by collecting blood from two healthy volunteers into different amounts of sodium and lithium heparin. As shown in Fig. 2, a lithium heparin concentration of 15 units/mL induced a nonsignificant increase in iMg^{2+}; the other curves showed a monotonous decrease with increasing heparin concentration. A comparison of iMg^{2+} measured in blood collected simultaneously into plain serum tubes and lithium heparin

Table 1. Calcium selectivity coefficients determined by the fixed interference method.

	$\log K^{Pot}_{Mg,Ca}$ after measurement of	
Ca^{2+}, mmol/L	40 sera	1000 sera
0.78	−0.47	−0.44
1.35	−0.72	−0.55
1.85	−0.70	−0.59

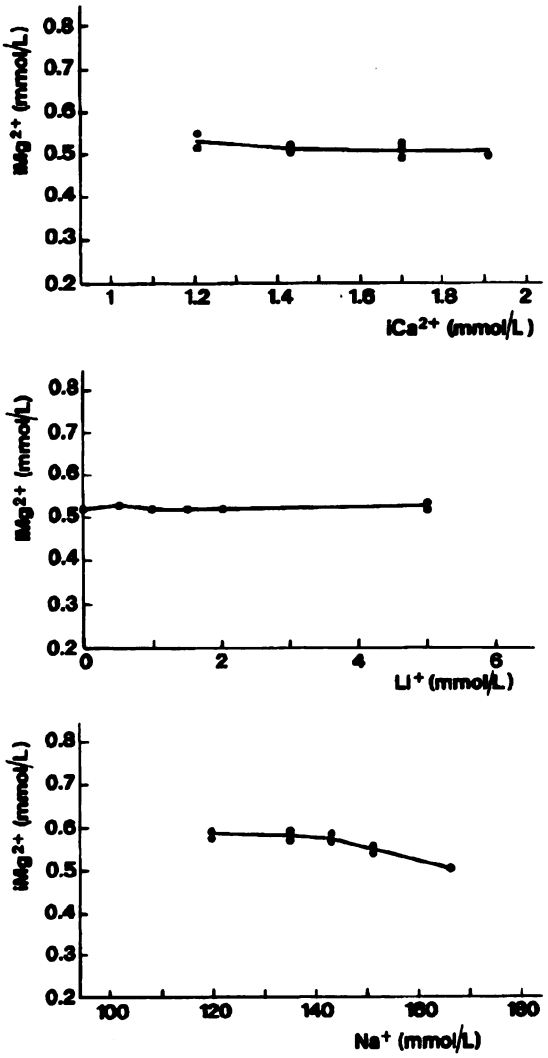

Fig. 1. Interference from Ca^{2+}, Na^+, and Li^+ on detection of iCa^{2+}-corrected iMg^{2+}, determined by addition of the interfering ions as their chloride salts to a buffered electrolyte solution; curves are drawn through the means of triplicates.

tubes (15 units/mL lithium heparin) showed significantly higher iMg^{2+} values in lithium heparin plasma (paired t-test, n = 39; average difference 0.021, t = 6.85, SD = 0.019, probability of equality P <0.0005). As expected, iCa^{2+} values detected in the lithium heparin tubes were slightly lower than in the serum samples.

Reference values for iMg^{2+} and the fraction of iMg^{2+} determined in 76 healthy volunteers (33 men, 43 women, median age 32 years, range 18–60) were 0.56 ± 0.05 mmol/L and 0.65 ± 0.04 (mean ± SD), respectively.

Discussion

The precision of the determination of iMg^{2+} was slightly less than reported for systems measuring iCa^{2+}, which are in the range 1.0–1.5% (*9, 10*). Given that the biological CV for iMg^{2+} is unknown, we were not able to calculate whether an analytical CV of 2–3% contributes significantly to the total variation.

Because no reference system is available for the measurement of iMg^{2+}, we could only estimate the accuracy of the method by comparing values for iMg^{2+} measured in aqueous solutions with values measured by AAS. The results of the linearity experiments did not indicate bias in the linear range, which therefore suggests adequate accuracy.

Linearity in the normal to high range of Mg^{2+} concentration (0.50–2.50 mmol/L) was excellent. The lower limit of the linear range (0.30 mmol/L) indicates a slight divergence from AAS values. If we assume that the distribution of Mg^{2+} in ionized and bound fractions does not change in hypomagnesemia, a value of 0.30 mmol/L for iMg^{2+} corresponds to a total Mg^{2+} of 0.46 mmol/L. Magnesium values <0.46 mmol/L are seen in ~2% of the Mg^{2+} requests in our laboratory, which makes this limitation a source of concern.

The apparently negative interference from Na^+ ions can be explained by the influence of the ionic strength. The activity coefficients for Mg^{2+} in the solution used,

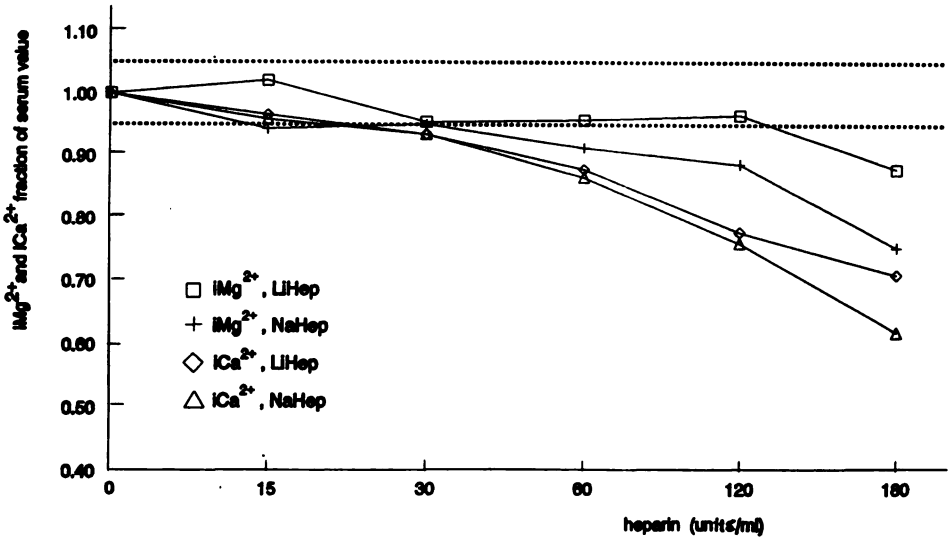

Fig. 2. Influence of sodium and lithium heparin on iMg^{2+} and iCa^{2+}, expressed as fractions of the simultaneously measured serum value of these ions.

Each data point represents the mean of duplicate determinations in plasma samples obtained from two healthy volunteers. The *dashed lines* show the 2 SD confidence interval of the iMg^{2+} serum value.

calculated from the Debye–Hückel equation, are 0.325 and 0.311 at NaCl concentrations of 140 and 160 mmol/L, respectively. That is, the increase in ionic strength decreases the Mg^{2+} activity by 4%. Because the ionic strength of plasma with an increased concentration of Na^+ will also be increased, this situation represents a phenomenon that is active in vivo. Correction for this negative bias is thus not necessary if one wishes to report a physiologically relevant activity-derived value, which is one of the advantages of ISE measurements (*11*).

The gradual decrease in calibration slope was the limiting factor for the lifetime of the electrode. When compared with the number of Mg^{2+} requests in our own laboratory (10–20 per day), a lifetime of 1000 samples is acceptable.

In our view, the recommended sample type for the determination of iMg^{2+} is serum, a matrix that contains no potentially interfering anticoagulants. Second best would be plasma heparinized with sodium or lithium heparin (maximal concentration 15 units/mL), which would enable measurement of iMg^{2+} in the same sample type recommended for analysis of iCa^{2+} (*12*).

We were not able to discern the cause of the positive bias (compared with serum) introduced when measuring iMg^{2+} in plasma heparinized with lithium heparin, 15 units/mL. Lithium interference was excluded by addition experiments. iCa^{2+} behaved as expected, with both sodium and lithium heparin causing a negative bias in comparison with serum. A possible explanation for the positive bias is the presence of preservatives in the lithium heparin blood-collection tubes. We realize that cellular metabolism may continue in the plain tubes during serum preparation (*13*); still, serum is our preference.

During the evaluation period, the Microlyte system functioned without serious problems. User-friendliness, speed, calibration frequency, and shelf life of calibrants all proved to be sufficient for "routine" analysis of iMg^{2+} in a clinical chemistry laboratory.

We conclude that the Microlyte 6 is a reliable and accurate system for the determination of iMg^{2+}. Its nonlinearity in the lower range, however, is a potential limitation to the assessment of the iMg^{2+} status in patients with severe hypomagnesemia.

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