Measurement of Serum Magnesium with a Centrifugal Analyzer

H. Khayam-Bashi, Tsan Z. Liu, and Vern Walter

We show how serum magnesium can be determined with a centrifugal analyzer. The method is based upon a manual procedure involving magnesium/calmagite complex formation in an alkaline reagent mixture. The assay, performed at 30 °C, requires 5 μl of serum in a final reaction volume of 405 μl, including 350 μl of reagent mixture. The stable color produced at 1 min is measured at 520 nm. Linearity studies showed that Beer's law was followed to 50 mg/liter. Average analytical recovery was 97.3%. Within-day studies showed CV's of 3.36% and 1.09%, compared to day-to-day variations of 5.32% and 3.04% at 18.8 and 46.6 mg of magnesium per liter, respectively. Correlation studies with the manual method, the Du Pont aca (methylthymol blue compleximetric) procedure, and atomic absorption spectroscopy showed correlation coefficients of 0.996, 0.976, and 0.950, respectively. Results compare excellently with those by common present methods. The method is fast, economical, reliable, and applicable to small specimen volumes.

Detection of hypomagnesemia requires a method that is more rapid, more sensitive at lower ranges, and more efficient for routine use with small quantities of specimens and reagents than those currently available to the clinical laboratory.

The classic procedure for measuring serum magnesium is to isolate magnesium as magnesium ammonium phosphate and then colorimetrically estimate phosphorus by the method of Fiske and SubbaRow (1). Unfortunately, this method is time consuming and not easily adapted to modern automation. Other techniques commonly used for this purpose are atomic absorption spectrometry, flame photometry, fluorometric analysis, spectrophotometric and colorimetric assays, or modifications of the magnesium ammonium phosphate procedure. Among the dye-binding compleximetric methods, those that involve methylthymol blue, titan yellow, and carboxyanilide dyes are used most often. Although these methods are effective, they are rather elaborate for routine laboratory use.

A relatively new manual method for serum magnesium quantitation, proposed by Gindler and Heth in 1971,1 involves the use of calmagite, a compound that is related to the carboxyanilide dyes.

\[ N=N-SO_3H \rightarrow Mg^{2+} \rightarrow Mg^{2+}\text{Calmagite Complex} \]

\[ \text{Calmagite (Blue)} \] (Pink)

This method, which was introduced commercially by the Pierce Chemical Co., Rockford, Ill. 61105, involves the reaction of serum magnesium with a reagent mixture containing calmagite.2 In this procedure, the pink magnesium/calmagite complex is measured spectrophotometrically.

The calmagite procedure involves only one reagent mixture which, because it contains EGTA [ethylenebis(oxyethyl)-enitriilo]tetraacetic acid], has shown negligible, if any, interference from calcium. The final color is stable, the reaction quickly reaches an end-point, the results are not affected by icteric serum or plasma, and no protein precipitation is necessary. Because of these advantages, the calmagite procedure appeared to be the most suitable method to adapt to an automated procedure.

The method presented here3 is based on the manual procedure developed by the Pierce Chemical Co. Our automated method is rapid, as effective in producing accurate serum magnesium quantitations as the more elaborate systems, and results correlate well with both the comparison methods and other current standard procedures.

Materials and Methods

We used CentrifiChem Model 300 centrifugal analyzer, supplied with an autopipetter and a computer adapted specifically to the Model 300 (Union Carbide Corp., Rye, N. Y. 10580). Calmagite reagents were purchased from Pierce Chemical Co. (Magnesium Rapid Stat Kit). The chemical compositions of the dye reagent and the base reagent used were identical to those recommended for the manual procedure:

Fig. 1. Relation between magnesium concentration and absorbance at 520 nm

![Absorbance vs Magnesium Concentration](image-url)

Fig. 2. Spectral scan pattern of the magnesium-calmagite complex

![Spectral Scan Pattern](image-url)

(a) **Dye reagent**, active ingredients, per liter: calmagite, 60 mg; KCl, 28 g; Bion NE-9, 1.08 g; Bion PVP, 10 g.²

(b) **Base reagent**, active ingredients, per liter: KCN, 2 g; KOH, 15.8 g; EGTA, 450 mg.² A 520-nm filter was used. Plastic disposable volumetric pipettes and containers were used throughout.⁴

The control serum was from a quality-control pool, QCI, used routinely in our laboratory, and also a toxic-concentration pool from Lederle Diagnostics (Los Angeles, Calif. 90051). Aqueous magnesium standards were made from magnesium iodate, Mg(IO₃)₂·4H₂O, to contain 20, 30, and 40 mg of magnesium per liter.

**Centrifchem Instrument Settings and Operational Procedure**

**Working reagent.** Prepare the working reagent freshly each day by mixing 10 volumes of dye reagent with one volume of base reagent.

**Instrument settings.**

(a) With computer (computer printout): Test code: magnesium; reaction type: 5 (stored blank, positive slope); precision: 1; unit: 0 (mg/dl); temperature: 30 °C; T₀ (time delay) = 30 s; ΔT = 60 s; mode: terminal; filter: 520 nm.

(b) Without computer (manual printout): Filter: 520 nm; temperature: 30 °C; terminal/rate: terminal; absorbance/concentration: concentration; calibrate/operate: calibrate; concentration factor: 122; auto/hold blank: hold water blank.

**Operational procedure.**

(a) Positions 0 and 1 are for reagent blanks. Place magnesium iodate standards in positions 2, 3, and 4 of the transfer disc in the order of 40, 30, and 20 mg/liter, respectively. Positions 5 through 29 are for the samples. Do not use the last sample plug.

(b) Preset a sample volume of 5 μl and a diluent volume of 50 μl in the autopipetter.

(c) Using the autopipetter, simultaneously pipet sample into the sample cavity and 350 μl of working reagent into the reagent cavity.

(d) If computer is used, push “spin” to start and wait for printout.

(e) If computer is not used, first wait for the calibrate value to print out, then adjust the concentration factor of 122 to the

---

Footnote:

⁴ With certain types of plastic labware, a calmagite precipitate sometimes appears at the edges of containers when the reagent mix is left standing for more than 15–20 min at room temperature. When this occurs, either containers of a different plastic composition should be used or the reagent should be kept in closed containers until just before use.
desired value, corresponding to the expected value of the standard. This should not vary more than ±5%. Turn the switch from "calibrate" to "operate" and push "print" to print out the results.

Experimental Studies

A spectroscale of the reagent-standard mixture was performed on a Beckman-25 Spectrophotometer (Beckman Instruments, Mountain View, Calif. 94043). The same reagent/magnesium ratio as that recommended for the manual calmagite method was used: 20 µl of the 40 mg/liter standard was mixed with 2 ml of the reagent mixture. The instrument was operated on the automatic scanning mode, scanning from 300–750 nm.

Sera that had been analyzed for magnesium by the manual calmagite procedure were used in recovery experiments. magnesium, 10 and 25 mg/liter, was added to these specimens, which were then further evaluated by the new automated procedure.

Correlation data were obtained on patients’ serum specimens received in the laboratory. We ran correlation tests with the Du Pont acu (Du Pont Instruments, Wilmington, Del. 198898), using a modification of the methylthymol blue compleximetric procedure; with the atomic absorption spectrometer Model 290 B (Perkin Elmer Instruments, Norwalk, Conn. 06856); and with the manual calmagite procedure recommended by Pierce Chemical Co.

Quantitation by atomic absorption is based upon the capability of magnesium atoms to absorb resonant energy of a wavelength of 285.2 nm. A 1 mg/liter working standard was prepared by mixing 0.1 ml of a 1.00 g/liter stock Mg2+ standard with 10 ml of 90 mmol/liter lanthanum oxide solution and 90 ml of de-ionized water. A blank also was prepared by mixing 10 ml of the lanthanum oxide solution and 90 ml of de-ionized water. Serum specimens were diluted 25-fold before the quantitation. Instrument settings were: wavelength, 285.2 nm; slit, 0.7 nm; source, hollow cathode, lamp current (4 mA); fuel, acetylene (reducing flame); fuel flow, 14.0; oxidizer, air. The atomic absorption spectrometer was standardized using the magnesium iodate standards. Magnesium concentration was based on the standard and was calculated by the following formula:

\[
\text{Reading} \times 1.0 \text{ mg/liter} \times 25 \text{ (diln factor)} = \text{Mg}^{2+}, \text{ mg/liter}
\]

The data were statistically evaluated as described by Barnett (2).

Results

The reaction mixture showed maximum absorption at 550 nm, with a second broad peak between 625–660 nm (Figure 1). A preliminary run using 520 and 550 nm for detection of the pink/violet color showed that the results were not significantly different at either of the two wavelengths; 520 nm was the wavelength we chose for all of these studies. The manual calmagite procedure calls for a 5-min incubation to reach a final endpoint. During our initial study, the instrument was run at intervals from 1–5 min with standards from 10–60 mg/liter. The results indicated that color yield was maximal by 1 min, and that the color intensity did not change between 1–5 min. This was true for all concentrations of magnesium used.

Linearity. Figure 2 shows the relationship between magnesium concentration and absorbance at 520 nm. Beer’s law was followed up to a concentration of 50 mg/liter, except that there may be a slight deviation from linearity at concentrations >45 mg/liter.

Precision and reproducibility. Ten repeated measurements were taken separately on each of two control sera. For QCI specimens, the mean was 18.8 mg/liter (SD, 0.6; CV, 3.36%). For the toxic-level pool the mean was 46.6 mg/liter (SD, 0.5; CV, 1.09%). Using the same control-sera pools, five or more repeated measurements were made on each specimen during seven days. The 37 measurements of the QCI pool averaged 18.7 mg/liter (SD, 1.0; CV, 5.32%). The standard error of the mean for these measurements was 0.1. For 36 different measurements on the toxic-level pool, the average was 46.6 mg/liter (SD, 1.4; CV, 3.04%), with a standard error of 0.2.

Correlation studies. We next correlated results by this method with those by the corresponding manual procedure, by a methylthymol blue method, which is adapted to the Du Pont acu, and by atomic absorption spectroscopy (Figure 3).

Analytical recovery. Known amounts of magnesium, ranging from 10 to 25 mg/liter, were added to sera that had been analyzed for magnesium content. The average analytical recovery was 97.3%. Of 13 specimens so supplemented, one showed a recovery of 93%, seven showed recoveries of 95–97%, and five showed recoveries of 100–101%.

Discussion

A within-run CV of 1–3% and a day-to-day CV of 3–5% can be routinely achieved. A run time of 1 min is sufficient. The data correlate well with results by the comparison method.

The method presented requires no deproteinization and no special optics, and only disposable plastic ware is used. Only one reagent mixture is used for this procedure, and the reagent is stable for at least 24 h. The method is sensitive and linear to 50 mg/liter, has a rapid turnaround time, requires only small volumes of specimens and reagents, and is practical and efficient for routine use in the clinical laboratory.

We thank G. Gotelli (U.C.S.F.) for Mg2+ measurements on the acu, and Ms. Susan Eastwood for editorial assistance.

References