The Automated Fluorometric Determination of Serum Magnesium

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A modified flow diagram and a simplified reagent have been developed for the automated fluorometric determination of serum magnesium by the Hill procedure (1).

This report presents a modification of the automated fluorometric determination of serum magnesium described by Hill (1). Significant alterations in the manifold, reagents, and sampling procedure have been introduced. The sensitivity of the procedure has been increased by incorporating recent technical improvements.

Experimental

Reagents

Acetate buffer 1 M, pH 4.4, containing 2.0 mg./ml. potassium oxalate

Potassium chloride 4% (w/v)

Buffered alcoholic 8-hydroxyquinoline To a solution of 3.0 gm. tris-(hydroxymethyl)aminomethane, in 20 ml. distilled or deionized (preferred) water, 50 ml. absolute ethanol is added with magnetic mixing. To the clear solution, 0.35 gm. 8-hydroxyquinoline (Fisher Certified No. 0-261) is added with mixing. When dissolved the solution is diluted to 100 ml. with absolute ethanol. The solution should be stored in an amber polyethylene bottle.

Magnesium standards The standards are prepared from magnesium oxide as described by Schacter (2) and cover the range from 0.5 to 4.0 mg. Mg++/100 ml. (0.41–3.28 mEq./L).

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Flow Diagram

The flow diagram is shown in Fig. 1. With the Sampler II,* specimens are sampled, diluted with buffered oxalate, dialyzed into potassium chloride solution, and mixed with the reagent. The resulting fluorescence is measured in a flow-cell fluorometer and recorded.

**Fig. 1.** Flow diagram for automated fluorometric serum magnesium determination.

Membrane material A Type C cupriphane dialysis membrane is used.

Fluorometer The Technicon fluorometer is used in this procedure. A Turner photofluorometer modified for automated operation can also be used.

Chart paper The chart paper used is ruled for per cent transmission; the chart speed is 18 in./hr.

Filters The primary (exciting) filter is a narrow-pass filter (Turner 110-812) with a peak at 405 mμ. It is a composite (Corning 7-51 and Wratten 2C). The secondary filter is a narrow-pass filter (Turner 110-822) with a peak at 525 mμ, color specification No. 58.

Aperture In this work a No. 4 aperture slit was used (see Operating Procedure) as was the 30X range setting. These conditions may be altered to accommodate local situations.

Operating Procedure

All solutions and reagents are aspirated for about 10 min. When the combined reagent stream passes through the flow cell, the fluorometer

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baseline is adjusted with the blank knob to the 1% line on the chart paper. A 4.0 mg./100 ml. magnesium standard is continuously aspirated and the fluorescence is recorded. The fluorescence response should be between 60 and 80 (arbitrary units). If less than 60 or above 80, the slits are replaced to bring the response within this range. The baseline is rechecked and readjusted to the 1% line if necessary. The analyst is referred to the fluorometer operating instruction manual for details.

Standards and specimens are then aspirated at either the 30 specimen/hr. rate (2:1 wash ratio) or the 40 specimen/hr. rate (1:1 wash ratio). (The 40 specimen per hour (1:1) sampling cam is not usually supplied with the Sampler II but can be obtained from Technicon.)

**Calibration Curve**

Standard magnesium solutions containing 0.5–4.0 mg. Mg\(^{++}\)/100 ml. (0.41–3.28 mEq./L.) are analyzed and the fluorescence response, in arbitrary units, is plotted against concentration. It is recognized that magnesium concentrations should be expressed in milliequivalents per liter for meaningful clinical interpretation. This expression is readily obtained by multiplying milligrams Mg\(^{++}\) per 100 ml. by 0.82. A typical calibration curve and the strip chart recording from which it was constructed are shown in Fig. 2.

![Typical calibration curve in automated fluorometric magnesium procedure.](image)
Results

From Fig. 2, it is evident that the fluorescence response (arbitrary units) is linear to 4.0 mg. Mg\(^{++}\)/100 ml. The results of analyses of several serum specimens submitted to the clinical biochemistry laboratory for determination of magnesium content, obtained by the modified automated procedure, agreed within 3% with the values obtained by the manual fluorometric procedure (2), and determined on a Turner Model 111 Photofluorometer.

Recovery of magnesium added to serum and determined by the present method varied from 92.6 to 97%.

Discussion

During the development of the present procedure several modifications were introduced. The fluorometric reaction with the buffered alcoholic 8-hydroxyquinoline reagent was simplified by combining all the ingredients in a single solution. The single solution was added to the dialyzate by a single Solvaflex\(^\ast\) manifold tube and mixed in one double mixing coil to insure complete reaction. No difference in response was noted when the Hill manifold and the modified manifold were compared. It was apparent that the new membrane material promoted better dialysis.

The modified reagent proved stable for at least 4 days at room temperature, although only 100-ml. quantities were prepared to insure fresh reagent. The reagent was checked daily by determinations of the deflection from the water baseline; it usually was between 20 and 25 units. The ability of the reagent to produce a fluorescence response between 60 and 80 units with the 4.0-mg. Mg\(^{++}\) standard served as an additional check on the reagent’s stability.

Good sensitivity was obtained in the present procedure with almost half the sample size used by Hill: 0.24 ml./min. as compared with 0.42 ml./min. This distinct advantage is in part the result of improved dialysis with the Type C membrane. The decreased dilution of the dialysis stream—e.g., 3.9 ml. reagent per minute as compared with 6.0 ml./min. in the Hill procedure, also contributed to the increased fluorescent response.

The potassium chloride content used in the dialyzer recipient solution was reduced to 4% because that concentration yielded less “noisy” baselines than the 5% recommended in the Hill procedure.

The combination of filters used here were those available in the Turner filter kit (Turner 110-839). The secondary filter, color specifi-

*Technicon Instruments Corporation.
cation No. 58, is a narrow-pass filter with a peak at 525 m\(\mu\), sufficiently close to the 530-m\(\mu\) peak determined spectrofluorometrically by Schachter (2).

Results obtained by further modification with the use of the aqueous 8-hydroxy-5-quinolinesulfonate reagent, which were reported later by Schachter (3), are under investigation; the findings will be reported shortly.

References