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TECHNICAL NOTE

Analysis of magnesium in tubular fluid using flameless atomic absorption spectrophotometry

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Despite the acknowledged importance of magnesium metabolism in many bodily functions, there have been relatively few direct studies of magnesium handling by the mammalian kidney. This is at least in part due to difficulties in accurate measurement of this ion in the picomole quantities present in fluid samples normally obtained from renal tubules by micropuncture. The only methodology in general use has been electron microprobe x-ray analysis [1, 2], but facilities and expertise to carry out this technique are not widely available.

Over the last ten years, various modifications of the technique of atomic absorption spectrophotometry, involving sample delivery by flameless furnace atomization, have been applied to the determination of sodium, potassium and calcium in microscopic droplets of fluid obtained from renal tubules [3–6]. We describe here the successful adaptation of this technique to the analysis of magnesium in tubular fluid samples, thereby opening up the potential for further micropuncture investigations of renal magnesium transport.

Methods

There are two stages involved in this procedure, namely sample preparation and microanalysis.

Sample preparation

Fluid samples obtained by micropuncture are first transferred onto the floor of siliconized glass cavity slides under water-equilibrated paraffin oil. Standard solutions are treated similarly.

Delivery of constant volumes of samples and standards into the graphite furnace atomizer is achieved by aspiration of fluid from one of the droplets under oil into a fine siliconized glass capillary filled with hydrated paraffin oil. A mark made on the outside of this capillary a short distance from its tip served as a reference point for fluid aspiration (volume approximately 0.4 nl). After drawing a little more oil into the tip to seal the sample, the capillary is lifted out of the oil on the sample slide and transferred to the floor of a graphite microboat for immediate transfer to the furnace for analysis. When another fluid is to be delivered in conjunction with the sample (see below), it is drawn up into the same capillary immediately following aspiration of the oil drop sealing the original sample (Fig. 1). The volume of the second fluid aspirated is identical to that of the sample, and is drawn up to the same mark. Delivery of the total fluid volume to the sample boat is achieved by rapid expulsion of both droplets together onto the floor of the boat under microscopic control. Immediately following this step, the microboat is transferred into the furnace and the analytical cycle initiated.

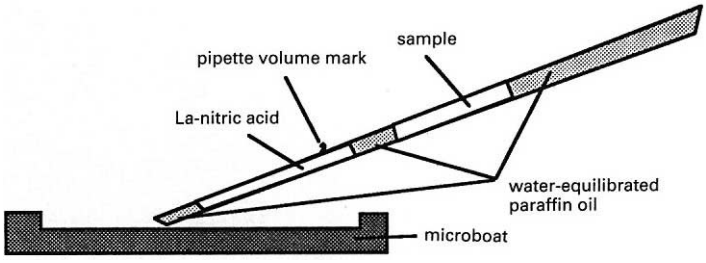

Fig. 1. *Microsample delivery system.* The sample (or standard) was sealed between oil columns, followed by aspiration of an equal volume (approx. 0.4 nl) of lanthanum oxide in nitric acid. Both fluid droplets were dispensed onto the floor of the graphite microboat immediately prior to analysis.

Microanalysis

The present method was developed using an atomic absorption spectrophotometer (Video 22 model, Instrumentation Laboratory, now Allied Analytic, Waltham, Massachusetts, USA), coupled with a graphite furnace atomizer (IL 755). The furnace unit is fitted with a rectangular pyrolytic graphite cuvette, designed for use with graphite microboats, which are reused.

The spectrophotometer is set with band width of 0.15 nm and an absorption wave length of 235.2 nm for magnesium. After purging with high purity argon, the furnace is set to dry the sample by bringing its temperature to 90°C over 10s, then to 110°C over 25s. Ashing follows this step, with the furnace temperature ramped to approximately 1200°C over 15s and maintained for another 25s. Atomization is then achieved by further ramping the temperature to 2250°C in 5s using the 'fast' heating mode, and maintaining it there for 5s. This is followed by an optional 'clean' cycle where the graphite furnace temperature is elevated to 2500°C to remove any residue that may remain in the cuvette before cooling down to the standby phase, ready for the next sample. The last step can be shown to avoid any 'carry-over' of magnesium from one sample to the next.

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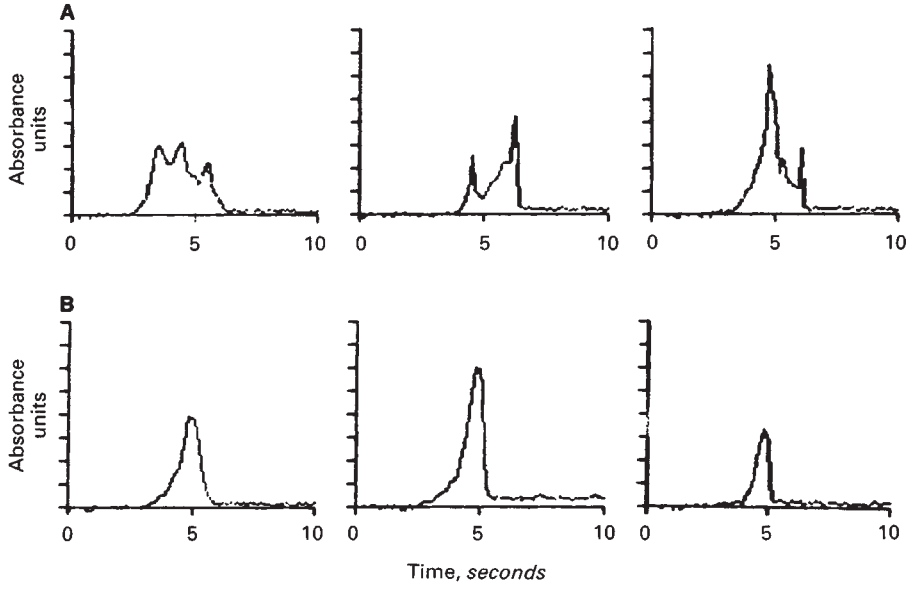

Fig. 2. *Absorption spectra.* **A.** Typical absorption spectra following pyrolysis of pure solutions of $MgCl_2$. **B.** Spectra obtained following the addition of 1N nitric acid to $MgCl_2$ standards.

The absorption spectrum is monitored throughout the atomization phase, triggered automatically by the furnace controller immediately on initiation of atomization. Peak height is determined electronically from the time course of the absorption spectrum, and is used to derive the quantity of magnesium present, and hence concentration.

Our early attempts to measure magnesium in undiluted subnanoliter volumes in this way were met with inconsistent absorption spectra. Spectra obtained using standard solutions containing $MgCl_2$ alone were often broad with multiple peaks, as shown in Figure 2A. This variability remained despite the use of different heating rates with various combinations of ashing and atomizing temperatures. This problems, thought to be due to the difficulty in atomizing magnesium as a halide compared to more oxidized salts, was eliminated by the delivery of some nitric acid together with the sample. Using the technique described above, an equal volume of 1 N HNO_3 was dispensed with each sample, resulting in the transformation of the absorption spectrum into a single well defined peak (Fig. 2B), which was found to be highly reproducible.

A second problem in the generation of consistent absorption spectra was encountered when phosphate was introduced into the test fluids. This caused reversion of the single absorption peak into multiple peaks similar to those depicted in the upper panel of Figure 2. This problem, previously recognized in conventional air-acetylene atomic absorption spectrophotometry, was overcome by inclusion of lanthanum ions in the acidic delivery medium. Lanthanum itself has no absorption at the wavelength set for magnesium, but eliminates the interfering effect of phosphate on the burn curve. The final carrier solution co-delivered with the tubular fluid sample or standard was thus lanthanum oxide (0.2%) in 1 N HNO_3. All subsequent results in this paper were obtained using this method.

Results

Figure 3A illustrates a typical standard curve obtained with pure solutions of $MgCl_2$ over a range (0 to 1.8 mmol/liter) likely to cover magnesium concentrations encountered in fluid from superficial cortical nephron segments. Over this range, calibra-

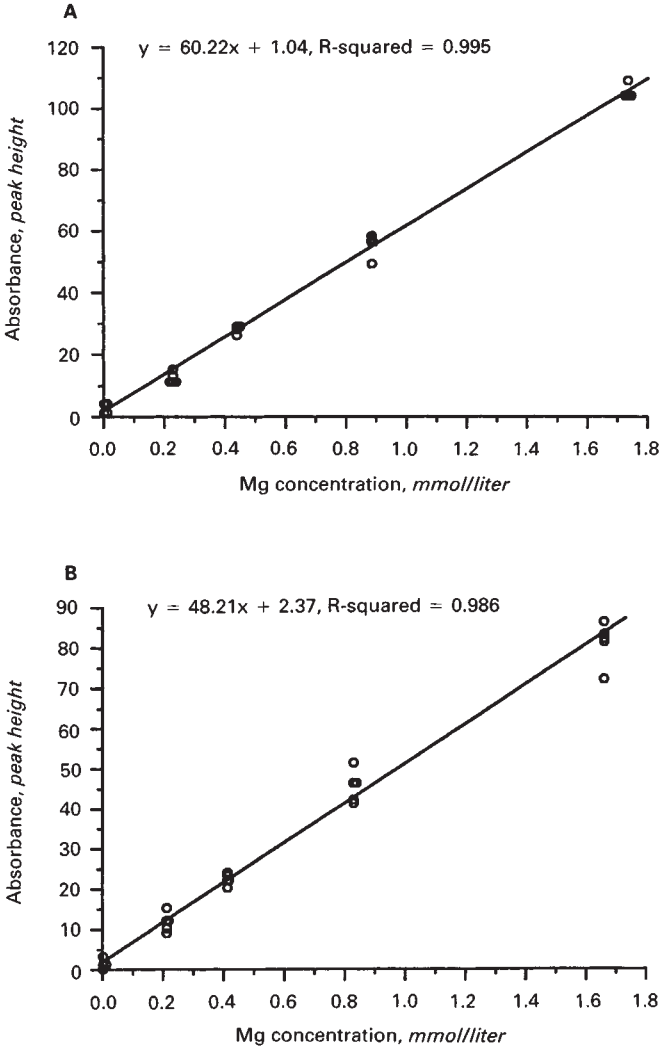

Fig. 3. *Magnesium standard curves.* **A.** Results obtained with solutions of pure $MgCl_2$. **B.** The calibration obtained when a 'cocktail' of other solutes was also present in the standard solutions (in mmol/liter: Na^+120, K^+10, Ca^{++}1, Cl^-120, SO_4^{--}1, $H_2PO_4^-$10, urea 250).

Table 1. Effect of presence of other solutes on the estimation of Mg in microsamples

	Concentration *mmol/liter*								
Solute	0	5	20	25	50	100	150	750	1000
None	1.04								
SD	0.06								
N	22								
Urea								1.05	1.02
SD								0.09	0.13
N								6	10
$CaCl_2$		1.09	1.28[a]						
SD		0.07	0.11						
N		6	10						
KCl							1.08		
SD							0.06		
N							5		
NaCl				1.02	1.13[a]	1.13[a]	1.14[a]		
SD				0.05	0.06	0.03	0.04		
N				5	5	13	6		
NaH_2PO_4			1.00	1.04	0.68[a]				
SD			0.01	0.03	0.12				
N			9	6	7				
Li_2SO_4		1.08			0.96[a]				
SD		0.03			0.14				
N		4			10				
$NaHCO_3$				1.05					
SD				0.06					
N				5					

The data shown are the Mg concentration estimates for a 1.0 mmol/liter Mg standard solution to which had been added each of the solutes shown in the left hand column at the concentrations shown along the top. SD = standard deviation; *N* = number of estimations.
[a] Signifies a significant difference ($P < 0.01$) from the estimate obtained in the absence of other salts

tion was essentially linear, as indicated by a coefficient of determination (R^2) of 0.99 or greater. The detection limit of the method was 0.02 mmol/liter corresponding to a sample mass of Mg of 8 picomoles. The standard deviation of a number of replicate estimates of Mg in a given fluid sample, a measure of the precision of the method, was 0.09 mmol/liter.

The influence of other solutes on the magnesium absorption spectrum was determined by the systematic incorporation of each solute at various concentrations in a 1.0 mmol/liter magnesium solution. These samples were then analyzed for magnesium against $MgCl_2$-only standards. Table 1 gives the estimated magnesium concentration of these solutions compared to the same solution without other added solutes. It can be seen that significant interactions were not encountered for any of the solutes tested in the range normally expected in cortical tubular fluid, with the exception of sodium which appeared to enhance the magnesium absorption signal at Na concentrations >50 mmol/liter. The extent of this interference, however, was constant over the range 50 to 150 mmol/liter, corresponding to what might be expected along the proximal and distal tubules under normal circumstances. It was thus possible to compensate for this error by inclusion of sodium in the standards at a fixed concentration within this range. This procedure did not affect the linearity of the standard curve for magnesium. In routine use, standards are actually employed containing a 'cocktail' of other solutes so as to mimic the usual composition of the tubular fluid being sampled. A typical calibration curve obtained under these conditions is shown in Figure 3B.

Table 2 illustrates some representative magnesium concentrations obtained by this method in distal tubular fluid samples obtained from normal Wistar rat kidneys under free flow conditions. The data demonstrate that continued magnesium absorption can be detected along the length of the superficial distal tubule, in agreement with data obtained by other authors using electron microprobe analysis for the determination of magnesium [7].

Table 2. Determination of Mg in distal tubule fluid samples

TF/P(inulin)	TF(Mg) *mmol/liter*	TF/UF(Mg)	FD(Mg) %
5.2	0.25	0.437	8.4
6.3	0.37	0.624	9.9
15.5	0.57	0.668	4.3
17.0	0.64	0.741	4.4

Abbreviations are: TF, tubular fluid; P, plasma; UF, plasma ultrafiltrate; FD, fractional delivery to the site of puncture (percent of filtered load).

Discussion

The purpose of this report is to demonstrate that atomic absorption spectrophotometry using flameless graphite furnace atomization of samples can be adapted to the determination of magnesium in picomole quantities. This enables subnanoliter samples of biological fluid, particularly renal tubular fluid, to be analyzed for magnesium routinely. Modifications of existing atomic absorption procedures were developed to circumvent the special problems associated with magnesium analysis, namely the failure of pure solutions of magnesium chloride to atomize with a discrete absorption peak, and the interfering effect of phosphates on the magnesium absorption spectrum. The first of these problems was overcome by acidification of the samples, and the second was eliminated by incorporation of lanthanum into the carrier solution.

While the present method involves the direct delivery of undiluted microsamples into the AA furnace, it has been shown by others that the instrumentation used here can also be employed (at least for Na and Ca) after predilution of samples to microliter volumes [5, 6]. This alternative approach, while not yet reported for Mg, has the advantage that diluted samples can be stored frozen pending analysis, and may be less subject to interfering effects from other solutes. On the other hand, predilution requires access to large volumes of ultrapure water and other facilities to avoid contamination, and involves higher costs in technical staff and disposables than does our method. Furthermore, the procedure described here is very similar to that already used by us (and others) to analyze tubular fluid samples for sodium and potassium [3, 8]. It is therefore feasible to proceed at a single sitting to the analysis of these elements following determination of magnesium, after simple replacement of the phototubes and resetting of the instrument analysis parameters and the furnace burn characteristics.

In comparison to the main alternative method for picomole analysis of magnesium, namely electron microprobe analysis, the present method has the advantages of being more generally available, requiring less specialized expertise in instrument operation, and requiring minimal sample handing and processing. However, as for any other microanalytical procedure, it is important that the standards used for analysis contain appropriate amounts of other solutes which are likely to be present in the biological samples to be analyzed. This will contribute substantially to eliminating errors which might be brought about by solute interactions during the atomization and analysis processes.

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