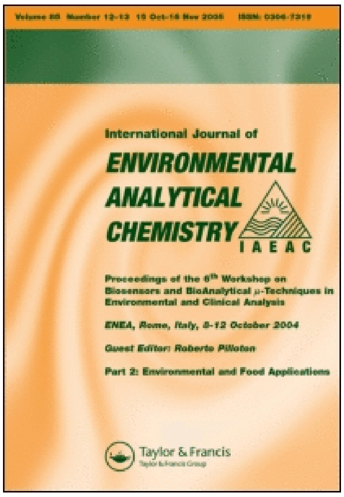

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Determination of Free Calcium and Magnesium Concentrations in Urine Samples by an Ion Exchange-Inductively Coupled Plasma Atomic Emission Spectroscopy Method

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DETERMINATION OF FREE CALCIUM AND MAGNESIUM CONCENTRATIONS IN URINE SAMPLES BY AN ION EXCHANGE-INDUCTIVELY COUPLED PLASMA ATOMIC EMISSION SPECTROSCOPY METHOD

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An ion exchange–inductively coupled plasma emission spectroscopy method was developed to measure free calcium and magnesium concentrations simultaneously in urine under non-trace ion exchange conditions. A single set of standards was sufficient for calibration of a given ion-exchange column over a range of concentrations of sodium, potassium, calcium, and magnesium. Zinc and urea at typical urine levels do not interfere. Analyses of four normal urines showed that from 40 to 66 percent of the calcium and magnesium was present in the free, or non-complexed, form.

KEY WORDS: Chemical speciation, urine, ion-exchange, ionized calcium, ionized magnesium, free ion measurement.

INTRODUCTION

Knowledge of free metal ion concentrations is often important in biological and clinical work. An ion exchange equilibration – atomic absorption method developed by Cantwell, Nielsen, and Hrudey[1] provides a unique way for their measurement. In this method, samples are passed through a small amount of strongly acidic cation exchange resin until ion exchange equilibrium is achieved between the resin and solution phases. The sorbed metal ions are then eluted from the resin with strong acid for determination. Under trace ion exchange conditions, that is, when the sought-for element occupies less than 1% of the total resin column capacity, the amount of metal ion in the resin phase is proportional to the free metal ion concentration in the solution phase. Trace conditions are typically maintained by providing a high concentration of univalent cations in the solution phase. For example, in 0.1 *M* $NaNO_3$, calibration plots for divalent metal ions are linear up to about 5×10^{-5} *M*. Because sodium and potassium concentrations are not normally high enough in urine to offer trace ion-exchange conditions for calcium and magnesium, which are typically present at millimolar levels, the amount of these metals present in the resin phase at equilibrium usually will not be directly proportional to their concentrations in the solution phase, i.e., the resin is not under trace ion-exchange conditions.

We report here a procedure in which the ion exchange equilibration method can be applied to the determination of free calcium and magnesium under non-trace conditions. In this method the four major cations present in urine (sodium, potassium, calcium, and magnesium) are sorbed to equilibrium on the resin phase, eluted, and determined by inductively coupled plasma (ICP) atomic emission spectroscopy (AES). Since the selectivity of the resin for metal ions is determined primarily by the ionic strength of the solution phase and the fraction of the resin phase containing the metal ions, the relationship between these factors was established by calibration with a set of standards containing the four metal ions over a range of concentrations expected for urine. The free metal ion concentrations can then be calculated by multiequilibrium regression.

THEORY OF METHOD

Background: For equilibration of a strong-acid type cation exchange resin with a solution containing a polyvalent metal ion M^{n+} and an electrolyte such as $NaNO_3$, the equilibrium reaction is (R^- = resin phase exchange sites)

$$M^{n+} + nRNa \rightleftharpoons R_nM + nNa^+ \tag{1}$$

The corresponding thermodynamic equilibrium constant K is

$$K = \frac{a_{R_nM} \bullet a^n_{Na^+}}{a_{M^{n+}} \bullet a^n_{RNa}} = \frac{[R_nM][Na^+]^n}{[M^{n+}][RNa]^n} \bullet \frac{\gamma_{R_nM} \bullet \gamma^n_{Na^+}}{\gamma_{M^{n+}} \bullet \gamma^n_{RNa}} \tag{2}$$

Cantwell *et al.*[1] showed that if the univalent ion concentration is kept constant and large relative to M^{n+}, then the fraction of resin in the R_nM form will be small, the activity coefficients and [RNa] in Equation (2) become essentially constant, and $[M^{n+}]$ becomes proportional to $[R_nM]$.

Ideally solutions under investigation will contain a constant high concentration of electrolyte that meets trace ion exchange conditions so that calibration plots are linear. In reality, electrolyte concentrations often vary from sample to sample and are not high enough to provide trace conditions. If the concentrations of electrolyte in samples are high enough to give trace conditions but differ from sample to sample, then a group of calibration curves is needed, each curve constructed with a different defined level of electrolyte concentration. This is inconvenient in practice. Alternatively, if electrolyte concentrations in samples are not high enough to provide trace conditions, then electrolyte can be added to bring the concentration up to a level where trace conditions prevail. However, this approach has two disadvantages. One is that the electrolyte concentration must be measured and adjusted before samples can be run through the column. This delay may cause problems in clinical studies; for example, some components in urine samples may precipitate. A second disadvantage is that the addition of sodium or potassium salts may disturb the original equilibria and the ionic strength. Since the most accurate free metal ion measurements are obtained when the original system is not disturbed, it is preferable that the measurements made insofar as possible under original conditions.

This work: A new calibration procedure applicable to non-trace ion exchange measurement was developed as follows. For the four major cations in urine, Na^+, K^+, Ca^{2+} and Mg^{2+}, the equilibria between a strongly acidic cation ion exchange resin and a urine sample in contact with it can be written as

$$Ca^{2+} + 2RNa \rightleftharpoons R_2Ca + 2Na^+ \tag{3}$$

$$Mg^{2+} + 2RNa \rightleftharpoons R_2Mg + 2Na^+ \tag{4}$$

$$Ca^{2+} + 2RK \rightleftharpoons R_2Ca + 2K^+ \tag{5}$$

$$Mg^{2+} + 2RK \rightleftharpoons R_2Mg + 2K^+ \tag{6}$$

Combining the thermodynamic constants for these equilibria with the ratios of the activity coefficients in the resin phase leads to four mixed constant expressions

$$K^{mix}_{Ca/Na} = \frac{[R_2Ca][Na^+]^2}{[Ca^{2+}][RNa]^2} \bullet \frac{\gamma^2_{Na+}}{\gamma_{Ca2+}} = K_{Ca/Na} \bullet \frac{\gamma^2_{RNa}}{\gamma_{R_2Ca}} \tag{7}$$

$$K^{mix}_{Mg/Na} = \frac{[R_2Mg][Na^+]^2}{[Mg^{2+}][RNa]^2} \bullet \frac{\gamma^2_{Na+}}{\gamma_{Mg2+}} = K_{Mg/Na} \bullet \frac{\gamma^2_{RNa}}{\gamma_{R_2Mg}} \tag{8}$$

$$K^{mix}_{Ca/K} = \frac{[R_2Ca][K^+]^2}{[Ca^{2+}][RK]^2} \bullet \frac{\gamma^2_{K+}}{\gamma_{Ca2+}} = K_{Ca/K} \bullet \frac{\gamma^2_{RK}}{\gamma_{R_2Ca}} \tag{9}$$

$$K^{mix}_{Mg/K} = \frac{[R_2Mg][K^+]^2}{[Mg^{2+}][RK]^2} \bullet \frac{\gamma^2_{K+}}{\gamma_{Mg2+}} = K_{Mg/K} \bullet \frac{\gamma^2_{RK}}{\gamma_{R_2Mg}} \tag{10}$$

Although activity coefficients in the resin phase cannot be determined, their ratios can be related to the metal ion fractions in the resin phase. Through trial and error the best fits for a set of standard solutions were obtained using the following equation set

$$\log(K^{mix}_{Ca/Na}) = a_1[RNa] + b_1[RK] + c_1[R_2Ca] + d_1[R_2Mg] + e_1 \tag{11}$$

$$\log(K^{mix}_{Mg/Na}) = a_2[RNa] + b_2[RK] + c_2[R_2Ca] + d_2[R_2Mg] + e_2 \tag{12}$$

$$\log(K^{mix}_{Ca/K}) = a_3[RNa] + b_3[RK] + c_3[R_2Ca] + d_3[R_2Mg] + e_3 \tag{13}$$

$$\log(K^{mix}_{Mg/K}) = a_4[RNa] + b_4[RK] + c_4[R_2Ca] + d_4[R_2Mg] + e_4 \tag{14}$$

where a_1 to e_4 are coefficients obtained by fitting the above equations to a set of standards using multiple regression. Since ammonium ion may be present in urine in significant amounts (0.02 to 0.06 *M*), it was also included in some calibration standards. Its inclusion is beneficial in that possible effects of ammonium ion on the resin equilibria are corrected for, and four variable terms can be used in equations (11) to (14) to give better fits. Once the coefficients have been gotten for a particular column, they can be used to calculate new mixed constants for unknown solutions.

EXPERIMENTAL

Reagents and solutions

All reagents were analytical grade or better. Distilled water was passed through a Barnstead NANOpure water system prior to use.

Stock solutions of 0.1 *M* $Ca(NO_3)_2$, 0.1 *M* $Mg(NO_3)_2$, 1.0 *M* $NaNO_3$, 0.5 *M* KNO_3, 1.0 *M* NH_4NO_3, 0.1 *M* $CaCl_2$, 0.1 *M* $MgCl_2$, 1.0 *M* NaCl, 0.5 *M* KCl and 1.0 *M* NH_4Cl were prepared from the corresponding salts. The purity of the calcium and magnesium salts was checked by titration with EDTA at pH 10 using Calmagite as indicator. A 0.125 *M* citrate solution was prepared by dissolution of 4.0552 g $K_3C_6H_5O_7 \cdot H_2O$ in about 60 mL of water, adjustment of the pH with 0.1 *M* HNO_3 to 6.5, then dilution to 100 mL. A 2 *M* solution of HNO_3 was used as eluent for the column. A 1000 ppm $Zn(NO_3)_2$ solution was prepared by dissolution of 0.4552 g of $Zn(NO_3)_2 \cdot 6H_2O$ in 100 mL of water.

Apparatus and procedure

The ion exchange column was constructed as described earlier[1], using 6.4 mg of 200–400 mesh Dowex 50 W × 8 strongly acidic cation exchange resin. The procedure for its use in a pumped flow system has been previously described[2]. In this work the earlier approach was modified in that the column eluent was not eluted directly into an atomic absorption instrument but was collected in 10-mL volumetric flasks for later aspiration into an ARL Model 34000 direct reader ICP atomic emission spectrometer. The modified flow system is shown in Figure 1.

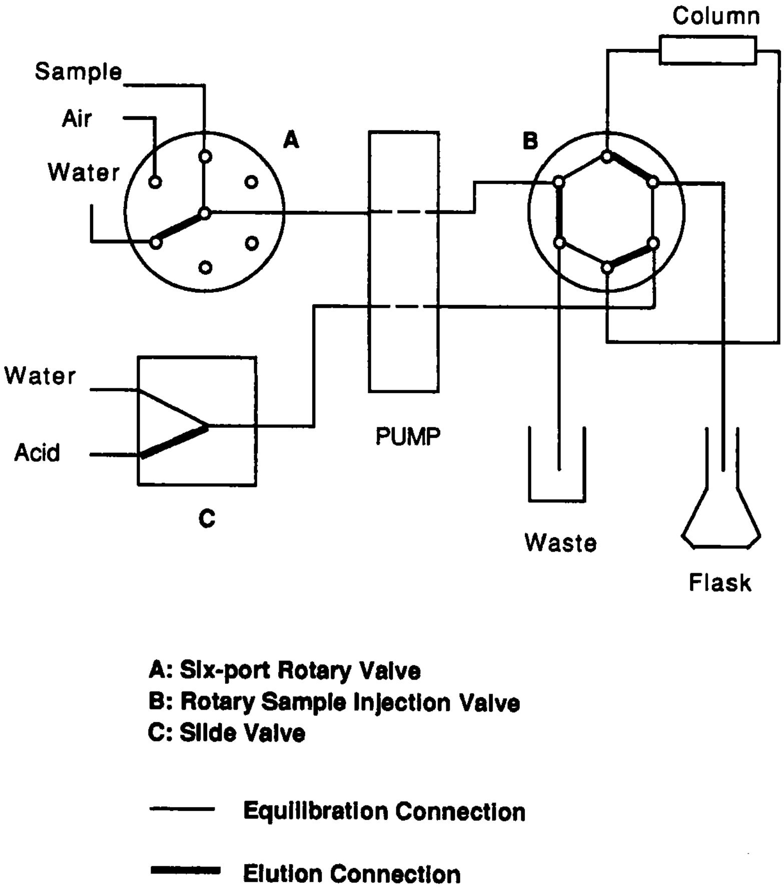

Figure 1 Flow system for free metal ion measurements.

Operation of the system involves five steps:

(1) The solution being analyzed is passed through the resin column until equilibrium is achieved (i.e., effluent from the column has the same concentration as the influent);

(2) Air is pumped through the column to clear most of the sample solution from the column and connecting tubes;

(3) Water is passed through the column to flush all traces of sample solution remaining in the column;

(4) 2 *M* HNO_3 is passed through the column to elute all cations on the resin; and

(5) Pure water is passed through the column to flush nitric acid from the system and prepare it for the next sample.

Preliminary studies of the column described here with samples containing normal concentrations of sodium, potassium, calcium and magnesium in urine showed that 3 minutes was sufficient for resin equilibration, and 40 seconds for elution. As a precaution, 5 minutes was used as the equilibration time and 1 minute for elution. Each of the other three steps was run for one minute.

To study the applicability of the method to real samples, fresh urine specimens were collected from four healthy volunteers and analyzed immediately. The pH of each sample was first measured, then an aliquot was analyzed for total S, P, Ca, Mg, Na and K by ICP-AES as follows:

(1) 2 mL of urine sample was delivered to a 10-mL volumetric flask, followed by addition of 1 mL of 2 *M* HNO_3 and dilution to 10 mL; this solution A was used for the total determination of S and P.

(2) 2 mL of solution A was delivered to a 10-mL volumetric flask, followed by addition of a 0.8 mL of 2 *M* HNO_3 and dilution to 10 mL. This solution B was used for total determination of Ca and Mg.

(3) 2 mL of solution B was delivered to a 10-mL volumetric flask, followed by addition of 0.8 mL of 2 *M* HNO_3 and dilution to 10 mL. This solution C was used for total Na and K determinations.

The free sodium and potassium ion concentrations were calculated from the ICP-determined values for totals of all the ions determined (all sulfur and phosphorus were assumed to be present as sulfate and phosphate) by computer calculation of the multiple equilibria involved. For this calculation the stability constants used were: for $NaSO_4^-$, log K = 0.68 ($\mu = 0$, 25°C); for KSO_4^-, log K = 0.79 (0, 25); for HSO_4^-, log K = 1.97 (0, 25); for $NaPO_4^{2-}$, log K = 0.75 (0.15, 37); for $NaHPO_4^-$, log K = 0.60 (0.2, 25); for NaH_2PO_4, log K = 0.114 (0.3, 37); for KPO_4^{2-}, log K = 0.60 (0.15, 37); for $KHPO_4^-$, log K = 0.48 (0.15, 37); for KH_2PO_4, log K = –0.20 (0.3, 37); for HPO_4^{2-}, log K = 12.35 (0, 25); for $H_2PO_4^-$, log K= 7.199 (0, 25); for H_3PO_4, log K = 2.148 (0, 25). For different urine samples, the ionic strength was taken as the total sodium and potassium concentrations, and all constants were adjusted to the ionic strength of each sample.

Calibration of the column

For calibration of the column, a set of 20 standard solutions containing varying amounts of the chloride salts of calcium, magnesium, sodium and potassium was prepared and analyzed to provide a broad representation of concentration ranges and ratios of each of the four cations in urine samples. As mentioned previously, ammonium chloride was also added to some of the standards. For these standards the free metal ion concentrations are considered to be the same as their total concentrations (Table 1).

After measuring the quantity of each metal ion sorbed onto the resin phase, the mixed constants of the metal ions for each standard were calculated from the free metal ion concentrations, the amount of metal ion on the resin phase, and the activity coefficient of each solution. The Davies equation in the form

$$\log(\gamma_i) = -AZ_i^2\left\{\frac{\sqrt{\mu}}{1-\sqrt{\mu}} - 0.3\,\mu\right\} \qquad (15)$$

was used to calculate the activity coefficients of each of the metal ions in solution. Here γ_i is the activity coefficient of ionic species i, A is a constant equal to 0.511 for aqueous solutions at 25°C, Z_i is the charge on ion i, and μ is the ionic strength of the solution.

The coefficients a_1 to e_4 in equations (11) to (14) were then calculated by multiple regression and these values, along with the metal ion concentrations in the resin phase, were used to calculate mixed selectivity constants for each solution. Tables 2 and 3 give results for experimental and calculated selectivity constants. The agreement indicates that the model used here for non-trace ion exchange equilibrium is satisfactory.

RESULTS AND DISCUSSION

Determination of free calcium and magnesium with citrate as the complexing ligand

Sample solutions containing known amounts of citrate, along with varying quantities of calcium, magnesium, sodium, and potassium nitrate, were prepared and analyzed to

Table 1 Composition of calibration standards for Dowex 50 W × 8 resin column used in this study (concentrations in mM).

Std.	*NaCl*	*KCl*	*$CaCl_2$*	*$MgCl_2$*	*NH_4Cl*
1	60	20	0.7	0.8	0
2	60	40	1.4	1.6	0
3	60	80	2.8	4.0	0
4	60	120	4.2	6.0	0
5	100	20	1.4	4.0	0
6	100	40	0.7	6.0	0
7	100	80	4.2	0.8	0
8	100	120	2.8	1.6	0
9	200	20	2.8	6.0	0
10	200	40	4.2	4.0	0
11	200	80	0.7	1.6	0
12	200	120	1.4	0.8	0
13	280	20	4.2	1.6	0
14	280	40	2.8	0.8	0
15	280	80	1.4	6.0	0
16	280	120	0.7	4.0	0
17	200	80	2.8	4.0	0
18	100	40	1.4	1.6	20
19	100	40	1.4	1.6	50
20	100	40	1.4	1.6	100

Table 2 Comparison of experimental and calculated K^{mix} values for calcium equilibria.

Std.	$K^{mix}_{Ca/Na}$ *Values*		$K^{mix}_{Ca/K}$ *Values*	
	Expt.	*Calc'd.*	*Expt.*	*Calc'd.*
1	636	699	153	170
2	731	753	181	191
3	803	801	224	216
4	859	827	250	237
5	728	695	162	153
6	721	734	181	189
7	761	747	207	207
8	784	780	260	260
9	620	622	145	144
10	662	645	170	158
11	647	646	228	227
12	653	682	245	255
13	553	564	135	141
14	570	575	173	164
15	627	629	200	199
16	656	650	238	236
17	702	682	206	206
18	706	709	197	199
19	734	720	211	211
20	729	739	234	233
Correl. coeff.	0.942		0.977	
Rel. std. err. of estimate, %	3.3		3.9	

Table 3 Comparison of experimental and calculated K^{mix} values for magnesium equilibria.

Std.	$K^{mix}_{Mg/Na}$ *Values*		$K^{mix}_{Mg/K}$ *Values*	
	Expt.	*Calc'd.*	*Expt.*	*Calc'd.*
1	256	277	62	67
2	282	296	70	75
3	307	309	86	83
4	333	319	97	91
5	269	260	60	57
6	267	260	67	67
7	321	310	87	86
8	312	313	104	104
9	237	241	55	56
10	263	260	67	64
11	250	256	88	90
12	274	274	103	103
13	232	235	57	59
14	256	238	74	68
15	241	245	77	77
16	251	255	91	92
17	272	270	80	82
18	281	283	79	79
19	290	289	83	85
20	297	298	95	94
Correl. coeff.	0.936		0.975	
Rel. std. err. of estimate, %	3.3		3.9	

compare measured with calculated values. The pH of each solution was measured, then the solution was pumped through the column to equilibration and the sorbed metal ions determined. In the calculations the free sodium and potassium concentrations were taken as their total concentrations, that is, Na^+ and K^+ complex formation with citrate at the pH values used here was considered to be negligible. The free calcium and magnesium concentrations were then determined by the procedure described previously, and compared to values calculated from total concentrations of sodium, potassium, calcium, magnesium, citrate, pH and stability constants from the literature[3]. Experimental and calculated results are compared in Table 4. For each sample, the first experimental value for free calcium or free magnesium was obtained through calculation with respect to sodium concentration in the sample according to equation (7) or (8), and the second with respect to potassium concentration according to equation (9) or (10). Agreement between the experimental and theoretical values was considered to be satisfactory for both free calcium and free magnesium concentrations in this system.

Investigation of zinc(II) and urea as possible interferences

In urine a number of inorganic and organic species are potential interferences with the proposed method. For example, polyvalent cations might sorb strongly onto the resin phase and replace calcium or magnesium. Zinc(II), which is present at ppm levels in urine, is an example. Another kind of interference might arise from activity coefficient effects caused by the 0.2 to 0.5 *M* concentrations of urea which occur in urine. Both zinc(II) and urea were therefore tested as possible interferences.

Sample solutions were prepared by measuring varying amounts of $Zn(NO_3)_2$ solution and solid urea, along with fixed amounts of sodium, potassium, calcium and magnesium chloride solutions, into 25-mL flasks. The final concentrations of sodium, potassium, calcium and magnesium salts were set at 0.100, 0.050, 1.7×10^{-3} and 2.0×10^{-3} *M* respectively. The samples were then analyzed using the proposed method. The results, Figure 2, show that over the range of concentrations found in normal urine samples, zinc and urea do not interfere with the measurement of free calcium and magnesium concentrations. Even though some zinc is sorbed onto the resin phase, thereby reducing the amounts of the four major ions on the resin, the calculated free calcium and magnesium concentrations are not significantly affected so long as the fraction of resin present in the major ion forms is not changed significantly. It appears that only when ions that are more strongly sorbed than Ca^{2+} and Mg^{2+} are present at concentrations of millimolar or higher will the accuracy of the method be affected.

Table 4 Determination of free calcium and magnesium species concentrations for a series of solutions in presence of citrate (all concentrations in mM).

Experimentally measured free ion concentrations		*Calculated free ion concentrations*		*Total metal ion added*	
Ca^{2+}	*Mg^{2+}*	*Ca^{2+}*	*Mg^{2+}*	*Ca^{2+}*	*Mg^{2+}*
2.5, 2.6	3.1, 3.3	2.5	3.3	3.2	4.0
2.9, 3.1	2.5, 2.8	3.1	2.8	4.8	4.0
3.7, 4.2	4.9, 5.6	4.0	5.2	4.8	6.0
1.7, 1.9	2.2, 2.4	2.0	2.7	3.2	4.0
2.5, 2.8	4.7, 5.4	2.6	5.2	3.2	6.0
3.1, 3.5	4.0, 4.6	3.4	4.5	4.8	6.0

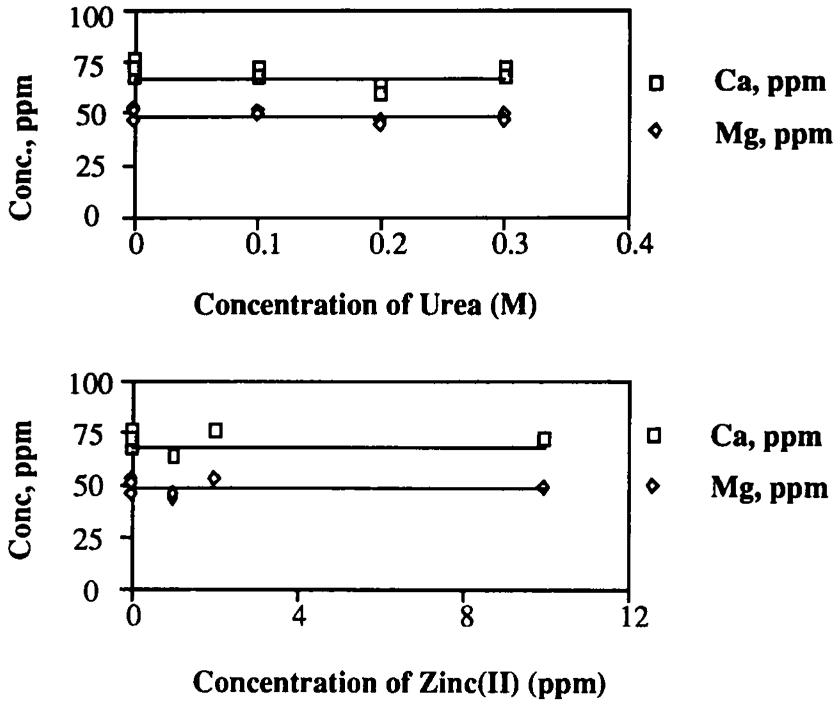

Figure 2 Effect of zinc(II) and urea on free Ca^{2+} and Mg^{2+} determinations.

Analysis of urine samples

From previous results we see that the method is able to measure free calcium and magnesium concentrations in the presence of citrate as a complexing ligand and that zinc(II) and urea do not interfere at typical concentrations in urine. The next step was to apply the method to real urine samples. Four normal urine specimens were therefore analyzed. Free sodium and potassium concentrations were calculated as before by correcting the total concentrations of these ions obtained by ICP-AES for the quantities tied up by sulfate and phosphate. This was done by a computer equilibration calculation, with the sulfate and phosphate concentrations obtained from S and P measurements by ICP-AES. About 5% of the sodium and potassium was present in bound form in these specimens.

From Table 5 we see that values for pH and total concentrations of sodium, potassium, calcium and magnesium vary widely, and that the fraction of the calcium and magnesium in free form varies from 40% to 66% over the four samples.

CONCLUSIONS

A method for the determination of free calcium and magnesium at millimolar concentrations has been described which permits their estimation under non-trace ion exchange conditions. The ionic strength of the sample need not be altered, enabling

Table 5 Measurements of free Ca^{2+} and Mg^{2+} in urine by proposed ion exchange — ICP method (n = 4, uncertainties are ± one standard deviation). Totals determined directly by ICP-AES.

Urine sample	*Na^+, M*	*K^+, M*	*Ca^{2+}, ppm*		*Mg^{2+}, ppm*	
	Total	*Total*	*Total*	*Free*	*Total*	*Free*
1 (pH 5.69)	0.251	0.062	316	126 ± 7	105	46 ± 3
2 (pH 6.29)	0.250	0.023	218	143 ± 18	45	29 ± 4
3 (pH 5.28)	0.219	0.051	154	94 ± 5	68	44 ± 2
4 (pH 5.74)	0.207	0.069	134	63 ± 2	126	67 ± 2

measurement of free metal ion concentrations without disturbing the original equilibria. By using a direct reading ICP-AES instrument as detector, the method also provides at the same time the total concentrations of calcium, magnesium, sodium, potassium, sulfate and phosphate.

Since the measurement involves a combination of ion exchange and ICP-AES, any di- or trivalent cation that can undergo ion exchange and can be determined by ICP-AES should be measurable by this method. This offers a versatile method for free metal ion determinations in complex environmental or biological solutions.

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