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Metal Analyses in Environmental and Pharmaceutical Samples by Capillary Electrophoresis with Methyl 3-Amino-3-(pyridin-3-yl)propanoate Dihydrochloride as a New Ion-Pairing Reagent

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Separation and determination of some common metal ions was achieved with methyl 3-amino-3-(pyridin-3-yl)propanoate dihydrochloride (MAPP) as an ion-pairing reagent and pyridine as a detectable counter-ion for indirect UV detection at 254 nm. The effects of the complexing reagent and chromophore concentrations, applied voltage, and organic solvent content on the separation were investigated. The optimized separation was carried out in a running electrolyte containing 16 mM MAPP and 20 mM pyridine at pH 4.0 and was successfully applied to the qualitative and quantitative analysis of Li^+, Na^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Ni^{2+}, and Zn^{2+} in pharmaceutical vitamin preparations and various water samples.

1. Introduction. – Determination of metal ions is of great interest in environmental, medical, and pharmaceutical sample analyses. Ion chromatography (IC) is a widely used analytical technique for metal analyses [1][2]. Capillary electrophoresis (CE) has a number of advantages over IC, *e.g.*, greater separation efficiency, higher selectivity, simplicity, and adaptability to a variety of different application conditions. The principle of separation by CE is based on differences in the mobilities of the analytes. The difficulty of the analysis of metal ions by CE lies in that most metal ions have roughly same mobility because of similar size and charge density. To modify the mobilities of alkali and alkaline-earth metal ions, either complexation or ion-pairing reagents like polyethers [3–7], carboxylic acids [8–10], 1,10-phenanthroline [11–13], 4-(2-pyridylazo)resorcinol (PAR) [14][15], 4-(2-thiazolylazo)resorcinol (TAR) [16–18], and pyridine carboxylic acids [19][20] have been used. In many cases, cationic and neutral surfactants [21–24], organic solvents [25][26], and polyelectrolytes [27–30] have been used in CE to improve the separation selectivity of metal ions. Because of the transparency of inorganic cations in the UV range, UV-absorbing cationic compounds that have similar electrophoretic mobilities, *e.g.*, imidazole [31–33], pyridine [34–37], aromatic amines [23][25][38][39], and Cu^{2+} [40] are widely used for the indirect detection of cations.

In this work, we investigated a new CE approach for the separation and determination of some common metal ions by means of a new ion-pairing reagent, methyl 3-amino-3-(pyridin-3-yl)propanoate dihydrochloride (MAPP), in the running electrolyte, and the method was applied to the analyses of pharmaceutical vitamin preparations and various water samples.

2. Results and Discussion. – 2.1. *Dependence on MAPP Concentration and pH.* In an applied electric field, all positively charged analytes are expected to migrate toward

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the cathodic end according to their effective and electrophoretic mobilities (EPMs), while MAPP would move in the opposite direction because of the carboxylate end group. However, cationic and neutral ion-pairs formed by MAPP ions with metal ions co-migrate in the electric field. Depending on the size and charge density of the metal ion, the concentration of ion-pairing reagent, and the degree of ion pairing, different metal ions exhibit different electrophoretic mobilities. The effect of MAPP on the separation of metal ions was, therefore, investigated by increasing its concentration in the running electrolyte containing 20 mM pyridine at pH 4.0. With increasing MAPP concentrations, the electro-osmotic mobility (EOM) decreased slightly up to 12 mM MAPP, and, at higher MAPP concentrations, remained reasonably constant, as shown in *Fig. 1*. The effect of the MAPP concentration (2.0–25.0 mM) on the effective mobilities of the investigated metal ions is shown in *Fig. 2*. As expected, the effective

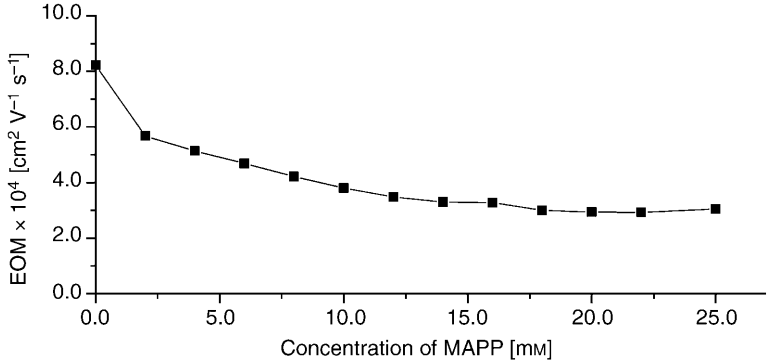


Fig. 1. *Dependence of EOM on MAPP concentration.* Electrophoresis conditions: 20 mM pyridine (pH 4.0); 15 kV; injection: 8 s at 0.8 p.s.i.; indirect detection at 254 nm.

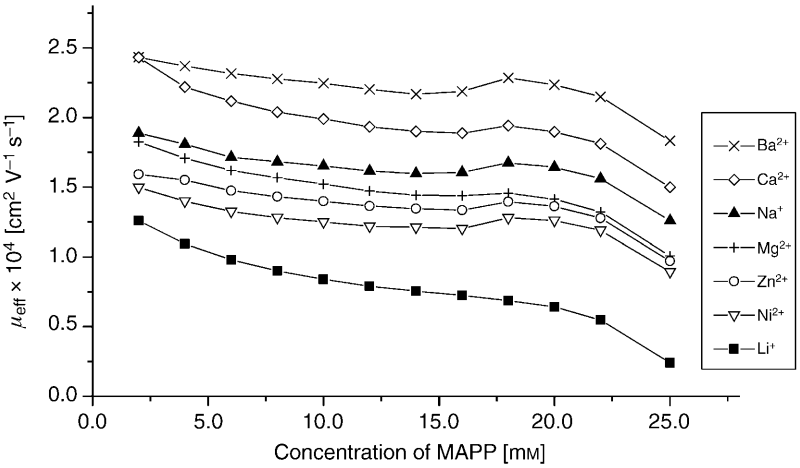


Fig. 2. *Dependence of EPMs on MAPP concentration.* Electrophoresis conditions: see *Fig. 1*.

mobilities of cations decreased with increasing MAPP concentration, since the mobilities of the ion pairs were smaller than those of the corresponding uncomplexed forms. Complete separation of Ba^{2+}, Ca^{2+}, Na^{+}, Mg^{2+}, Zn^{2+}, Ni^{2+}, and Li^{+} was achieved by the addition of 5.0 – 18.0 mM MAPP, with optimal separation observed at 16.0 mM. Depending on the objectives of the application and the sample composition, different concentrations of MAPP can be used to provide selective resolution between the analytes. For example, in the case of Zn^{2+}-free samples, analysis with higher MAPP concentrations can improve resolution, especially between Ba^{2+}/Ca^{2+} and Na^{+}/Mg^{2+} ion pairs.

It is necessary for the pH to be acidic for sufficient protonation of the visualization reagent, and *Timerbaev* [25] reported an upper pH limit of *ca.* 5 to prevent metal hydrolysis. For this reason, the effect of pH on the separation was investigated in a running electrolyte containing 16 mM MAPP, and pH 4.0 was found to be the best working pH (not shown). At lower pH values, *e.g.*, 3.0, EOM decreased but deterioration of the resolution of some peaks, due to peak tailing, was observed. At pH 5.0, the EOM increased slightly and resolutions decreased.

2.2. *Dependence on Pyridine Concentration*. The effect of the concentration of pyridine used as the visualizing agent for the indirect detection of metal ions on the separation is shown in *Fig. 3*. 20 mM was chosen as optimal pyridine concentration. The separation between Mg^{2+}/Zn^{2+} and Zn^{2+}/Ni^{2+} ion pairs could not be achieved at lower concentrations of pyridine. No improvements were observed at 25 mM, especially in terms of the effective mobilities of the metal ions, but the increased viscosity of the running electrolyte led to a decrease in peak sharpness and an increase in the analysis time.

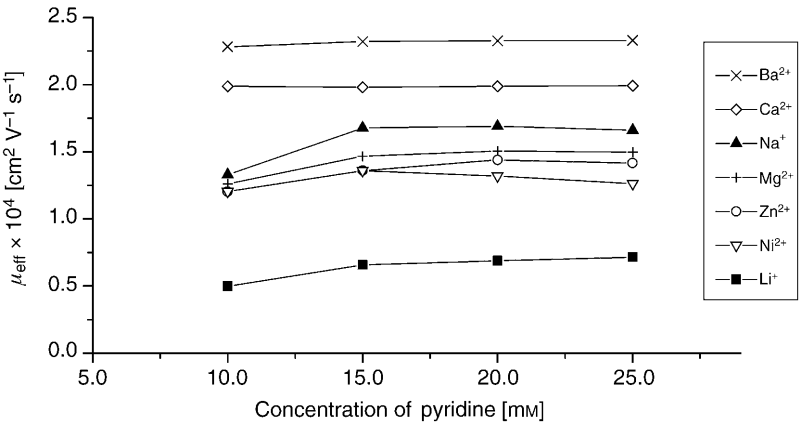


Fig. 3. *EPMs as a function of pyridine concentration.* Electrophoresis conditions: 16 mM MAPP (pH 4.0); 15 kV; injection: 8 s at 0.8 p.s.i.; indirect detection at 254 nm.

2.3. *Dependence on Voltage.* Separation voltages ranging from 10 to 30 kV were applied from the anodic side, and the effective mobilities of metal ions were investigated to find optimal voltage conditions (*Fig. 4*). The mobilities of all metal ions except Li^{+} increased slightly from 10 to 15 kV, then remained effectively constant up to 30 kV. The effect of voltage (10 – 30 kV) on the resolution (R_s) of investigated metal

ions is shown in *Table 1*. R_s Values were calculated as $2(t_2 - t_1)/(w_1 + w_2)$, where t_1 and t_2 are the migration times and w_1 and w_2 are the peak widths of the analytes. For the choice of the optimal voltage, the resolution of the Ni^{2+}/Li^+ pair can be omitted since it has the largest value at all voltages. Thus, the best voltage for the overall separation of the remaining metal ion pairs, *i.e.*, 15 kV, was selected as the working voltage.

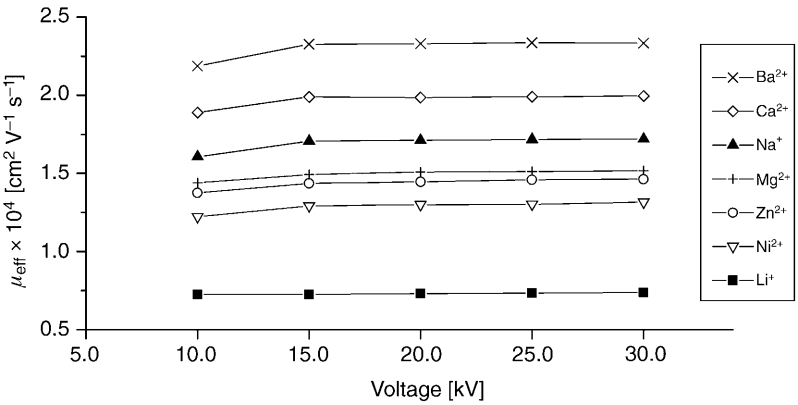


Fig. 4. *Dependence of EPMs on voltage*. Electrophoresis conditions:16 mM MAPP + 20 mM pyridine (pH 4.0); injection: 8 s at 0.8 p.s.i.; indirect detection at 254 nm.

Table 1. *Dependence of Resolution* (R_s) *of Metal Ion Pairs on Voltage* (V [kV])

V [kV]	R_s [a])					
	Ba^{2+}/Ca^{2+}	Ca^{2+}/Na^+	Na^+/Mg^{2+}	Mg^{2+}/Zn^{2+}	Zn^{2+}/Ni^{2+}	Ni^{2+}/Li^+
10	1.82	1.87	2.06	0.84	1.25	5.44
15	1.92	2.01	1.96	0.87	1.69	4.98
20	1.92	1.84	1.90	0.75	1.06	5.02
25	1.76	1.63	1.75	0.84	1.38	6.04
30	2.20	2.01	1.84	0.72	1.05	5.65

[a]) R_s calculated as $2(t_2 - t_1)/(w_1 + w_2)$, where t is migration time and w is peak width.

2.4. *The Presence of an Organic Modifier.* MeOH, MeCN, and acetone over a concentration range of 0–20% (v/v) were investigated as organic modifiers. The effects of each solvent on the separation are shown in *Fig. 5*. The EOM decreases sharply (from 3.27×10^{-4} to 2.47×10^{-4}, 3.17×10^{-4}, and 2.41×10^{-4} cm^2 V^{-1} s^{-1} for MeOH, MeCN, and acetone, respectively) when 5% (v/v) of organic solvent is added to the running electrolyte and continues to decrease slightly (down to 2.03×10^{-4}, 2.64×10^{-4}, 2.01×10^{-4} cm^2 V^{-1} s^{-1} for MeOH, MeCN, and acetone, respectively) by increasing the modifier concentration up to 20% (v/v), as expected, considering the increased viscosity of the electrolyte solution. Electrophoretic mobilities of metal ions and the resolution of peaks, especially between Mg^{2+}/Zn^{2+} and Zn^{2+}/Ni^{2+} pairs, were decreased by the addition of organic solvent. The use of an organic modifier is, therefore, not recommended.

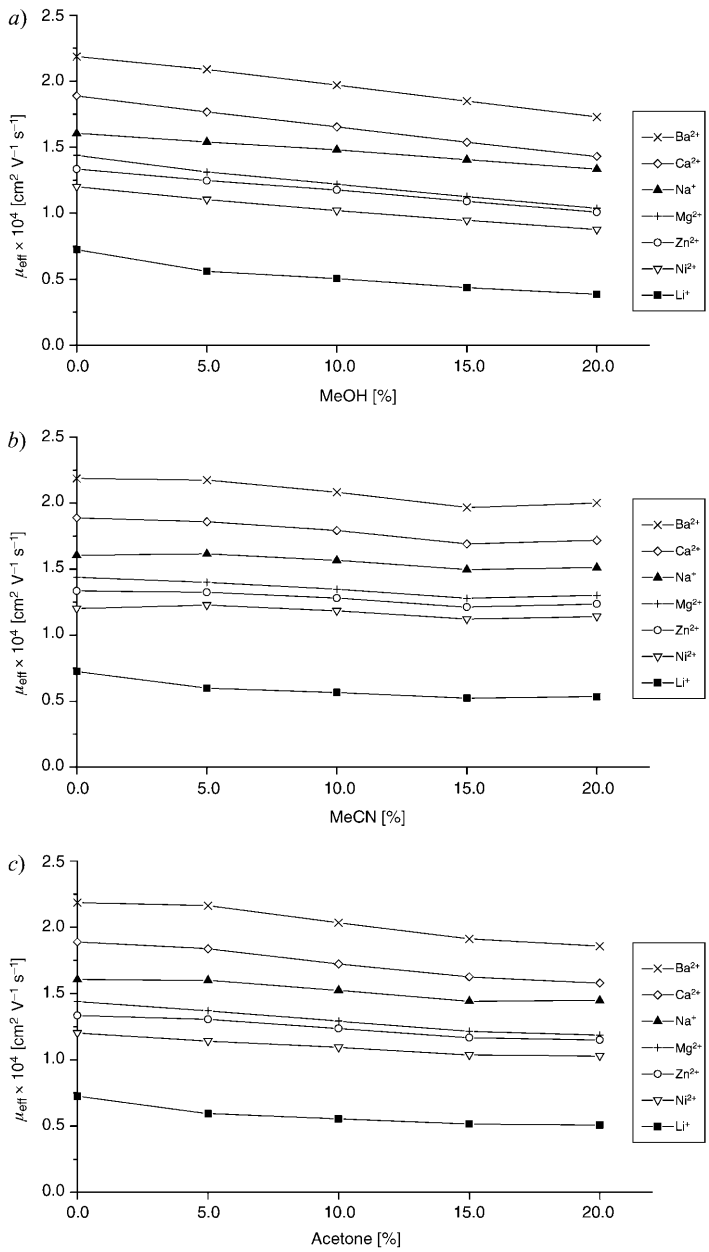


Fig. 5. *Effect of addition of* a) *MeOH,* b) *MeCN, or* c) *acetone on the separation*

3. Quantification. – The separation of seven metal ions under optimized conditions, *i.e.*, 16 mM MAPP, 20 mM pyridine (pH 4.0), and 15 kV, is shown in *Fig. 6*. The limits of detection (LODs), relative standard deviations (RSDs (%)), and regression lines are

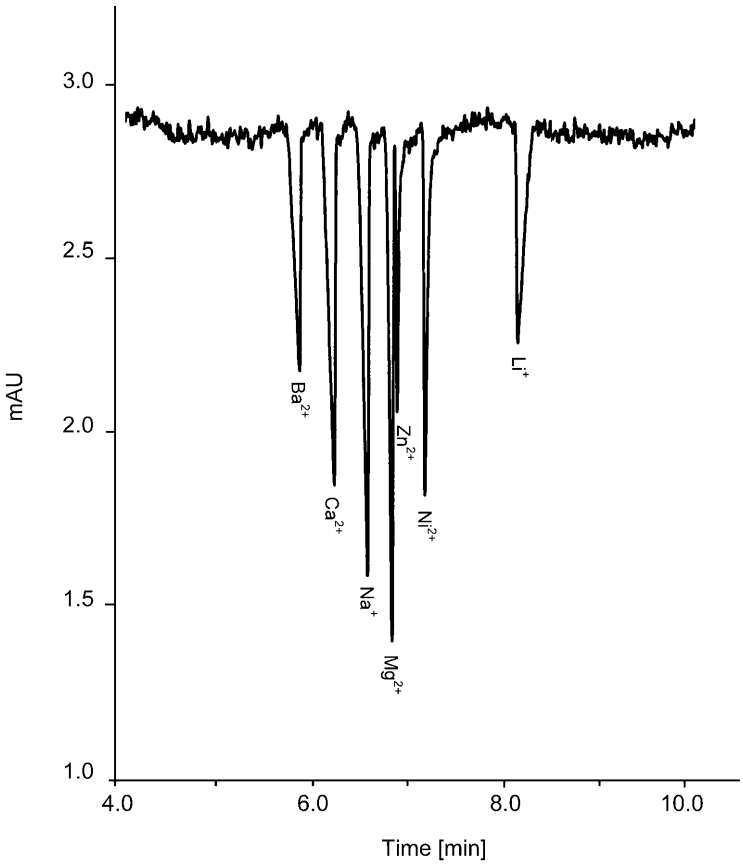


Fig. 6. *Electropherogram of metal ions* (Mg^{2+}, Ba^{2+}, Ca^{2+}, Na^{+}, Zn^{2+}, Ni^{2+}, and Li^{+}). Running electrolyte: 16 mM MAPP + 20 mM pyridine (pH 4.0); 15 kV; injection: 8 s at 0.8 p.s.i.; indirect detection at 254 nm; [Mg^{2+}] in the sample = 0.3 mM; remaining ions at 0.4 mM.

Table 2. *Analytical Characteristics of the Method*

Metal ion	LOD [mg/l][a])	RSD% [b])		Regression-line parameters[c])	R^2 [d])
		t [min]	Peak area [AU]		
Ba^{2+}	11.5	0.83	3.43	$y = 0.07 \times 10^3\, x - 4.82 \times 10^2$	0.9985
Ca^{2+}	2.28	0.87	0.85	$y = 0.30 \times 10^3\, x + 2.70 \times 10^2$	0.9997
Na^{+}	1.05	0.93	2.12	$y = 0.58 \times 10^3\, x - 0.65 \times 10^2$	0.9983
Mg^{2+}	0.77	0.96	4.93	$y = 0.50 \times 10^3\, x - 1.95 \times 10^2$	0.9997
Zn^{2+}	9.81	0.95	4.58	$y = 0.10 \times 10^3\, x - 3.85 \times 10^2$	0.9983
Ni^{2+}	6.45	0.98	1.35	$y = 0.18 \times 10^3\, x - 4.16 \times 10^2$	0.9989
Li^{+}	0.62	1.18	4.98	$y = 1.35 \times 10^3\, x - 1.63 \times 10^2$	0.9997

[a]) Limits of detection; signal/noise = 3. [b]) Relative standard deviation (RSD%); $n = 10$. [c]) y = corrected peak area [AU], x = conc. [mg/l]. [d]) Correlation coefficient.

reported in *Table 2*. In the equations of calibration graphs, y is the corrected peak area (arbitrary units), x is the concentration in mg/l, and R^2 is the correlation coefficient. The method was applied to the analysis of pharmaceutical vitamin-mineral tablets and water samples to evaluate the qualitative and the quantitative performance. *Table 3* gives the quantitative results obtained with five replicates of each sample. Recoveries of metal ions were estimated by adding a known amount of each metal ion into the sample. Vitamin tablet 1 was diluted 1:50 (v/v) for Zn^{2+} and 1:400 (v/v) for Ca^{2+} and Mg^{2+} analyses since the Zn^{2+} content was lower than the LOD under the higher dilution conditions used for Ca^{2+} and Mg^{2+} (*Fig. 7*). *Fig. 8* shows the analysis of interstitial water of a lacustrine surface sediment. The electropherograms of all samples gave migration times for the metal ions identical to those in *Fig. 7*.

Table 3. *Quantitative Sample Analysis Results*

Sample	Metal ion	Dilution factor	Quantity [mg/l][a])	Listed amount [mg/l][b])	Added amount [mg/l][c])	Total amount [mg/l][a])	Recovery [%]
Vitamin tablets							
1	Ca^{2+}	1:400	21.6 ± 0.7	20.0	10	31.2 ± 0.5	98.7
	Mg^{2+}	1:400	9.5 ± 0.3	10.0	10	19.4 ± 0.4	99.4
	Zn^{2+}	1:50	14.5 ± 0.4	15.0	10	23.9 ± 0.6	97.6
2	Ca^{2+}	1:400	6.3 ± 0.5	5.13	10	16.4 ± 0.3	100.6
	Na^{+}		1.94 ± 0.03	n.l.	10	12.6 ± 0.5	105.5
	Mg^{2+}		2.5 ± 0.2	2.12	10	12.4 ± 0.3	99.7
3	Ca^{2+}	1:4000	9.4 ± 0.2	10.0	10	19.7 ± 0.4	101.7
	Na^{+}		10.9 ± 0.4	n.l.	10	20.8 ± 0.5	99.5
Tap-water samples							
1	Ca^{2+}	1:1	20.6 ± 0.3	–	10	30.8 ± 0.3	100.6
	Na^{+}		4. 9 ± 0.3	–	10	14.8 ± 0.2	99.4
	Mg^{2+}		3.8 ± 0.2	–	10	13.6 ± 0.2	98.2
2	Ca^{2+}	1:1	22.4 ± 0.4	–	10	32.2 ± 0.5	99.3
	Na^{+}		10.1 ± 0.2	–	10	19.6 ± 0.2	97.5
	Mg^{2+}		3.72 ± 0.08	–	10	13.5 ± 0.5	98.4
Sediment[d])	Ca^{2+}	1:1	52.4 ± 0.5	–	10	62 ± 1	99.6
	Na^{+}		3.6 ± 0.2	–	10	13.6 ± 0.2	100.3
	Mg^{2+}		10.2 ± 0.2	–	10	19.7 ± 0.3	97.5

[a]) The results are based on analysis of five replicates. [b]) Declared by the manufacturer; n.l. = not listed. [c]) Added to samples to facilitate estimation of metal ion recovery. [d]) Supernatent interstitial water from a centrifuged lacustrine surface sediment sample.

4. Conclusions. – A simple and effective CE method for the analysis of metal ions based on the use of a new ion-pairing reagent, MAPP, has been developed. Pyridine was used for indirect UV detection of metal ions. Complete separation of the investigated metal ions could be performed in less than 4 min with good reproducibility and high peak symmetry. The potential of the proposed method was demonstrated by its application to the analysis, without any tedious sample preparation, of metal ions commonly found in environmental water and pharmaceutical vitamin samples. The

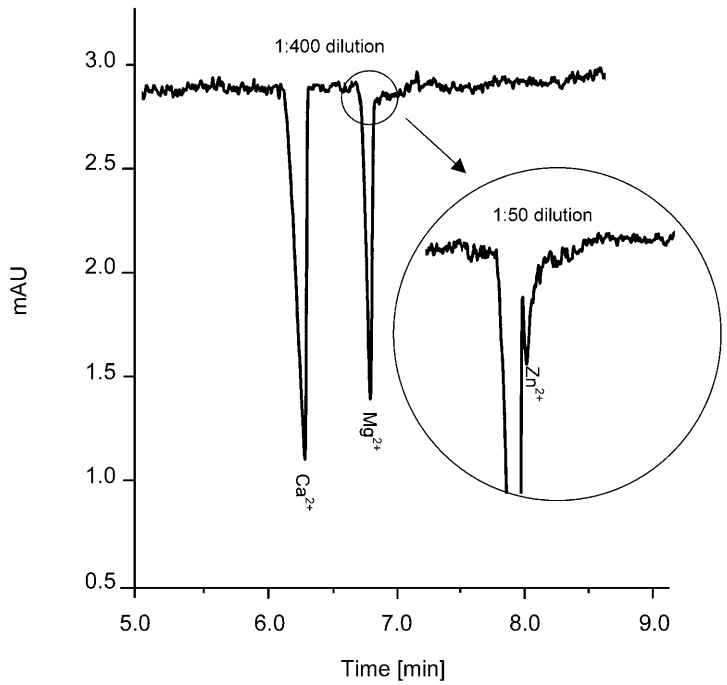


Fig. 7. *Electropherogram for vitamin tablet 1.* Electrophoresis conditions: see *Fig. 6.*

results obtained from the vitamin analyses are in good agreement with the contents cited by the manufacturers.

Experimental Part

1. *General.* All chemicals were purchased from *Fluka* (Buchs, Switzerland). Solns. of alkali (Li^+, Na^+), alkaline-earth (Mg^{2+}, Ca^{2+}, Ba^{2+}), and transition (Zn^{2+}, Ni^{2+}) metal ions were prepared from reagent-grade chloride or bromide salts. Vitamin-mineral preparations were obtained from local pharmacies in Switzerland and Turkey. Electrophoresis was performed on a *Beckman P/ACE MDQ* system (Fullerton, CA, USA) equipped with a photodiode-array UV detector from *Beckman Technologies* with detection at 254 nm. The system was controlled with *32 Karat*™ software; an uncoated fused silica capillary of 50 μm I.D. × 375 μm O.D., with total and effective lengths of 57 and 50 cm, resp. (*Agilent*, Switzerland) was used at 25°. ^{1}H- and ^{13}C-NMR Spectra: *Bruker DRX-500 Larmor*-frequency spectrometer; in D_2O, δ in ppm. MS: *Finnigan MAT SSQ-7000* mass spectrometer with an ESI interface: in *m*/z.

2. *Sample Preparation.* Stock solns. of each cation (10 mM) were prepared and diluted to the specified concentrations with deionized water before analysis. Vitamin-mineral tablets were dissolved in 25 ml deionized water and diluted to appropriate concentrations for CE analyses. Surface sediment samples from Motte Lake (France) were centrifuged at 6000 r.p.m. for 6 min before analysis of the supernatant.

3. *Methyl 3-Amino-3-(3-pyridinyl)propanoate Dihydrochloride* (*MAPP*, **5**). MAPP was synthesized as summarized in the *Scheme.* Nicotinic acid (= pyridine-3-carboxylic acid, **1**; 15 g, 0.12 mol) was dissolved in concentrated H_2SO_4 (0.33 mol) and refluxed with MeOH (100 ml) for 2 h to give the methyl ester **2** [41]; **2** (0.1 mol) was then converted to the β-keto ester **3** with sodium methoxide (0.15 mol) [42], and then NH_3 gas (0.24 mol) was bubbled through the soln. to give the enamine **4** [43]. Compound **4** was reduced by reaction with $NaBH_4$ (0.2 mol) in AcOH (1.56 mol) and THF (100 ml) [43] to give 9.35 g of the desired product **5** (43% yield). ^{1}H-NMR (500 MHz, D_2O): 3.34 (*dd*, 1 H); 3.39 (*dd*, 1 H); 3.74 (*s*, 3 H); 5.21 (*t*, 1 H); 8.22 (*m*, 1 H); 8.81 (*m*,

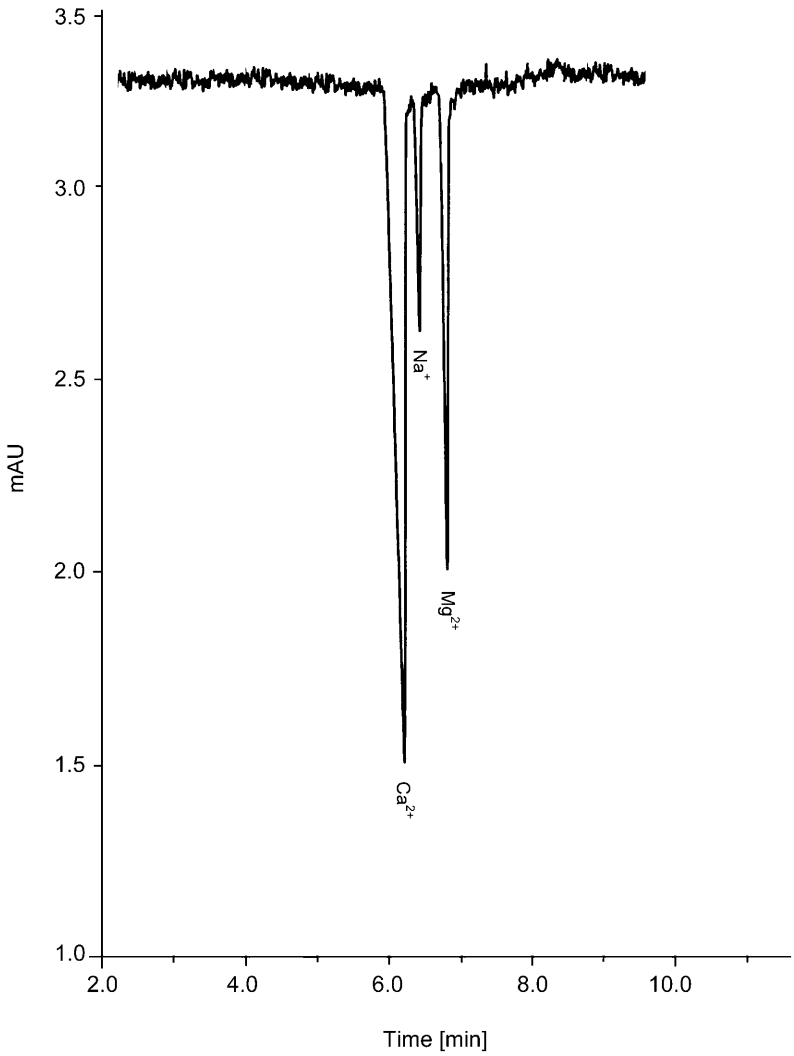
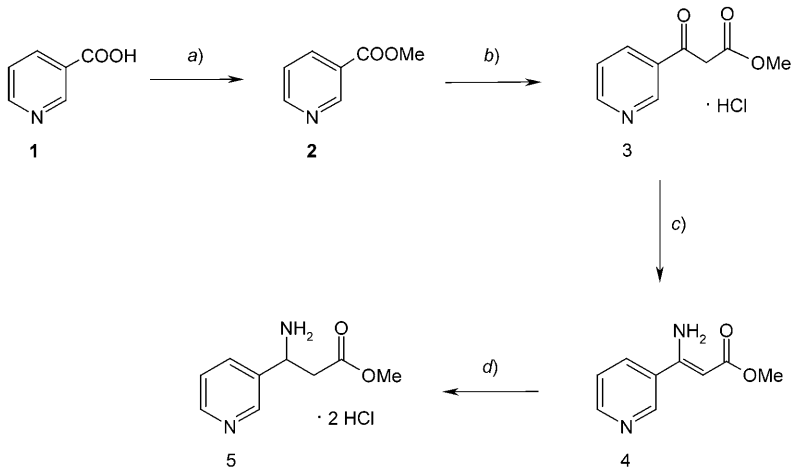


Fig. 8. *Electropherogram obtained for a sample of interstitial water from a lacustrine surface sediment.* Electrophoresis conditions: see *Fig. 6*.

1 H); 8.94 (*m*, 1 H); 9.05 (*s*, 1 H). ^{13}C-NMR/DEPT (125 MHz, D_2O): 171.1 (C=O); 145.5 (CH); 142.9 (CH); 141.5 (CH); 135.5 (C); 128.1 (CH); 52.9 ($CHNH_2$); 48.5 (MeO); 36.8 (CH_2). ESI-MS: 181 (*M*H$^+$, free base).

4. *Electrophoresis.* A new fused-silica capillary was rinsed with MeOH, 1M HCl, 1M NaOH, 0.1M NaOH, deinonized water, and running electrolyte for 5, 5, 30, 10, 5, and 10 min, resp. Between injections, the capillary was rinsed with running electrolyte for 2 min. pH Values were adjusted with 0.1M HCl. Hydrodynamic injection was performed at 0.8 p.s.i (1 p.s.i = 6894.76 Pa) for 8 s, and the applied voltage was set at 15 kV at the anode.

Scheme. *Synthesis of MAPP*



a) H_2SO_4, MeOH, 65°. *b*) NaOMe, MeCOOEt. *c*) Toluene, i-PrOH, HCOOH, 65°, NH_3. *d*) $NaBH_4$, AcOH, THF, HCl, – 5°.

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