# Determination of ammonium and metal ions by capillary electrophoresis–potential gradient detection using ionic liquid as background electrolyte and covalent coating reagent

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## Abstract

A capillary zone electrophoresis (CZE)–potential gradient detection (PGD) method coupled with field-amplified sample injection was developed to determine alkali metal, alkaline-earth metal, nickel, lead and ammonium ions. The capillary surface was coated with dialkylimidazolium-based ionic liquid and thus the electroosmotic flow (EOF) of the capillary was reversed. The buffer composed of 7.5 mM lactic acid, 0.6 mM 18-crown-6, 12 mM $\alpha$-cyclodextrin ($\alpha$-CD); it was adjusted to pH 4.0 by 1-hexyl-3-methylimidazolium hydroxide. The 11 cations were baseline separated within 14 min with $5.1–18.9 \cdot 10^4$ plates (for 40-cm-long capillary) in separation efficiency, and the detection limits were in the range of 0.27–7.3 ng/ml. The method showed good reproducibility in terms of migration time with RSD $\leq 0.90\%$ for run-to-run and $\leq 1.65$ for day-to-day assessment.
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## 1. Introduction

Capillary zone electrophoresis (CZE) is an increasingly used technique in the separation of ionic species because of its high resolution, short analysis time, little buffer consumption and simplicity in operation. Separation of metal ions by CZE has been actively studied because of the real-world requirements [1–8]. The chelating reagents such as lactic acid, melonic acid and tartaric acid can be added into the separation buffer to modify the mobilities of alkaline-earth metal ions, while inclusion complex reagents such as 18-crown-6 ether [4–7] and poly(ethylene glycol) [9,10] were used to separate the co-migrated ammonium and potassium ions, and to improve the resolution between some alkaline-earth metal ions.

Optical detection is commonly used in capillary electrophoresis (CE). Because most of metal ions are UV or fluorescence inactive, they have to be detected by indirect mode in which imidazole, nicotinamide, pyridine, benzylamine and benzimidazole have been used as background visualization ions [5–8,11–13]. However, indirect detection usually results in lower sensitivity than the direct mode [14,15]. Furthermore, optical detection presents a disadvantage for CE because the optical path length (diameter of the capillary) is generally less than 100 µm in order to favor better dissipation of Joule heat during the separation process. Potential gradient detection (PGD) is generally a conductivity detection method operated under d.c. mode. Although there are variations in the design of the working cell [16,17], they all work based on changes of the electric field strength during electrophoresis.

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Because the electric field strength is inversely proportional to the ionic mobility, a change in signal will be generated when the sample zone of different mobility than the buffer passes through the detecting electrodes. It is a universal detecting method for charge-carrying species. Like the optical method, the direct PGD method, in which the mobility of the analyte is higher than that of the background co-ion, offers higher detection sensitivity than the indirect mode. Although PGD is the most straightforward and simple method to be applied [14], it is not widely used possibly due to difficulties in achieving high sensitivity so far.

In this study, the separation behavior of metal and ammonium ions by CZE was investigated in the electrolyte system containing 1-hexyl-3-methylimidazolium (HMIM) based ionic liquid as background co-ion and lactic acid as chelating reagent, 18-crown-6 ether as inclusion reagent for the analytes in an IL-coated capillary. PGD was employed to detect the metal ions with addition of α-cyclodextrin (α-CD) into run buffer modifying the mobility of HMIM. Baseline separation of the 11 analytes ($Li^+$, $Na^+$, $K^+$, $Cs^+$, $Mg^{2+}$, $Ca^{2+}$, $Sr^{2+}$, $Ba^{2+}$, $NH_4^+$, $Ni^{2+}$, $Pb^{2+}$) were accomplished within 14 min with detection limits as low as sub-ppb level using the FASI technique.

## 2. Experimental

### 2.1. Chemicals

Imidazole and 3-chloropropyl-trimethoxysilane (CPT-MS) were purchased from Fluka (Buchs, Switzerland). 1-Bromohexane was product of Aldrich (Milwaukee, WI, USA). Dimethyl sulfoxide (DMSO), used as electroosmotic flow (EOF) marker, was obtained from Merck (Darmstadt, Germany). The ultrapure water in the experiments was prepared by a Milli-Q system (Bedford, MA, USA). α-CD was purchased from Sigma (Louis, MO, USA). 1-Hexyl-3-methylimidazolium bromide (HMIMBr) was synthesized by modifying the procedure described by Dzyuba and Bartsch [18]. It was converted to hydroxide form using Sigma Dowex I ion-exchange resin. The 1.0 mg/ml metal cation stock solutions were made by dissolving the appropriate weights of the nitrate salts.

### 2.2. Apparatus

Experiments were conducted on two CE systems. For the CE–UV system, a laboratory built system equipped with a Prince CE System (Lauerlabs, Emmen, The Netherlands) and a Linear Instrument (Reno, NV, USA) UVIS 200 detector was employed. For the CE–PGD system, an IA-P1 (CE Resources, Singapore, Republic of Singapore) was employed. Electropherograms were recorded with CSW17 software (DataApex, Prague, Czech Republic). Electrophoresis was conducted in fused silica capillaries of 50 μm I.D. × 360 μm O.D. (Polymicro Technologies, Phoenix, AZ, USA).

All solutions were filtered with 0.20 μm Minisart (Goettingen, Germany) filters and degassed in an ultrasonic bath.

### 2.3. Capillary coating

The imidazolium based ionic liquid was covalently coated onto the capillary surface by the procedures below. The fresh capillary was flushed with 1 M sodium hydroxide for 2 h, followed by deionized water and 1 h of 1 M hydrochloric acid; afterwards it was rinsed with deionized water and methanol for 10 min consecutively. It was flushed with nitrogen gas and heated to 120 °C overnight. In a nitrogen-filled glove box, CPTMS was filtered and introduced into the capillary by positive pressure. The capillary was sealed on both ends and kept at 90 °C for 15 h. After that, it was flushed with nitrogen to drive out the CPTMS residue and then rinsed with toluene. At room temperature, excess imidazole was dissolved in toluene; the supernatant was filtered and introduced into the capillary. The capillary was sealed and kept at 90 °C for 4 h, then it was rinsed with toluene, dichloromethane progressively and consequently dried with nitrogen under 70 °C for 1 h. It was rinsed with 1-bromohexane for 10 min, then sealed and heated under 80 °C for 10 h. The pretreated capillary was rinsed successively with 10 min of toluene, 10 min of methanol and 30 min of deionized water. It was coupled to a PGD cell afterwards.

### 2.4. Sample injection

For the hydrodynamic injection, the metal ions dissolved in run buffer were injected into the capillary by 50 mbar, 5 s. For the IL-coated capillary coupled with PGD, FASI was employed. The targets were dissolved in 50-fold diluted run buffer. Run buffer diluted to 1/200 was injected into the capillary by 50 mbar, 100 s as "water plug" that would be removed by EOF during FASI. The injection end was then dipped into the sample vial and a voltage of 4 kV was applied to electrokinetically inject the sample into the capillary. During this period, the metal ions migrated into the capillary under the positive electric field, while the injected plug was pushed out from the capillary inlet by the reversed EOF. The injection procedure was monitored by the PGD detector, which would indicate increasing signal with the pushing out of the plug of low conductivity and introduction of the sample. When the signal reached 90% of the maximum, the voltage was increased to 8 kV till all the analytes had been detected. For the same sample and procedure, the time passed before the baseline reached 90% of the maximum was recorded and the quantitative runs were time-controlled.

## 3. Results and discussion

### 3.1. Effect of buffer pH

Lactic acid is a weak complex reagent to the divalent and some of the monovalent ions such as lithium with a p$K_a$ value
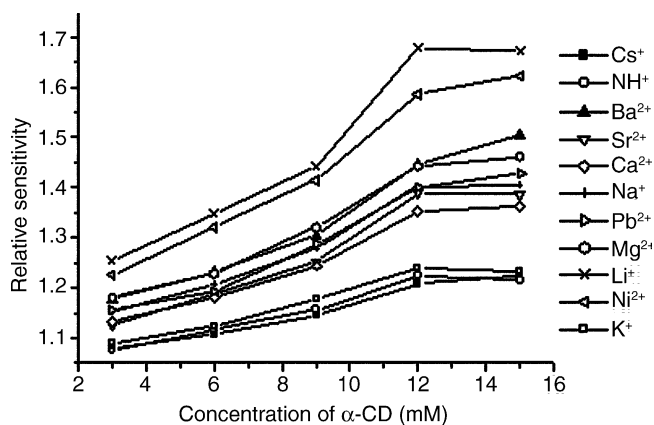
Fig. 1. Influence of α-CD on detection sensitivity of ions; buffer: 7.5 mM lactic acid, 0.6 mM 18-crown-6, added by desired concentration of α-CD and adjusted to pH 4.0 by 100 mM HMIM hydroxide; capillary: 40 cm; applied voltage: 8 kV; injection: 50 mbar, 15 s; detection: PGD.
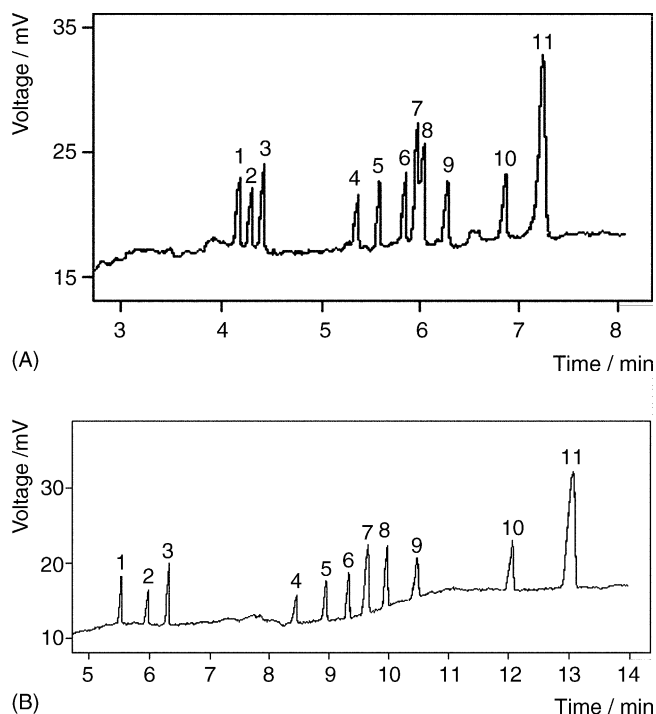


Fig. 2. Comparison of bare and IL-coated capillaries; capillaries: (A) bare silica; (B) IL-coated; buffer: 7.5 mM lactic acid, 0.6 mM 18-crown-6, 12 mM α-CD, adjusted to pH 4.0 by 100 mM HMIM hydroxide; peaks (concentrations in μg/ml): 1, $Cs^+$ (2.5); 2, $NH_4^+$ (0.1); 3, $K^+$ (0.5); 4, $Ca^{2+}$ (0.5); 5, $Sr^{2+}$ (1); 6, $Na^+$ (0.5); 7, $Pb^{2+}$ (5); 8, $Mg^{2+}$ (0.5); 9, $Ba^{2+}$ (2.5); 10, $Ni^{2+}$ (2.5); 11, $Li^+$ (0.5); others as in Fig. 1.

of 3.86 [19]; change of pH will have influence on the degree of complexation of the metal ions. As expected, the mobilities of magnesium, calcium, barium, lead and nickel ions decreased with the increasing buffer pH, which is attributed to the ion–lactate complex formed. Variation in pH would change the migration orders of some analytes, for example, sodium, calcium and lead: at pH 3.3, calcium and lead co-migrated followed by sodium; from 3.5 to 4.3, they migrated in the order of calcium, lead, sodium; as pH further increased, sodium migrated out between the other two cations; at pH 4.8, the order changes to be sodium (merged with calcium) and lead. The best resolution of the analytes was obtained at pH 4.0.

### 3.2. Effect of α-CD

Fig. 1 illustrates the variation of peak height ratio (of analyte in the α-CD containing buffer over that in non-α-CD buffer) versus concentration of α-CD. It can be seen that the peak height ratio increases significantly with α-CD concentration till 12 mM, from concentration of 15 mM, there was no notable amplification of the peak heights (not shown). Introduction of α-CD did not bring obvious influence on the mobilities or migration order of the metal ions. This may be because the complexing ability of α-CD to 18-crown-6 or metal ion is very weak [20]. We also found that peak symmetry and resolution deteriorated with increasing α-CD concentration due to the increasing mobility mismatch between analytes and co-ion; however, all the peaks were baseline resolved in the IL-coated capillary. Also, the analysis time slightly lengthened from ca 12.8 min of unmodified buffer to ca. 13.1 min in buffer containing 12 mM α-CD.

### 3.3. Influence of ionic-liquid coating

Resolution of the analytes in the IL-coated capillary significantly improved (Fig. 2); the shoulder-merging peaks of

magnesium and lead in the bare silica capillary were baseline separated. It was also determined that the separation efficiency of the 40-cm-long capillary, in terms of plate number, increased from $3.48–14.9 \cdot 10^4$ plates with bare capillary (excluding the peaks of magnesium and lead) to $5.1–18.9 \cdot 10^4$ plates with the IL-coated capillary probably due to the repulsive force between the cations and the positively charged capillary surface [21]. Although still showed drifting, the baseline was more stable than that of uncoated capillary. The IL coating favors low detection limit; however, the analysis time was longer.

The coating stability was assessed from the migration time variation of the last peak. An IL-coated capillary was used for electrophoresis for 9 days and the run buffer was left in it when no experiment was carried out. At the 26th day, the capillary was tested again with the same buffer and the migration time of lithium changed from 13.07 (1st day) to 12.80 min, suggesting the gradual degradation of the coating. Nevertheless, the separation efficiency of the analytes was still acceptable. The coating procedure was also tested in which a long capillary was coated following procedures in Section 2.3. After reaction, the capillary was cut into four pieces. The EOFs of the four pieces were measured and the relative standard derivation of these EOFs was 5.6%.

## 4. Conclusion

The resolution of the metal ions was greatly improved in the ionic-liquid coated capillary in which the EOF was reversed. With the buffer employed, the ions can be baseline separated. The FASI–CZE–PGD method developed can separate and detect the 11 ions with lower LOD than the conventional indirect optical detection method and with acceptable reproducibility.

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