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Simultaneous separation of inorganic anions and cations by capillary zone electrophoresis

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Abstract

A new capillary electrophoretic approach for simultaneous separation of fast anions and cations is demonstrated. Indirect UV detection at 214 nm in conjunction with electromigration sampling from both ends of the capillary was developed. Two electrolyte systems based on imidazole-nitrate and copper(II)-ethylenediamine-nitrate were investigated for the simultaneous separation of chloride, sulphate, hydrocarbonate, potassium, ammonium, calcium, sodium and magnesium ions. Experimental parameters that were evaluated included a nature of UV chromophore, pH of electrolyte, a nature of complexing agent. The method permits the excellent separation of three anions and five cations in only 4 min using electrolyte system containing 2.5 mmol l⁻¹ Cu(NO₃)₂, 5 mmol l⁻¹ ethylenediamine and 1 mmol l⁻¹ fumaric acid at pH 8.5 adjusted with tetraethylammonium hydroxide. © 1998 Elsevier Science B.V.

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1. Introduction

A simultaneous determination of inorganic anions and cations in a single analysis is both advantageous and necessary. The use of different methods, one for the anions and other for the cations, is time consuming.

Ion chromatography (IC) has been widely used in different operation modes for the simultaneous analysis of both inorganic anions and cations. The first technique is carried out on a dual-channel instrument in which the anions and cations are separated and determined via separate channels [1–3]. The term ‘simultaneous’ is hardly appropriate for this tech-

nique. Using this approach, two separate pumps, eluents, columns and detectors are required. A tandem operation [4–7] of anion- and cation-exchange columns in a single-channel IC system requires specialized hardware and sets stringent constraints on the choice of the mobile phase composition.

Another technique uses a single ion-exchange column with mixed anion- and cation-exchange capacity [8–10] but these columns cannot compete with conventional separation techniques with respect to selectivity and resolution.

The third technique deals with a development of suitable mobile phases. For example, complexation in the mobile phase can be used [11–13]. In this case metal ions are converted into anionic complexes and

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are separated together with other anions in an anion-exchange column. However, the determination of alkali and alkaline-earth cations using this technique is complicated because these cations exhibit very low complexation ability.

In recent years the use of capillary zone electrophoresis (CZE) for the analysis of ionic analytes has grown significantly. Because of its higher resolution, shorter analysis time, lower consumption of reagents, and greater simplicity in operation compared to IC [14], CZE has received a great deal of attention for the determination of inorganic ions. Numerous applications of CZE have been reported for the determination of alkali and alkaline-earth metals [15–17] and common inorganic anions [18–20] in various aqueous samples. However, as the separation by CZE is based on the difference in electrophoretic mobilities of the analytes, the determination of fast anions and cations in a single run under conventional CZE conditions is not possible.

In this paper, we report on the new CZE approach for a simultaneous separation of highly to moderately mobile anions and cations based on the electromigration sample injection from both ends of the capillary and indirect UV detection. Electrolyte systems based on imidazole-nitrate and copper(II)-ethylenediamine-nitrate have been developed for the simultaneous separation of NH_4^+ , K^+ , Na^+ , Ca^{2+} , Mg^{2+} , Cl^- , SO_4^{2-} and HCO_3^- ions.

2. Experimental

2.1. Instrumentation

Separations were performed on a P/ACE 2100 apparatus (Beckman Instruments Inc., Fullerton, CA, USA) equipped with a UV detector with wavelength filters (200, 214, 230 and 254 nm). Fused-silica capillaries (Polymicro Technology, Phoenix, AZ, USA) of 75 μm I.D. \times 375 μm O.D. and 90 cm and 72 cm total length (50 cm to the detector) were used. Samples were introduced by electromigration injection (5 kV; 5 s). System Gold software was used for data acquisition. Indirect UV detection was employed at 214 nm. All experiments were conducted at 25°C.

2.2. Reagents and solutions

All chemicals used were of analytical-reagent grade. Deionized water was obtained by passing distilled water through a Waters Milli-Q water-purification system (Millipore, Eschborn, Germany). Stock solutions (0.01 mol l^{-1}) of analyte ions were prepared from inorganic salts. Stock solutions and samples were diluted to the desired concentration with water before introduction.

Imidazole, ethylenediamine (En), tetramethylammonium chloride (TMACl), tetraethylammonium chloride (TEACl) and tetrabutylammonium hydroxide (TBAOH) were purchased from Sigma (Sigma, St. Louis, MO, USA). Dionex OnGuard A cartridges in the hydroxide form were used to convert TMACl and TEACl to hydroxides.

The working electrolytes were prepared fresh daily. All electrolyte solutions were filtered through a 0.45 μm membrane filter and degassed by ultrasonication.

2.3. Procedures

The capillary was rinsed with 1.0 mol l^{-1} sodium hydroxide and water for 5 min, then equilibrated with carrier electrolyte for 20 min at the beginning of each day. Between all electrophoretic separations the capillary was rinsed for 2 min with carrier electrolyte.

3. Results and discussion

In capillary zone electrophoresis separation is based on differences in electrophoretic mobility of analytes in the presence of an electric field. Consequently, separation of fast inorganic cations and anions in a single run is impossible by using conventional CZE.

In this work, we have investigated a new approach for a simultaneous CZE separation of highly to moderately mobile anions and cations based on electromigration sample introduction from both ends of the capillary. This approach requires the capillary with the detector window placed approximately in the middle of the capillary (Fig. 1). Two vials are filled with a sample solution. Then both capillary

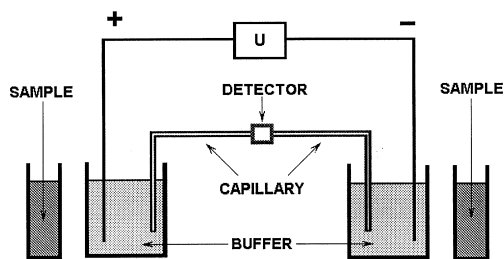


Fig. 1. Schematic diagram of CZE system used.

ends are soaked in the sample vials and electromigrative injection is performed. Using such system only the cations are injected from anodic capillary end and anions from cathodic end. After applying the voltage both cations and anions migrating in the opposite directions from different capillary ends will reach the detector. The migration times of cations with respect to anions can be additionally optimized by changing the ratio of the capillary lengths before and after detector window.

3.1. Separation in acidic electrolyte

The main problem in this approach is the selection of the electrolyte for the simultaneous indirect UV detection of both cationic and anionic analytes.

The use of indirect UV detection is required since alkali, alkaline-earth metal ions and common inorganic anions have no UV absorbance. The non-absorbing sample co-ions will displace by charge displacement the UV-absorbing electrolyte ions, which will induce a negative peak whose area is proportional to the sample co-ion concentration. Optimal detection sensitivity is observed under following conditions. Firstly, the electrophoretic mobilities of the UV chromophore and of the analytes should be almost identical for best peak symmetry. Secondly, the molar extinction coefficient of the UV chromophore should be high to achieve high detection sensitivity and to maximize the dynamic detection range. Thirdly, the transfer ratio, defined as the number of UV-absorbing ions of the electrolyte additive displaced by each analyte co-ion, should be maximized.

For the simultaneous indirect detection of cations and anions the carrier electrolyte must contain two UV chromophores – one cationic and the other

anionic. These conditions limit the choice of potential electrolyte components.

In our work two UV-absorbing cations – imidazole and copper(II) – were selected and compared for indirect detection of cationic analytes. The separation results with these co-ions have been reported for alkali and alkaline-earth cations [17,21]. Both these chromophores are well suited as an electrolyte because their electrophoretic mobilities are closely matched to many metal cations. In addition, imidazole and copper exhibit spectral characteristics required for indirect UV detection at 214 nm. The pK_{a1} value of imidazole is 6.9, therefore sensitive detection can be achieved in the pH range below 6. The pH of the copper-based electrolyte must also be kept in acidic medium because Cu^{2+} will hydrolyse at pH greater than 5.

Most of the separations of inorganic anions are performed in the presence of a chromate-based electrolyte [18]. However, at 214 nm, the UV absorption of chromate is at a minimum. Good candidates for background electrolytes for indirect detection at 214 nm are strongly absorbing nitrate and molybdate ions, but nitrate has a higher mobility in the selected pH range. In addition, chromate and molybdate anions cannot be used in copper based electrolyte because of the low water solubility of $CuCrO_4$ and $CuMoO_4$. So in our work we used nitrate containing electrolytes.

The migration times of tested ions in the investigated electrolytes are compared in Table 1. Both

Table 1
Migration times of ions observed in imidazole-nitrate and copper-nitrate electrolyte systems

Ion	Migration time (min)	
	4 mmol l ⁻¹ imidazole-NO ₃ ⁻	4 mmol l ⁻¹ Cu(NO ₃) ₂
Cl ⁻	4.08	4.25
SO ₄ ²⁻	4.16	4.41
K ⁺	3.23	3.42
NH ₄ ⁺	3.24	3.42
Ca ²⁺	4.25	4.88
Na ⁺	4.48	5.21
Mg ²⁺	4.52	5.27

Experimental conditions: electrolyte pH 4.5 was adjusted with HNO₃; capillary, 40 cm from anodic and 50 cm from cathodic end to detector; applied voltage, 25 kV; electromigration (5 s, 5 kV) injection from both ends of the capillary; indirect UV detection at 214 nm.

electrolyte systems show a similar separation ability, but the detection sensitivity for cations with the copper-nitrate electrolyte are about 3–4 times lower owing to the smaller molar absorptivity. For this reason, an imidazole-nitrate solution was selected as the electrolyte in this work.

The separation of NH_4^+ and K^+ is difficult due to their identical electrophoretic mobilities. The use of 18-crown-6 to solve this problem has been described in several publications [16,17], therefore we have not investigated this way.

Electrolyte pH plays an important role in CZE, because it affects both the overall charge of the analytes and the EOF. Using 5 mM of imidazole and 2 mM of HNO_3 , the natural pH of the electrolyte was 7.4. In order to examine the effect of pH on separation, all other experimental conditions were kept constant except of the different amounts of sulphuric acid used to adjust the pH of the electrolyte below 6.0. The effect of the pH on the migration times of six ions can be seen in Fig. 2. As the pH is lowered, the migration times of the anions decrease, whereas for the cations the opposite effect is ob-

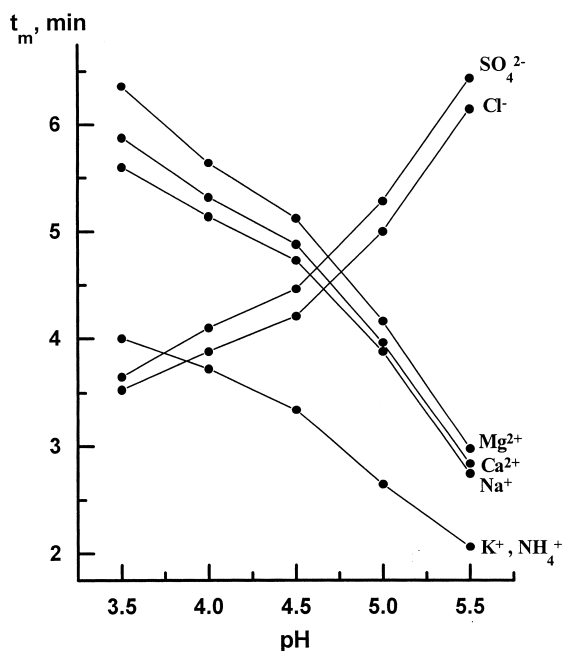


Fig. 2. Effect of electrolyte pH on migration of the ions. Electrolyte, 5 mmol l^{-1} imidazole, 2 mmol l^{-1} HNO_3 ; pH was adjusted with sulphuric acid; other conditions as in Table 1.

served. Since all the ions investigated are fully ionized in the pH range studied, the change in the migration times as the pH is decreased reflects the decrease in the EOF. Good resolution of ions studied is observed in the pH range between 4.0 and 4.5, but the presence of sulphate in the electrolyte causes a system peak in the baseline at the migration time corresponding to sulphate. For this reason the influence of several other acids on the separation of tested ions was investigated. This includes phosphoric, acetic, malonic, oxalic, tartaric and fumaric acids at pH 4.0. In the presence of phosphoric or acetic acids, Na^+ and Mg^{2+} could not be resolved. When tartaric acid was used, the resolution of Ca^{2+} and Mg^{2+} was poor and in the presence of oxalic acid the migration times of these cations significantly increased. The best separation of cations was obtained at pH 4.0 in the presence of malonic or fumaric acids, but the malonic acid caused a system peak directly after a peak of sulphate and, therefore, could influence the determination of sulphate. Because of this interference, malonic acid was not studied further. Fig. 3 shows an example of the simultaneous separation of anions and cations ob-

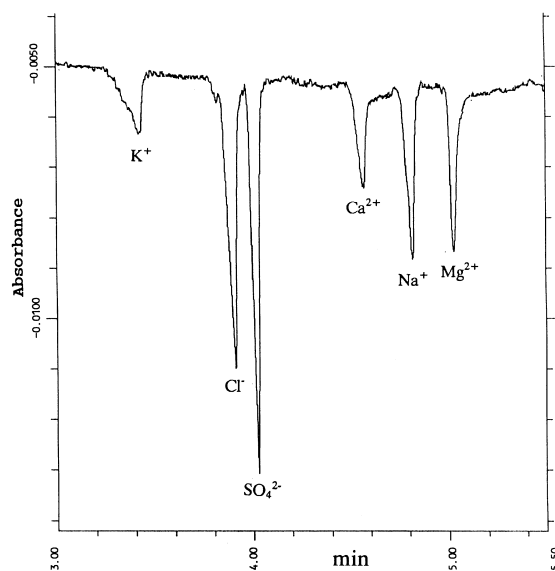


Fig. 3. Example of electropherogram for a mixture containing K^+ ($2 \cdot 10^{-4}$ mol l^{-1}), Cl^- ($2 \cdot 10^{-4}$ mol l^{-1}), SO_4^{2-} ($1 \cdot 10^{-4}$ mol l^{-1}), Ca^{2+} ($1 \cdot 10^{-4}$ mol l^{-1}), Na^+ ($2 \cdot 10^{-4}$ mol l^{-1}), and Mg^{2+} ($1 \cdot 10^{-4}$ mol l^{-1}). Electrolyte, 5 mmol l^{-1} imidazole, 2 mmol l^{-1} HNO_3 ; pH 4.0 was adjusted with fumaric acid; other conditions as in Table 1.

tained under optimum conditions. Baseline resolution of all components was achieved in less than 5 min. We also noted that other anions such as F^- and $H_2PO_4^-$ have relatively low mobilities so they do not interfere in the separation.

3.2. Separation in alkaline electrolyte

Alkaline electrolyte offers the possibility to detect hydrocarbonate, which under optimized above conditions exists as neutral molecules and, consequently, can not be determined. Moreover, at $pH > 8$ a separation of potassium and ammonium can be achieved by optimization of the pH-value of the electrolyte, so the addition of a crown-ether as proposed in [22] is not necessary. However, most of the cationic UV chromophores cannot be used in the alkaline medium because of their deprotonation.

In this work we have introduced copper(II) chelate with ethylenediamine (En) as cationic UV chromophore at the first time. This complex has following advantages. Firstly, $CuEn_2^{2+}$ exhibits a strong UV absorption over a wide wavelengths range below 260 nm. For example, the molar extinction coefficient of this cation at 254 nm is 3820, at 230 nm-6340, and at 214 nm-4400 $l/mol \cdot cm$. Secondly, the mobility of $CuEn_2^{2+}$ is only slightly lower than mobilities of common metal cations over a wide pH range, therefore detection can be achieved also in the alkaline medium (pH 8–10).

Preliminary experiments showed that hydrocarbonate, a weak acid, exhibits a low mobility in the pH range 8.0–8.5 and elutes far after the others analytes of interest. For this reason the capillary length after detector window was shortened from 40 cm to 22 cm. This change decreased significantly the migration times for anions (positive power supply) without a significant change of those for cations.

In order to determine the best separation conditions, a series of $2.5 \text{ mmol l}^{-1} Cu(NO_3)_2$ and $5 \text{ mmol l}^{-1} En$ carrier electrolytes with different pH values were tested. Fig. 4 illustrates the effect of electrolyte pH on the migration times of analytes. The results show that a good separation of K^+ and NH_4^+ ions is obtained at $pH \geq 8.5$. The migration times of calcium and magnesium decreases from pH 8.0 to 8.5 and increases when the pH is further

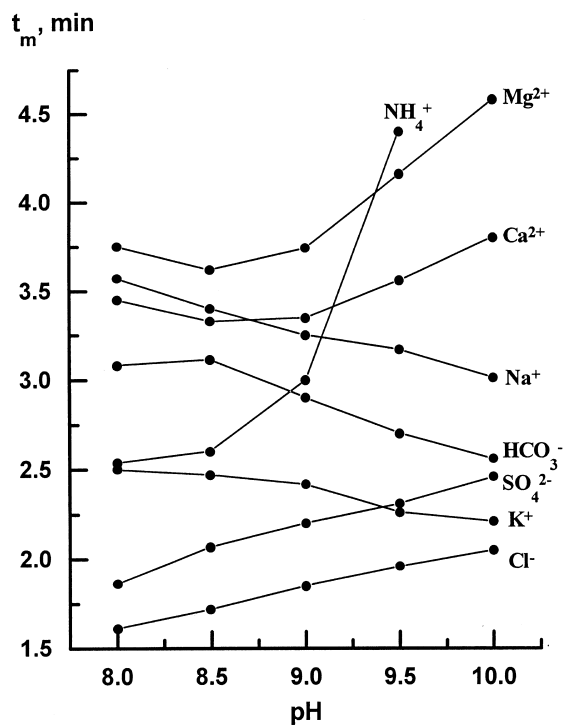


Fig. 4. Effect of electrolyte pH on migration of the ions. Electrolyte, $2.5 \text{ mmol l}^{-1} Cu(NO_3)_2$, $5 \text{ mmol l}^{-1} En$; pH was adjusted with En; capillary, 50 cm from anodic and 22 cm from cathodic end to detector; applied voltage, 25 kV; electromigration (5 s, 5 kV) injection from both ends of the capillary; indirect UV detection at 214 nm.

increased. The explanation of this effect probably is a partial formation of hydroxides. This also results in the significant peak broadening for these analytes. However, in the optimum pH range 8.0–8.5 the resolution between Ca^{2+} and Na^+ is poor. Fumaric acid therefore was also tested as a complexing agent in order to improve the separation selectivity of these cations.

The main problem in optimizing the separation with alkaline electrolyte was the selection of the base for the adjusting the pH after addition of weak complexing acid. Such bases as NaOH, KOH or NH_3 do not fit because they cause system peaks at the times corresponding to the migration times of sodium, potassium and ammonium ions, respectively. Several organic bases i.e. En, TMAOH, TEAOH and TBAOH were therefore studied. Good results were achieved by using TEAOH because the mobilities of

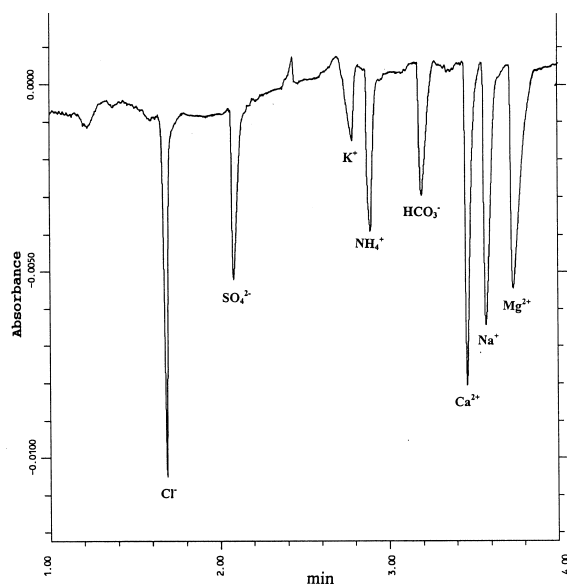


Fig. 5. Example of electropherogram for a mixture containing Cl^- ($2 \cdot 10^{-4} \text{ mol l}^{-1}$), SO_4^{2-} ($5 \cdot 10^{-5} \text{ mol l}^{-1}$), K^+ ($2 \cdot 10^{-4} \text{ mol l}^{-1}$), NH_4^+ ($3 \cdot 10^{-4} \text{ mol l}^{-1}$), HCO_3^- ($1 \cdot 10^{-4} \text{ mol l}^{-1}$), Ca^{2+} ($1 \cdot 10^{-4} \text{ mol l}^{-1}$), Na^+ ($2 \cdot 10^{-4} \text{ mol l}^{-1}$), and Mg^{2+} ($1 \cdot 10^{-4} \text{ mol l}^{-1}$). Electrolyte, $2.5 \text{ mmol l}^{-1} \text{ Cu}(\text{NO}_3)_2$, $5 \text{ mmol l}^{-1} \text{ En}$; 1 mmol l^{-1} fumaric acid, pH 8.5 was adjusted with TEAOH; other conditions as in Fig. 4.

investigated cations are higher than that of the TEA^+ , hence this co-ion shows a system peak at the time longer than the migration times of analytes.

On the basis of the performed experiments, the electrolyte containing $2.5 \text{ mmol l}^{-1} \text{ Cu}(\text{NO}_3)_2$, $5 \text{ mmol l}^{-1} \text{ En}$ and 1 mmol l^{-1} fumaric acid at pH 8.5 adjusted with TEAOH was found to give the best compromise between peak resolution, separation time and acceptable baseline noise. Fig. 5 shows an electropherogram of a mixed cation-anion standard under optimum conditions. The quantifications of ions in real samples will be an aim of forthcoming investigations.

4. Conclusions

This is the first report of the simultaneous CZE

separation of fast inorganic anions and cations, based on the electromigrative sample injection from both ends of the capillary. Electrolyte systems based on imidazole-nitrate and copper(II)-ethylenediamine-nitrate have been developed for indirect UV detection at 214 nm. The feasibility of the separation of chloride, sulphate, hydrocarbonate, potassium, ammonium, sodium, calcium and magnesium in a single run has been demonstrated.

The method reported in this paper is not optimized at this time for quantitation of ions. In further investigations therefore more emphasis will be paid to the quantitative aspects of the proposed method and to the analysis of different water samples.

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