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Utilisation of crown ethers in microemulsion electrokinetic chromatography for the separation of inorganic cations

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Abstract

A new method to influence the separation selectivity of inorganic cations in capillary electrophoresis is presented. This method combines the use of certain crown ethers to form complexes with a specific cation (changing its ionic radius/charge ratio and thereby its electrophoretic mobility) with partitioning of the crown ether/analyte complex between an aqueous phase and a pseudo-stationary phase, such as the oil droplet of a microemulsion. Several microemulsions, including uncharged oil droplets and oil droplets with different degrees of surface charge were tested to evaluate their ability to improve the separation of the selected analytes.

Keywords: Microemulsion electrokinetic chromatography; Crown ether; Inorganic cations; Separation selectivity

1. Introduction

Since the first instruments for capillary zone electrophoresis (CZE) became available commercially, this separation technique has gained increased interest and has found its way into a large number of application areas in analytical chemistry [1,2]. These fields of application range from the separation of macromolecules in biological materials [3] to the analysis of small inorganic ions [4]. Major benefits of CZE are the lack of a stationary phase, which can be deteriorated by matrix components in complex samples, as well as the quite simple (and easy to understand) mechanism involved in the separation of the analytes. In many cases this electrophoretic separation mechanism can be regarded as complementary with respect to the commonly employed separation techniques based on chromatographic principles. Nevertheless, this simple separation mechanism can also be seen as a drawback. Due to the lack of chromatographic interactions in CZE, all solutes are solely separated according to their difference in electrophoretic mobility, which is governed by their ionic radius/charge ratio. In the case of matching ionic radius/charge ratios or if uncharged analytes are present, co-migration occurs unless additional chromatographic interactions are introduced. Examples for such approaches are capillary electrochromatography (CEC) where a true stationary phase is employed [5,6], or micellar electrokinetic chromatography (MEKC) [7,8] and microemulsion electrokinetic chromatography (MEEKC) [9,10] where so-called pseudo-stationary phases are present and distribution between a mainly aqueous phase and micelles or oil-droplets, respectively, occurs. Another way to resolve co-migrating analytes that does not rely on the introduction of an additional separation mechanism, is to change either the ionic radius or the charge state of one of these analytes [11]. In the simplest case this can be done by variation of the pH of the carrier electrolyte leading to changes in the degree of dissociation of the solutes (if possible). A more sophisticated approach to achieve resolution of co-migrating analytes is their selective complexation affecting both the ionic radius and the charge of a particular molecule [12]. A typical example of this is the addition of a crown ether (commonly 18-crown-6 (18C6)) to overcome the co-migration of K^+ and NH_4^+ when the widely-used imidazole electrolyte is employed [13,14]. This crown ether (18C6) selectively forms a complex with

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the K^+ ion and thereby increases its ionic radius [15]. The separation of K^+ and NH_4^+ is therefore dependant on the increase in ionic radius for the complexed K^+ ion (primarily governed by the size of the 18-crown-6 molecule), as well as the formation constant of the respective complex. Unfortunately, if at all, these two parameters can only be varied in very narrow confines leading to a limited control of separation selectivities.

In general, electrophoretic techniques with pseudostationary phases are employed to enable the separation of uncharged analytes [16], but some reports describing their use for the separation of small ions also exist. Focusing on the separation of inorganic cations, the use of complexing agents and auxiliary separation processes to resolve comigrating analytes by capillary electrophoretic techniques has been reviewed recently by Boyce and Haddad [17]. The works presented in that review paper dealt largely with the electrophoretic separation of metal ions after their complete (pre-capillary) complexation with different complexing agents.

In the present paper a new approach for the separation of inorganic cations, in particular K^+ and NH_4^+ based on the use of crown ethers together with a microemulsion pseudostationary phase is described. The separation of these analytes occurs due to a combined separation mechanism, that of an increase in ionic radius for the complexed ion and the distribution of the hydrophobic complex between the aqueous phase and the oil phase of the microemulsion.

2. Experimental

2.1. Instrumentation

All experiments were performed on an Agilent 3D CE system (Agilent, Waldbronn, Germany), with a diode array detector. Injection was done by application of a pressure of 50 mbar for 5 s. A separation voltage of 30 kV was used with the capillary thermostatted at 25 °C. Indirect UV detection was employed with a 5 mM imidazole buffer as the probe. A detection wavelength of 390 nm was used with the reference wavelength set at 214 nm. As imidazole does absorb at 214 nm but not at 390 nm positive peaks are obtained for the analytes.

2.2. Materials and reagents

Fused silica capillaries (75 μm i.d. × 360 μm o.d.) obtained from Polymicro Technologies (Phoenix, AZ, USA) were used. Capillaries with a total length of 63 cm and an effective length of 54.5 cm were employed. New capillaries were conditioned by flushing with 1 M NaOH for 20 min followed by 0.1 M NaOH for 10 min and water for 10 min. Between runs the capillary was flushed for 1 min with 0.1 M NaOH, 1 min with water and finally 4 min with the BGE. Water was purified using a Milli-Q (Millipore, Bedford, MA, USA) system. KCl, $MgCl_2$, NH_4Cl, $CaCl_2$, Tris(hydroxymethyl)aminomethane (Tris), and acetic acid were obtained from Merck (Darmstadt, Germany). Imidazole, dicyclohexano-18-crown-6 (DCH-18C6), benzo-18-crown-6 (B-18C6), dibenzo-18-crown-6 (DB-18C6) and sodium dodecylsulfate (SDS) were obtained from Fluka (Buchs, Switzerland); polyoxyethylene(23)lauryl ether (Brij-35) and 18-crown-6 (18C6) were obtained from Aldrich (Milwaukee, WI, USA), octane, hexane, butanol, ethyl acetate and *N*,*N*-dimethylformamide were obtained from J.T. Baker (Deventer, The Netherlands) and $NaClO_4$ was obtained from Riedel-de Haën (Seelze, Germany). Sudan Red III was obtained from Carl Roth (Karlsruhe, Germany). All chemicals were of analytical grade. Hydrogen dodecylsulfate (HDS) was prepared by exchanging the sodium of SDS for protons by ion exchange (Dowex AG 50W-X8, 200–400 mesh, H^+ form).

2.3. Preparation of microemulsions

A 5 mM imidazole buffer was prepared by titrating an imidazole solution to pH 4.5 with acetic acid (unless otherwise stated). Where Tris–HDS was used as the surfactant, HDS was added to the imidazole solution and titrated to pH 4.5 with Tris. The microemulsion was prepared by mixing 0.8 g of octane, 6.6 g butanol, 0.0529 g 18C6 (or 0.0624 g B-18C6 or 0.0746 g DCH-18C6), 2 g total of Brij-35 and SDS, and sonicating for approximately 5 min. 90.6 g of imidazole buffer was then added and sonicated again for 30 min. The microemulsions containing just Brij-35 and no SDS generally required at least 24 h to clear. All microemulsions were filtered prior to use through a 0.45 μm filter. Standards were prepared in the running buffer.

2.4. Determination of the electroosmotic flow (EOF) and effective mobilities

The EOF was determined by recording the migration time of *N*,*N*-dimethylformamide (λ = 230 nm) due to its high polarity and low inclusion into the stationary phase. The following equation was used to calculate the EOF:

$$\mu_{\mathrm{eof}} = \frac{L_{\mathrm{T}} L_{\mathrm{D}}}{V t_{\mathrm{m}}}$$

where μ_{eof} is the mobility of the EOF, L_{T} and L_{D} the total length and the length of the capillary from the injection end to the detector respectively, V the applied voltage and t_{m} the migration time observed for the selected EOF marker (DMF). The effective mobilities (μ_{eff}) of the analytes were calculated by taking t_{m} at the top of the peak, and using $\mu_{\mathrm{eff}} = \mu - \mu_{\mathrm{eof}}$.

The mobility of the oil droplet was measured by recording the t_{m} of the highly hydrophobic Sudan Red III (λ = 525 nm), which should be almost exclusively incorporated into the pseudostationary phase.

3. Results and discussion

According to the principal intention of this work a number of experimental setups were tested to evaluate the influence of different parameters on the separation of a model mixture of inorganic cations (including the critical pair K^+/NH_4^+, as well as Ca^{2+} and Mg^{2+}). Several types of separation systems were investigated, including an aqueous electrolyte, micellar and microemulsion systems in combination with various complexing agents. In addition to these basic parameters, some other factors were evaluated such as the type of surfactant used for the micellar or microemulsion system and the use of organic solvents as modifiers in the aqueous phase of the electrolyte system.

3.1. Effect of the separation system

As expected, a purely aqueous carrier electrolyte based on imidazole without the addition of a crown ether did not show any separation for the critical pair K^+/NH_4^+. To rule out any direct interaction with the pseudo-stationary phase, such as the oil droplets present in microemulsions, the experiment was repeated employing microemulsion systems without the crown ether present. Octane was used as the oil-phase, with one system containing only Brij-35 as the stabilizing surfactant and one employing a mixture of two surfactants, namely SDS and Brij-35. Once again no separation of the two co-migrating analytes was observed. Utilising an aqueous electrolyte with 2 mM crown ether [0.0529%, 0.0624% or 0.0746% (all w/w) for 18C6, B-18C6 or DCH-18C6, respectively] as the complexing agent, differences in the effective mobility between K^+/NH_4^+ were found to be in the range of 6% (for DCH-18C6) and approximately 10% (for 18C6 and B-18C6). In a separation system including an additional pseudo-stationary phase the following situation is encountered. Besides the first equilibrium:

$$K^+_{(\text{free,aq})} + E^-_{(\text{free,aq})} \rightleftharpoons \{K^+_{(\text{complexed})}E^-\}_{(\text{aq})} \quad (1)$$

where E^- is the anionic counterion that is closely associated with the complexed cation to maintain electroneutrality, a second equilibrium exists, namely:

$$\{K^+_{(\text{complexed})}E^-\}_{(\text{aq})} \rightleftharpoons \{K^+_{(\text{complexed})}E^-\}_{(\text{pseudo-stationary phase})} \quad (2)$$

This second equilibrium represents the partition of the crown ether/analyte complex between the aqueous phase of the electrolyte and the micelles (or oil droplets of the microemulsion) and is mainly governed by the hydrophobicity of the employed crown ether. Comparing the results for different systems all including DCH-18C6 (the most suitable crown ether for the selected separation problem, as discussed in detail in the following paragraph of the manuscript), an increase in separation was observed when a system with a pseudo-stationary phase (either micelles or oil droplets) was used. This can clearly be seen from Table 1, comparing the mobilities obtained with an aqueous electrolyte, a MEKC and a MEEKC system. As can be deduced from these data, the microemulsion showed a higher increase in separation than the micellar system. This is in accordance with the literature reporting that due to the decreased rigidity of the oil droplets (compared to conventional micelles) hydrophobic analytes can more easily penetrate and enter the organic core of this type of pseudo-stationary phase [18]. An additional factor influencing the differences in migration between the complexed and the non-complexed species is the mobility of the oil droplets. Using uncharged surfactants such as Brij-35, the pseudo-stationary phase is transported with the EOF, whereas the addition of a negatively-charged surfactant like SDS leads to a mobility of the oil droplets directed toward the anodic end of the capillary which is counterdirected to the cathodic EOF. A microemulsion with uncharged oil droplets (Brij-35 as the surfactant) led to a slight increase in separation (compared to the purely aqueous system) with a difference in the effective mobilities (μ_{eff}) of less than 10% for K^+ and NH_4^+. Introducing negative charges by exchanging some Brij-35 with SDS, resulted in an increase in separation ($\Delta\mu_{\text{eff}} > 15\%$) for these two ions (shown in Fig. 1), with a Brij-35:SDS ratio of 1.5:0.5. The decrease in separation observed with the SDS microemulsion for the doubly-charged Ca^{2+} and Mg^{2+} ions may be due to dominating electrostatic interactions of these ions with the SDS present. The broad fronting of the NH_4^+ peak in Fig. 1a is not observed if a separation system based on 18C6

Table 1
Effective mobilities and the percent difference in μ_{eff} of K^+ and NH_4^+, with various separation systems

Separation system	μ_{eff} ($\times 10^{-5}$ cm^2/V s)		Percent difference in μ_{eff}
	K^+	NH_4^+	
Buffer[a], DCH-18C6[b]	65.8	69.7	5.6
Brij MEKC[c], DCH-18C6[b]	61.6	67.1	8.2
Brij MEEKC[d], DCH-18C6[b]	51.1	56.6	9.7
Brij/SDS (3:1) MEEKC[b], DCH-18C6[b]	40.0	47.1	15.1
Brij/Tris–HDS (1:1) MEEKC[d], DCH-18C6[b]	28.7	41.3	30.5

[a] 5 mM imidazole, pH 4.5.
[b] 0.0746% DCH-18C6.
[c] 2% total surfactant in 5 mM imidazole (pH 4.5).
[d] 2% total surfactant, 0.8% octane, 6.6% butanol in 5 mM imidazole (pH 4.5).

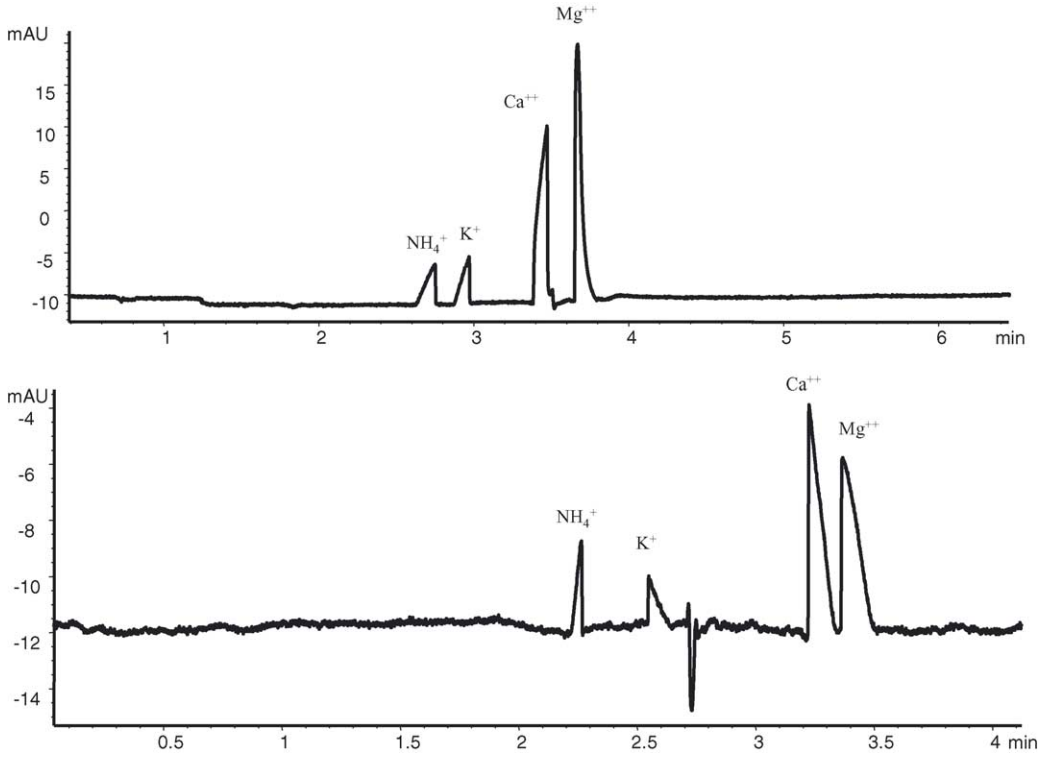

Fig. 1. Separation of four inorganic cations using two different microemulsions. Capillary: 63 cm (55 cm to detector) × 75 μm i.d. Microemulsion buffer: 0.8% (w/w) *n*-octane, 6.6% (w/w) *n*-butanol, 90.6% (w/w) 5 mM imidazole buffer (pH 4.5), 2 mM DCH-18C6 and, in (a) 2% (w/w) Brij-35, and in (b) 0.5% (w/w) SDS and 1.5% (w/w) Brij-35. Peaks: (1) NH_4^+, (2) K^+, (3) Ca^{2+}, (4) Mg^{2+} (all 1 mM).

as crown ether is used. A possible explanation for this effect is, that peak shapes are also influenced by the fact that separation in one case mainly takes place in the aqueous phase (18C6) whereas if a more hydrophobic crown ether is employed (e.g. DCH-18C6) an additional equilibrium namely the distribution of the complex between the aqueous phase and a pseudostationary phase exists. A secondary effect of the addition of SDS to the system is a change in the anionic counterion from acetate (present in the imidazole buffer) to dodecylsulfate. Dodecylsulfate would dominate as the counterion due to its excess concentration and greater ion-pairing ability than acetate. This could have a significant effect on the transport of the complexed cation into the stationary phase (see Eq. (2)), which requires an anionic counterion to accompany it. Thus a more lipophilic counteranion would allow greater partitioning into the oil stationary phase. A possible way to investigate this hypothesis is the design of almost identical separation systems which only differ in the nature of the predominantly present anionic solute. For this reason a microemulsion containing ClO_4^- instead of acetate was prepared. In the Hofmeister series of increasing polarisability of anions it can be seen that ClO_4^- and CH_3COO^- lie at opposite ends of the polarisability spectra, when it comes to simple anions [19]:

$$CH_3COO^- < Cl^- < NO_3^- < I^- < SCN^- < ClO_4^- \quad (3)$$

SDS, with its long hydrophobic chain as well as a strong charge, belongs to a different group of compounds, namely surfactants. Hence they congregate at the oil/water interface. Instead of CH_3COOH used to adjust the pH of the imidazole buffer added to the Brij-35 microemulsion, $HClO_4$ was employed, with a final concentration of 0.032 mM required to bring the pH to 4.5. An extra 0.12 g of $NaClO_4$ was added resulting in a total of 2.032 mM ClO_4^- concentration, to ensure that it was in excess compared to the Cl^- counterion of the standard cations. No change was seen in the separation between K^+ and NH_4^+, suggesting that the nature of the anion does not play a crucial role in this process.

A further increase in separation may be achieved by a higher concentration of SDS leading to additional negative charges on the surface of the oil droplets and thereby a more pronounced migration counterdirected to the EOF. Unfortunately this increase in SDS concentration is accompanied with detection interference due to the high concentration of Na^+ ions present. For this reason the Na^+ content of SDS was reduced by exchanging the Na^+ ions with the less mobile Tris-H^+ ions, which are significantly less competing with the analytes with respect to the exchange of the probe molecules in indirect detection. Employing this system with 1% Tris–HDS, a further increase in separation ($\Delta\mu_{eff} > 30\%$) could be observed. A similar effect was also found for the MEKC system. The mobility of the oil droplets was measured by sudan red III added to the standards

Table 2
Effective mobilities and the percent difference in μ_{eff} of K^+ and NH_4^+, employing different crown ethers

Separation system[a]	μ_{eff} ($\times 10^{-5}$ cm^2/V s)		Percent difference in μ_{eff}	log $P_{o/w}$[b]
	K^+	NH_4^+		
Brij/SDS (3:1) MEEKC, 18C6	46.1	52.4	12.1	−0.81
Brij/SDS (3:1) MEEKC, B-18C6	46.9	52.9	11.3	2.28
Brij/SDS (3:1) MEEKC, DCH-18C6	40.0	47.1	15.1	2.79

[a] 2% total surfactant, 0.8% octane, 6.6% butanol, 0.0529% 18C6 (or 0.0624% B-18C6 or 0.0746% DCH-18C6) in 5 mM imidazole.
[b] Data calculated using $P_{o/w}$ estimation software [15].

and was (for $n = 3$) $-10.34\ (\pm 0.03) \times 10^{-5}$ cm V^{-1} s^{-1} and $-15.28\ (\pm 0.02) \times 10^{-5}$ cm V^{-1} s^{-1} for the 0.5:1.5 and 1:1 SDS:Brij-35 ratio microemulsion, respectively.

3.2. Effect of the nature of the crown ether

As already mentioned above, different crown ethers have been included in this study. 18C6 is the most-commonly used complexation agent for the separation of K^+ and NH_4^+ in CZE. In addition to that, three other crown ethers were investigated, namely B-18C6, DCH-18C6 and DB-18C6 all with extra hydrophobic groups to increase the partitioning into the oil droplets. This is reflected in their calculated log $P_{o/w}$ values [20] as can be seen from Table 2. The perfect choice of crown ether would be that which shows a far better solubility in the oil phase than in the water phase. Of the available crown ethers, DB-18C6 was ruled out as it was insufficiently soluble in the small amount of octane used in the microemulsion. 18C6 was too soluble in the water phase [as indicated by the log $P_{o/w}$ (octanol–water partition coefficient) values], so it did not partition sufficiently into the oil phase. Interestingly, with its $P_{o/w}$ value lying between the extrema (18C6 on the one side and DCH-18C6 and DB-18C6 on the other side), B-18C6 was found to behave more like 18C6 than the more hydrophobic species. As can be seen from the data in Table 2, DCH-18C6 showed best results with respect to the desired effects. Okada and Sugaya [21] reported that complexation of cations increased from 62 to 90% by increasing the crown ether concentration from 4 to 7 mM. Two 0.5:1.5 Brij-35:SDS microemulsions were prepared with 6 and 8 mM (0.1116 and 0.1489 g, respectively, in 50 g electrolyte) DCH-18C6. The addition of 6 mM DCH-18C6 resulted in a mobility difference of 42.5% between K^+ and NH_4^+ compared to just 15% obtained with the original separation system containing 2 mM of the complexing agent. The 8 mM crown ether microemulsion resulted in an even greater separation, with the K^+ peak overlapping with the Ca^{2+} peak. Thus, the concentration of the crown ether is an important parameter in the selectivity control of this system.

3.3. Other parameters effecting separation

Partition of the crown ether or the crown ether/analyte complex between the aqueous phase and the oil droplets can be influenced by several changes in the separation system. As mentioned above, crown ethers with a different degree of hydrophobicity were studied in this work. In addition to that, other parameters exist that can be used to modify partition behaviour. One possible approach is to change the organic solvent forming the core of the oil droplets. A microemulsion containing hexane was chosen for its lower log $P_{o/w}$ than octane (3.29 and 4.27, respectively), possibly allowing greater partitioning of the crown ether. No significant difference was noted, which may be due to an insignificant difference in hydrophobicity, or may simply be in agreement with previous findings that the nature of the oil has only little effects on the separation in MEEKC [22]. Another possibility is to add an organic solvent in order to influence the solubility of hydrophobic substances (in this case the crown ether) in the aqueous part of the microemulsion. In accordance with earlier investigations focusing on the separation mechanisms in MEEKC, 2-propanol and methanol were chosen for this purpose [23]. Although amounts up to 20% of these solvents were added, no significant change in the separation selectivity was observed.

4. Conclusions

The method proposed in this work allows the improved separation of co-migrating analytes employing a combination of two effects; firstly, the introduction of selective complexing agents and secondly, the partition of these complexes between the two phases of the employed MEKC or MEEKC systems. Further changes in separation selectivity can be obtained by controlling the migration velocity of the pseudo-stationary phase compared to that of the bulk flow (i.e. the EOF) present in the selected system. This concept is described here for the separation of inorganic cations combining an MEEKC system with crown ether complexation, however, it is applicable to a range of analytes with complexing agents that show some degree of hydrophobicity. For indirect UV detection as done in the present work there is certainly a loss of sensitivity due to the presence of relatively high concentrations of cations of the detergent. In case of analytes that are suited for direct UV detection, this drawback no longer exists.

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