Optimization of conductivity detection of low-molecular-mass anions in capillary zone electrophoresis

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Abstract

The optimization of background electrolyte compositions for capillary zone electrophoresis in combination with conductivity detection focusing on maximal detector response is discussed. A theoretical approach pointing out the influence of the electrolyte co- and counter-ion mobilities on the detector signal has been developed. Using this model, running buffer compositions providing optimum S/N ratios for the selected analytes could be calculated. The results derived from these examinations have been verified by experimental investigations, namely the determination of inorganic and organic anionic solutes. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Although the majority of commercially available instruments for capillary zone electrophoresis (CZE) are still equipped with spectrophotometric detectors and in particular with UV absorbance detectors, conductivity detection (CD) has gained an increasing importance especially in the analysis of inorganic anions and other low-molecular-mass anionic solutes like carboxylic acids. A number of papers have been published, dealing with the application of CZE in combination with CD for the determination of these solutes in a variety of matrices [1–14]. For this purpose, different detector configurations featuring suppressed [15,16] and non-suppressed CD have been developed. Since an end-column, non-sup-

pressed conductivity detector for CZE has become commercially available, an intensified use of this detection method can be observed [6].

Comparing indirect UV detection and CD for the determination of low-molecular-mass anionic solutes, the latter technique can be regarded as superior with respect to its improved detection sensitivity leading to lower limits of detection (LODs) and quantification (LOQs) [17]. A number of papers on the development and optimization of background electrolytes (BGEs) for the analysis of the solutes mentioned above using indirect UV detection have been published [18–25]. Some of these works describe methodologies for carrier electrolyte optimization procedures in general [18–22], whereas others focus on the theoretical background of indirect UV detection [23–25]. Regarding the use of CD in combination with CZE, no sufficient investigations discussing the influence of carrier electrolyte ingredients, namely the electrolyte co-ion and counter-ion, can be found.

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In this paper the various factors which should be considered in selecting an appropriate BGE in order to optimize the sensitivity as well as the signal-to-noise (S/N) ratio of CD in CZE will be discussed. For this purpose a mathematical description of the detection signal as a function of the mobilities of the carrier electrolyte co-ion and counter-ion was derived, based on the theory developed for CD. The results taken from these theoretical considerations have been verified using different BGE compositions for the analysis of a set of low-molecular-mass anionic compounds including inorganic anions and carboxylic acids as model substances.

2. Theoretical

The operating principles of CD in combination with CZE can best be described by comparison of the conductance of a typical BGE with that of a sample zone during their migration through the detection cell. Throughout this work we restricted our considerations to the detection of anionic solutes, but the detector response equations presented in this manuscript are equally applicable to cationic analytes. The following expression can be used to calculate the conductivity G of an electrolyte solution containing a single species being partially dissociated into the ions E^+ and E^-:

$$G = \frac{(\lambda_{E^+} + \lambda_{E^-})c_E \alpha_E}{10^{-3}K} \tag{1}$$

where λ_X is the limiting equivalent ionic conductance of the ion X, c_x and α_x stand for the concentration and the degree of ionization, respectively and K refers to the cell constant of the detector. For a typical running buffer composition used in CZE with CD (prepared for example from 3-[*N*-morpholino]-2-hydroxypropanesulfonic acid (MOPSO) and Tris in a neutral pH range) the following equation is valid for the conductance of the BGE G_{BGD}

$$G_{BGD} = \frac{\lambda_{E^+} c_{E^+}^C \alpha_{E^+} + \lambda_{E^-} c_{E^-}^C \alpha_{E^-}}{10^{-3}K} \tag{2}$$

where the superscript C refers to the carrier electrolyte. Assuming that the running buffer consists of

two completely dissociated species, Eq. (2) can be simplified as follows:

$$G_{BGD} = \frac{\lambda_{E^+} c_{E^+}^C + \lambda_{E^-} c_{E^-}^C}{10^{-3}K} \tag{3}$$

The conductance of the sample zone G_{SMPL} on the other hand can be expressed in the following way:

$$G_{SMPL} = \frac{\lambda_{E^+} c_{E^+}^S + \lambda_{E^-} c_{E^-}^S + \lambda_{A^-} c_{A^-}^S}{10^{-3}K} \tag{4}$$

where the superscript S refers to the sample zone and A^- to the anionic analyte. The influence of the corresponding cation A^+ can be neglected because it migrates in the opposite direction as soon as an appropriate voltage is applied and thereby cannot reach the detector. A relation between the concentrations c of E^+ and E^- in the carrier electrolyte and the sample zone can be established as follows:

$$c_{E^+}^S = c_{E^+}^C + (1 - k_A)c_{A^-}^S \tag{5}$$

$$c_{E^-}^S = c_{E^-}^C - c_{A^-}^S k_A \tag{6}$$

where k_A is the transfer ratio (which is the number of equivalents of the carrier electrolyte co-ion which is going to be displaced by each equivalent of the analyte ions) [23] of the analyte anion A^-. Substituting Eqs. (5) and (6) into Eq. (4) leads to:

$$G_{SMPL} =$$
$$\frac{\lambda_{E^+}\left[c_{E^+}^C + (1-k_A)c_{A^-}^S\right] + \lambda_{E^+}\left(c_{E^-}^C - c_{A^-}^S k_A\right) + \lambda_{A^-} c_{A^-}^S}{10^{-3}K} \tag{7}$$

The signal measured by the detector, namely the difference in conductance between sample zone and BGE, ΔG can be expressed as follows:

$$\Delta G = G_{SMPL} - G_{BGD}$$
$$= \frac{c_{A^-}^S\left[\lambda_{E^+}(1 - k_A) - \lambda_{E^-} k_A + \lambda_{A^-}\right]}{10^{-3}K} \tag{8}$$

From this equation it can be deducted that ΔG depends on the limiting equivalent ionic conductance of the analyte ion A^-, of the carrier electrolyte co-ion E^-, of the counter-ion E^+ and the transfer ratio k_A. Considering the situation encountered in CZE it should be taken into account that k_A is

determined by the Kohlrausch regulating function (KRF) [26]:

$$KRF = \sum_i \frac{c_i z_i}{|\mu_i|} \tag{9}$$

Using this Eq. (9), assuming monovalent ions only, the following equation can be derived:

$$k_A = \frac{\mu_{E^-}(\mu_{A^-} + \mu_{E^+})}{\mu_{A^-}(\mu_{E^-} + \mu_{E^+})} \tag{10}$$

where μ_x refers to the absolute value of the mobility of the ion X. As the limiting equivalent ionic conductance λ_x is a function of μ_x, is has to be substituted by:

$$\lambda_x = F\mu_x \tag{11}$$

where F refers to the Faraday constant. Regarding Eq. (8), substituting λ_X by the term taken from Eq. (11), k_A by the term taken from Eq. (10) and assuming a known value for μ_{A^-} or λ_{A^-}, respectively, ΔG can be expressed as a function of the mobilities of the carrier electrolyte co-ion E^- and counter-ion E^+:

$$\Delta G(\mu_{E^+}, \mu_{E^-}) =$$

$$\frac{c_A^S \cdot \left\{ \mu_{E^+} F \left[1 - \frac{\mu_{E^-}(\mu_{A^-} + \mu_{E^+})}{\mu_{A^-}(\mu_{E^-} + \mu_{E^+})} \right] - \mu_{E^-} F \frac{\mu_{E^-}(\mu_{A^-} + \mu_{E^+})}{\mu_{A^-}(\mu_{E^-} + \mu_{E^+})} + \lambda_{A^-} \right\}}{10^{-3} K} \tag{12}$$

Eq. (12) finally allows to calculate the magnitude of the detected signal depending on the mobilities of the BGE ions and therefore can be used as a tool to find an optimum carrier electrolyte composition (E^- and E^+) for a maximum detector response ΔG for a given analyte A^-.

3. Experimental

3.1. Instrumental

The CE instrument employed was a Crystal 310 (Thermo Bioanalysis, Santa Fe, CA, USA) equipped with a Crystal 1000 conductivity detector (Thermo Bioanalysis) connected to a HP 3359 data acquisition system (Hewlett-Packard, Palo Alto, CA, USA). Separations were carried out using Con Cap I fused-

silica capillaries (Thermo Bioanalysis) with an effective length of 70 cm and an inner diameter of 50 μm. Before each analysis the capillary was rinsed with running buffer for 3 min. Injection was performed in a hydrodynamic mode at the cathodic side by applying a pressure of 25 mbar for 0.2 min. A potential of -30 kV was used for separation of anionic or $+30$ kV for cationic species.

3.2. Reagents and samples

For all solutions 18 MΩ high-purity water obtained from a Milli-Q System (Millipore, Marlborough, MA, USA) was used. Carrier electrolytes were prepared from MOPSO, LiOH, NaOH, KOH, triethylamine (TEA), arginine (Arg) and Tris (all purchased from Sigma, St. Louis, MO, USA). Tetradecyltrimethylammoniumbromide (TTAB) (Merck, Darmstadt, Germany) was used for electroosmotic flow (EOF) reversal. Standard solutions were made by dissolving the appropriate salts or carboxylic acids (purity>99%) in high purity water. For measuring the mobilities of the electrolyte counterions a pH 6.0 running buffer consisting of 30 mM 2-(N-morpholino)ethanesulfonic acid (MES) and 30 mM histidine (His) was employed.

4. Results and discussion

For the calculations based on Eq. (12), chloride has been selected as a model substance to demonstrate the influence of the BGE composition on the magnitude of the detector response achieved in CZE with CD. Assuming that the concentration of chloride in the sample zone is 1 mM, its mobility – 0.711 cm^2 kV^{-1} s^{-1}, the cell constant of the detector K 75 cm^{-1} and the Faraday constant 96.485 C mol^{-1}, ΔG can be plotted as a function of the absolute values of the carrier electrolyte co-ion and counter-ion mobilities. The resulting data can be seen in Fig. 1. Regarding the influence of the electrolyte co-ion it can be observed that (as might be expected) a larger value for ΔG is obtained at an increased difference in mobilities between the analyte ion and the electrolyte co-ion. On the other hand, an inverse situation is encountered with respect to the electrolyte coun-
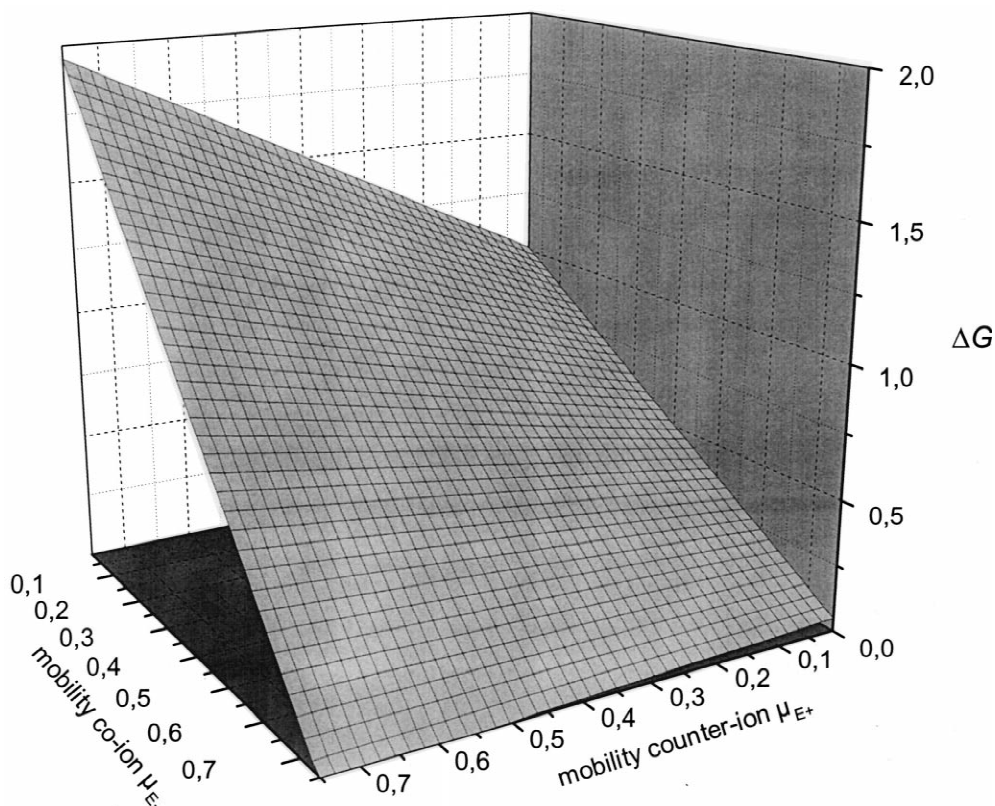
Fig. 1. Difference in conductance ΔG (in μS) calculated for chloride from Eq. (12) as a function of the absolute value of the electrolyte co-ion and counter-ion mobility (in cm^2 kV^{-1} s^{-1}).

ter-ion as higher counter-ion mobilities lead to increased detector response.

To understand this fact it is necessary to study the dependence of the transfer ratio k_A as a function of the mobilities of the carrier electrolyte ions E^- and E^+ (Eq. (10)) shown in Fig. 2. As can be seen from this plot, for chloride as analyte increased values for k_A are obtained with high mobilities of the electrolyte co-ion and low mobilities of the electrolyte counter-ion. Whereas in indirect UV detection high transfer ratios are desirable to achieve a maximum difference between the UV absorbance of the BGE and the sample zone, the inverse situation is encountered in the case of direct CD. The signal achieved in CD is composed of the conductance provided by the electrolyte co-ion and counter-ion plus the conductivity of the sample ion. Regarding the sample zone, the decrease in conductivity resulting from the displacement of the carrier elec-

trolyte co-ion (and in case of k_A values >1 also resulting from displacement of the excess electrolyte counter-ion which is necessary to maintain electroneutrality) must be compensated by the analyte. Therefore high transfer ratios k_A values lead to a decreased detector response related to the increased need in compensation of the conductance provided by the displaced electrolyte ions. Summing up the results obtained from Figs. 1 and 2, a carrier electrolyte consisting of a low-mobility co-ion and a medium to high-mobility counter-ion would be desirable for a maximum signal intensity.

Nevertheless an additional fact has to be taken into account. In general, a high detector response does not yet guarantee an optimum result; instead a high signal intensity combined with a low baseline noise is desired, in order to detect even very low concentrations of the selected solute. Assuming that the baseline noise is directly proportional to G_{BGD}, the
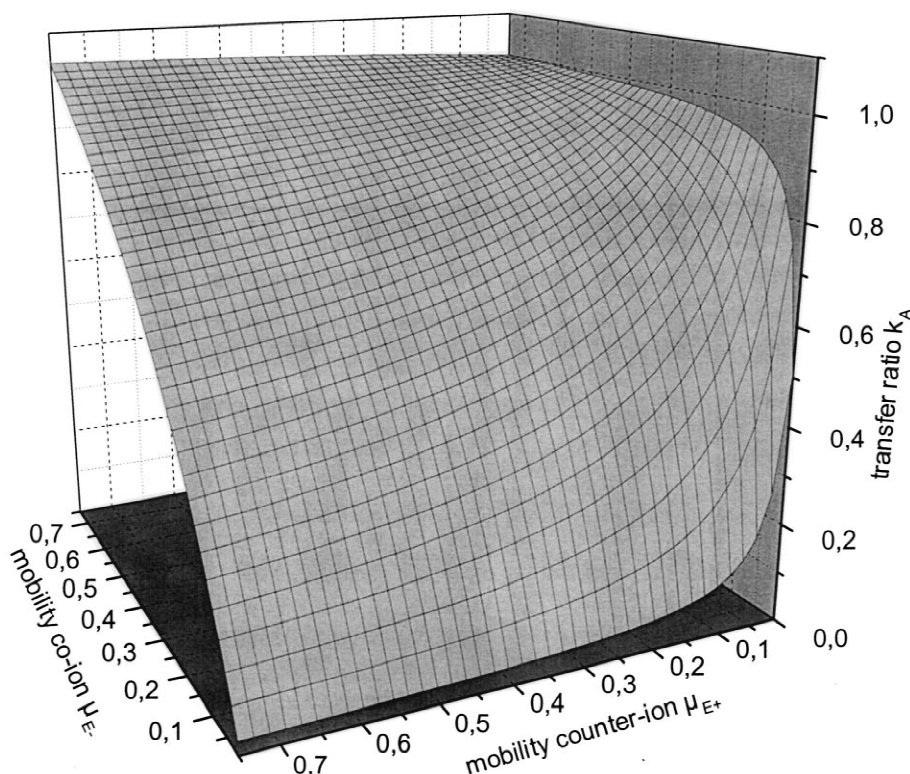
Fig. 2. Transfer ratio k_A calculated for chloride from Eq. (10) as a function of the absolute value of the electrolyte co-ion and counter-ion mobility (in cm^2 kV^{-1} s^{-1}).

term ΔG divided by G_{BGD} must be maximized. This term can be calculated from the following expression:

$$\frac{\Delta G(\mu_{E^+}, \mu_{E^-})}{G_{BGD}(\mu_{E^+}, \mu_{E^-})}$$

$$= \frac{c_{A^-}^S \left\{ \mu_{E^+} F \left[1 - \frac{\mu_{E^-} - (\mu_{A^-} + \mu_{E^+})}{\mu_{A^-} - (\mu_{E^-} + \mu_{E^+})} \right] - \mu_{E^-} F \frac{\mu_{E^-} - (\mu_{A^-} + \mu_{E^+})}{\mu_{A^-} - (\mu_{E^-} + \mu_{E^+})} + \lambda_{A^-} \right\}}{\mu_{E^+} F c_{E^+}^C + \mu_{E^-} F c_{E^-}^C}$$

(13)

Fig. 3 is obtained by plotting the results from Eq. (13) in the same way as those from Eq. (12) in Fig. 1. It can be seen from this plot, that after replacing ΔG by a parameter expressing the S/N ratio namely $\Delta G/G_{BGD}$, the optimum carrier electrolyte for the detection of chloride consists of a low-mobility co-ion as well as a low-mobility counter-ion.

Whereas the conclusions concerning the influence of the electrolyte co-ion mobility on the detector response drawn from the theoretical part of this paper can be regarded as obvious [11,12,17], no experimental investigations focusing on the electrolyte counter-ion can be found in the literature. For this reason we prepared a set of carrier electrolytes listed in Table 1, consisting of MOPSO, the electrolyte co-ion, 0.15 mM TTAB for EOF reversal and a pH of 7.0, adjusted by a variety of basic substances (KOH, NaOH, LiOH, TEA, Tris and Arg) providing electrolyte counter-ions within a wide range of mobilities, to justify the theoretical results by experimental data. The value for G_{BGD} (in μS) shown in this table was directly taken from the detector display. Fig. 4 presents the electropherograms for a set of anionic solutes (chloride to valerate) employing the BGEs described above; it is obvious that less mobile electrolyte counter-ions provided increased S/N ratios. This fact matches with the theoretical considerations expressed in Eq. (13) and plotted in Fig. 3. It can be deduced, that these increased S/N
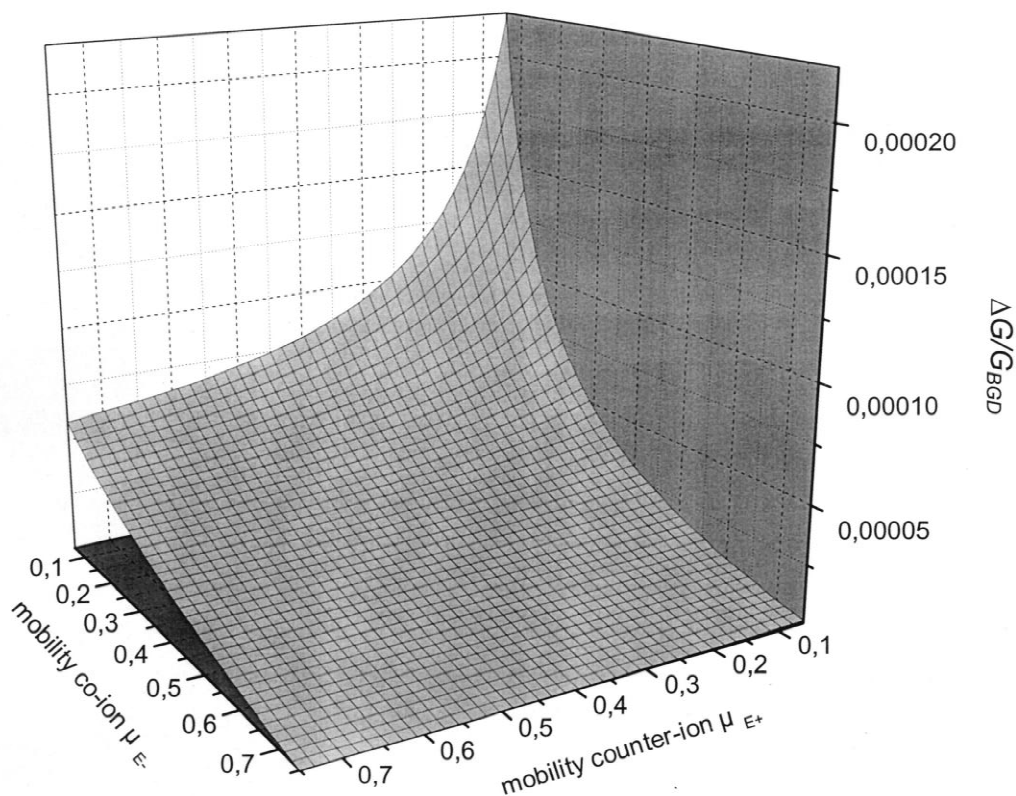
Fig. 3. $\Delta G/G_{BGD}$ calculated for chloride from Eq. (13) as a function of the absolute value of the electrolyte co-ion and counter-ion mobility (in cm^2 kV^{-1} s^{-1}).

ratios can be exclusively assigned to the fact that the decrease in detector response caused by low-mobility counter-ions (as can be seen from Fig. 1) is over-compensated by the lower background conductivity (leading to a lower baseline noise) of BGEs prepared from these less mobile species. The correlation between peak areas and the mobilities of the electrolyte counter-ions employed can be seen from Table 2, demonstrating this relationship using the peak obtained for formate as example and a number of different electrolyte counter-ions covering a wide range of electrophoretic mobilities.

Table 1
Carrier electrolyte compositions employed in this work

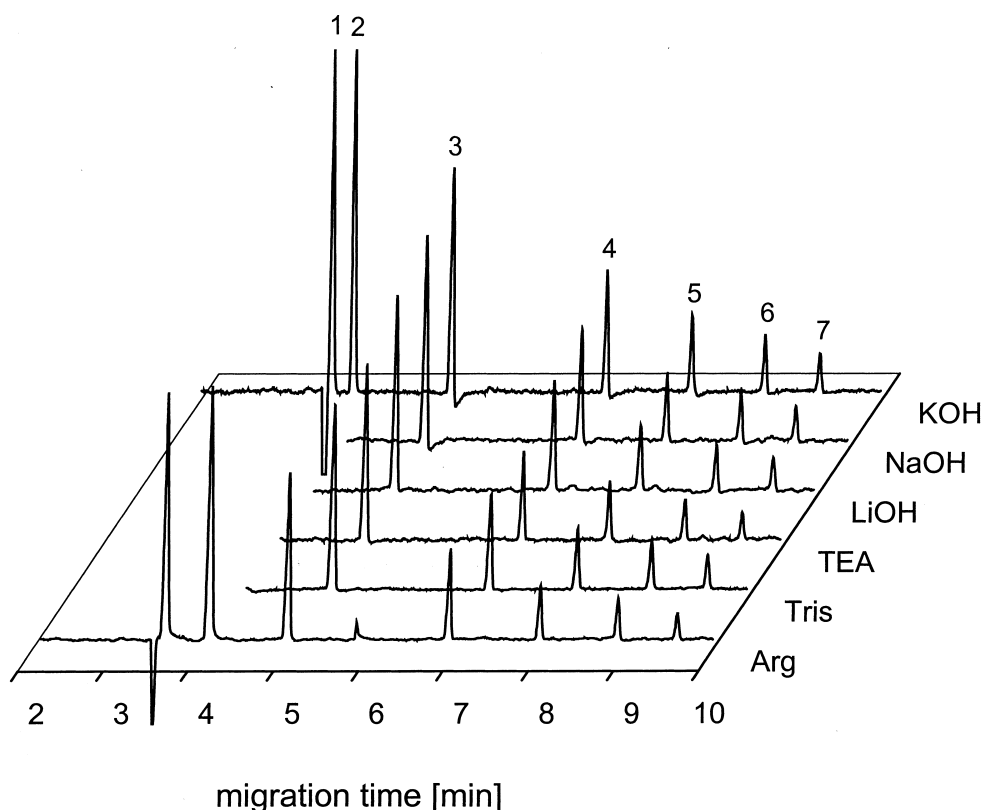| c(MOPSO) (mmol/l)	Counter-ion	c(counter-ion) (mmol/l)	μ(counter-ion) (cm^2 kV^{-1} s^{-1})	G_{BGD} (μS)
25	K$^+$	14	0.693	15.7
25	Na$^+$	14	0.474	12.5
25	Li$^+$	14	0.363	11.1
25	TEA$^+$	14	0.297	10.0
25	Tris$^+$	16	0.259	9.2
25	Arg$^+$	15	0.229	8.3

Fig. 4. Electropherograms obtained for a standard mixture of anions (10 mg/l each) using different carrier electrolyte compositions (to increase the clearness of this plot the first minutes of the electropherograms 2–5 are not shown). Carrier electrolyte: 25 mM MOPSO, 0.15 mM TTAB, pH adjusted to 7.0 with Arg, Tris, TEA, LiOH, NaOH and KOH. Peaks: 1=chloride; 2=sulfate; 3=formate; 4=acetate; 5=propionate; 6=butyrate; 7=valerate.

4. Conclusion

The results obtained in this work indicate that the theoretical considerations derived for the description of CD in combination with CZE provide a useful tool for carrier electrolyte optimization. Corresponding to the theoretical predictions presented in this paper, our experiments verified that BGEs made from low-mobility co- and counter-ions provide optimum results regarding the S/N ratios obtained for a number of selected analytes. Less mobile electrolyte counter-ions lead to a decrease in detector response; nevertheless this effect is overcompensated by the fact that the S/N ratio is improved by the low background conductivity of running buffers prepared from low-mobility co- as well as counter-ions.

Table 2
Peak areas obtained for a 10 mg/l formate standard solution as a function of the electrolyte counter-ion mobility

Counter-ion	μ(counter-ion) (cm^2 kV^{-1} s^{-1})	Peak area (arbitrary units)
K$^+$	0.693	117 865
Na$^+$	0.474	120 203
Li$^+$	0.363	123 032
TEA$^+$	0.297	132 369
Tris$^+$	0.259	143 382
Arg$^+$	0.229	146 518

Acknowledgements

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