Optimisation of separation selectivity in capillary zone electrophoresis of inorganic anions using binary cationic surfactant mixtures

Anthony H. Harakuwe, Paul R. Haddad*, Wolfgang Buchberger

Department of Chemistry, University of Tasmania, GPO Box 252C, Hobart, Tasmania 7001, Australia

Abstract

Mixtures of different cationic surfactants such as dodecyltrimethylammonium bromide and tetradecyltrimethylammonium bromide can be used as carrier electrolyte additives in order to manipulate separation selectivity and resolution of inorganic anions in capillary zone electrophoresis. The migration order is dependent on both the total surfactant concentration and the ratio of the two surfactants. The optimisation of separation selectivity is demonstrated for a nine-anion standard mixture as well as for complex matrices such as Bayer liquor.

1. Introduction

While the majority of applications of capillary zone electrophoresis (CZE) continue to deal with the separation of large biomolecules, this technique has also become increasingly important for the determination of inorganic and low-molecular-mass organic ions [1]. It evens holds out the prospect of becoming an alternative to the well-established technique of ion chromatography.

Separation selectivity of CZE depends on differences in the effective mobilities of the species to be separated. Several ways can be exploited for manipulation of separation selectivity: changing the pH of the carrier electrolyte for species undergoing protonation or deprotonation reactions; adding ion-pairing reagents or complexing reagents in order to affect charge and size of the species; use of organic solvents in the carrier electrolyte for changing the hydration and size of the ions; employment of pseudostationary phases such as micelles or polymeric ions in the carrier.

CZE of inorganic anions is generally done in a coelectroosmotic mode with injection at the cathodic side and detection at the anodic side [2–4]. This arrangement requires the reversal of the electroosmotic flow (EOF) which can be achieved by addition of cationic surfactants such as hydrophobic quaternary ammonium ions (EOF modifiers) to the carrier electrolyte [2,5]. The reversal of the EOF is caused by the formation of hemimicelles on the inner surface of the fused-silica capillary. On the other hand, EOF modifiers are potential ion-pairing reagents and can be useful for the optimisation of the separation selectivity as well.

Previous work [6] has demonstrated that different alkylammonium ions can have different

---

* Corresponding author.

effects on the migration order of inorganic anions. It seems that all papers published until now on the separation of inorganic anions have included the use of a single surfactant in the carrier electrolyte and the employment of mixtures of different surfactants has not yet been reported. Therefore, this study was undertaken in order to evaluate the extent to which the use of more than one cationic surfactant in the carrier electrolyte can be employed to optimise separation selectivity and resolution. Chromate was chosen as the carrier electrolyte itself because this is a well-established system allowing universal indirect UV detection [7].

2. Experimental

2.1. Instrumentation

The CZE instrument employed was a Quanta 4000 (Waters, Milford, MA, USA) interfaced to a Maxima 820 data station (Waters). Separations were carried out using a polyimide-coated fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA), measuring 60 cm × 75 μm I.D., effective length 52 cm. Injections were performed hydrostatically by elevating the sample at 10 cm for 30–45 s at the cathodic side of the capillary. The running voltage was −20 kV and indirect UV detection at 254 nm was used.

2.2. Reagents and procedures

The carrier electrolyte consisted of 5 mM sodium chromate (6.5 mM chromate for the Bayer liquor sample) containing varying amounts of dodecyltrimethylammonium bromide (DTAB) and tetradecyltrimethylammonium bromide (TTAB) and adjusted to pH 8.8 with potassium hydroxide. Both DTAB and TTAB were obtained from Aldrich (Milwaukee, WI, USA) and were dried at 100°C for 1 h before use. All other chemicals used were of analytical grade. All solutions were prepared in water treated with a Millipore (Bedford, MA, USA) Milli-Q water-purification apparatus. Standard anion mixtures containing 10 μg/ml of each

anion were prepared from appropriate stock solutions.

Before starting a series of injections, the capillary was conditioned by the following flushing sequence: water, ethanol, water, 5 min each; 0.5 M potassium hydroxide, 8 min; water, 5 min; carrier electrolyte, 10 min.

3. Results and discussion

3.1. Selectivity effects in mixed EOF modifiers

The employment of two EOF modifiers such as DTAB and TTAB (as used throughout this work) leads to the necessity of optimising two variables, namely the total concentration of the quaternary ammonium salts as well as the ratio of the two different compounds. Fig. 1 shows the dependence of the electrophoretic mobilities of a series of inorganic anions on the total concentration of EOF modifier for a ratio of 1:1 between DTAB and TTAB. An interpretation of these results must take into account the critical micelle concentrations (CMCs) of DTAB and TTAB, which are 15 and 3.5 mM, respectively [8]. It might be concluded that in the range below 7 mM total concentration of the 1:1 mixture the changes in separation selectivity are mainly due to ion-pairing effects, whereas above this concentration ions establish an equilibrium between the aqueous phase and the micelles (which may include micelles formed by ions of both surfactants individually or as mixtures).

The concept of two different equilibria existing below and above the CMC has also been proposed by Kaneta et al. [9] who reported the separation of five anions with direct UV detection in a phosphate–Tris buffer containing cetyltrimethylammonium chloride. The authors also calculated ion-association constants for the equilibrium existing below the CMC as well as distribution coefficients for the concentration range above the CMC. On the other hand, it should be pointed out that the results obtained above the CMC are not necessarily to be described as a distribution of anions between the aqueous phase and the micelles but could be due
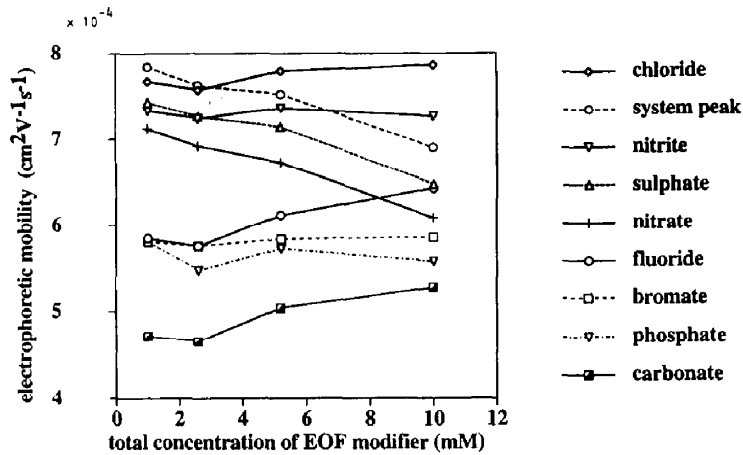
Fig. 1. Dependence of electrophoretic mobilities of anions on the total concentration of the EOF modifier using 1:1 mixtures of DTAB and TTAB.

to some ion-pairing between the anions and the micelles as well with an equilibrium constant different from that for the interaction between the anions and the monomeric surfactant. In this case, the micelles would act in the same way as polymer ions, which have been reported as additives to the buffer in order to optimise separation selectivity [10]. Whatever the detailed mechanism of these interactions might be, our results indicate that upon addition of cationic surfactants, the separation selectivity changes gradually from the migration order to be expected from consideration of the molar conduc-

tivities of the ions to a migration order resembling in part that occurring in ion-exchange chromatography.

Once an appropriate total concentration of surfactant has been chosen, variation of the TTAB:DTAB ratio can be used for fine-tuning the separation. Fig. 2 shows the dependence of electrophoretic mobilities on the ratio of TTAB and DTAB at a total concentration of 2.6 mM. A computer-assisted iterative optimisation procedure developed initially for reversed-phase chromatography [11] was found to be quite helpful for this final optimisation step. This
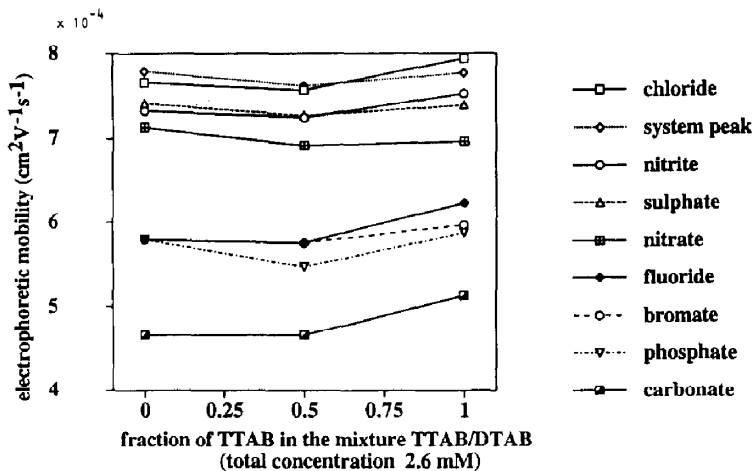

Fig. 2. Dependence of electrophoretic mobilities of anions on the ratio of TTAB and DTAB (total concentration 2.6 mM).

procedure was initiated using two experiments in which desired concentrations of TTAB and DTAB alone were used as EOF modifier. Migration times were calculated for all intermediate mixtures of linear combinations of the two limiting concentrations by assuming a linear relationship between migration time and TTAB:DTAB ratio. Using an appropriate optimisation criterion, the optimal ratio was then predicted and verified by an additional experiment. If these experimental data differed from the predicted data, the additional experimental data were used to establish an improved function for the dependence of migration times on the ratio and a new prediction performed. The optimal separation of a mixture of nine anions is shown in Fig. 3.

Besides the effects on separation selectivity, the TTAB:DTAB ratio also affects the analysis time due to its influence on the electroosmotic flow. Both salts are effective in reversing the EOF with TTAB exhibiting the predominant effect when combined with DTAB. The electroosmotic mobility calculated for a chromate carrier electrolyte containing only DTAB or TTAB at a concentration of 2.6 mM was $1.58 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1} for DTAB and $2.48 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1} for TTAB.

A mentioned above, the reversal of the EOF is due to the formation of hemimicelles of TTAB and DTAB at the inner surface of the fused-silica capillary, which causes the ζ potential to reverse its sign. Therefore, conditioning of the capillary is crucial. Our investigations indicated that inappropriate conditioning can have serious effects not only on the EOF but also on the quantification of some ions, especially phosphate. Phosphate almost completely disappeared (especially at levels below 10 ppm) if the flushing procedure described in the experimental part did not include the final purge with the carrier electrolyte for 10 min. Apparently, phosphate tended to adsorb to the bare silica surface. If the capillary was conditioned with the carrier electrolyte, the active sites of the surface became saturated with TTAB and DTAB and phosphate was no longer lost by adsorption.

3.2. Applications of mixed EOF modifiers

The optimisation strategies described in this paper have been applied to a range of different applications including the analysis of fluoride in Bayer liquors which are alkaline, high ionic strength solutions from the Bayer process for extraction of alumina from bauxite. Initial experiments [12] had indicated that a chromate electrolyte containing 0.5 mM TTAB was incapable of resolving the fluoride peak from formate and succinate present in the complex sample. The results given above suggested the employment of higher concentrations of TTAB–DTAB mixtures which would move the fluoride to considerably shorter migration times. On the other hand, it was mentioned earlier that at higher concentrations of the EOF modifier the separation selectivity tends towards that of ion-exchange chromatography. Therefore, one would expect that the resolution between fluoride and formate would not improve considerably at higher concentrations of the surfactants, as fluoride and formate show similar retention times in ion chromatography. Nevertheless, organic acids turned out to behave in a quite
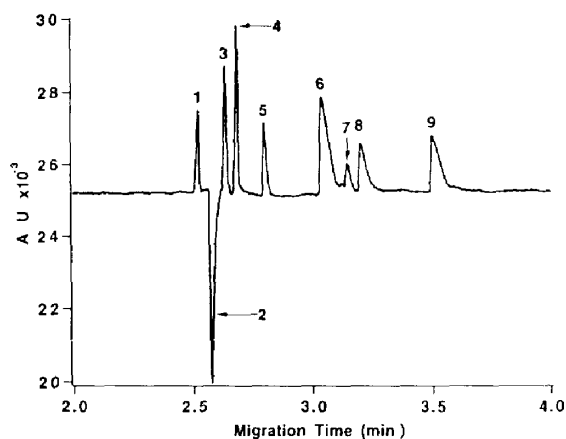

Fig. 3. Optimised separation of a mixture of nine inorganic anions. Conditions: electrolyte contained 2.35 mM TTAB, 2.65 mM DTAB and 5 mM chromate at pH 8.8. Sample was introduced by hydrostatic injection (30 s at 10 cm) and separated using 20 kV from a negative power source. Detection was in the indirect UV mode at 254 nm. Solute concentrations: 10 ppm. Peaks: 1 = chloride; 2 = system peak (bromide); 3 = nitrite; 4 = sulfate; 5 = nitrate; 6 = fluoride; 7 = bromate; 8 = phosphate; 9 = carbonate.
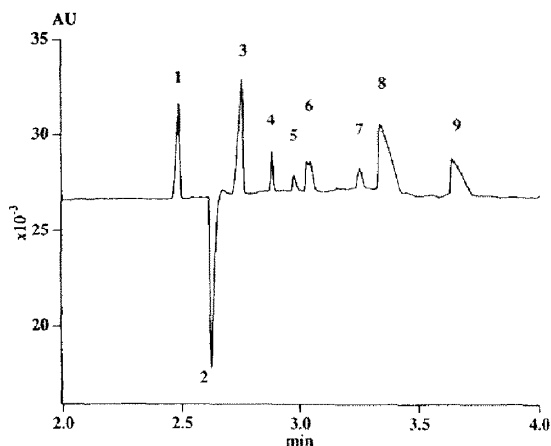
Fig. 4. Separation of 1:1000 (v/v) diluted Bayer liquor using an electrolyte comprising 5 mM TTAB and 1 mM DTAB. Other conditions: 6.5 mM chromate, pH 8.84, 45 s sampling time, −20 kV separation voltage and indirect UV detection at 254 nm. Peaks: 1 = chloride; 2 = system peak; 3 = sulfate; 4 = oxalate; 5 = fluoride; 6 = formate + malonate; 7 = succinate + tartrate; 8 = carbonate; 9 = acetate.

different way, as shown in the electropherogram given in Fig. 4. Fluoride is well separated from organic acids using 5 mM TTAB–1 mM DTAB (for this application, the chromate concentration of the carrier electrolyte had been increased from 5 to 6.5 mM in order to get a more efficient focusing of the sample components by sample stacking).

4. Conclusions

In conclusion, the use of mixtures of different quaternary ammonium salts as well as variation of the total concentration of these salts are efficient approaches to the optimisation of separation selectivity, resolution and analysis time in the determination of inorganic and low-molecular-mass organic anions. Computer-assisted procedures are likely to gain importance in the optimisation process and require only a small number of experiments in order to find an optimum for a given separation.

Acknowledgement

References

[1] P. Jandik and G. Bonn, Capillary Electrophoresis of Small Molecules and Ions, VCH, New York, 1993.
[2] W.R. Jones and P. Jandik, J. Chromatogr., 546 (1991) 445.
[3] W.R. Jones and P. Jandik, J. Chromatogr., 608 (1992) 385.
[4] W.R. Jones, J. Chromatogr., 640 (1993) 387.
[5] K.D. Altria and C.F. Simpson, Chromatographia, 24 (1987) 527.
[6] W. Buchberger and P.R. Haddad, J. Chromatogr., 608 (1992) 59.
[7] P. Jandik and W.R. Jones, J. Chromatogr., 546 (1991) 431.
[8] E.J. Fendler and J.H. Fendler, in V. Gold (Editor), Advances in Physical Organic Chemistry, Academic Press, London, 1970, p. 276.
[9] T. Kaneta, S. Tanaka, M. Taga and H. Yoshida, Anal. Chem., 64 (1992) 798.
[10] S. Terabe and T. Isemura, Anal. Chem., 62 (1990) 652.
[11] P.J. Schoenmakers, A.C.J.H. Drouen, H.A.H. Billiet and L. de Galan, Chromatographia, 15 (1982) 688.
[12] S. Vanderaa and P.R. Haddad, presented at 6th International Symposium on High Performance Capillary Electrophoresis, San Diego, CA, 1994, poster P-509.