

Effects of carrier electrolyte composition on separation selectivity in capillary zone electrophoresis of low-molecular-mass anions

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ABSTRACT

A systematic investigation was carried out on the influence of carrier electrolyte composition on separation selectivity in capillary zone electrophoresis of inorganic anions. Chromate, chloride, sulphate or nitrate was used as the carrier electrolyte, each containing a quaternary ammonium salt to reverse the electro-osmotic flow. The concentration and nature of the carrier electrolyte salt affected the separation order only to a minor degree. Much more pronounced effects could be achieved by adding organic solvents to the carrier electrolyte or by using different quaternary ammonium salts. Chemical modification of the inner wall of the fused-silica capillary did not affect the migration order significantly. There is no indication of retention by effects similar to ion-interaction chromatography as long as capillaries with an inner diameter of 75 μm are used. Nevertheless, such effects might play an important role for capillaries with diameters of 10 μm or less.

INTRODUCTION

Recently, capillary zone electrophoresis (CZE) has been demonstrated to be a useful technique for high-efficiency separations of inorganic anions [1–4]. A typical carrier electrolyte consists of sodium chromate containing a reagent to reverse the electro-osmotic flow (EOF). Anions are separated according to their mobility under the influence of an applied potential of 10–30 kV. Detection can be achieved by indirect UV absorption. Under these conditions, high-efficiency separations of anions are possible with plate numbers greater than 300 000 per metre. For certain samples CZE might be a better technique than ion chromatography (IC), which

is now well established for the analysis of ionic species.

Unlike IC, little information is available on the parameters affecting separation selectivity in CZE of low-molecular-mass anions. Jones and Jandik [3] have reported small selectivity changes by varying the concentration of chromate or of the EOF modifier. Further data would make the optimization of CZE separations much easier, but unfortunately these data are still awaited. This paper reports a more systematic investigation into separation selectivity in CZE. Different EOF modifiers as well as different carrier electrolytes were tested. Totally aqueous electrolytes, but also mixtures with organic solvents, including methanol, acetonitrile, acetone, tetrahydrofuran and ethylene glycol, were used. Furthermore, the applicability of capillaries with hydrophobic phases bonded to fused silica was investigated.

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EXPERIMENTAL

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 an AccuSep fused-silica capillary (60 cm \times 75 μ m I.D., Waters) or a CElect-H2 capillary (70 cm \times 75 μ m I.D., Supelco, Bellefonte, PA, USA). The effective length of the capillaries (from the point of sample introduction to the point of detection) was 8 cm shorter than the total length. Injection was performed hydrostatically by elevating the sample at 10 cm for 20 s at the cathodic side of the capillary. Direct or indirect UV detection at 214 nm or indirect UV detection at 254 nm was used.

The carrier electrolytes were prepared from sodium chromate tetrahydrate, potassium nitrate, potassium chloride or sodium sulphate and an EOF modifier such as dodecyltrimethylammonium bromide, tetradecyltrimethylammonium bromide, hexadecyltrimethylammonium bromide (all obtained from Fluka, Buchs, Switzerland) or tetradecyltrimethylammonium chloride (prepared by passing a 5 mM solution of the corresponding bromide salt through a column filled with a strong anion-exchange resin). The electrolytes were prepared either in Milli-Q water or in water-organic solvent mixtures. Analytical-grade ethylene glycol and high-performance liquid chromatography (HPLC)-grade methanol, acetonitrile, tetrahydrofuran and acetone were obtained from Merck (Darmstadt, Germany). Samples were prepared in Milli-Q water.

All migration times were normalized to that of nitrate in order to obtain reproducible results. A normalization to a neutral electro-osmotic marker was less feasible because in some cases the electro-osmotic flow was very small, which resulted in excessively long migration times for the marker.

RESULTS AND DISCUSSION

Concentration effects

For investigating selectivity effects in CZE, a test mixture containing ten anions (thiosulphate, bromide, chloride, iodide, sulphate, nitrite, nitrate, chlorate, thiocyanate and fluoride) was used throughout this work. The separation of several of these anions has been reported in the literature [3]

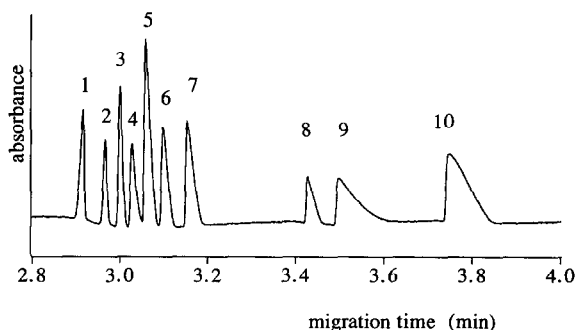


Fig. 1. Separation of a standard mixture of inorganic anions using standard conditions (see text). Peaks: 1 = thiosulphate; 2 = bromide; 3 = chloride; 4 = iodide; 5 = sulphate; 6 = nitrite; 7 = nitrate; 8 = chlorate; 9 = thiocyanate; 10 = fluoride. Indirect UV detection at 254 nm.

and was carried out with chromate as the carrier electrolyte and a proprietary EOF modifier. Our own experiments started with a carrier electrolyte of 5 mM chromate and 0.5 mM tetradecyltrimethylammonium bromide. This quaternary ammonium salt was introduced by Altria and Simpson [5,6] for reversing the EOF in the capillary. The separation was carried out at a voltage of 20 kV, resulting in an electric current of approximately 20 μ A. Throughout this paper these conditions will be referred to as standard conditions. A typical electropherogram obtained under standard conditions is given in Fig. 1.

Varying the chromate concentration within a range from 1 to 7 mM influenced the separation only to a minor degree since the migration order of only iodide, sulphate and nitrite was affected. By decreasing the chromate concentration, the order gradually changed to sulphate/nitrite/iodide, whereas by increasing the concentration the migration order became iodide/sulphate + nitrite (co-migrating).

When the EOF modifier concentration was varied over the range 0.2–0.8 mM, the separation was affected only with respect to the migration order of iodide, sulphate and nitrite. Decreasing the concentration resulted in a migration order iodide/sulphate + nitrite (co-migrating), whilst increasing the concentration led to a migration order sulphate/nitrite/iodide. More pronounced effects have been reported by Jones and Jandik [3] by varying their proprietary EOF modifier up to 5 mM. This concentration range was not investigated in this work.

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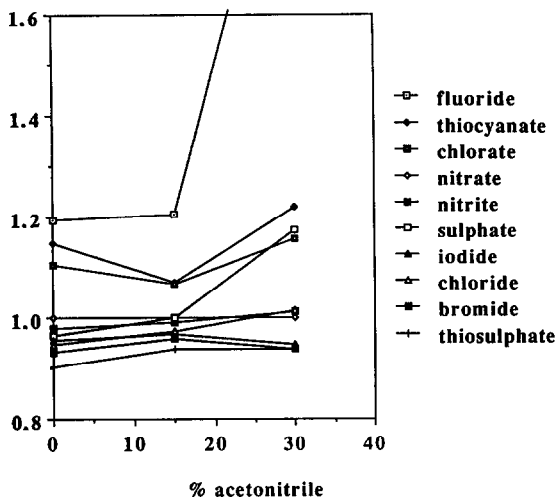


Fig. 2. Effect of acetonitrile in the carrier electrolyte on migration order.

Organic modifier effects

In a series of experiments the influence of organic solvents in the carrier electrolyte was investigated. Up to 30% methanol, acetonitrile, tetrahydrofuran, acetone or ethylene glycol was used. As can be seen from the results given in Figs. 2-6, migration order is strongly influenced by the content of organic solvent in the carrier electrolyte. Obviously, there are some general trends for all organic modifiers (at 30% modifier the migration order sulphate/nitrate is reversed; the resolution between at least two peaks of the triplet thiosulphate/bromide/chloride decreases; the relative migration time of nitrite tends to increase, thereby eventually reversing the migration order nitrite/nitrate), but the data obtained so far do not allow the establishment of a relationship between the nature of the organic solvent and its influence on the electrophoretic mobility of different ions. Furthermore, organic solvents caused a general increase in the migration times of all ions, which can be attributed to two facts: on the one hand, the organic solvent decreases the electrical conductivity, thereby decreasing the current (a carrier electrolyte prepared in 30% organic solvent decreased the current to 10-14 μ A, depending on the solvent); on the other hand, the organic solvent possibly decreases the amount of EOF modifier ad-

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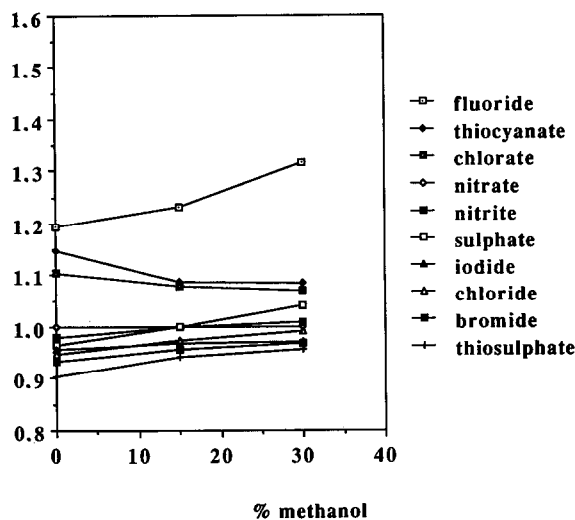


Fig. 3. Effect of methanol in the carrier electrolyte on migration order.

sorbed onto the inner wall of the fused-silica capillary, which results in a lower EOF to the anode. The following numbers are the factors by which the migration time of nitrate is increased in a carrier electrolyte with 15 and 30% organic solvent: 1.4 and 1.9 (methanol), 1.2 and 3.0 (acetonitrile), 1.5 and 3.3

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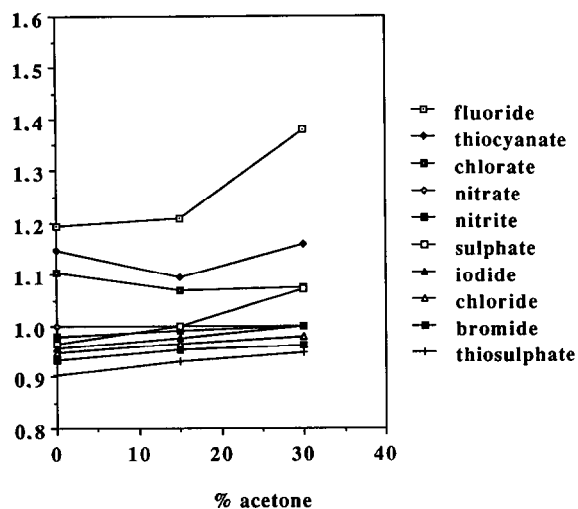


Fig. 4. Effect of acetone in the carrier electrolyte on migration order.

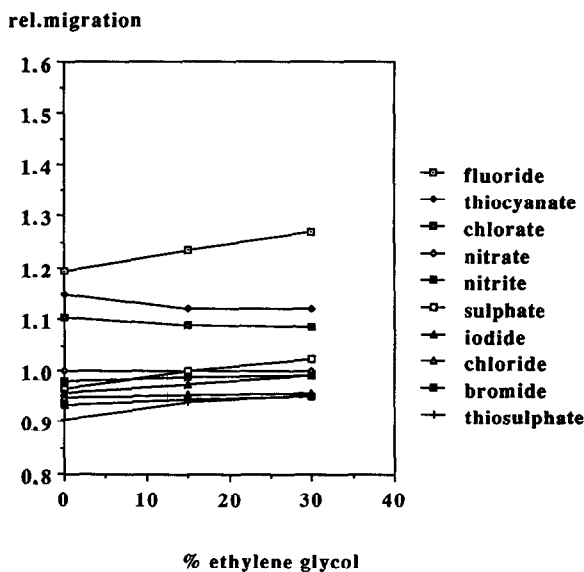


Fig. 5. Effect of ethylene glycol in the carrier electrolyte on migration order.

(tetrahydrofuran), 1.4 and 2.2 (acetone), 1.6 and 2.4 (ethylene glycol).

A typical electropherogram obtained with a carrier electrolyte containing 30% methanol is given in Fig. 7.

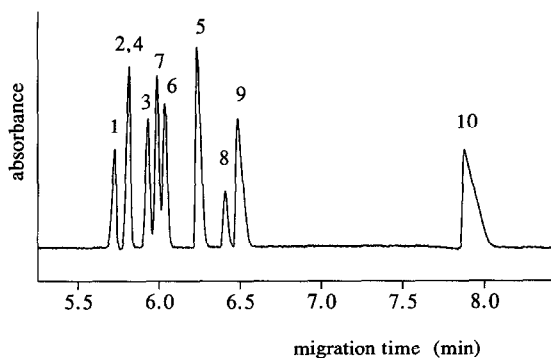


Fig. 7. Separation of a standard mixture of inorganic anions using a carrier electrolyte containing 30% methanol. All other conditions and peaks as for Fig. 1.

Nature of the carrier electrolyte

Next, several other salts were used instead of chromate in the carrier electrolyte. These included potassium chloride, potassium sulphate and potassium nitrate. All other conditions remained the same as the standard conditions, except the detection, which was carried out at 214 nm either in the direct mode or in the indirect mode. This low wavelength necessitated the use of the EOF modifier as the chloride salt form instead of the bromide salt.

The potassium chloride electrolyte yielded a migration order and migration times similar to those obtained under standard conditions. Direct UV detection was employed for the separation of bromide, iodide, nitrite, nitrate and thiocyanate. The migration times (relative to nitrate) were 0.943 (bromide), 0.964 (iodide), 0.996 (nitrite) and 1.104 (thiocyanate). The use of a potassium sulphate electrolyte instead of potassium chloride resulted in a similar separation with migration times (relative to nitrate) of 0.928 (bromide), 0.951 (iodide), 0.984 (nitrite) and 1.118 (thiocyanate). The only advantage of a chloride or sulphate carrier electrolyte with direct UV detection at 214 nm might be the increased detection selectivity if UV-absorbing anions must be determined in a matrix which does not absorb at 214 nm but would interfere with indirect detection modes.

The situation is somewhat more complicated if potassium nitrate is used as the carrier electrolyte. There can be positive as well as negative peaks depending on the UV absorption ratio of the sample anion and the carrier electrolyte anion. Nitrate in

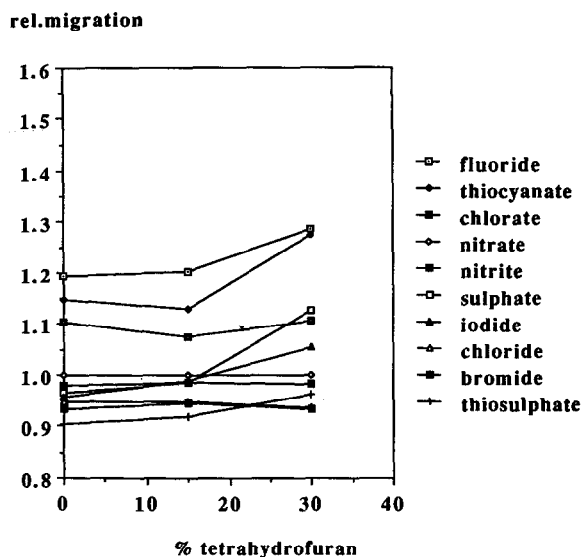


Fig. 6. Effect of tetrahydrofuran in the carrier electrolyte on migration order.

the sample should give no signal. In our experiments the injection of nitrate resulted in a positive peak at the migration time of chloride. The reason for this peak is not clear at the moment. The elution order for the other anions was the same as for standard conditions, but peak shape was worse. Therefore, nitrate as a carrier electrolyte does not offer any advantages and was not investigated further.

EOF modifier effects

An important component of the carrier electrolytes investigated is the EOF modifier, a quaternary ammonium salt which reverses the EOF to the anode. The influence of the nature of the EOF modifier on separation selectivity was studied by using three alkyltrimethylammonium bromides with different chain lengths of the alkyl group. The results are given in Fig. 8. Again, several pronounced changes in the migration order could be observed, especially with respect to the ions thiosulphate, iodide and thiocyanate. A further effect that was noted was that the average migration times were roughly doubled when using the EOF modifier with the shorter alkyl group, whereas the use of the EOF modifier with the longer alkyl group did not change the migration times significantly.

Huang *et al.* [4] have reported that hydrophobic quaternary ammonium salts, such as tetradecyltrimethylammonium bromide, attach to the inner surface of fused-silica capillaries, thereby shielding the negative charge of the silica and influencing the electro-osmotic flow in an opposite direction. On the other hand, the adsorbed quaternary ammonium salt might also act as an anion exchanger. Such phenomena are well known from ion-pair (or ion-interaction) liquid chromatographic analysis of anions using a reversed-phase material as the stationary phase and an eluent containing a quaternary ammonium salt. Recently, Pfeffer and Yeung [7] have demonstrated the use of ion-pairing effects for CZE separation of the anions of isomeric aminonaphthalenesulphonic acids. These anions were separated by differences in retention rather than by differences in electrophoretic mobility. The separation was therefore described as electrochromatography. Analogous to this, additional ion-exchange effects might be expected to occur for the CZE separations of inorganic anions described in this paper. Any modification of the inner fused-silica wall of

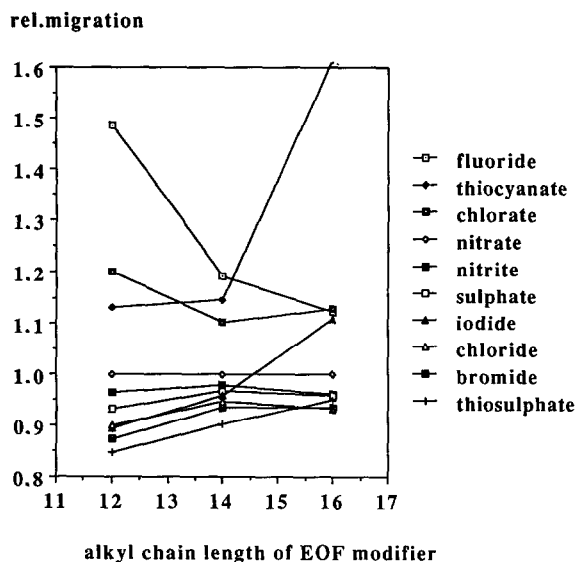


Fig. 8. Effect of alkyl chain length of the EOF modifier on migration order.

the capillary can influence the amount of adsorbed EOF modifier, thereby influencing the EOF as well as any ion-exchange mechanism. These effects were studied by using a commercially available capillary (CElect-H2), which is modified by C_{18} on the inner wall (unfortunately, data on the extent of the modification, such as carbon loading, are not available).

Capillary effects

A C_{18} -modified CElect-H2 capillary was tested using the chromate electrolyte as described for the standard conditions as well as using this electrolyte in 30% ethylene glycol. It was necessary to increase the voltage to 30 kV in order to obtain reasonable migration times. The separations obtained were exactly the same as those obtained with the bare fused-silica capillary. One might expect that the more hydrophobic wall would adsorb a larger amount of the EOF modifier, thereby eventually increasing the EOF to the anode. Contrary to this, the voltage had to be increased by 50% to achieve the same migration times. There is no simple explanation at hand for this fact. The separation order remained the same, which would mean that anion-exchange effects are either non-existent or exactly the same for both capillaries. Another argument against an anion-exchange mechanism is the fact that the use of organic solvents in the carrier elec-

trolyte influenced the migration order in a way quite different from the effects that would be expected from ion-interaction chromatography.

Nevertheless, a closer look at the electropherograms obtained for unmodified and modified capillaries revealed some interesting details. The peak shapes obtained for iodide and thiocyanate were more asymmetrical than can be explained by the mismatch of electric mobility of sample anion and carrier electrolyte anion. In ion-interaction chromatography, iodide and thiocyanate would be strongly retained if tetradecyltrimethylammonium bromide was used as the ion-interaction reagent. Therefore, it can be concluded that in CZE there is some interaction between the adsorbed reagent and iodide as well as thiocyanate, leading to peak tailing. On the other hand, this effect could not be used to manipulate the retention of these solutes, because the inner diameter of the capillaries used in this work is too large to allow efficient mass transfer between the solution and the wall of the capillary. In the experiments of Pfeffer and Yeung [7] mentioned above, this problem was overcome by the use of capillaries with only 10 μm inner diameter, which allows an efficient mass transfer and chromatographic retention. The fact that some interactions between inorganic anions and the adsorbed EOF modifier are evident from our electropherograms suggests that further experiments with 10- μm capillaries would be interesting. In this case, a careful choice of the carrier electrolyte will be necessary. Chromate will not necessarily be the best carrier

electrolyte, since it acts as a strong eluent in IC chromatography, thereby reducing the ion-exchange effects of the EOF modifier. Electrolytes acting as weak eluents in ion chromatography could be preferable.

The results presented in this paper indicate that the use of organic solvents in the carrier electrolyte is one of the most efficient ways to change the retention order and to optimize the separation selectivity, as long as capillaries with an inner diameter much larger than 10 μm are used. This concept has been found useful for the analysis of anions in samples containing small amounts of iodide in the presence of a large excess of sulphate. Under standard conditions, the large sulphate peak makes the accurate evaluation of the iodide peak area difficult. Organic solvents such as ethylene glycol can improve the resolution of these two peaks dramatically. Details of these applications will be reported in another paper.

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