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Peak shapes in open tubular ion-exchange capillary electrochromatography of inorganic anions

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Abstract

An experimental study of parameters influencing peak shapes in ion-exchange open tubular (OT) capillary electrochromatography (CEC) was conducted using adsorbed quaternary aminated latex particles as the stationary phase. The combination of separation mechanisms from both capillary electrophoresis and ion-exchange chromatography results in peak broadening in OT-CEC arising from both these techniques. The sources of peak broadening that were considered included the relative electrophoretic mobilities of the eluent co-ion and analyte, and resistance to mass transfer in both the mobile and stationary phases. The parameters investigated were the mobility of the eluent co-ion, column diameter, separation temperature and secondary interactions between the analyte and the stationary phase. The electromigration dispersion was found to influence peak shapes to a minor extent, indicating that chromatographic retention was the dominant source of dispersion. Improving the resistance to mass transfer in the mobile phase by decreasing the capillary diameter improved peak shapes, with symmetrical peaks being obtained in a 25 μm I.D. column. However, an increase in temperature from 25°C to 55°C failed to show any significant improvement. The addition of *p*-cyanophenol to the mobile phase to suppress secondary interactions with the stationary phase did not result in the expected improvement in efficiency.

Keywords: Peak shapes; Open tubular ion-exchange capillary electrochromatography; Electrochromatography; Inorganic anions

1. Introduction

Open tubular (OT) chromatographic separations have proved to be more successful when conducted in the gaseous phase (i.e., capillary gas chromatography) than in the liquid phase [i.e., open tubular liquid chromatography (OT-LC)]. The fact that OT-LC has not been widely accepted into analytical practice can be attributed to the much lower diffusion rates of analytes in the liquid phase when compared to the gas phase. As a result the potentially highly efficient separations and short analysis times in open tubular columns can be realised only in very narrow tubes (with diameters usually in the order of 10 μm or smaller) [1], where the phase ratio is very low and serious detection problems arise. An alternative to narrow bore capillaries is the use of elevated temperatures, as demonstrated recently by Liu et al. [2,3], where the increase in temperature

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results in faster diffusion of the analytes so that wider capillaries can be used to obtain efficient separations without loss of column capacity or detection sensitivity. However, elevated temperatures impose instrumental complications and are not applicable when the analytes are thermally unstable.

A further factor contributing to peak broadening in OT-LC is the parabolic flow velocity profile present when using pressure-driven flow. Advances in electroseparation methods, particularly the development of capillary electrophoresis (CE) in the mid 1980s and of capillary electrochromatography (CEC) in the late 1990s, offers the alternative of moving the liquid through the capillary by electroosmosis. Electro-driven separations have the advantage of minimising peak spreading because the electroosmotically-driven flow has an almost flat velocity profile across the capillary under typical operating conditions. In 1974 Pretorius et al. [4] demonstrated the use of electroosmosis to pump solvents in liquid chromatography using thin-layer stationary phases, open tubes and packed columns, and showed that plate height could be reduced by a factor of 25. Tsuda et al. [5] demonstrated the separation of several aromatic compounds using a 30 μm I.D. ODS capillary. Theoretical plate heights were 29-times lower using electroosmotic flow than the calculated plate height using laminar flow. Martin and co-workers [6,7] have presented theoretical considerations on the benefits of using electroosmosis and showed that to obtain the same efficiency, the tube diameter could be 1.73-times larger when using electroosmosis in comparison to laminar flow. The authors noted that the main advantage was that the detector volume could be increased by a factor of 3 without any loss in separation performance. Recently, Schurig and co-workers [8,9] have shown the potential advantages of using OT-CEC when comparing different open tubular chromatographic methods (gas chromatography, supercritical fluid chromatography, OT-LC and OT-CEC) for the separation of enantiomers of hexobarbital, using a 50 μm I.D. capillary coated with Chirasil-DEX. Apart from a longer analysis time, OT-CEC was superior in chiral separation factor, peak resolution and efficiency to all the other techniques.

In addition to a potential improvement in efficiency, electro-driven separation methods present interesting opportunities for separating charged analytes by influencing the separation selectivity. This involves combining the separation mechanisms from CE and chromatography, so that analytes are separated both on the basis of differences in their electrophoretic mobilities and through chromatographic interactions with the stationary phase. The combination of these two separation mechanisms potentially enables the separation selectivity to be changed from that of one technique to that of the other, with a range of intermediate selectivities also being possible. Recently, we have demonstrated that ion-exchange interactions can be introduced to a CE separation by coating the capillary wall with very small diameter (75 nm) anion-exchange latex particles and we have used this approach to manipulate the separation selectivity of inorganic anions in OT-CEC [10]. It was shown that by using typical ion chromatography (IC) eluents of varying elution strengths, the separation selectivity could be changed from a separation resembling the IC elution order to one resembling the CE migration order. Regarding the separation efficiency, a principal disadvantage in this approach was the poor peak symmetry and broad peaks observed for analytes such as I^-, SCN^- and $S_2O_3^{2-}$, which interacted strongly with the stationary phase.

A review of OT-CEC literature indicates that while the efficiency for OT-CEC is substantially better than for OT-LC [8,11–13], in most cases it is not comparable to that obtainable by CE [14,15]. An exception to this generalisation is the work of Guo and Colón [16,17] who reported 500 000 plates/m in the separation of polycyclic aromatic hydrocarbons using an 13 μm I.D. open tubular column. A sol–gel stationary phase was used and this provided a larger surface area and hence more capacity than monolayer type stationary phases prepared by conventional methods. In contrast, Liu et al. [11] obtained low efficiencies when separating substituted benzenes in 10 μm I.D. columns prepared with an adsorbed layer of cetyltrimethylammonium bromide (CTAB). Efficiencies for propylbenzene were reported to be 32 000 plates/m, but no plate count was given for butylbenzene, which showed a significantly poorer peak shape. Similarly, in our previous work [10] the high efficiencies expected for OT-CEC separations were not observed for strongly interacting analytes, which typically showed efficiencies of 10 000 theoretical plates/m. However, it should be noted that the

majority of this work was performed using 75 μm I.D. capillaries.

Many of the fundamental concepts in OT-CEC, such as resistance to mass transfer, can be transferred from OT-LC, so that the same principles will apply, such as the expected reduction in plate height with decreasing capillary diameter. Also, an increase in temperature can be expected to improve efficiency in an OT-LC system, but it is unclear whether this will be beneficial in OT-CEC due to the adverse influence of temperature on the electrophoretic component in the separation mechanism. A number of possible mechanisms may be involved in the observed peak broadening in OT-CEC including electromigration dispersion for charged solutes, column overloading and resistance to mass transfer in the stationary phase, so that it would not be appropriate to suggest that these adverse effects are only due to the relatively large column diameter and resulting resistance to mass transfer in the mobile phase. This paper presents an experimental study aimed to provide a better understanding of parameters influencing the separation efficiency in the separation of inorganic anions in OT-CEC so that the full potential of the method can be realised. Various approaches, often employed in OT-LC, are used to investigate the potential sources inefficiency in OT-CEC.

2. Experimental

2.1. Instrumentation

Separations were carried out on a HP ^{3D}CE instrument (Hewlett-Packard, Waldbronn, Germany) using fused-silica capillaries (Polymicro, Phoenix, AZ, USA) of varying diameters (75 μm, 50 μm and 25 μm I.D.), having a length of 50.0 cm, 41.5 cm to detector. Injection was performed by applying 10 mbar pressure for 5 s to the anodic side of the capillary. Detection was by direct absorbance at 210 nm. All separations were carried out at −25 kV and at 25°C unless otherwise stated.

2.2. Reagents

Quaternary ammonium latex particles (AS5A type) with an approximate size of 75 nm were supplied as an 11% (w/v) aqueous suspension from Dionex (Sunnyvale, CA, USA). Dialysis tubing with a molecular mass (M_r) cut-off of 12 000 and Dowex MR-3C mixed bed ion-exchange resin, both obtained from Sigma–Aldrich (Milwaukee, WI, USA), were used for purification of the latex.

Analytical-grade tris(hydroxymethyl)amino methane (Tris) was obtained from Sigma–Aldrich and used without further purification. Standards of 10 m*M* Br^-, I^-, NO_2^-, NO_3^-, SCN^-, $S_2O_3^{2-}$ and CrO_4^{2-} were prepared from sodium or potassium salts of analytical grade. Background electrolytes (BGEs) were prepared by titration of Tris with the corresponding acid (HCl, H_2SO_4, citric, or $HClO_4$) to a pH of 8.05. All mobile phases were degassed by application of a vacuum for 1 min.

2.3. Preparation of the open tubular capillary columns

Columns were prepared by coating a bare fused-silica capillary with cleaned aminated latex particles using the method reported previously [10]. Briefly, the capillary was rinsed with water for 10 min, followed by 0.1 *M* HCl for 10 min and 1 *M* NaOH for 60 min. The cleaned suspension of AS5A particles was then flushed through the capillary at 50 mbar for 20 min and left to sit for a further 20 min. This was repeated a further two times. The capillary was then flushed at 50 mbar with water for 30 min, before flushing with water at 1 bar for 60 min.

3. Results and discussion

3.1. Sources of band dispersion in OT-CEC

In our previous studies of OT-CEC [10] broad, tailed peaks were typically obtained for strongly interacting analytes, such as I^-, SCN^- and $S_2O_3^{2-}$. In order to improve these peak shapes an understanding of the possible sources of peak tailing in both the CE and IC separation mechanisms is necessary. An important source for zone broadening in CE is the electromigration dispersion resulting from differences in mobility between the co-ion (in this case, the eluent ion) and the analyte [18]. This results in analytes that have a mobility greater than the co-ion

giving fronted peaks, those that have a mobility less than the co-ion giving tailed peaks, and those having a mobility equal to that of the co-ion giving symmetrical peaks. It should be noted that tailed peaks in CE due to electromigration dispersion appear triangular in shape in contrast to the typical curved tailed peak shape often seen in chromatography. In IC, inefficiency is characterised generally by the Van Deemter equation, which identifies contributions due to inefficiency from three sources: flow inequalities in the column packing, axial diffusion in the mobile phase, and resistance to mass transfer effects in both the stationary and mobile phases [19]. Since the column used in this study was an open tubular column with an adsorbed monolayer of stationary phase [20], only the mass transfer effects in the mobile and stationary phases are likely to have significance [21]. Resistance to mass transfer in the stationary phase may arise from diffusion in the stationary phase (Ref) and/or slow adsorption/desorption kinetics (ref). In this case, as the support for the latex particles has a similar charge to that of the analytes, diffusion in the stationary phase does not contribute significantly to inefficiency [19]. With regard to the kinetics of adsorption/desorption, it has been shown by Fornstedt et al. [22] that when there are two mechanisms contributing to retention, then even if the second mechanism only influences retention to a minor extent, the influence on inefficiency can be quite significant. For the polarisable anions examined in this case, Pohl and co-workers [23–25] have shown that secondary interactions are involved in their separation on agglomerated ion-exchange columns and these interactions result in poor peak shapes. To overcome this, the addition of *p*-cyanophenol to the eluent has been used in IC to block adsorptive sites, leaving only ion-exchange interactions to control retention.

To summarise, the main dispersion mechanisms in question are: (i) electromigration dispersion and the resistance to mass transfer in both the (ii) mobile and (iii) stationary phase. The corresponding experimental parameters which influence these effects are: (i) differences in mobility between the eluent co-ion and the analyte, (ii) capillary diameter and separation temperature, and (iii) the influence of secondary interactions. These parameters were investigated and are discussed below.

3.2. Electromigration dispersion

In our previous study [10], it was demonstrated that the apparent mobility of an analyte anion could be reduced significantly due to the presence of ion-exchange interactions with the stationary phase, especially when the eluent contains a relatively weak competing ion. Therefore, the migration through the capillary of an analyte with strong ion-exchange interactions (e.g., I^-) will be influenced more by the stationary phase than that for an analyte with weak ion-exchange interactions (e.g., Cl^-).

When trying to foresee the effect of chromatographic retention on the electromigration dispersion, an assumption that the electrophoretic mobility of the analyte (rather than its effective mobility) governs the electromigration dispersion leads to the conclusion that no adverse effect on peak shape should occur as the ion-exchange interaction, and hence retention, of analyte ions increases. On the other hand, if it is assumed that the effective mobility of the analyte governs the electromigration dispersion, then a greater electromigration dispersion should be observed for I^- than Cl^-, due to the slower migration of Cl^-. Because of the complexity of this phenomenon and a lack of clear evidence from the literature, the significance of electromigration dispersion in OT-CEC was examined experimentally.

In order to investigate the influence of the mobility of the electrolyte competing ion (co-ion) on peak shape, formate was used as the electrolyte competing ion since it has a low absolute value of mobility ($-54.6\cdot10^{-9}$ $m^2/V\ s$) and is a weak ion-exchange competing ion. An electrolyte was prepared containing 5 m*M* of formate and 10 m*M* Tris (pH 8.05). The electrophoretic mobility of formate is nearly identical to that of BrO_3^-, which also has a low ion-exchange interaction. According to the above principles governing electromigration dispersion [18], peaks that migrated before BrO_3^- should be fronted, while those that migrated after BrO_3^- should be tailed. As can be seen from Fig. 1, NO_2^- (peak 1) which migrated before BrO_3^- (peak 2) did not exhibit the triangular shape characteristic of fronted peaks in CE, and also clearly showed chromatographic tailing, indicating that some other factors were involved. Fig. 1 also shows that there was a large difference in peak shape between analytes, with Br^- (peak 3) and NO_3^-

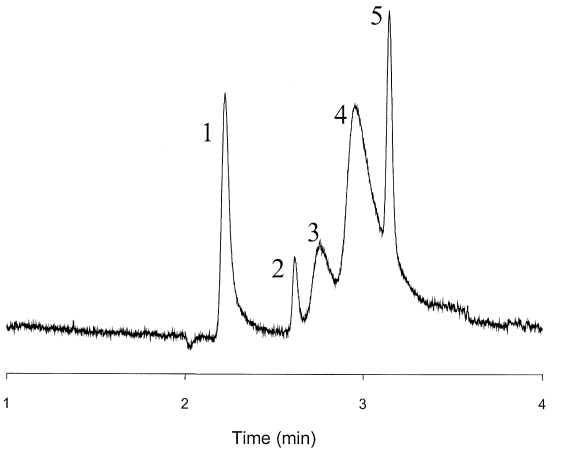

Fig. 1. Separation of five inorganic anions in an AS5A coated capillary using 5 m*M* formate–10 m*M* Tris (pH 8.05) as the electrolyte. Conditions: 50 cm capillary (41.5 cm to detector)×75 μm I.D., −25 kV, injection of 0.2 m*M* at 5 mbar for 10 s. Peaks: 1=NO_2^-, 2=BrO_3^-, 3=Br^-, 4=NO_3^-, 5=IO_3^-.

(peak 4) giving considerably broad peaks and IO_3^- (peak 5) and BrO_3^- (peak 2) giving quite sharp peaks. BrO_3^- and IO_3^- have low ion-exchange selectivity coefficients and as such, their migration was dominated by their electrophoretic mobility. This should result in BrO_3^- having a near symmetrical peak shape, and IO_3^- showing slight electrophoretic tailing. If electromigration dispersion is the major source of peak broadening, then any peak migrating between BrO_3^- and IO_3^- should have a peak shape better than IO_3^- but worse than BrO_3^-. However, the peaks for Br^- and NO_3^-, which due to their ion-exchange interaction migrated between BrO_3^- and IO_3^-, were broad and this indicates that it was not the electrophoretic component but the chromatographic component of the separation mechanism which was the source of the poor peak shapes.

3.3. Resistance to mass transfer in the mobile phase

There are several approaches in OT-LC which are commonly used to improve the resistance to mass transfer in the mobile phase. The first approach, as proposed by Knox and Gilbert [1] is to reduce the distance that the analyte must diffuse to reach the stationary phase. They have shown that in order to obtain a higher efficiency OT-LC separation than the corresponding packed column LC separation, it is necessary to use OT columns of 10 μm or less due to the slow diffusivity of analytes in solution. The second approach is to increase the rate at which the analytes diffuse, which can be achieved by increasing the operating temperature. Liu et al. [2] reported that an increase in column temperature from 25°C to 200°C resulted in an improvement in efficiency similar to that achieved by decreasing the capillary diameter from 50 μm to 12 μm. Recently Pyo et al. [26] have studied the effect of increased temperature on ion-exchange-OT-LC separations of inorganic anions and found that increasing temperature caused a significant improvement in efficiency.

3.3.1. Effect of capillary diameter

To examine the effect of changing capillary diameter on peak shape, experiments were performed in 25, 50 and 75 μm I.D. AS5A coated capillaries using perchlorate as the competing ion. According to Knox and Gilbert [1], reducing the capillary diameter should reduce the contribution to inefficiency from resistance to mass transfer in the mobile phase. However, changing the diameter of the capillary also influences the effective ion-exchange capacity of the column [10], so a direct comparison of peak shapes in the different columns is difficult. In order to gain some insight into the influence of capillary diameter, it is necessary to ensure that analytes interact with the stationary phase to the same extent in the capillaries of different diameters. This requires different concentrations of competing ion to be used in the electrolyte which, in turn, changes other factors which influence peak shape such as Joule heating, the extent of stacking, the speed of the analysis due to viscosity changes in the electrolyte, kinetics of adsorption/desorption, etc. To ensure that analytes had a similar ion-exchange interaction in each capillary and to enable some semi-quantitative comparison to be made, the concentration of perchlorate in the electrolyte was adjusted so that $S_2O_3^{2-}$ (peak 8), SCN^- (peak 7) and CrO_4^{2-} (peak 9) migrated in sequence. Fig. 2 shows the separations in 75 μm (10 m*M* ClO_4^-), 50 μm (30 m*M* ClO_4^-) and 25 μm (50 m*M* ClO_4^-) AS5A coated capillaries. While we recognise that changing the concentration of competing ion will have some influence on peak shape, we

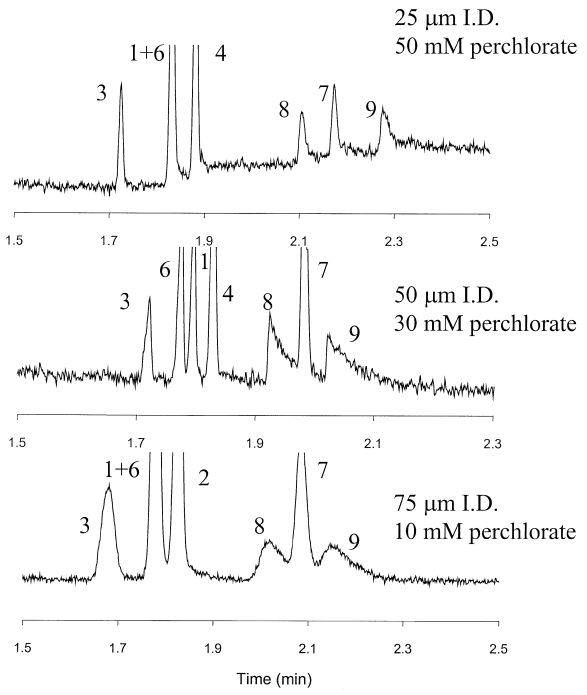

Fig. 2. Comparison of separations in different diameter capillaries with approximately the same ion-exchange interaction obtained by changing the competing ion (perchlorate) concentration. Peaks: 6=I^-, 7=SCN^-, 8=$S_2O_3^{2-}$, and 9=CrO_4^{2-}. Other conditions as in Fig. 1.

believe this will mainly affect the width of the peak and should exert only a minor influence on peak tailing. Comparing the peaks in the different diameter capillaries (Fig. 2) shows that the peaks for CrO_4^{2-} and $S_2O_3^{2-}$ in the 25 μm I.D. capillary were much narrower than in the larger diameters and they also showed less chromatographic tailing. This suggested, as expected from theory, that the resistance to mass transfer in the mobile phase contributed significantly to the observed inefficiency. However, it must be noted that using narrow capillaries is not a viable option when using UV absorbance detection because of the significant reduction in detection sensitivity due to the shorter path length.

3.3.2. Effect of temperature

To examine the influence of changing the temperature on OT-CEC separations, the separation of Br^-, NO_2^-, NO_3^- and I^- in 10 m*M* Cl^- was investigated at temperatures between 25 and 55°C. It should be noted that these values represent the temperature at which the capillary column was set and does not include any change in temperature from Joule heating. In addition, instrumental limitations allowed only about 70% of the capillary length to be thermostated and the buffer reservoirs were maintained at room temperature. Fig. 3 shows that as the temperature was increased, the migration times decreased due to a decrease in viscosity. The separation at 55°C was complete in nearly half the time required for the same separation at 25°C, with a subsequent loss in resolution between Br^-, NO_2^- and NO_3^-. Increasing the temperature from 25–55°C appeared to have a beneficial effect on separation efficiency with efficiencies for the I^- peak improving from 5000 to 10 000 plates/m. Similar results were reported by Jakubetz et al. [8] who observed an increase in efficiency (6600 to 10 000 plates/m) when the temperature was increased from 20 to 60°C when separating enantiomers of hexobarbital by OT-CEC. Whilst increasing the temperature resulted in higher efficiencies and better peak shapes, it is questionable as to whether the improvement in peak shapes is solely due to an improvement in resistance to mass transfer in the mobile phase or whether the are influences from kinetics, etc.

In order to make a semi-qualitative judgement, it was necessary to compare peak shapes of the same analyte at different temperatures and at approximate-

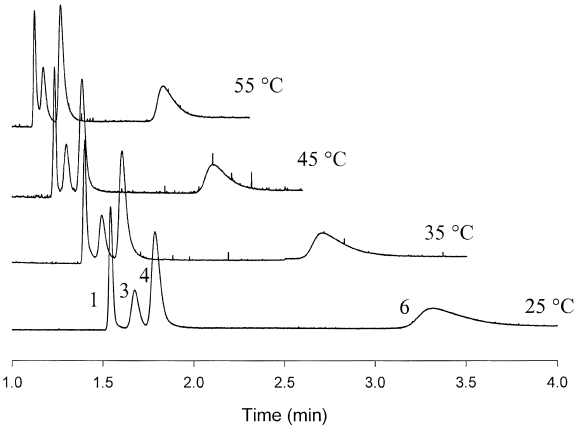

Fig. 3. Separation of inorganic anions in 75 μm at different temperatures in 10 m*M* Cl^-–20 m*M* Tris (pH 8.05) at different temperatures. Other conditions as in Fig. 1.

ly the same retention time. This was accomplished by adjusting the ionic strength of the electrolyte at 25°C such that the I^- peak migrated at approximately the same time as at 55°C when using a 10 m*M* Cl^- electrolyte. Again, while we recognise that increasing the concentration of the electrolyte will have other influences on the separation efficiency, an indication of the effect on peak shape may be inferred. It can be seen from Fig. 4 that there was no significant improvement in peak shape for I^- (peak 6) indicating that there was little benefit from using elevated temperatures. This result is in contrast to findings in OT-LC where it has been shown that an increase in temperature did have a significant influence on peak shape [2,26]. In discussing possible reasons for this difference, calculations presented by both Liu et al. [2] and Pyo et al. [26] can be used to estimate the decrease in capillary diameter which would be equivalent in terms of efficiency gains to benefits achieved by an increase in temperature. If it is assumed that the actual capillary temperature was changed from 25°C to 55°C, this correlates to changing the capillary diameter from 75 μm I.D. to 55 μm I.D. If we now consider that the buffer was not pre-heated before entering the capillary, then a loss in efficiency of as much as 30% may arise from the ''cold point'' effect [2]. Recalculating the corresponding capillary diameter to account for this reduced influence of temperature gives the equivalent capillary diameter as 65 μm I.D. Considering that changing the capillary diameter from 75 μm to 50 μm I.D. (Fig. 2) did not improve peak shape significantly, it is expected that the improvement in using a 65 μm I.D. capillary would be insignificant. It should also be noted that the increase in temperature will also result in broader peaks due to an increase in longitudinal diffusion which may partly offset any improvement in mass transfer, and that the influence of temperature on the kinetics of adsorption and desorption on separation efficiency are not clear.

3.4. Resistance to mass transfer in the stationary phase

The resistance to mass transfer in the stationary phase is made up of contributions from diffusion in the stationary phase and the kinetics of adsorption/desorption. As discussed previously in Section 3.1, only the kinetics of adsorption/desorption, which are generally considered not to be a problem in IC,

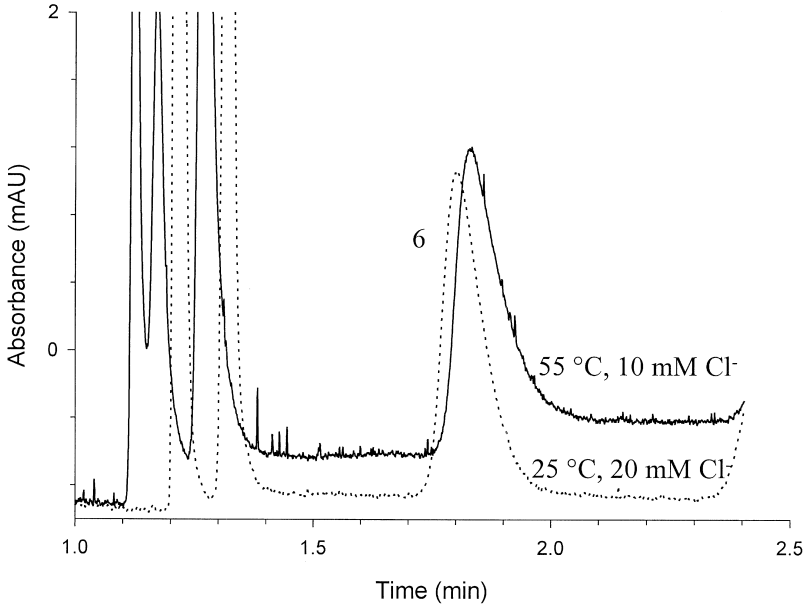

Fig. 4. Comparison of peak shape for I^- (peak 6) at 55°C, 10 m*M* Cl^- and 25°C, 20 m*M* Cl^-–40 m*M* Tris (pH 8.05). Other conditions as in Fig. 1.

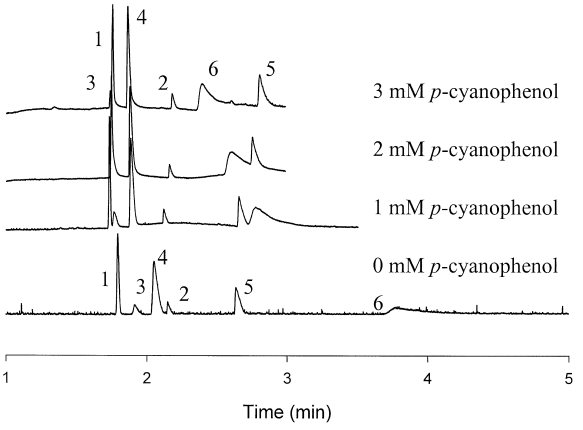

Fig. 5. Influence of the addition of *p*-cyanophenol to a 10 m*M* Cl^-–20 m*M* Tris (pH 8.05) BGE on the separation selectivity of inorganic anions in an AS5A coated capillary. Other conditions as in Fig. 1.

should have an influence on peak shape. Fornstedt et al. [22] have shown that peak tailing is usually caused when the kinetics of a secondary retention mechanism are much slower than those of the primary retention mechanism. Pohl and co-workers [23–25] have shown that secondary interactions are involved in the separation of polarisable anions on agglomerated ion-exchange columns and these result in peak shapes typically being poor. To overcome this, the addition of *p*-cyanophenol to the eluent has been used in IC to block adsorptive sites, leaving only ion-exchange interactions to control retention. The addition of *p*-cyanophenol also influences the separation of other ions, such as Br^- and NO_3^- [23] which also interact with the stationary phase via $\pi-\pi$ type interactions. Fig. 5 shows that with increasing additions of *p*-cyanophenol, the migration times of the strongly retained analytes, such as I^- and SCN^- were reduced quite significantly, indicating that secondary retention equilibria contributed to the retention of these analytes. To determine the influence of *p*-cyanophenol on peak shapes, similar retention was required in electrolytes with and without *p*-cyanophenol. Again it must be reiterated that while this will not provide conclusive results, it may provide some insight into probable causes. Fig. 6 shows the separation of several polarisable inorganic anions, with the retention adjusted by the addition of *p*-cyanophenol so that I^- had approximately the same degree of ion-exchange interaction. It can be seen that there was no improvement in peak

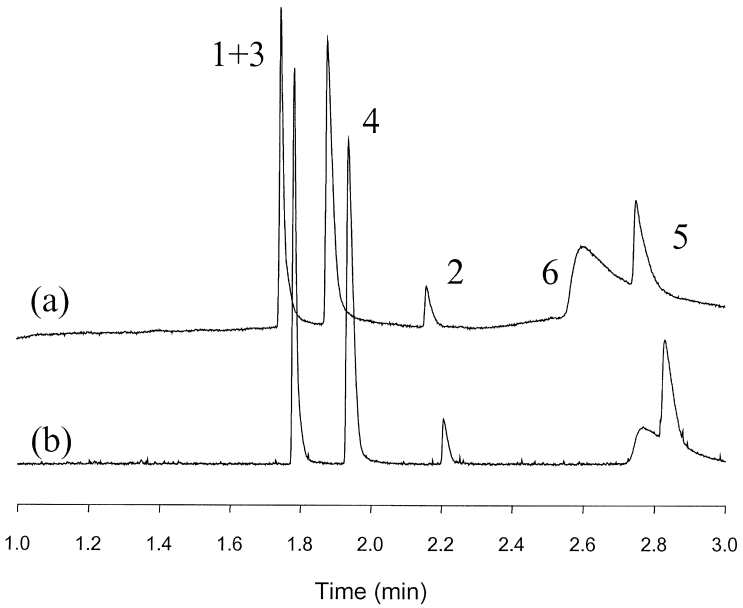

Fig. 6. Comparison of peak shapes with and without the addition of *p*-cyanophenol to the BGE. (a) 2 m*M* *p*-cyanophenol+10 m*M* Cl^-–20 m*M* Tris, (b) 20 m*M* Cl^-–40 m*M* Tris. Other conditions as in Fig. 1.

shape, and in fact the separation without the addition of *p*-cyanophenol appears to be slightly better. This is in contrast to expectations from IC, and may be explained by the differences in ionic strength used to obtain a similar retention so that a suitable comparison could be made.

An alternative method to determine the influence of secondary equilibria effects is to compare the separation of polarisable and non-polarisable analytes that have similar mobilities and similar retention. For this purpose, the peak shapes of I^- and SO_4^{2-} were compared when both analytes had a migration time of around 4 min. Indirect absorbance detection was necessary to detect SO_4^{2-} and Fig. 7 shows the separation of SO_4^{2-} and other UV-transparent anions using an AS5A coated capillary and NO_3^- as the absorbing competing ion in the electrolyte. Comparing the peak for I^- in 10 m*M* Cl^- (peak 6, Fig. 3, 25°C) to that of SO_4^{2-} in 2.5 m*M* nitrate (peak 5, Fig. 7), several things are apparent. The first is the SO_4^{2-} peak has a slower migration time, hence a stronger interaction with the stationary phase than I^- ($k'=0.9$ and 0.8, respectively). Secondly, even with this stronger interaction the peak shape of SO_4^{2-} is very symmetrical and exhibits none of the tailing observed for I^-. Similarly well shaped peaks were observed under the same conditions for PO_4^-. This suggests that the poor peak shapes obtained for the polarisable anions are not characteristic of the OT-CEC approach, but are characteristic of the analytes and their interaction with the stationary phase. Suitable stationary phases that are not able to interact with polarisable analytes through $\pi-\pi$ interactions should result in superior peak shapes than those observed in this work.

A further possibility to explain poor peak shapes is column overloading arising as a result of the small amount of stationary phase present on the column wall. To investigate this, the concentration of an analyte showing considerable ion-exchange interaction with the stationary phase (CrO_4^{2-}) was varied over two-orders of magnitude (0.01–1.0 m*M*) and the results are shown in Fig. 8. It can be seen that when 1 m*M* CrO_4^{2-} was injected, there is evidence of overloading in the faster migration time of the peak maximum and also the peak is much broader than the other concentrations. However, it can be concluded that under the given injection conditions, 0.1 m*M* concentrations of the analytes should not cause significant column overloading and this factor is therefore not responsible for the observed peak shapes.

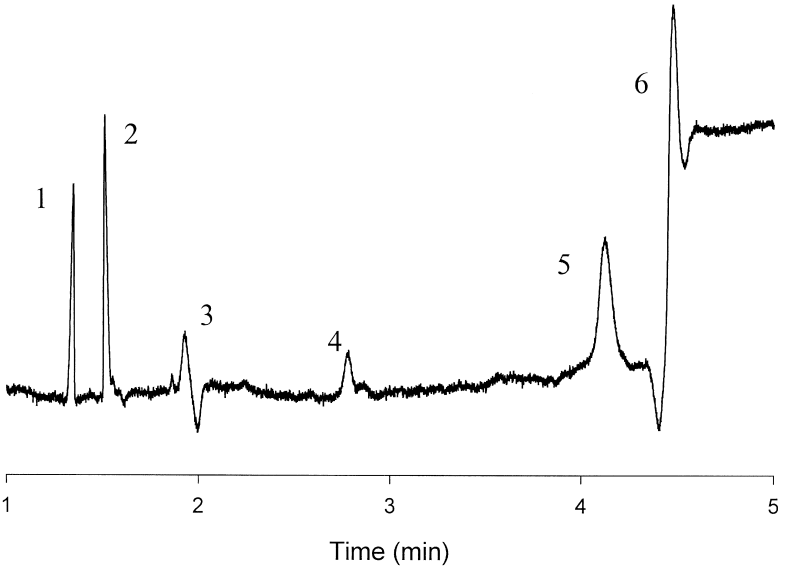

Fig. 7. Separation of inorganic anions using indirect UV detection in an AS5A coated capillary. Conditions: 2.5 m*M* nitrate, 5.0 m*M* diethanolamine (pH 9.0), −25 kV, injection of 1 m*M* ions for 2 s at 10 mbar. Peaks: 1=Cl^-, 2=F^-, 3, 4=system peaks, 5=SO_4^{2-}, 6=EOF.

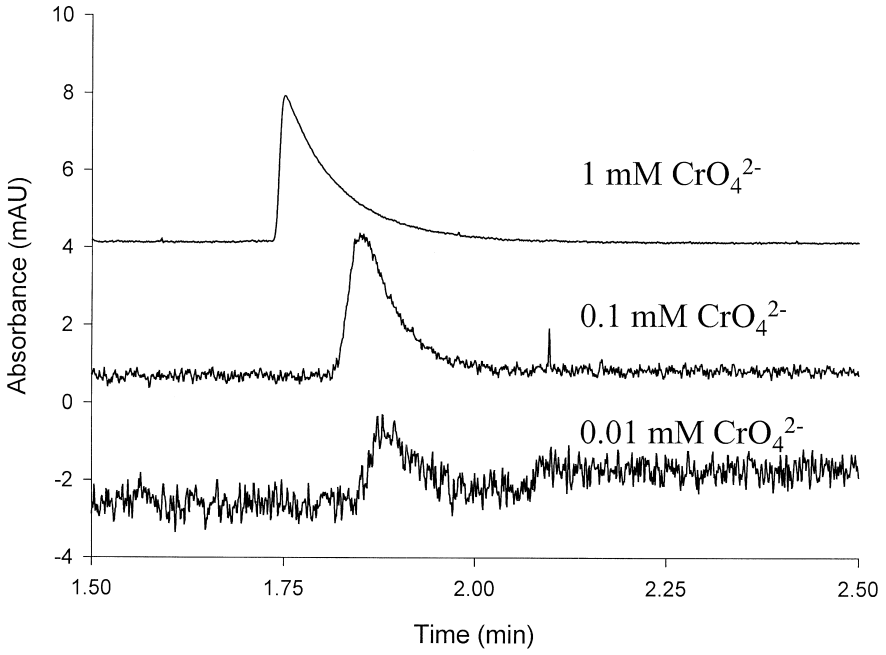

Fig. 8. Injection of decreasing amounts of chromate in an AS5A coated capillary. BGE: 10 m*M* ClO_4^-–20 m*M* Tris (pH 8.05) detection at 254 nm. Scale adjusted for 0.1 m*M* CrO_4^{2-} (×5) and 0.01 m*M* CrO_4^{2-} (×5). Other conditions as in Fig. 1.

4. Conclusions

Contributions to peak shape in open tubular capillary electrochromatography arise from both electrophoretic and chromatographic sources. While the electrophoretic component (electromigration dispersion) is not the major contribution to band broadening in this case, appropriate competing ion mobility is still important. The resistance to mass transfer in the mobile phase can be improved by decreasing the capillary diameter, which results in a significant reduction in peak tailing, especially for polarisable anions. However, this is offset by a reduction in detection sensitivity when using UV absorbance detection. The effect of the separation temperature was inconclusive, possibly because of the small range of temperatures studied and the presence of the ''cold point'' effect. The addition of *p*-cyanophenol to the mobile phase partially suppressed secondary interactions with the stationary phase, but no improvement in peak shape was observed possibly due to incomplete suppression of the secondary interactions. Symmetrical peaks for well retained non-polarisable analytes such as sulfate were observed in a 75 μm column. It remains unclear what causes this different tendency to peak broadening between the polarisable and non-polarisable anions and this deserves further investigation.

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