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Optimisation of the separation of anions by ion chromatography–capillary electrophoresis using indirect UV detection

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Abstract

The separation of a complex mixture of inorganic and organic anions by ion chromatography–capillary electrophoresis using a cationic polymer added to the background electrolyte and indirect UV detection has been studied. The addition of unmodified polymer to an electrolyte suitable for indirect detection resulted in the appearance of a system peak due to the counter-anion on the polymer and while the position of the analytes relative to this system peak could be changed, this was found to be an unacceptable approach for mixtures of large numbers of analytes. Although conversion of the polymer to replace the counter-ion with the indirect UV detection probe ion simplified the system, this approach restricted the flexibility of the system because the probe and polymer concentration were necessarily linked. This limitation could be overcome by selecting the appropriate type of probe ion, with probes having a low ion-exchange selectivity coefficient providing greater retention of analytes than probes with a high ion-exchange selectivity coefficient. Three electrolyte systems with different probes (benzoate, chromate and phthalate) were modelled using a previously derived migration equation and this was used to optimise the electrolyte composition to enable the separation of a mixture of 24 inorganic and organic anions within 7 min. The electrolyte composition was then optimised for the analysis of anions in Bayer liquor with the final separation selectivity being substantially improved for selected key analytes.

Keywords: Ion chromatography–capillary electrophoresis; Optimization; Indirect detection; Mathematical modelling; Background electrolyte composition; Electrochromatography; Electrokinetic chromatography; Inorganic anions; Organic acids; Poly(diallyldimethalammonium chloride)

1. Introduction

The combination of ion chromatography (IC) and capillary electrophoresis (CE) for the separation of small ions has received considerable attention due to the potential to manipulate the separation selectivity by varying the contribution of the two different separation mechanisms. In IC, analyte ions are separated primarily by differences in ion-exchange interactions with a solid stationary phase, whereas CE separation is based on differences in electrophoretic mobility. The separation order of certain analyte ions in IC is often quite different to that occurring in CE. For example, halide anions migrate in the order $F^- < Cl^- < Br^- < I^-$ in IC and in the order $I^- \cong Br^- < Cl^- < F^-$ in CE, so a combination of these two techniques should enable the separation

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selectivity to be varied considerably if the contribution of each separation mechanism can be controlled.

IC and CE can be combined in a number of ways, including using an ion-exchange stationary phase packed into a CE capillary (i.e. using capillary electrochromatography, CEC) [1,2] or adsorbed onto the capillary wall in open tubular CEC columns [3]. Both of these methods have the limitation that the ion-exchange capacity of the column cannot be varied easily. A simpler way is to add a soluble ionic polymer to the background electrolyte (BGE) in a conventional CE system. Such a polymer acts as a ''pseudo-stationary phase'' (since it will move according to electrophoretic principles) and provides many of the advantages of packed capillaries. However, a soluble pseudo-stationary phase has the added advantage that the column capacity can be changed simply and effectively by preparing a new BGE with a different concentration of the pseudo-stationary phase.

The use of a soluble polymer as a method to combine IC and CE can be seen as a variant of electrokinetic chromatography (EKC), and was first demonstrated by Terabe and Isemura [4,5] who added either polybrene or poly(diallyldimethylammonium chloride) to alter the mobilities of isomeric organic acids possessing almost identical electrophoretic mobilities. Both the type and concentration of the polymer were shown to influence the mobility of analyte ions. Stathakis and Cassidy [6,7] extended this technique to enable the separation of UV-transparent ions. Polymer was added to the BGE with a UV-absorbing counter-ion (either chromate or benzoate) being employed to act as an indirect detection ''probe'' ion and also as the ion-exchange competing ion. As expected from IC theory, the use of a competing ion with a low ion-exchange selectivity coefficient (e.g., benzoate) enabled more interaction of the analytes with the polymer than a probe with a high ion-exchange selectivity coefficient (e.g., chromate). Analytes that had high ion-exchange selectivity coefficients interacted more with the polymer than analytes with low selectivity coefficients, and subsequently the change in their observed mobility was larger. Separations of anionic metal complexes, inorganic anions, and mixtures of the two have been shown by Krohkin and co-workers [8–10]. They studied the influence of three different polymers and found the interaction of analytes with the polymers increased in the order polybrene< poly(diallyldimethylammonium chloride)<poly(*N*-ethyl-4-vinylpyridinium bromide). It was also demonstrated that the selectivity could be changed by varying either the concentration of the polymer, the type of polymer, or the concentration of competing ion.

Ion chromatography–capillary electrophoresis (IC–CE) was introduced recently by Li et al. [11] who showed that the mobility of anions could be influenced substantially by the addition of polymer to the BGE, even in the presence of a high concentration of salt (typically 50–150 m*M* NaCl). In this case, the addition of a high concentration of salt dominated the ion-exchange competing ion effect, so the influence of the counter-anion added with the polymer became insignificant. This meant that the concentration of salt and polymer could be changed independently, which was a major advantage over previously demonstrated methods. Even greater flexibility was shown when the nature of the salt anion was changed, giving three independent parameters that could be varied to influence the separation selectivity.

Recently, we developed a mathematical retention model that enabled the polymer and salt concentration to be optimised simultaneously on the basis of only five initial experiments [12]. Agreement between experimental and observed mobilities was excellent ($r^2 > 0.97$) in electrolytes containing one of four different competing ions, ranging from fluoride (which has a low ion-exchange selectivity coefficient) to sulfate (which has a high ion-exchange selectivity coefficient). The benefits of IC–CE were illustrated when the model was used to optimise the separation of a mixture of 16 inorganic and organic anions using only five initial experiments.

While indirect UV detection using a soluble polymer IC–CE system has been reported previously, there has been no comprehensive study involving the optimisation of all three variable parameters, namely the type and concentration of the ion-exchange competing ion and the concentration of the added polymer. The aim of the present study was to establish the conditions under which IC–CE using indirect UV detection can be employed in the separation of a complex mixture of inorganic anions.

2. Experimental

2.1. Instrumentation

The CE instrument used was a Hewlett-Packard ^{3D}CE (Hewlett-Packard, Waldbronn, Germany). Separations were carried out using a Polymicro (Phoenix, AZ, USA) fused-silica capillary [50.0 cm (41.5 cm to detector)×50 μm I.D. unless otherwise noted]. Injection was performed by applying a 50 mbar pressure for 5 s to the cathodic side of the capillary unless otherwise stated. Detection was by indirect UV absorbance at 214 nm (benzoate as probe) or 254 nm (chromate and phthalate as probes).

2.2. Reagents

Analytical-grade tris(hydroxymethyl)amino methane (Tris) and L-histidine were obtained from Sigma–Aldrich (Milwaukee, WI, USA) and were used without further purification. Poly(diallyldimethalammonium chloride) (PDDAC) with a molecular mass of 400 000–500 000 was obtained from Aldrich as a 20% (w/v) solution.

Anion standards of 10 m*M* concentration (Br^-, Cl^-, NO_2^-, NO_3^-, SO_4^{2-}, oxalate, ClO_4^-, ClO_3^-, malonate, formate, F^-, BrO_3^-, citrate, succinate, tartrate, glutarate, adipate, IO_3^-, acetate, propanoate, butanoate, isovalerate, caproate, caprylate) were prepared from the acid form or sodium or potassium salts of analytical-reagent grade. Samples with a concentration of 0.5 m*M* of each anion were prepared in water.

Stock solutions of BGE containing 500 mequiv of probe (chromate, phthalate or benzoate) were prepared by titration of Tris to a pH of 7.70 with the acid form of the probe to give a Tris-probe stock. PDDAC was converted to the probe form by following the procedure of Cassidy and Stathakis [6]. Briefly, PDDAC was passed through an anion-exchange column previously conditioned with hydroxide. Column effluent was collected in a flask with the appropriate concentration of probe in the acid form giving a stock solution of PDDA probe.

BGEs were prepared by mixing appropriate concentrations of Tris probe and PDDA probe to give the desired concentration of polymer and probe. All electrolytes were buffered using 10 m*M* of the ampholytic buffer histidine at a pH of 7.70. All BGEs were degassed by vacuum sonication and filtered through a 0.45 μm filter before use.

3. Results and discussion

3.1. Requirements for indirect UV detection in IC–CE

Indirect detection in CE has been reviewed recently and the following guidelines for appropriate electrolyte composition have been suggested [13]. First, the electrolyte should be buffered in order to provide sufficient ruggedness and reproducibility. This must be performed without introducing any co-ions that can compete with the probe and cause both a reduction in detection sensitivity and the introduction of a system peak. Second, the electrophoretic mobility of the probe should match that of the analytes, and when a selection of analytes with a range of mobilities are to be analysed the probe concentration should be maximised to minimise electromigration dispersion. Third, the molar absorptivity of the probe should be maximised to give the best possible detection sensitivity.

A recent study by Boyce et al. [14] employed the above guidelines for indirect UV detection in ion-exchange capillary electrochromatography using an open tubular ion-exchange CEC column. In this case, in addition to the requirements already indicated, the probe must also function as the ion-exchange competing ion. It was found that indirect UV detection in ion-exchange CEC was feasible but due to the limited ion-exchange capacity of open tubular columns the separation selectivity could not be varied to any significant extent using a single probe ion. A series of probes with differing ion-exchange selectivity coefficients was deemed necessary and using an appropriate selection it was demonstrated that the separation selectivity could be changed from predominantly IC in nature to predominantly CE. Electromigration dispersion was observed to be significant when low concentrations of probes were employed in order to maximise analyte retention.

Indirect detection in IC–CE has similar requirements to those encountered by Boyce et al. [14], but should offer greater flexibility because of the ability

to vary the ion-exchange capacity of the column. Unlike the situation encountered using open tubular columns, low concentrations of competing ion are unlikely to be required because stronger analyte retention can be obtained by increasing the ion-exchange capacity rather than lowering the eluotropic strength of the eluent.

3.2. Potential of adding unmodified PDDAC to the BGE

Indirect detection in CE is best accomplished by having only one co-ion in the electrolyte, namely the probe itself [13]. However, the polymer selected for use in this study was obtained in the chloride form. Nevertheless, it would be desirable if the unmodified polymer could be added to a BGE containing a UV-absorbing probe. The introduction of an additional co-anion (in this case, Cl^-) would cause a reduction in detection sensitivity and the occurrence of a system peak. The extent to which both of these detrimental factors occurs is dependent on the relative mobilities of both the probe and the co-ion. The occurrence and position of the system peak are of great consequence since this peak will interfere with the separation of surrounding peaks. The position of the system peak generated when two co-ions are present in the BGE is given by [15]

$$\mu_s = (\mu_{A1} - \mu_{A2})x - \mu_{A1} \quad (1)$$

where μ_s is the mobility of the system peak, μ_{A1} is the mobility of the first co-ion (higher mobility), μ_{A2} is the mobility of the second co-ion (lower mobility), and x is the mole fraction of the first co-ion. A mixture of equivalent concentrations of two co-ions will give a system peak having a mobility which is the average of the mobilities of the two co-ions. In IC–CE the addition of polymer to the BGE will influence the observed mobility of the analyte anions and it should be theoretically possible to change the position of the analytes relative to the system peak.

To examine the potential of this approach, a two-probe electrolyte containing CrO_4^{2-} and phthalate was prepared using equivalent concentrations of each probe, giving a system peak with mobility between those of the two probes. These UV-absorbing anions were selected for this investigation instead of Cl^-, the form in which the polymer was obtained, so that analytes migrating before and after the system peak could be visualised [15]. The influence of [$PDDA^+$] on the position of the system peak and the peaks of the analytes was determined by adding $PDDA^+$–CrO_4^{2-} to the BGE while keeping the total concentration of CrO_4^{2-} and phthalate constant at 2.5 m*M* each. Fig. 1 shows that as [$PDDA^+$] was increased, the position of the system peak moved to lower mobilities. With 0.8% $PDDA^+$ added to the BGE, the system peak had the lowest mobility and it can be seen that it no longer interfered with the analysis of F^-, although the system peak then interfered with

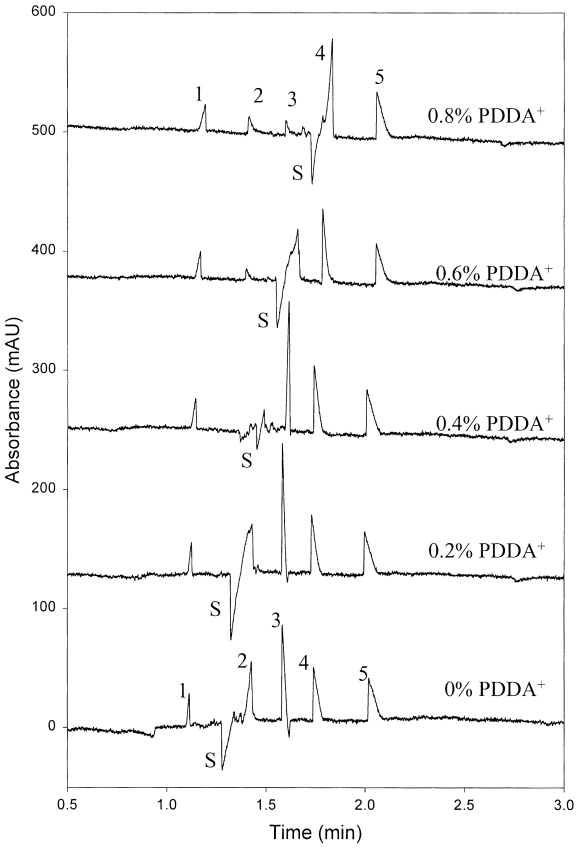

Fig. 1. Influence of adding PDDA on the position of the system peak in the separation of inorganic anions using a multiple probe BGE. All electrolytes contain 2.5 m*M* phthalate and 2.5 m*M* CrO_4^{2-} prepared from phthalate–Tris (pH 7.70), PDDA–CrO_4^{2-} and Na_2CrO_4 to give the appropriate concentration of $PDDA^+$ and were buffered with the addition of 10 m*M* histidine. Separation was performed in a 50.0 cm capillary (41.5 cm to detector)×75 μm I.D. with a voltage of −30 kV. Peaks are: 1=Cl^-, 2=F^-, 3=C_1-SO_3^-, 4=C_2-SO_3^-, 5=C_5-SO_3^-, S=system peak.

the analysis of ethanesulfonate. Whilst it might be possible to improve the final separation by varying the relative ion-exchange selectivity coefficients of the analytes and probes, the approach of using two co-ions was considered to be unsuitable when trying to separate a large number of analytes.

3.3. Indirect detection using a single probe anion

The above investigation suggested that addition of unmodified PDDAC to BGEs used for indirect detection was undesirable. It was therefore necessary to change the counter-ion of the polymer to one that could act both as a suitable probe and as an ion-exchange competing ion. This was achieved by first converting the polymer to the hydroxide form using the method of Stathakis and Cassidy [6] and then titrating this with the acid form of the probe. It should be noted that the polymer was not stable in the hydroxide form for extended periods and immediate titration of the polymer after elution from the ion-exchange column used to convert it to the hydroxide form was necessary in order to prevent decomposition of the polymer.

Conversion of the polymer to the probe form restricts the flexibility of the IC–CE system demonstrated by Fritz and co-workers [11,12] because the concentration of probe and polymer are invariably linked. This therefore restricts the size of the available experimental space because the probe concentration can never be lower than the concentration required to satisfy electroneutrality with the polymer. A higher concentration of probe can be employed by adding the probe as an alternative form (such as Na^+).

To examine the requirements for indirect UV detection in IC–CE, three different competing ions, CrO_4^{2-}, phthalate and benzoate, were selected on the basis of their relative ion-exchange strengths and the experimental space was defined between 0 and 0.64% $PDDA^+$ and 10–40 mequiv of probe (5–20 m*M* for CrO_4^{2-} and phthalate, and 10–40 m*M* for benzoate). The influence of the variation of each of these parameters is discussed below in further detail.

3.3.1. Variation of polymer concentration

Increasing the concentration of polymer in the BGE is equivalent to increasing the column ion-exchange capacity in IC. This will result in a decrease in the observed mobility of the analytes due to a higher degree of interaction with the polymer. Fig. 2 shows the influence of increasing [$PDDA^+$] on the observed mobilities of 16 analyte anions using phthalate as the competing ion. It can be seen that some analytes were influenced by the addition of $PDDA^+$ more than others, for example the mobility of oxalate was reduced substantially in comparison to acetate. This enabled the separation selectivity to be varied. Data for all 24 analyte anions were obtained but eight analytes are not included in Fig. 2 to improve the clarity of the figure.

3.3.2. Variation of probe concentration

The influence of increasing the concentration of the competing ion in an ion-exchange system is well established and results in a decrease in interaction between the analytes and the stationary phase, causing analytes to be eluted earlier. The same situation occurs in IC–CE, with interaction between the analytes and the polymer being suppressed at higher concentrations of competing ion, resulting in an increase in observed mobilities. Fig. 3 shows this influence using a constant polymer concentration of

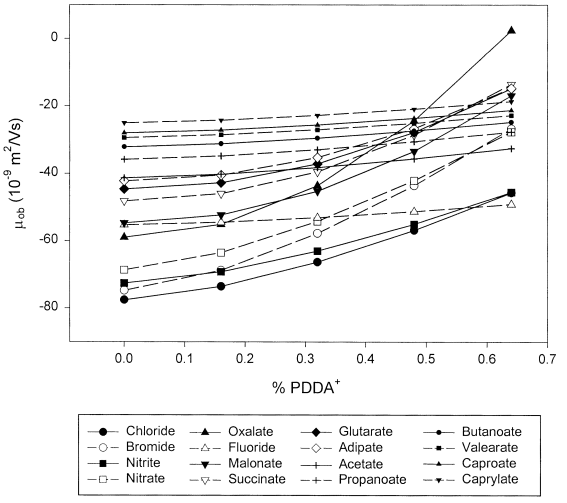

Fig. 2. Influence of increasing the concentration of $PDDA^+$ on the separation selectivity of selected anions in a phthalate electrolyte. The electrolyte was prepared from phthalate–Tris (pH 7.70) and phthalate–$PDDA^+$ to give a total concentration of 20 m*M* phthalate and was buffered with 10 m*M* histidine. Other conditions as in Fig. 1.

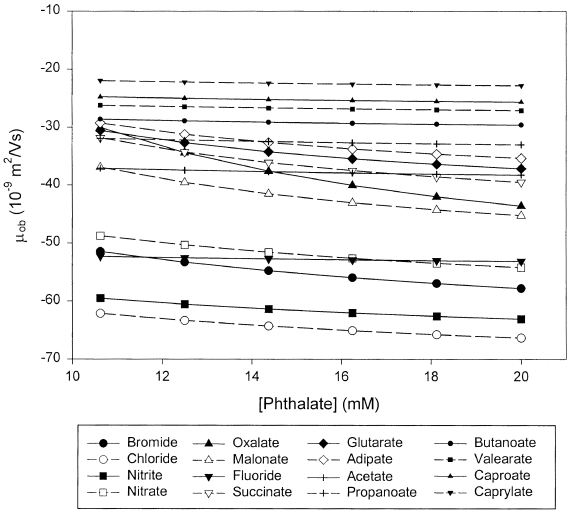

Fig. 3. Influence of increasing the concentration of phthalate on the separation selectivity of selected anions in a BGE containing 0.40% $PDDA^+$. Other conditions as for Fig. 2.

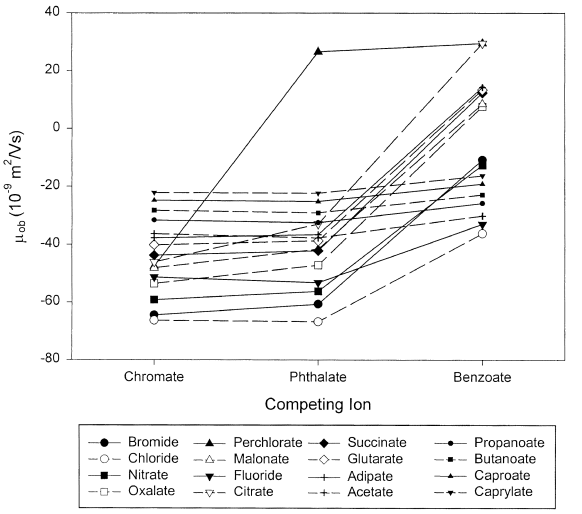

Fig. 4. Influence of varying the ion-exchange strength of the probe on the observed mobilities of selected anions. All electrolytes contain 0.32% $PDDA^+$ and the probe concentration was 20 m*M* (CrO_4^{2-} and phthalate) or 40 m*M* (benzoate). Other conditions as for Fig. 2.

0.40% and 10–20 m*M* phthalate as the competing ion.

3.3.3. Variation of the ion-exchange selectivity coefficient of the probe

The interdependence of the polymer and competing ion concentrations restricts the accessible BGE compositions and means that the ion-exchange selectivity coefficient of the probe becomes an important parameter. Fig. 4 shows changes in observed mobility for 16 analyte anions using three different competing anions and equivalent concentrations of polymer. Chromate has the highest ion-exchange selectivity coefficient and it can be seen that analytes exhibited the highest observed negative mobilities in this BGE, indicating a low level of interaction with the polymer. Conversely, analytes exhibited the lowest observed negative mobilities in the benzoate BGE, indicating a high degree of interaction with the polymer. Phthalate has an intermediate ion-exchange selectivity coefficient, and observed mobilities of the analytes values fell between those for chromate and benzoate. It should be noted that the mobility of ClO_4^- was unchanged when going from the phthalate to benzoate systems because both of these competing ions were ineffective at modifying the interactions of ClO_4^- with the polymer under the conditions used.

The significance of being able to vary the ion-exchange selectivity coefficient of the probe becomes important when practical considerations regarding BGE composition are made. For example, in a chromate BGE with 0.64% $PDDA^+$ added there is only minimal interaction of most of the analytes with the polymer. More interaction can be obtained by increasing the polymer concentration further, however this is not a good option for several reasons. First, as the concentration of polymer increases, the concentration of chromate increases concomitantly and the baseline becomes noisier because of the increased background absorbance. It is also important that the background absorbance remains within the linear range of the detector. Second, the increase in ionic strength of the BGE will result in a higher current and increased Joule heating, leading to a noisier baseline and a reduction in separation efficiency. Third, the viscosity of the BGE increases with increasing polymer concentration until it becomes impractical to add more due to increased analysis and flushing times. Changing the competing ion from CrO_4^{2-} to benzoate would establish a higher degree of interaction between the analytes and the polymer without any of the above problems outlined.

3.4. Optimisation of electrolyte conditions

The above discussion indicates that the separation selectivity and hence resolution between analytes can be adjusted by changing the concentration of polymer, the concentration of competing ion, and/or the type of competing ion. However, when a large number of analytes is to be separated, selecting the appropriate electrolyte conditions can be difficult. Recently, Breadmore et al. [12] demonstrated that the separation selectivity in IC–CE can be optimised using five initial experiments to solve the following migration equation:

$$\mu_{ob} = \frac{1}{1 + (w/V_{mp})(K'_{A,E})^{1/y}(Q/y)^{x/y}[E]^{-x/y}}\left(\frac{b_{mp}}{\sqrt{I}}\right) + \frac{(w/V_{mp})(K'_{A,E})^{1/y}(Q/y)^{x/y}[E]^{-x/y}}{1 + (w/V_{mp})(K'_{A,E})^{1/y}(Q/y)^{x/y}[E]^{-x/y}}\left(\frac{b_{sp}}{\sqrt{I}}\right) \quad (2)$$

where μ_{ob} is the observed mobility of the analyte, w is the mass of the polymer, V_{mp} is the volume of the mobile phase, $K_{A,E}$ is the selectivity coefficient of analyte A over eluent E, Q is the ion-exchange capacity of the polymer, y is the charge of the eluent anion, x is the charge of the analyte anion, [E] is the concentration of eluent anion, b_{mp} is the constant relating the influence of ionic strength on electrophoretic mobility, I is the ionic strength and b_{sp} is the influence of ionic strength on the mobility of the polymer. In Eq. (2), w/V_{mp} is defined by the concentration of $PDDA^+$ added to the electrolyte (as %, w/v), Q is likewise defined by the concentration of the polymer and can be estimated from the number of repeat units of polymer added. The other parameters, x, y, [E] and I are defined by the particular analyte under consideration and the conditions used. The remaining parameters, $K'_{A,E}$, b_{mp} and b_{sp}, are not defined and need to be determined using non-linear regression applied to experimental data measured under defined conditions.

In order to optimise the BGE composition, the constants in Eq. (2) need to be determined. To do this, a data set comprising the observed mobilities of the 24 analyte anions determined at five BGE compositions (termed the primary data set) was acquired. The primary data set was acquired using 0% $PDDA^+$/10 mequiv probe, 0% $PDDA^+$/40 mequiv probe, 0.64% $PDDA^+$/40 mequiv probe, 0.32% $PDDA^+$/25 mequiv probe, and 0.16% $PDDA^+$/33 mequiv of probe. Values for the system constants in Eq. (2) for each analyte are shown in Table 1 for three probe ions. A secondary data set containing five randomly selected data points over the experimental area showed excellent correlation between the experimental and predicted observed mobilities with correlation coefficients greater than 0.97 being obtained for the three electrolyte systems. It should be noted that values for the selectivity coefficient for perchlorate and citrate could not be obtained in the benzoate electrolyte using the primary data set due to complete association of this analyte with the polymer, even at the lowest concentrations of polymer employed in this study.

From Table 1 it can be seen that the selectivity coefficients were highest when benzoate was used as the competing ion and lowest when CrO_4^{2-} was used. Analytes therefore showed significantly more interaction with the polymer in benzoate BGEs than the phthalate and chromate BGEs, which is also apparent from Fig. 4. Several trends are evident from Table 1, especially for the weakly interacting carboxylic acids. For example, when considering the monocarboxylic acids, selectivity coefficients for the benzoate BGE decreased as the carbon chain length increased up to C_5 (valerate) and then increased slightly for C_6 and C_8. This trend can be explained in terms of steric hindrance between the analyte and the $PDDA^+$ functional group increasing with analyte size, up to the point where sufficient carbon chain length is reached to cause secondary, hydrophobic interactions with the polymer. A similar reduction in selectivity coefficients is observed for the dicarboxylic acids, oxalate (C_2) through to adipate (C_6). This again is probably due to the increased size of the analyte molecule, coupled with the increased distance between the two anionic groups on the analyte.

Optimisation of the separation conditions for the separation of all 24 analyte anions was possible using the determined system constants and a suitable resolution criterion. In this case, the choice of probe was restricted to chromate and phthalate because there was excessive retention of perchlorate and citrate in the benzoate BGE. As the goal was to

Table 1
Values of constants (for analytes arranged in order of decreasing electrophoretic mobility) in Eq. (2) as determined from non-linear regression

Analyte	CrO_4^{2-}, $b_{sp} = 3.01$		Phthalate, $b_{sp} = 2.98$		Benzoate, $b_{sp} = 2.58$	
	$K_{A,E}$	b_{mp}	$K_{A,E}$	b_{mp}	$K_{A,E}$	b_{mp}
Bromide	0.167	−75.1	0.372	−75.5.1	2.27	−72.8
Chloride	0.071	−73.3	0.132	−72.4	1.43	−70.7
Nitrite	0.069	−74.8	0.107	−74.6	1.47	−72.0
Nitrate	0.111	−72.8	0.259	−72.4	2.02	−69.0
Sulfate	2.64	−67.1	3.21	−67.7	18.3	−66.8
Oxalate	3.373	−67.4	7.43	−67.0	19.3	−68.8
Perchlorate	4.17	−64.4	8.64	−67.0	–	−64.3
Chlorate	0.066	−61.1	0.121	−64.2	1.51	−63.9
Malonate	2.07	−56.6	3.03	−54.2	16.7	−52.5
Formate	0.025	−54.5	0.018	−56.3	0.419	−55.8
Fluoride	0.015	−54.5	0.009	−56.3	0.867	−51.6
Bromate	0.035	−54.5	0.031	−55.9	0.817	−53.4
Citrate	2.94	−53.4	14.1	−52.5	–	−55.6
Succinate	1.73	−51.5	1.89	−52.1	14.5	−54.2
Tartrate	2.00	−50.7	2.08	−50.7	18.3	−53.9
Glutarate	1.27	−46.8	1.29	−46.9	13.0	−41.6
Adipate	1.11	−43.9	1.07	−44.1	12.8	−41.6
Iodate	0.011	−39.0	0.004	−39.6	0.318	−35.8
Acetate	0.006	−38.5	0.006	−38.37	0.298	−36.7
Propanoate	0.006	−33.7	0.006	−34.9	0.275	−31.9
Butanoate	0.005	−30.2	0.004	−31.2	0.269	−30.7
Valearate	0.006	−27.8	0.003	−28.5	0.257	−26.3
Caproate	0.005	−26.6	0.003	−27.0	0.272	−25.0
Caprylate	0.004	−23.9	0.003	−24.1	0.278	−22.4
r^2	0.990		0.979		0.986	
Slope	1.02		1.04		1.05	
Intercept	0.864		1.13		1.56	

separate all of the anions, the minimum resolution criterion was selected:

$$r_{min} = \min(R_{s(i,i+1)}) \quad (3)$$

where $R_{s(i,i+1)}$ is the resolution between adjacent peaks. This criterion takes the value of the resolution between the worst resolved peak pair in the entire separation. The highest criterion value will therefore be when the resolution between the worst pair is at its greatest. Fig. 5 shows the resolution surface for all 24 ions using a chromate BGE. The use of CrO_4^{2-} as the competing ion was found to provide a higher criterion value than phthalate, due largely to the higher separation efficiency of the high mobility inorganic anions as a result of lower electromigration dispersion in this electrolyte. The optimum separation conditions were at 0.28% $PDDA^+$ and 16.25 m*M* CrO_4^{2-}, and the optimised separation is shown in Fig. 6. It can be seen that all 24 analytes were well separated and the total analysis time was under 7 min. The analytes are numbered in order of decreasing electrophoretic mobility and the elution sequence in Fig. 6 indicates that the separation selectivity differed significantly from that obtainable by conventional CE.

3.5. Optimisation and separation of components of Bayer liquor

The determination of anions in Bayer liquor was investigated as a potential application where the advantage of being able to manipulate the separation selectivity would be beneficial. Analytes of interest

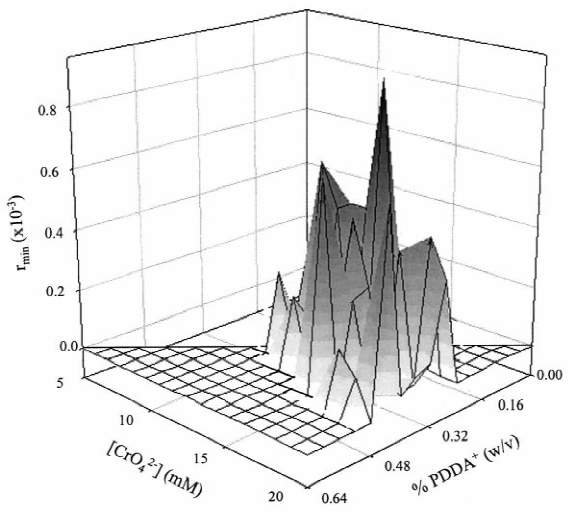

Fig. 5. Minimum resolution surface response for the separation of 24 anions in a CrO_4^{2-}–$PDDA^+$ BGE. Optimum separation conditions are at 0.28% $PDDA^+$ and 16.25 m*M* CrO_4^{2-}. Conditions as in Fig. 2.

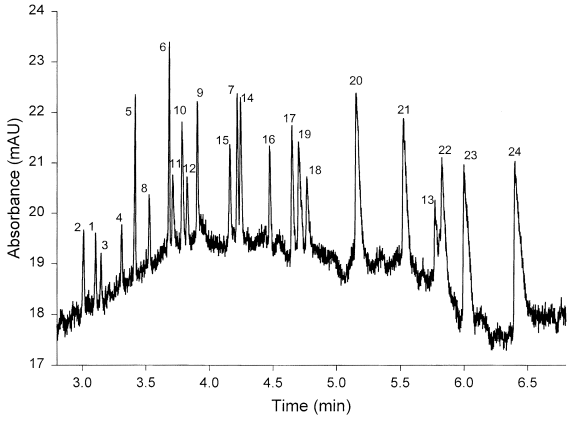

Fig. 6. Optimised separation of 24 anions by IC–CE using indirect UV detection with CrO_4^{2-} as the competing ion/probe. Conditions are 0.28% $PDDA^+$ and 16.25 m*M* CrO_4^{2-} buffered with 10 m*M* histidine (pH 7.70). Peaks are numbered according to their capillary zone electrophoretic migration order. Other conditions as in Fig. 2. Peaks are: 1=Br^-, 2=Cl^-, 3=NO_2^-, 4=NO_3^-, 5=SO_4^{2-}, 6=oxalate, 7=ClO_4^-, 8=ClO_3^-, 9=malonate, 10=formate, 11=F^-, 12=BrO_3^-, 13=citrate, 14=succinate, 15=tartrate, 16=glutarate, 17=adipate, 18=IO_3^-, 19=acetate, 20=propanoate, 21=butanoate, 22=isovalerate, 23=caproate, 24=caprylate.

in Bayer liquor are chloride, oxalate, fluoride, formate, acetate and sulfate. This application is often considered to be problematic by CE due to the high pH of the sample and the high sample ionic strength, both of which result in poor separation efficiencies. A further problem is the potential co-migration of several analytes, particularly, fluoride/formate and chloride/oxalate/sulfate. The application of IC–CE can potentially eliminate this problem by manipulation of the separation selectivity to improve the resolution of important peak pairs. In optimising the electrolyte conditions for this analysis, the concentration of chromate was restricted to >15 m*M* as preliminary studies by CE indicated that this provided sufficient ionic strength for analyte stacking to occur for the Bayer liquor sample. The optimum separation conditions identified using the same approach outlined above and again employing the minimum resolution criterion were 20 m*M* CrO_4^{2-}

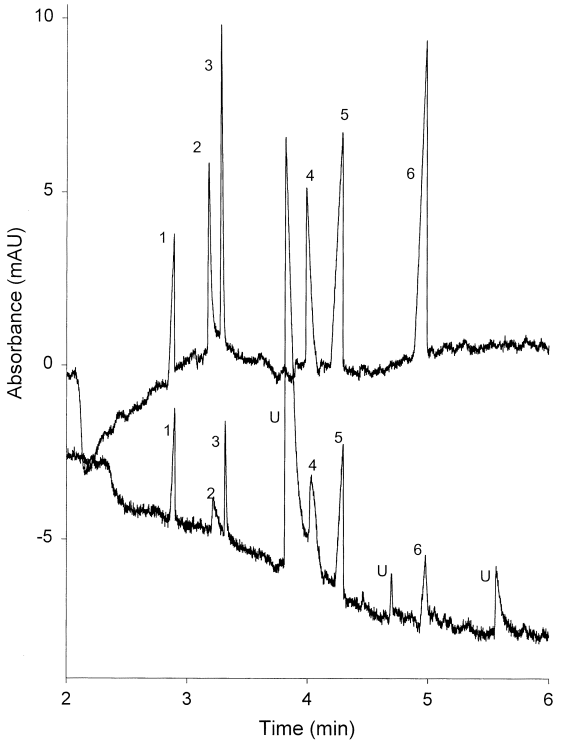

Fig. 7. Separation of anions found in Bayer liquor. Top trace is a standard solution, while the bottom separation is a real sample. Electrolyte composition contains 0.55% $PDDA^+$ and 20 m*M* CrO_4^{2-}. Other conditions as in Fig. 2. Peaks are: 1=Cl^-, 2=F^-, 3=formate, 4=acetate, 5=sulfate, 6=oxalate.

and 0.55% $PDDA^+$. The separation of synthetic and actual Bayer liquor samples obtained under these conditions are shown in Fig. 7. It can be seen that peak shapes were well maintained in the standard sample and the resolution between all peak pairs was satisfactory. Application to the real sample gave a similar selectivity to the standard but co-migration of acetate with an unknown peak occurred, possibly CO_3^{2-}. Whilst this example illustrates the potential of IC–CE to analyse complex samples, a further potential advantage of the possibility for identification of unknown peaks (such as that appearing in the real sample) is enhanced by the fact that the separation selectivity can be changed substantially by varying the electrolyte conditions and comparison with known standards in different BGEs would provide substantial supporting evidence for analyte identification.

4. Conclusions

It has been shown that IC–CE using indirect UV detection can be used for the separation of complex mixtures of inorganic and organic anions. The separation selectivity can be adjusted by varying the electrolyte composition, with changes in probe concentration, probe type and polymer concentration having a significant influence on selectivity. Conversion of the polymer to the probe form is required to remove the interference of a system peak created when unmodified polymer is added to the electrolyte. Although this modification restricts the concentration of both polymer and probe that can be employed, the ability to change the ion-exchange selectivity coefficient of the probe more than compensates for this limitation. Analyte migration in three different electrolyte systems (benzoate, phthalate and chromate) was modelled with a previously described migration equation and this enabled the electrolyte composition for the separation of a mixture of 24 inorganic and organic anions to be optimised. Further potential of IC–CE was demonstrated by separating target anions in Bayer liquor with a separation selectivity differing substantially from that attainable in conventional CE.

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