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Review

Use of ionic polymers as stationary and pseudo-stationary phases in the separation of ions by capillary electrophoresis and capillary electrochromatography

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Abstract

One of the problems with capillary electrophoresis is a lack of versatility regarding manipulation of the separation selectivity. A new and potentially universal concept is to introduce an ion-exchange component into a separation so that the migration of analyte ions is influenced by both their electrophoretic mobilities and their chromatographic properties. This may be accomplished by use of capillaries filled with or coated with solid ion-exchange polymers, or by addition of a soluble ionic polymer to the background electrolyte to create a pseudo-stationary phase. While each of these methods achieves the same result, they are not competitive, but rather complementary as the problems associated by one approach are overcome by the others. Recent highlights in the field are used to illustrate the flexibility that this approach provides to electrophoretic separation of ions.

Keywords: Reviews; Ion chromatography–capillary electrophoresis; Electrochromatography; Stationary phases, electrochromatography; Capillary electrophoresis–ion chromatography; Inorganic anions; Organic acids; Inorganic cations

Contents

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1. Introduction

The importance of being able to separate and quantify inorganic and small organic ions was fully realized only after ion chromatography (IC) came into general use in the early 1980s. In modern IC an ionic mobile phase (the eluent) is pumped through an ion-exchange column, causing sample ions to move at different rates and thus be separated from one another. Separation is achieved by differential interaction of analytes with an oppositely charged stationary phase via an ion-exchange mechanism. Detection is most commonly accomplished by either suppressed or non-suppressed conductivity. IC is rugged and dependable and has relatively good chromatographic efficiency [1,2].

Capillary electrophoresis (CE) is a more recent method in which ions are separated in an open-tubular capillary that is filled with a pH-buffered electrolyte called the background electrolyte (BGE). The capillary is generally fused-silica 50- or 75 μm I.D. and 50 cm or more in length. Application of a potential up to 30 kV causes sample ions to move toward the electrode of opposite charge and through a detector. Separation is based on differences in migration velocities of the sample ions, which result from a combination of electrophoretic (analyte specific) and electroosmotic (analyte non-specific) migration. Generally, the separation efficiency in CE can be as much as 10 times greater than that achieved in IC (as expressed in theoretical plates) and CE can potentially provide much faster separations than IC [3–5].

One of the problems with CE is a lack of versatility compared to IC regarding manipulation of the separation selectivity. In IC, selectivity (and hence resolution) can be changed by varying the characteristics of the stationary phase or the composition of the eluent. In CE, selectivity manipulation is more problematic and the normal approach to improving resolution is to extend the residence time of the analytes in the capillary by adjusting both the direction and magnitude of the electroosmotic flow (EOF) [6,7]. Since the electroosmotic migration is the same for all analytes, changes in EOF do not affect selectivity and other means must be sought to modify the electrophoretic migration of sample ions relative to one another. In the separation of metal cations this has been accomplished by adding a ligand to the BGE that will complex the cations to varying degrees and thereby enhance the resolution. Separation of as many as 27 cations in a single run has been accomplished by partial complexation with hydroxyisobutyric acid (HBIA) or lactic acid [8]. For anions, the situation is more difficult. Changes in pH can be an effective way to enhance differences in electrophoretic mobility of some ions, but this is restricted to ionizable analytes and the mobility change occurs only over a relatively narrow pH range. The addition of one or more organic solvents to the BGE may affect the solvation of analyte ions to different extents and thus alter their relative mobilities [9], but in many instances, analytes are affected in a similar manner and thus the potential for selectivity manipulation is limited. The addition of a surfactant to form micelles may alter the net electrophoretic mobilities of analyte ions by hydrophobic interaction, but this is limited to hydrophobic ions which can partition into the internal cavity of the micelle.

A new and potentially more universal concept is to introduce an ion-exchange component into the separation so that the migration of analyte ions is influenced by both their electrophoretic mobilities and their chromatographic properties. This is an attractive approach because the affinities of ion exchangers for analyte ions are often completely different from the order of migration based on their

electrophoretic mobilities. For example, in CE the migration order of the halide anions is: I^-, Br^-> Cl^->F^- while in ion-exchange chromatography the migration order is reversed: F^->Cl^->Br^->I^-. This difference arises from the different separation mechanisms: in CE, analytes are separated on the basis of their size-to-charge ratio, while in IEC analytes are separated predominantly on their electrostatic interactions with the functional groups on the ion exchanger and the establishment of the following equilibrium (shown for anions):

$$A^{x-} + SP\text{–}E^{y-} \rightleftharpoons E^{y-} + SP\text{–}A^{x-} \quad (1)$$

where A^{x-} is the analyte anion, E^{y-} is the eluent anion, SP is the stationary phase, x is the charge on the analyte anion, and y is the charge of the eluent anion. A combination of IEC and CE should therefore provide separation selectivities intermediate between those of the two techniques used alone and also offer the possibility of ''tuning'' the separation to give a desired selectivity by controlling the relative contributions of the electrophoretic and chromatographic mechanisms to the separation.

A simple method to accomplish this goal is the addition of a surfactant of opposite charge to the analytes into the BGE at a concentration sufficient to form a micelle [3,4,10]. Sample ions are retarded by forming ion pairs with the surfactant molecules in the micelles which move electrophoretically in the opposite direction to the ions (and hence can be referred to as a pseudo-stationary phase). Some success has been obtained with this approach [11–13], but there are several limitations. Firstly, precipitation may occur between the analyte ions and the surfactant. Secondly, the analytes can interact with both micelles and surfactant monomers which move electrophoretically at different speeds, leading to band broadening. Thirdly, the extent to which this method can be used to change the separation selectivity of analyte ions is rather limited because the interactions with the micelle are relatively weak, so only small changes are usually reported [12].

A new alternative to surfactants is the use of a soluble ionic polymer added to the BGE, which while utilizing a similar separation mechanism, provides greater separation efficiencies [14,15]. In this system, analyte ions undergo ion-exchange competition with the other ions in the BGE (such as the counter-ion of the charged polymer or a further ion added deliberately), with the result that the migration of the analyte ions slows when they are bound to the slow-moving polymer. The names ''ion-exchange electrokinetic chromatography'' [14], ''ion electrokinetic chromatography'' [16,17] and ''ion chromatography–capillary electrophoresis (IC–CE)'' [18] have been suggested for separations of this type.

Capillary electrochromatography (CEC) using solid stationary phases packed into a capillary has also recently become an active research area for separation of both neutral and ionic analytes and functions by combining chromatographic and electrophoretic separation mechanisms. In contrast to the methods discussed above using soluble polymers, packed-capillary CEC uses a heterogeneous stationary phase to provide a ''true'' chromatographic system. This approach has the advantage that pre-existing knowledge and chromatographic phases can be employed.

The purpose of the present review is to acquaint the reader with some of the recent developments in the separation of ions resulting from the use of ionic polymers, especially the use of both soluble and solid polymers, for the separation of small ions. In order to provide appropriate perspective, the use of surfactants for selectivity manipulation has also been included. The major criterion for selection of material for this review has been that the method discussed must involve both electrophoretic and ion-exchange effects in the separation. In some cases a further effect (usually hydrophobic interactions between the polymer and sample ions) may also be operative. Cations as well as anions are included as analytes, but it should be recognized that selectivity manipulation of cations is typically achieved by complexation effects rather than ion-exchange and as such, most of the examples presented here deal with separation of inorganic or relatively small organic anions.

2. Surfactants as pseudo-stationary phases

Surfactants were one of the first types of pseudo-stationary phases introduced to CE and as such there has been considerable work on the use of surfactants

for selectivity control for inorganic anions. In this approach, a surfactant of opposite charge to the analytes is added to the BGE. Both monomeric and aggregated surfactant molecules (micelles) are present when the concentration of surfactant is greater than the critical micelle concentration (CMC). As analytes migrate through the column, they can interact with both the monomeric and micellar forms of the surfactant, with the overall net mobility of the analyte ($\mu_{A,net}$) given by:

$$\mu_{A,net} = x_A(\mu_A + \mu_{EOF}) + x_{mon}(\mu_{EOF}) + x_{mic}(\mu_{mic} + \mu_{EOF}) \quad (2)$$

where μ_A and μ_{mic} are the electrophoretic mobilities of the free analyte, the analyte–monomer complex and the analyte–micelle complex, respectively, μ_{EOF} is the electroosmotic flow and x_A, x_{mon} and x_{mic} are the mole fractions of free analyte, monomer-associated analyte and micelle-associated analyte. It is assumed that the mobility of the micelle does not change when analytes are associated, and the interaction with monomeric surfactant molecules occurs so that the complex is neutral, and as such will have no electrophoretic mobility and will migrate solely with the EOF.

It should be emphasized at this point, that the micelles and analytes are oppositely charged and therefore will move in opposite directions. The contribution of the monomeric surfactant to the overall migration depends on the ion-pair formation constant but not the total concentration of surfactant (if this is above the CMC) because the concentration of monomer remains constant. On the other hand, the contribution of the micelles to the overall migration depends on both the distribution coefficient of the analyte into the micelle and also on the concentration of surfactant when above the CMC.

The use of surfactants for the selectivity manipulation of ions in CE was pioneered by Jones and Jandik [4] who were the first to examine the effect of adding a cationic EOF modifier on the migration of inorganic anions. They found that increasing the concentration of cationic EOF modifier (in this case, the proprietary reagent Waters OFM anion BT) up to 5.0 m*M* resulted in a decrease in analyte mobility, particularly for nitrate, sulfate and bromide. They proposed that ion-association between the anions and the long chain quaternary ammonium compound in the EOF modifier was responsible.

Kaneta et al. [10] observed greater changes than those observed by Jones and Jandik when they added high concentrations of the cationic surfactant cetyltrimethylammonium chloride (CTAC) to the electrolyte for the separation of inorganic anions. They found that when CTAC was added below its critical micelle concentration (CMC, 1.0 m*M*) the migration order resembled that based on electrophoretic mobilities, with only iodide showing some interaction with the surfactant monomers. However, at 25 m*M* CTAC they found considerable interaction of all analyte anions (bromide, iodide, nitrate, bromate and iodate) with the micelles, resulting in a complete reversal of separation selectivity. They observed that the manner in which the mobility changed with surfactant concentration depended on whether the surfactant was added above or below the CMC, suggesting that the association constants with the micelles and monomers differed. Equations were derived to estimate ion-association constants with the monomers and distribution coefficients into the micelle.

The effect of varying the chain length of the surfactant and its influence on the separation of ions was examined by Buchberger and Haddad [9]. The addition of dodecyltrimethylammonium bromide (DTAB, C_{12}), tetradecyltrimethylammonium bromide (TTAB, C_{14}) and hexadecyltrimethylammonium bromide (HTAB, C_{16}) all reduced the mobilities of inorganic anions, with a more pronounced effect being observed for the longer chain length surfactants. It was also noted that the different surfactants influenced the migration of each anion in a different manner. This was subsequently exploited by using combinations of DTAB and CTAB to modulate the separation selectivity of inorganic and small organic anions [13]. The migration order was found to be dependent on both the total concentration of surfactant used and also on the ratio of the two surfactants. This approach was used to optimize the separation of anions in Bayer liquor [11].

The use of different EOF modifiers and their effect on the separation of anions was examined by the groups of Galceran et al. [19] and François et al. [20]. Low concentrations in the range 0–0.8 m*M*

Table 1
Separations of inorganic and small organic ions using an ionic surfactant as the pseudo-stationary phase

Analytes	Chromatographic phase	Electrolyte	Column dimensions	Detection	Efficiency (plates/m)	Ref.
Thiosulfate, bromide, chloride, sulfate, nitrite, nitrate, molybdate, azide, tungstate, monofluorophosphate, chlorate, citrate, fluoride, formate, phosphate, phosphite, chlorite, galactartrate, carbonate, acetate, ethanesulfonate, propionate, propanesulfonate, butyrate, butanesulfonate, valerate, benzoate, L-glutamate, pentanesulfonate and D-gluconate	Waters OFM anion BT (0.5 m*M*)	5 m*M* sodium chromate	520 mm×50 μm I.D. (600 mm total)	Indirect UV, 254 nm	NS	[4]
Bromide, nitrate, bromate, iodide and iodate	CTAC (0.2–25 m*M*)	20 m*M* phosphate–Tris buffer, pH 7.0	300 mm×50 μm I.D. (500 mm total)	Direct UV, 214 nm	NS	[10]
Fluoride, thiocyanate, chlorite, nitrite, nitrate, sulfate, iodide, chloride, bromide and thiosulfate	DTAB, TTAB and HTAB (0.5 m*M*)	5 m*M* sodium chromate	520 mm×75 μm I.D. (600 mm total)	Indirect UV, 254	NS	[9]
Bromide, chloride, sulfate, nitrite, nitrate, fluoride, formate, carbonate, acetate, propionate, butyrate, valearate	Waters OFM anion BT (0.075 m*M*)	5 m*M* chromate, 0, 3 or 5% (v/v) 1-butanol	625 mm×75 μm I.D. (700 mm total)	Indirect UV, 254 nm	NS	[61]
Chromate	TTAB (0–90 m*M*)	20 m*M* phosphate, pH 10	330 mm×50 μm I.D. (530 mm total)	Direct UV, 237 nm	NS	[60]
Chloride, nitrite, sulfate, nitrate, fluoride, bromate, phosphate and carbonate	Mixture of DTAB (2.65 m*M*) and TTAB (2.35 m*M*)	5 m*M* sodium chromate adjusted to 8.8 with NaOH	520 mm×75 μm I.D. (600 mm total)	Indirect UV, 254 nm	NS	[13]

Table 1. Continued

Analytes	Chromatographic phase	Electrolyte	Column dimensions	Detection	Efficiency (plates/m)	Ref.
Chloride, sulfate, oxalate, fluoride, formate, malonate, succinate, tartrate, phosphate, carbonate, acetate and citrate	Mixture of DTAB (1 m*M*) and TTAB (5 m*M*)	7.5 m*M* sodium chromate, adjusted to pH 9.1 with NaOH	520 mm×75 μm I.D. (600 mm total)	Indirect UV, 254 nm	NS	[11]
Iodide, nitrite, nitrate and thiocyanate	DTAB (50 m*M*)	18 m*M* borate, 30 m*M* phosphate, 10% propanol, pH 7.0	530 mm×50 μm I.D. (760 mm total)	Direct UV, 235 nm	100,000–125,000	[61]
Nitrite, nitrate and bromide	CTAC (1.1 m*M*)	20 mm tetraborate, pH 8.94	255 mm×50 μm I.D. (500 mm total)	Direct UV, 200 nm	NS	[62]
Bromide, chloride, sulfate, nitrite, nitrate and phosphate	HMB (0.4 m*M*) TTAB (0.2 m*M*) CTAB (0.02 m*M*) HDMB (2×10^{-4} m*M*)	5 m*M* sodium chromate, pH 8.0	500 mm×50 μm I.D. (720 mm total)	Indirect UV, 254 nm	NS	[19]
Bromide, chloride, nitrite, sulfate, nitrate, chlorate, fluoride and phosphate	HMB (0.1 m*M*) DMB (0.1 m*M*) TTAB (0.1 m*M*) THPB (0.1 m*M*)	5 m*M* potassium dichromate, 1.6 m*M* triethanolamine, pH 8	400 mm×75 μm I.D. (470 mm total)	Indirect UV, 260 nm	NS	[20]
1-Naphthalenesulfonic acid, 2-naphthalenesulfonic acid,	1,5-bis(triethylammonium) propane (10 m*M*)	10 m*M* phosphate, pH 7	Celect-N coatint (Supelco)	Direct UV, 230 nm	NS	[63]
1,5-naphthalenedisulfonate acid, 1,6-naphthalenedisulfonate,	1,5-bis(triethylammonium) pentane (10 m*M*)		500 mm×50 μm I.D. (720 mm total)			
2,6-naphthalenedisulfonate, 2,7-naphthalenedisulfonate,	1,5-bis(trimethylammonium) heptane (10 m*M*)					
1,4-naphthalenedicarboxylic acid, 2,3-naphthalenedicarboxylic acid,	TMAB (10 m*M*)					
2,6-naphthalenedicarboxylic acid, phthalate, isophthalate, and terephthalate	TBAB (10 m*M*)					
AD-1, AD-2, AD-3 and AD-4	25 m*M* TBAB	10 m*M* sodium tetraborate, pH 9.2	500 mm×50 μm I.D. (720 mm total)	Direct Vis, 550 nm	NS	[64]
1-Naphthalenecarboxylic acid, 2-naphthalenecarboxylic acid,	Brij-35 (30 m*M*)	10 m*M* sodium tetraborate	500 mm×50 μm I.D. (750 mm total)	Direct UV, 230 nm	NS	[65]
1-naphthalenesulfonic acid, 2-naphthalenesulfonic acid,	Brij-58 (30 m*M*)					
2,3-naphthalenedicarboxylic acid, 2,6-naphthalenedicarboxylic acid,	Brij-78 (30 m*M*)					
1,5-naphthalenedisulfonate acid, 2,6-naphthalenedisulfonate, phthalate, isophthalate, and terephthalate						

NS, not stated; CTAC, cetyltrimethylammonium chloride; CTAB, cetyltrimethylammonium bromide; DTAB, dodecyltrimethylammonium bromide; TTAB, tetradecyltrimethylammonium bromide; HTAB, hexadecyltrimethylammonium bromide; HMB, hexamethonium bromide; HDMB, hexadimethrine bromide; TMAB, tetramethylammonium bromide; TBAB, tetrabutylammmonium bromide; DMB, decamethonium bromide; THPB, tributylhexadecylphosphonium bromide.

were sufficient to provide a reversed, stable EOF for all the surfactants studied, but changes in selectivity were only slight due to the low concentration of modifier added. The effect of adding higher concentrations of TTAB was examined and it was observed that the mobility of the anions became lower and resolution between crucial peak pairs was improved, but only this particular surfactant was employed at these higher concentrations.

Massart and co-workers were able to predict migration behavior of anions in the presence of added CTAB at concentrations both below [21] and above the CMC [22]. Using a central composite experimental design the migration of anions as a function of both CTAB concentration and pH was given in a single three-dimensional plot. Micellar partitioning was taken into account above the CMC (1–5 m*M* CTAB). Of the ions studied, the migration of iodide was the most affected by CTAB.

While the use of surfactants for selectivity manipulation has been investigated quite thoroughly, analyte–surfactant interaction has only been moderated by varying the type and concentration of the surfactant. In IC, the composition of the eluent, particularly the salt and organic modifier content are known to greatly influence retention and it is anticipated that variation of these parameters would affect analytes differently, thus enabling selectivity variation.

It is apparent from these examples that surfactants can be used to modify the electroosmotic mobility of anions. However, there are some limitations in the effectiveness of this technique. Solubility and current considerations limit the surfactant to a relatively low concentration, which is problematic when weakly-interacting analytes are examined. The restricted time window within which the analytes must migrate also limits the potential applications. Precipitation of the analyte–surfactant ion-pair may also occur and in many instances the changes in the electroosmotic mobility encountered are rather small. A further limitation is the different interactions occurring between analytes and the monomeric and micellar forms of the surfactant. Table 1 summarizes some methods using surfactants as pseudo-stationary phases for the separation of inorganic and small organic ions.

3. Capillaries filled with solid ion-exchange polymers

CEC is a new and exciting technique that has developed rapidly in the last decade. Originally introduced by Pretorius et al. [23] it generated much interest from a chromatographic perspective because the mobile phase is propelled through the chromatographic bed by EOF rather than by pressure. This results in a relatively flat flow profile in CEC, providing high separation efficiencies. It also means that columns can be packed with small particles because there is no back-pressure generated when using EOF. From an electrophoretic perspective, the progression from CE to CEC is attractive because of the ability to change the separation selectivity by controlling both the chromatographic and electrophoretic mechanisms. As the chromatographic phase is stationary (in contrast to moving surfactant and soluble-polymer pseudo-stationary phases) the overall net mobility of the analyte ($\mu_{A,net}$) is given by:

$$\mu_{A,net} = x_A(\mu_A + \mu_{EOF}) \quad (3)$$

where μ_A is the electrophoretic mobility of the free analyte, μ_{EOF} is the mobility of the EOF and x_A is the mole fraction of free analyte.

There are several distinct advantages of this type of CEC compared with the use of pseudo-stationary phases. Firstly, the migration time window into which analytes must be eluted is potentially infinite due to the use of a fixed chromatographic stationary phase. Secondly, solubility problems encountered when using a surfactant or polymeric electrolyte additive are obviated when a solid stationary phase is used. Finally, a fixed stationary phase can be better suited than a pseudo-stationary phase to alternative forms of detection, such as conductivity or mass spectrometry.

CEC columns are generally prepared by packing chromatographic particles into the capillary and retaining these particles with frits. While the analytical promise for this method is excellent, there are several problems regarding its practical implementation. Firstly, it is challenging to pack small particles (typically less than 5 μm in diameter) into a capillary having an internal diameter of 50–100 μm. Second-

ly, the formation of homogenous and reproducible frits is difficult and frits introduce weak points into the column where the possibility of breakage is increased. Thirdly, the presence of different physical zones in the capillary (open section, frits and column bed) can result in substantial differences in EOF between these zones which often results in the formation of bubbles unless high pressure (5–12 bar) is applied to both ends of the column during the separation. Fourthly, packed capillary columns are prone to overheating, resulting in the column bed drying out. Low ionic strength buffers and/or low field strengths are therefore required and this places significant constraints on the ability to control the relative contributions of CE and ion-exchange in the ion-exchange–CEC (IE–CEC) separation.

Monolithic columns are considered by many to be a more practical alternative to packed columns in that they offer many of the same advantages as packed columns but without most of the problems [24]. Monolithic columns are formed by generation of a continuous polymeric network inside the capillary [25] and can be classified according to whether the stationary phase is a (i) molded porous polymer [26], (ii) molded porous sol–gel [27], (iii) particle-fixed continuous bed [28], or (iv) microfabricated monolithic bed [29]. While each method has particular advantages, they all have the common advantage that no frits are needed to retain the packing in the column. As a result, there is often no need to use overpressure to stop bubble formation. However, the development of monolithic columns faces considerable challenges, particularly with regard to column fabrication. There are still major problems regarding stability, reproducibility and heterogeneity of the different types of columns.

While CEC can be performed using either packed or monolithic columns, the majority of the work presented to date has used packed capillaries, especially utilizing silica-based packings [30–34]. These packings are undesirable in many instances because of their limited working pH range and somewhat poorly defined surface characteristics. The advantages of using a polymeric ion-exchange packing for IE–CEC were demonstrated by Hilder et al. [30]. Capillaries were packed with a Dionex AS9-HC material with the packing held between two frits made from sintered porous silica. While the ability to use polymeric material greatly increased the variety of stationary phases that could be used, perhaps the most significant advantage was the low back-pressure of the column. This enabled the column to be flushed very quickly (<1 min) and also allowed the system to be used in a pressure-assisted mode (pCEC) where pressure was applied to the column inlet only. This enabled the two separation mechanisms (IEC and electrophoresis) to be controlled more independently of each other than if EOF alone was used to propel the eluent. Varying the concentration of the eluent anion could be further used to manipulate the chromatographic component of the separation (Fig. 1), while the electrophoretic component could be changed by varying the separation voltage. The contributions of these two mechanisms were optimized using an artificial neural network (ANN), resulting in the separation of eight UV-absorbing anions in 2.3 min under optimal conditions. In this case the columns could also be used without any applied overpressure, making the technique simple and enabling it to be used in commercial instruments which do not include a high pressure source.

While direct photometric detection was used for evaluating the potential of IE–CEC for the analysis of inorganic anions, relatively few inorganic anions are UV absorbing. Indirect UV detection in packed column CEC has also been investigated for the detection of UV-transparent anions [31,35], but was also found to cause some problems with baseline stability when using a polymer packing material (Dionex AS9-SC). In contrast, conductivity detection (using a capacitively coupled contactless conductivity detector), with detection taking place through the packed bed, was shown to be a more general and more sensitive detection method for these analytes. Table 2 lists some details of CEC methods using capillaries packed with ion-exchange polymers for the separation of inorganic and small organic ions.

4. Capillaries coated with ion-exchange polymers

The use of capillaries in which the stationary phase is attached to the surface of the capillary forming an open-tubular (OT) column can provide a

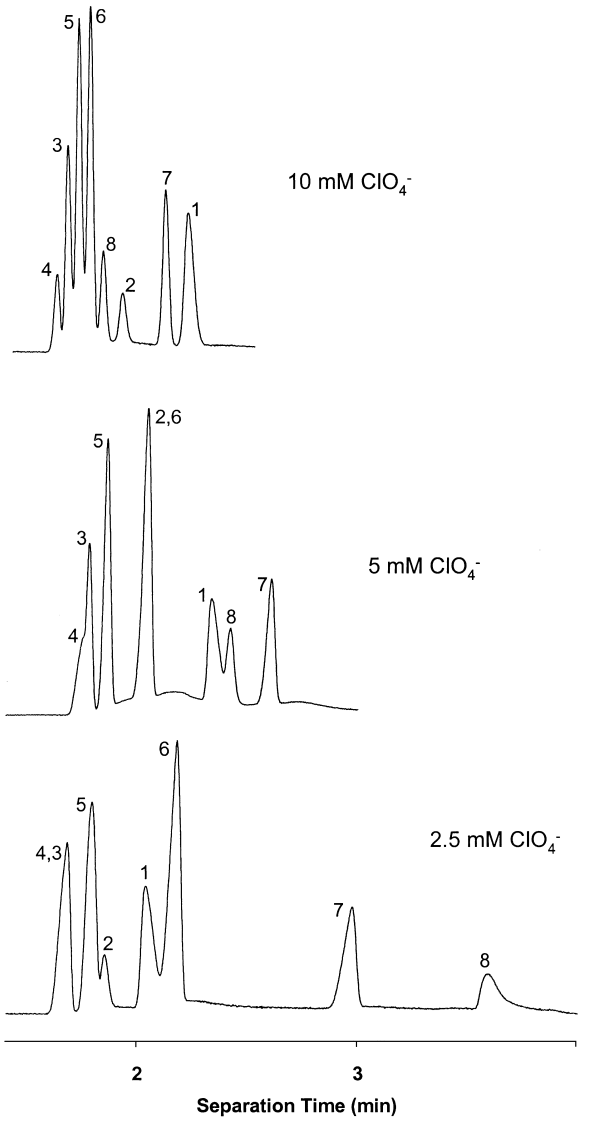

Fig. 1. Effect of mobile phase concentration on separation selectivity. Mobile phase: 2.5–10 m*M* $HClO_4$ (titrated to pH 8.05 with Tris). Flow is a combination of 10 bar pressure and EOF (−10 kV). Capillary: 75 μm I.D.×25 cm packed with Dionex AS9-HC (26 cm to detector, 34.5 cm total). Detection: direct UV, 214 nm. Peaks are (all 0.2 m*M*): 1, IO_3^-; 2, BrO_3^-; 3, NO_2^-; 4, Br^-; 5, NO_3^-; 6, I^-; 7, SCN^-; 8, CrO_4^{2-}. Reproduced from Ref. [30] with permission.

similar migration mechanism to packed and monolithic columns (as given by Eq. (3)), without many of the associated problems. They offer advantages similar to those achieved using OT columns instead of packed columns for HPLC, especially the potentially higher separation efficiency resulting from the reduction of the eddy diffusion term in the Van Deemter equation [36,37]. Moreover, OT columns are particularly attractive for CEC because they are instrumentally simple to use because there is no need to use high pressure and there are no problems with bubbles. There are also several disadvantages of OT columns. Firstly, the ion-exchange capacity of the column is much lower than a packed column, making OT columns more prone to overloading. Secondly, narrow capillaries are needed to obtain high separation efficiency due to the slow diffusion rates occurring in liquid phases. Thirdly, detection is more problematic due to the smaller column dimensions. Even with these limitations, OT–CEC offers an attractive alternative to packed and monolithic columns and Table 3 summarizes details of some methods using this approach.

The problem of low column capacity can be overcome by increasing the surface area of the capillary wall [38,39]. While this has been done successfully by etching the surface, an alternative approach is to adsorb particles of a high-capacity ion-exchange material onto the surface in a similar manner to that used in the construction of latex-agglomerated stationary phases used in IC. This approach was first used for IE–CEC by Gjerde and Yengoyan [40] who adsorbed small cationic particles onto fused-silica capillaries and analyte anions were shown to be separated in capillaries prepared in this way by a combination of chromatographic and electrophoretic components. However, methods to control the migration by changing the relative contributions of the two separation mechanisms were not discussed.

The soluble polymer, poly(ethyleneimine) (PEI) was used by Nutku and Erim [41] to coat a capillary which was then used for the separation of inorganic anions. As PEI contains a tertiary amine, the ion-exchange capacity is dependent on the pH. Minor ion-exchange interaction was observed at pH 8, with more interaction observed when the pH was lowered to pH 5, but even in the latter case only small changes in mobility were observed. Better performance was obtained by adding PEI to the electrolyte and this approach is discussed further in Section 5. Since then, PEI-coated capillaries have been used for

Table 2
Separations of inorganic and small organic ions using columns filled with solid (packed or monolithic) ion-exchange polymers

Analytes	Chromatographic phase	Electrolyte	Column dimensions	Detection	Efficiency (plates/m)	Ref.
Iodate, bromate, nitrite, bromide, nitrate, iodide, thiocyanate, and chromate	IonPac AS9-HC (9 μm latex agglomerated alkyl quaternary ammonium)	(a) 5 m*M* sulfuric acid titrated with Tris to pH 8.05 (b) 2.5 m*M* carbonate, 2.5 m*M* bicarbonate, pH 10 (c) 5 m*M* perchloric acid titrated with Tris to pH 8.05	250 mm×75 μm I.D. (360 mm total)	Direct UV, 214 nm	NS	[30]
Iodate, bromate, nitrite, bromide, nitrate, iodide, thiocyanate, and chromate	IonPac AS9-HC (9 μm latex agglomerated alkyl quaternary ammonium)	9 m*M* perchloric acid titrated with Tris to pH 8.05	250 mm×75 μm I.D. (360 mm total)	Direct UV, 214 nm	NS	
Iodate, bromate, nitrite, bromide, nitrate, iodide, thiocyanate, and chromate	IonPac AS9-HC (9 μm latex agglomerated alkyl quaternary ammonium)	2.5 m*M* hydrochloric acid titrated with Tris to pH 8.05, −30 kV at 1.3 min	85 mm×75 μm I.D. (360 mm total)	Direct UV, 214 nm	NS	
Iodate, bromate, nitrite, bromide, nitrate, iodide, thiocyanate, and chromate	IonPac AS9-HC (9 μm latex agglomerated alkyl quaternary ammonium)	2.5 m*M* sulfuric acid titrated with Tris to pH 8.05	85 mm×75 μm I.D. (360 mm total)	Direct UV, 214 nm	NS	
Iodate, bromate, nitrite, bromide, nitrate and iodide	IonPac AS90SC (13 μm latex agglomerated alkyl quaternary ammonium)	(a) 10 m*M* hydrochloric acid titrated with Tris to pH 8.05 (b) 5 m*M* sulfuric acid titrated with Tris to pH 8.05 (c) 5 m*M* perchloric acid titrated with Tris to pH 8.05	243 mm×75 μm I.D. (338 mm total)	Direct UV, 214 nm	NS	[35]
Chloride, fluoride, bromide, nitrate and sulfate	IonPac AS90SC (13 μm latex agglomerated alkyl quaternary ammonium)	10 mm *p*-toluenesulfonic acid titrated with Tris to pH 8.05	259 mm×75 μm I.D. (347 mm total)	Indirect UV, 254 nm and direct CD (using C^4D)	NS	
Chloride, fluoride, bromide, nitrate and sulfate	IonPac AS90SC (13 μm latex agglomerated alkyl quaternary ammonium)	2.5–50 m*M* *p*-toluenesulfonic acid titrated with Tris to pH 8.05	259 mm×75 μm I.D. (347 mm total)	Indirect UV, 254 nm and direct CD (using C^4D)	NS	
Chloride, fluoride, nitrite, bromide, nitrate, phosphate, sulfate and iodide	IonPac AS90SC (13 μm latex agglomerated alkyl quaternary ammonium)	10 mm *p*-toluenesulfonic acid titrated with Tris to pH 8.05	259 mm×75 μm I.D. (347 mm total)	Indirect UV, 254 nm and direct CD (using C^4D)	NS	

NS, not stated; Tris, tris(hydroxymethyl)amino methane; CD, conductivity detection; C^4D, capacitively-coupled contactless conductivity detector.

Table 3
Separations of inorganic and small organic ions using polymeric ion-exchange open tubular columns

Analytes	Chromatographic phase	Electrolyte	Column dimensions	Detection	Efficiency (plates/m)	Ref.
Nitrite, nitrate, bromide, iodide and thiocyanate	Fused silica coated with PEI	20 m*M* Tris pH 8	760 mm×75 μm I.D. (630 mm total)	Direct UV, 210 nm	NS	[41]
Tartaric, malic, citric, lactic, succinic and acetic acids	Fused silica coated with PEI	20 m*M* 2,4-dihydroxybenzoic acid, pH 4.9	455 mm×75 μm I.D. (630 mm total)	Indirect UV, 249 nm	NS	[43]
4-Nitrophenol, 3-nitrophenol, 4-chlorophenol, 3-chlorophenol and 2-chlorophenol	Fused silica coated with PEI	25 m*M* borate, pH 9.2	580 mm×75 μm I.D. (730 mm total)	Direct UV, 210 nm	NS	[42]
Chloride, sulfate, nitrite, nitrate, phosphate and carbonate	Copolymerisation of trimethylammoniumstyrene chloride with vinyl groups covalently bound to the wall	5 m*M* chromate, pH 7.7	595 mm×50 μm I.D. (670 mm total)	Indirect UV, 254 nm	NS	[50]
Bromide, iodide, chromate, nitrite, nitrate, thiocyanate and molybdate	Copolymerisation of trimethylammoniumstyrene chloride with vinyl groups covalently bound to the wall	40 m*M* borate, pH 9.0	900 mm×50 μm I.D. (900 mm total)	Direct 210 nm	NS	
Bromide, chloride, sulfate, nitrate, fluoride and phosphate	Reactive polyamide	2.25 pyromellitic acid, 1.6 m*M* triethanolamine, pH 7.9 adjusted with sodium hydroxide	550 mm×75 μm I.D. (600 mm total)	Indirect UV, 250 nm	NS	[49]
o-Nitrobenzoic acid, citraconic acid, *m*-nitrobenzoic acid, *o*-toluic acid, benzoic acid, *p*-toluic acid, *p*-hydroxybenzoic acid and *p*-aminobenzoic acid	Thermal immobilisation of poly(vinyl amine) via internal crosslinking	50 m*M* sodium acetate, pH 4.0	320 mm×50 μm I.D. (400 mm total)	Direct UV, 254 nm	NS	[48]
o-Nitrobenzoic acid, citraconic acid, *m*-nitrobenzoic acid, *o*-toluic acid, benzoic acid, *p*-toluic acid, *p*-hydroxybenzoic acid and *p*-aminobenzoic acid	Immobilised poly(vinyl amine) derivatised with *N*,*N*,*N*-trimethylaminoacrylamide	50 m*M* sodium acetate, pH 4.0	320 mm×50 μm I.D. (400 mm total)	Direct UV, 254 nm	NS	
Bromide, iodide, nitrite and nitrate	Fused silica capillary coated with AS5A latex particles (alkyl quaternary ammonium)	10 m*M* hydrochloric acid titrated with Tris to pH 8.05	415 mm×75 μm I.D. (500 mm total)	Direct UV, 214 nm	NS	[46]
Bromide, iodide, nitrite, nitrate, thiosulfate, thiocyanate and chromate	Fused silica capillary coated with AS5A latex particles (alkyl quaternary ammonium)	20–1000 m*M* hydrochloric acid titrated with Tris to pH 8.05	415 mm×75 μm I.D. (500 mm total)	Direct UV, 214 nm	NS	
Bromide, iodide, nitrite, nitrate, thiosulfate, thiocyanate and chromate	Fused silica capillary coated with AS5A latex particles (alkyl quaternary ammonium)	(a) 15 m*M* hydrochloric acid titrated with Tris to pH 8.05 (b) 15 m*M* sulfuric acid titrated with Tris to pH 8.05 (c) 15 m*M* perchloric acid titrated with Tris to pH 8.05	415 mm×75 μm I.D. (500 mm total)	Direct UV, 214 nm	NS	
Bromide, iodide, nitrite, nitrate, thiosulfate, thiocyanate and chromate	Fused silica capillary coated with AS5A latex particles (alkyl quaternary ammonium)	2.5–100 m*M* perchloric acid titrated with Tris to pH 8.05	415 mm×75 μm I.D. (500 mm total)	Direct UV, 214 nm	NS	
Chloride, bromide, fluoride, bromate, chlorate, iodate, sulfate and sulfite	Fused silica capillary coated with AS5A latex particles (alkyl quaternary ammonium)	3 m*M* nitric acid titrated with DEA to pH 9.2	415 mm×75 μm I.D. (500 mm total)	Indirect UV, 214 nm	13,000–50,000	[44]

NS, not stated; TBA, tetrabutylammonium; Tris, tris(hydroxymethyl)amino methane; PEI, poly(ethyleneimine); DEA, diethanolamine.

the separation of substituted phenols [42] and organic acids in fruit juices and wine [43].

Recently, Breadmore and co-workers [44–46] have reported the selectivity manipulation of inorganic anions by varying the electrolyte composition when capillaries were coated with ~70 nm diameter anion-exchange latex particles. The migration order of analytes was found to be strongly dependent on the type and concentration of the electrolyte anion, and for UV absorbing anions, selectivity control could be achieved by varying only the eluent concentration (Fig. 2). When the method was applied to indirect UV detection, the concentration of the eluent anion could be varied over a small range only and selectivity manipulation was achieved more easily by changing the nature of the eluent anion rather than its concentration, as can be seen in Fig. 3. In order to understand the retention mechanism applicable in both the direct and indirect UV detection systems, a theoretical equation was derived to explain the manner in which observed mobilities changed with varying electrolyte compositions [47]. Excellent agreement was obtained between experimental and theory.

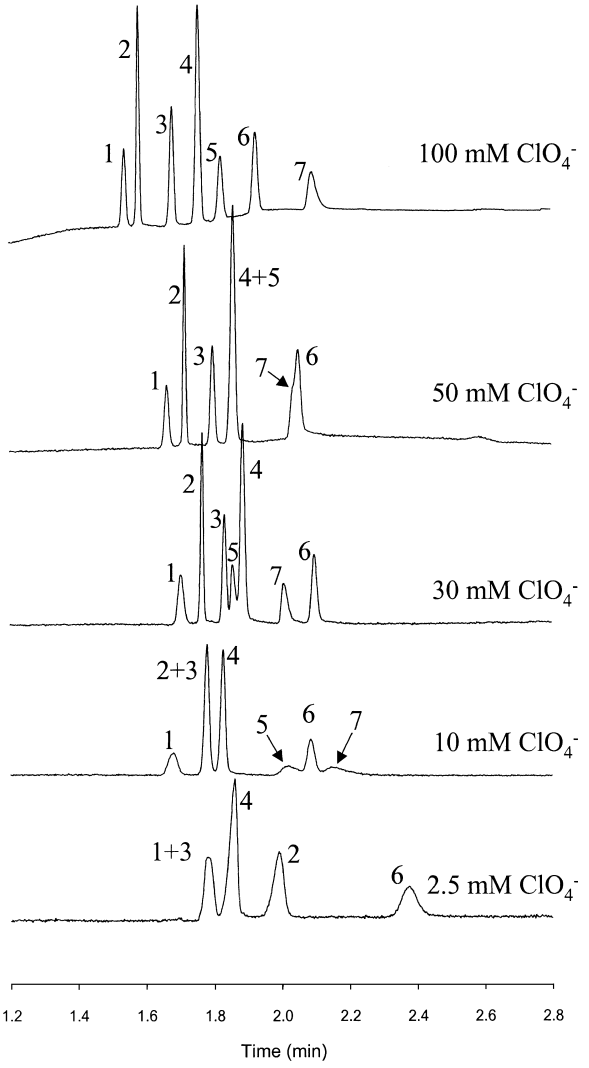

Fig. 2. Separation of seven inorganic anions in an AS5A-coated capillary using different concentrations of perchlorate in the BGE. All separations at −25 kV. Direct detection at 214 nm. Peaks are: 1, Br^-; 2, I^-; 3, NO_2^-; 4, NO_3^-; 5, $S_2O_3^{2-}$; 6, SCN^-; 7, CrO_4^{2-}. Reproduced from Ref. [46] with permission.

The distinction between CE and OT–CEC is not always straightforward, as evidenced in a report by Chiari et al. [48] who made OT columns by adsorbing the cationic polymer poly(vinylamide) onto the capillary wall. While the capillary wall had a positive charge, no ion-exchange interaction with the wall was observed, with the positive surface charge used solely to provide a reversed EOF and to thus avoid the need to add a cationic surfactant. Similar approaches have been reported by Burt et al. [49] and Finkler et al. [50]. While not strictly CEC, the potential for ion-exchange interactions exists and modification of the electrolyte conditions may be sufficient to facilitate this.

5. Soluble ionic polymers

5.1. Introduction and principles

In earlier sections methods were described in which an ion-exchange component was introduced in the separation of ions by the use of surfactants to form micelles, electroosmotic eluent propulsion in packed capillaries, or the use of capillaries with embedded ion-exchange particles. Perhaps the ultimate technique is to use soluble ionic polymers as a ‘‘stationary’’ phase. This is accomplished simply by adding a soluble ionic polymer to the capillary electrolyte of an ordinary CE set-up. The capillary is open at both ends, thus eliminating the need for frits.

The use of soluble ionic polymers as a pseudo-

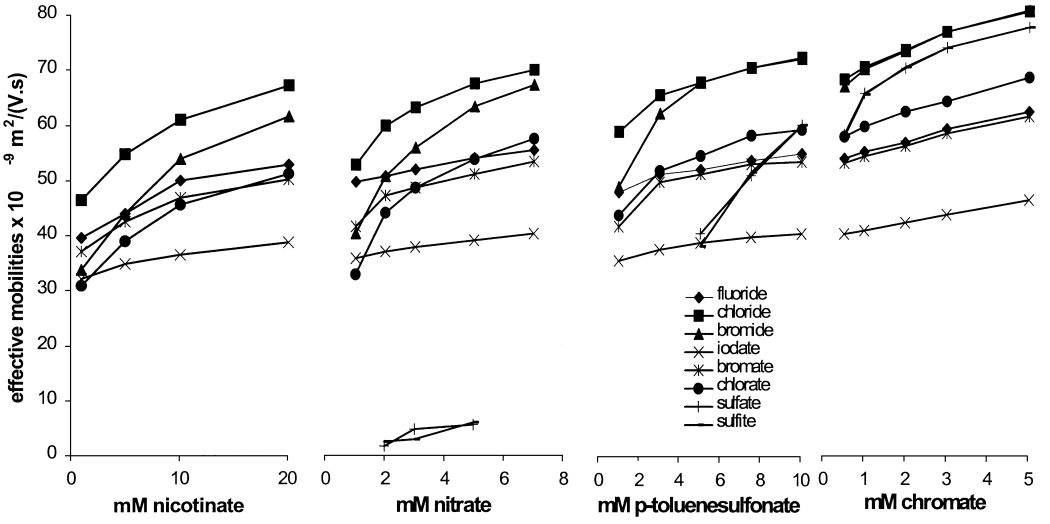

Fig. 3. Plots of eluent concentration versus effective mobility for eight inorganic ions and four indirect UV detection eluents (nicotinate, chromate, *p*-toluenesulfonate, nitrate). Reproduced from Ref. [44] with permission.

stationary phase offers substantial advantages over surfactants, with the most notable being the fixed structure of the polymer molecule in solution compared to the transient and concentration-dependent nature of the micelles formed when surfactants are used. This means that when soluble ionic polymers are used, analytes interact with only one form of pseudo-stationary phase, leading to higher separation efficiencies. There are several other benefits which arise from the use of soluble ionic polymers. Firstly, organic modifiers can be used with soluble polymers at much higher concentrations than with micelles. Secondly, because the soluble polymer is present throughout the BGE, mass transfer and eddy diffusion effects are virtually eliminated since analytes are not required to diffuse in order to interact with an ion-exchange site, nor to undergo a transition from a liquid mobile phase into a solid stationary phase. Finally, variation of the nature and ion-exchange capacity of the pseudo-stationary phase can be accomplished simply by changing the composition of the BGE, making this a much more flexible approach than using solid stationary phases.

Separation of anions is accomplished by adding a soluble polymer containing positively-charged quaternary ammonium groups to the BGE in a CE apparatus. When a potential is applied, the bulky polymer migrates slowly toward the cathode while the more mobile sample anions migrate toward the anode. In doing so, the anions interact in varying degrees with the positively charged groups on the polymer.

This interaction can be expressed as an equilibrium in which each sample anion spends a certain time fraction as the free anion (f_A) and the remaining time fraction ($1-f_A$) associated with the ionic polymer. The net anionic mobility is towards the anode, and is given by the equation:

$$\mu_A\,(\text{net}) = f_A\,\mu_A - (1 - f_A)\,\mu_p + \mu_{eo} \qquad (4)$$

where μ_A (net) is the net anionic mobility, μ_A is the electrophoretic mobility of the free anion, μ_p is the electrophoretic mobility of the polymer, and μ_{eo} is the electroosmotic mobility. Thus, differences in the net mobilities of anions to be separated are based on differences in their relative fractions in free (f_A) and associated forms ($1-f_A$) as well as on any differences in electrophoretic mobilities of the free anions.

5.2. Separation of anions using soluble cationic polymers

The use of cationic polymers to influence the migration of ions was first introduced by Terabe and Isemura in 1990 [14] and since then, numerous

Table 4
Separations of inorganic and small organic ions using a water-soluble polymer as the pseudo-stationary phase

Analytes	Chromatographic phase	Electrolyte	Column dimensions	Detection	Efficiency (plates/m)	Ref.
1-naphthalenesulfonic acid, 2-naphthalenesulfonic acid, 2,6-naphthalenedisulfonate acid, 2,7-naphthalenedisulfonate, 1,6-naphthalenedisulfonate acid, 1,5-naphthalenedisulfonate acid and 1,7-naphthalenedisulfonate acid	DEAE dextran (2% w/v) and PDDAC (0.3% w/v)	50 m*M* phosphate, pH 7.0	500 mm×50 μm I.D. (750 mm total)	Direct UV, 210 nm	NS	[14]
Benzoate, *o*-, *m*-, and *p*-aminobenzoic acid, 1-napthoic acid, *o*-, *m*-, and *p*-hydroxybenzoic acid, 2-naphthoic acid and 1-naphthalenesulfonate	PDDAC (0.3% w/v)	50 m*M* phosphate, pH 7	500 mm×50 μm I.D. (750 mm total)	Direct UV, 210 nm	NS	[15]
Bromide, chloride, fluoride, nitrite, nitrate, phosphate and sulfate	PDDPi chromate (0.2% w/v) PDDPy chromate (0.17% w/v) HDM chromate (0.30% w/v) DEAED chromate (0.55% w/v)	5 m*M* sodium chromate, pH 8.0	520 mm×75 μm I.D. (600 mm total)	Indirect UV, 254 nm	NS	[51]
Benzoate, salicylate, 3-methylsalicylate, 3,5-dihydroxybenzoate	PDDPi chloride (0.2% w/v)	5 m*M* phosphate, pH 6.8	530 mm×77 μm I.D. (600 mm total)	Direct UV, 214 nm	100,000–300,000	[52]
Iron(III) EDTA, oxalate, citrate and EDTA	PDDPi chromate (0.23% w/v)	5 m*M* chromate, pH 8.4	530 mm×77 μm I.D. (600 mm total)	Indirect UV, 254 nm	NS	
Chloride, bromide and sulfate	PDDPi chromate (0.154% w/v)	5 m*M* chromate, pH 8.0	530 mm×77 μm I.D. (600 mm total)	Indirect UV, 254 nm	NS	
Malonate, lactate, acetate, succinate and citrate	PDDPi chromate (0.154% w/v)	5 m*M* chromate, pH 8.0	530 mm×77 μm I.D. (600 mm total)	Indirect UV, 254 nm	NS	
Bromide, chloride, iodide, nitrite, nitrate, sulfate, perchlorate, fluoride, phosphate, carbonate, acetate, PAR, cobalt(II) PAR, nickel(II) PAR and iron(II) PAR	Polybrene (0.05% w/v)	5 m*M* potassium chromate, pH 8.90	460 mm×50 μm I.D. (600 mm total)	Indirect UV, 254 nm for first 2 min, direct Vis 490 nm after 2 min	Up to 400,000	[17]
Nitrite, bromide, nitrate, iodide and thiocyanate	PEI (0–0.1% w/v)	20 m*M* acetate, pH 5	630 mm×75 μm I.D. (760 mm total)	Direct UV, 210 nm	NS	[41]
4-Nitrophenol, 3-nitrophenol, 2-chlorophenol, 3-chlorophenol and 4-chlorophenol	PEI (0.5% w/v)	20 m*M* Tris, pH 8.3	600 mm×75 μm I.D. (750 mm total)	Direct UV, 210 nm	NS	[56]
Nitrate, iron(III) CDTA, manganese(II) CDTA, cadmium(II) CDTA, palladium(II) CDTA, nickel(II) CDTA, cobalt(II) CDTA, zinc (II) CDTA and coper(II) CDTA	PDDAC (50 m*M*)	3 m*M* sodium sulfate, 10 m*M* sodium acetate, pH 7.0	450 mm×50 μm I.D. (500 mm total)	Direct UV, 210 nm	220,000–300,000	[53]
Bromide, iodide, nitrite, nitrate, chromate, thiocyanate and molybdate	PDDAC (0.05% w/v)	150 m*M* lithium sulfate, 20 m*M* borate, pH 8.5	325 mm×50 μm I.D. (400 mm total)	Direct UV, 214 nm	NS	[18]
Benzoate, benzoatesulfonate, *p*-toluenesulfonate, *p*-aminobenzoate, *p*-hydroxybenzoate, 2-naphthalenesulfonate, 1-naphthalenesulfonate, 3,5-dihydroxybenzoate and 2,4-dihydroxybenzoate	PDDAC (0.3–1.0% w/v)	150 m*M* lithium sulfate, 20 m*M* borate, pH 8.5	325 mm×50 μm I.D. (400 mm total)	Direct UV, 214 nm	NS	
Benzoate, benzoatesulfonate, *p*-toluenesulfonate, *p*-aminobenzoate, *p*-hydroxybenzoate, 2-naphthalenesulfonate, 1-naphthalenes 3,5-dihydroxybenzoate and 2,4-dihydroxybenzoate	PDDAC (0.8% w/v)	(a) 150 m*M* lithium sulfate and 20 m*M* borate (c) 150 m*M* sodium sulfate and 20 m*M* borate (d) 150 m*M* sodium chloride and 20 m*M* borate	325 mm×50 μm I.D. (400 mm total)	Direct UV, 214 nm	NS	

Table 4. Continued

Analytes	Chromatographic phase	Electrolyte	Column dimensions	Detection	Efficiency (plates/m)	Ref.
Bromide, nitrite, nitrate, chromate, iodide, molybdate, phthalate, 1,2,3-benzenetricarboxylate, 1,2-benzenedisulfonate, terephthalate, isophthalate, benzoate, *p*-toluenesulfonate, 1,3,5-benzenetricarboxylate, 2-naphthalenesulfonate, 1-naphthalenesulfonate, 3,5-dihydroxybenzoate and 2,4-dihydroxybenzoate	PDDAC (0.3% w/v)	120 m*M* lithium sulfate, 20 m*M* borate, pH 8.5	325 mm×50 μm I.D. (400 mm total)	Direct UV, 214 nm	195,000–429,000	
Cobalt(II) HBED, aluminium HBED, manganese(III) HBED, lead(II) HBED, iron(III) HBED, cadmium(II) HBED, copper(II) HBED, zinc(II) HBED, nickel(II) HBED	PDDA hydroxide (50 m*M*)	10 m*M* sodium tetraborate, 0.1 m*M* HBED adjusted to pH 10 with acetic acid	455 mm×50 μm I.D. (500 mm total)	Direct UV, 242 nm	NS	[55]
Bismuth(III) HBED, scandium(III) HBED, indium(III) HBED, cobalt(III) HBED, gallium(III) HBED, aluminium(III) HBED, chromium(III) HBED, manganese(III) HBED, lead(II) HBED, iron(III) HBED, cadmium(II) HBED, palladium(II) HBED, cobalt(II) HBED, copper(II) HBED, zinc(II) HBED, nickel(II) HBED, manganese(II) HBED, vanadate	PDDA hydroxide (55 m*M*)	10 m*M* sodium tetraborate, 0.1 m*M* HBED adjusted to pH 10 with acetic acid	455 mm×50 μm I.D. (500 mm total)	Direct UV, 242 nm	NS	[54]
Copper(II) EDDHA	PDDAC (10 m*M*)	5 m*M* sodium sulfate, 10 m*M* tetraborate, pH 9.2	455 mm×50 μm I.D. (500 mm total)	Direct UV, 242 nm	NS	
Bromide, iodide, nitrite, nitrate, chromate, thiocyanate, molybdate, bromate,	(a) PDDAC (0.05% w/v)	(a) 10 m*M* hydrochloric acid titrated with Tris to pH 8.05 and 150 m*M* sodium fluoride	415 mm×75 μm I.D. (500 mm total)	Direct UV, 214 nm	NS	[47]
phthalate, 1,2-benzenedisulfonate, iodate, benzenesulfonate, benzoate,	(b) PDDAC (0.35% w/v)	(b) 10 m*M* hydrochloric acid titrated with Tris to pH 8.05 and 110 m*M* sodium chloride				
p-toluenesulfonate, 2-naphthalenesulfonate, 3,5-dihydroxybenzoate	(c) PDDAC (0.30% w/v)	(c) 10 m*M* hydrochloric acid titrated with Tris to pH 8.05 and 50 m*M* sodium sulfate				
Bromide, iodide, nitrite, nitrate, chromate, thiocyanate, molybdate, bromate, phthalate, 1,2-benzenedisulfonate, iodate, benzenesulfonate, benzoate, *p*-toluenesulfonate, 2-naphthalenesulfonate, 3,5-dihydroxybenzoate	PDDAC (0.9% w/v)	10 m*M* hydrochloric acid titrated with Tris to pH 8.05 and 80 m*M* sodium fluoride	415 mm×75 μm I.D. (500 mm total)	Direct UV, 214 nm	NS	
Bromide, chloride, nitrite, nitrate, sulfate, oxalate, perchlorate, chlorate, malonate, formate, fluoride, bromate, citrate, succinate, tartrate, glutarate, adipate, iodate, acetate, propanoate, butanoate, isovalerate, caproate and caprylate	PDDA chromate (0.28% w/v)	16.25 m*M* chromic acid titrated with Tris to pH 7.70 and 10 m*M* histidine, pH 7.70	415 mm×75 μm I.D. (500 mm total)	Indirect UV, 254 nm	NS	[58]
Chloride, fluoride, formate, acetate, sulfate and oxalate	PDDA chromate (0.55% w/v)	20.00 m*M* chromic acid titrated with Tris to pH 7.70 and 10 m*M* histidine, pH 7.70	415 mm×75 μm I.D. (500 mm total)	Indirect UV, 254 nm	NS	

NS, not stated; DEAE dextran, (diethylamino)ethyldextran; PDDA, poly(diallyldimethylammonium); PDDPi, poly(1,1-dimethyl-3,5-dimethylenepiperidinium); PDDPy, poly(1,1-dimethyl-3,5-dimethylenepyrrolidinium), HDM, hexadimethrine; DEAED, diethylaminodextran; EDTA, ethylenediaminetetraacetic acid; CDTA, *trans*-1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid; PAR, 4-(2-pyridylazo)resorcinolate; PEI, poly(ethyleneimine); HBED, *N*,*N*-bis(hydroxybenzyl)ethylenediamine-*N*,*N*-diacetic acid; EDDHA, ethylenediaminedi(*o*-hydroxyphenylacetic acid).

publications have appeared in this field as summarized in Table 4. A cationic polymer, either 2% (diethylamino)ethyldextran (DEAE dextran) or 0.3% poly(diallyldimethylammonium chloride) (PDDAC), was added to the BGE. Both the analyte anions and the polymer ions are subject to electrophoresis but in opposite migration directions. Due to the bulkiness of the polymer, migration of anions attached to the polymer is slower than the free analyte anions. Even anions having identical electrophoretic mobilities will migrate with different velocities if their ion-pair formation constants are different. Complete resolution of 2- and 1-naphthalenesulfonic acid was obtained in only 8 min with PDDAC. This mixture gave only a single peak without PDDAC. Six isomeric naphthalenedisulfonic acids were separated with 2% DEAE dextran added to the BGE.

In a subsequent paper [15], separation of carboxylate and dicarboxylate anions was studied using PDDAC or polybrene bromide as the cation polymer. Fig. 4 compares the change in velocity relative to benzoic acid as the standard for monocarboxylic acids as a function of PDDAC concentration in the BGE. The relative change is also shown for 0.3% polybrene. The use of differential velocity ($V_{sample} - V_{std}$) eliminates the effect of electroosmotic velocity and indicates the effect of just the polymer addition on the electrophoretic velocity. A similar plot was made for dicarboxylic acids. The ion-exchange effect induced by polymer in the BGE resulted in much better resolution of carboxylic acid anions.

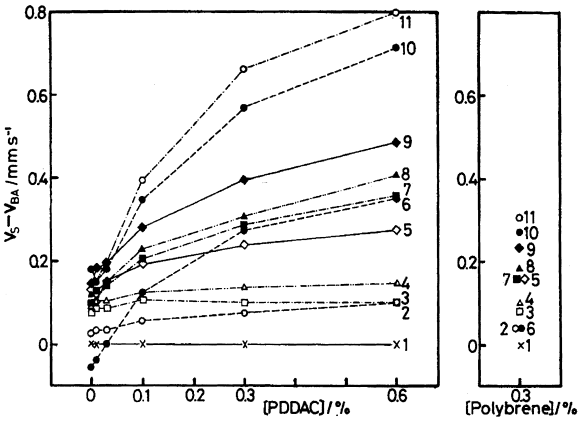

Fig. 4. Dependence of relative migration velocities of monobasic acids on the concentration of PDDAC. 1, benzoic acid (BA, the standard solute of the migration velocity); 2, *o*-; 3, *m*-; 4, *p*-aminobenzoic acids; 5, 1-naphthoic acid, 6, *o*-; 7, *m*-; 8, *p*-hydroxybenzoic acids; 9, 2-naphthoic acid, 10, 2-; 11, 1-naphthalenesulfonic acids. The relative velocities in a 0.3% polybrene solution are also shown. Capillary 750 mm×50 μm I.D. (500 mm to detector). Buffer solution, 50 m*M* phosphate buffer (pH 7.0) applied voltage 20 kV. Reproduced from Ref. [15] with permission.

The name ion-exchange electrokinetic chromatography (IE–EKC) was used to describe the technique, as ''the separation principle is based on differences in ion-pair formation constants, but not on electrophoretic mobilities; therefore the technique should be classed as a chromatographic method''. While it is true that in the case in question, separation could not be achieved without the chromatographic component, the overall separation mechanism was a combination of both electrophoresis and chromatography, so that classification solely as a chromatographic technique could be misleading.

Stathakis and Cassidy [51] examined the flexibility of using a polymeric pseudo-stationary phase by examining the extent to which the chromatographic component could be varied by changing the composition of the electrolyte. Four different polymers [poly(1,1-dimethyl-3-5-dimethylpiperidinium) chromate (PDDPiCr), poly(1,1-dimethyl-3-5-dimethylenepyrrolidinium) chromate (PDDPyCr), hexadimethrene chromate (HDMCr) and dimethylaminodextran chromate (DEADCr)] were examined with PDDPyCr being the most promising. Variation of the polymer concentration was found to decrease mobilities of analytes due to interactions with the oppositely charged polymers, with the greatest changes in mobility being observed for those analyte ions which show strong retention in IC using strong base anion-exchange stationary phases. The method was applied to the determination of bromide, chloride and sulfate in potash using the interaction of sulfate with the polymer to improve its resolution from bromide and chloride. A subsequent communication [52] examined the separation of organic and inorganic anions, where benzoate was used as the polymer counter-ion instead of chromate. This resulted in greater interaction of the analytes with the polymer because of the lower ion-exchange selectivity coefficient of benzoate in comparison to chromate.

The separation of inorganic anions and anionic metal complexes using cationic polymers as elec-

trolyte additives has been extensively studied by Krohkin et al. They have examined the separation of metal complexes of 4-(2-pyridylazo)resorcinolate (PAR) [16,17], *trans*-1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′tetraacetic acid (CDTA) and ethylenediamine tetracetic acid (EDTA) [53], ethylenediaminedi(*o*-hydroxyphenylacetic acid) (EDDHA) [54] and more recently, *N*,*N*-bis(hydroxybenzyl)-ethylenediamine-*N*,*N*-diacetic acid (HBED) [54,55]. In all cases they found the addition of a cationic polymer improved the separation providing better resolution between many of the complexes. The authors called their method ion electrokinetic chromatography.

The effect of adding poly(ethyleneimine) (PEI) to the electrolyte on the migration of inorganic anions [41] and phenols [56] has been reported by Erim and Nutku. In both cases, the addition of an ionic polymer to the electrolyte improved the resolution of the analytes, and high concentrations could be employed to make the analytes migrate after the EOF. However, under these conditions, the baseline became unstable, peak shapes deteriorated, the analysis time was prolonged, and the resolution was similar to that obtained at lower concentrations.

5.3. Use of soluble polymers at higher salt concentration

5.3.1. Principles

The groundwork for a major advance in using a soluble polyelectrolyte to modify the CE separation of ions was laid by the discovery that sharp analyte peaks could be obtained with a BGE containing a high salt concentration. It had been commonly thought that even a moderately high ionic concentration in the BGE would lead to Joule heating and serious peak distortion. However, Ding et al. [57] obtained very satisfactory separations of both inorganic and organic anions in electrolyte solutions as high as 5 *M* sodium chloride. The key to success was to have the salt concentration in the BGE at least three times higher than that of the sample.

When a soluble cationic polymer P^+Cl^- is added to the BGE, a sample anion A^- undergoes the following ion-exchange equilibrium in solution:

$$P^+Cl^- + A^- \rightleftharpoons P^+A^- + Cl^- \quad (5)$$

The equilibrium constant (K) for this reaction is:

$$K = \frac{[P^+A^-][Cl^-]}{[A^-][P^+Cl^-]} \quad (6)$$

At a fixed concentration of P^+Cl^-, a conditional constant, K', may be written as follows:

$$K' = K[P^+Cl^-] \quad (7)$$

Combining Eqs. (3) and (4), and rearranging:

$$\frac{[A^-]}{[P^+A^-]} = \frac{[Cl^-]}{K'} \quad (8)$$

The electrophoretic migration will depend primarily on the fraction of sample anion that is present as the free anion (see Eq. (4)). This is true because the free anion will migrate rapidly toward the anode, while the fraction associated with the ion exchanger will move only very slowly in the opposite direction. Eq. (8) shows that a higher concentration of salt (Cl^- in this example) will repress the ion-exchange and lead to more rapid migration. Conversely a higher K or a higher concentration of polymer will increase the ion-exchange and slow down the migration of the sample anion.

After enumerating these principles, Li et al. [18] published a comprehensive paper on the use of a soluble ionic polymer to separate anions by a mechanism involving both ion chromatography and capillary electrophoresis. Conditions for separation were studied using a mixture of seven inorganic anions and later with a second mixture of organic anions. Preliminary experiments were performed with each of several polymers added to the BGE at a pH of 8.5. A relatively high concentration (120–150 m*M*) of sodium chloride or lithium sulfate was found to markedly improve the sharpness of the anion peaks. Direct detection at 214 nm was employed.

Fig. 5 shows a baseline separation of seven inorganic anions using 0.05% PDDAC in the BGE to provide an ion-exchange component to the separation. In the absence of PDDAC it was not possible to separate bromide and iodide because their electrophoretic mobilities are almost identical. Other polymers tested did not perform as well as PDDAC. PEI, polyacrylamide and polyvinylpyrrolidone failed to provide complete resolution of the test mixture.

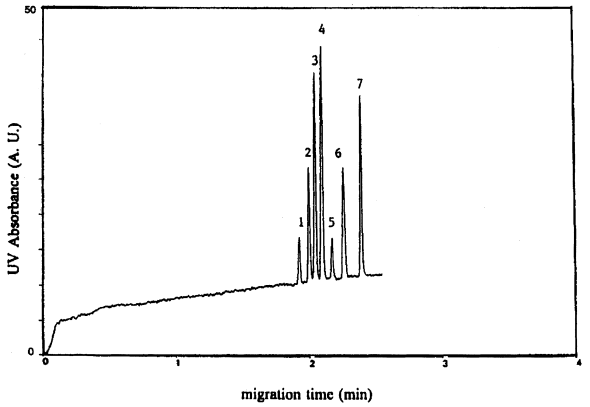

Fig. 5. Separation of inorganic anions. Electrolyte: 150 m*M* Li_2SO_4, 20 m*M* borate, 0.05% PDDAC, pH 8.5; other conditions as specified in experimental section. Peaks: 1, Br^-; 2, I^-; 3, NO_2^-; 4, NO_3^-; 5, CrO_4^{2-}; 6, SCN^-; 7, MoO_4^{2-}. Reproduced from Ref. [18] with permission.

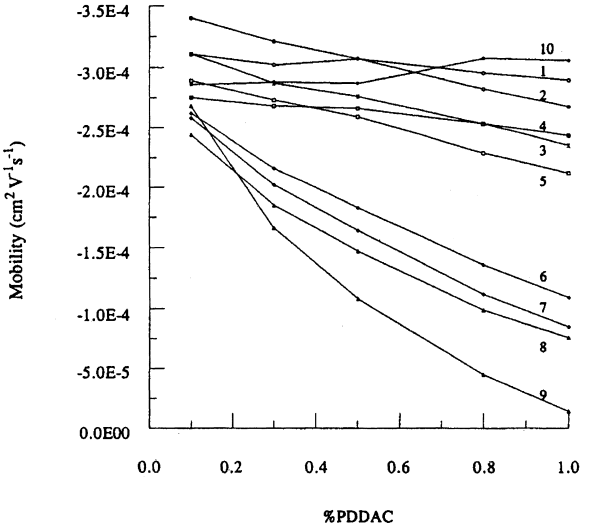

Fig. 6. Effect of PDDAC concentration on EOF and electrophoretic mobilities of organic anions. Electrolyte contains 150 m*M* Li_2SO_4, 20 m*M* borate and PDDAC at pH 8.5. Other conditions as specified in experimental section. Samples: 1, benzoate; 2, benzenesulfonate; 3, *p*-toluenesulfonate; 4, *p*-aminobenzoate; 5, *p*-hydroxybenzoate; 6, 2-naphthalenesulfonate; 7, 1-naphthalenesulfonate; 8, 3,5-dihydroxybenzoate, 9, 2,4-dihydroxybenzoate; 10, water for EOF. Amount injected was 20 ppm for each anion. Reproduced from Ref. [18] with permission.

Hexadimethrene bromide (polybrene) gave much poorer separations than PDDAC.

5.3.2. Effect of BGE pH

When a cationic polymer such as PDDAC is added to the BGE, the capillary surface becomes coated with some of the polymer. This changes the charge on the capillary surface from negative to positive and the direction of EOF from cathodic to anodic. The adsorbed polymer layer is remarkably stable and the EOF is almost constant over a broad pH range. In an electrolyte containing 150 m*M* lithium sulfate, 0.05% PDDAC and 20 m*M* of a pH buffer, the following EOF values were measured [18]:

pH 2.3 anodic EOF $=2.46\times10^{-4}$ ($cm^2\ V^{-1}\ s^{-1}$)

pH 5.0 anodic EOF $=2.65\times10^{-4}$

pH 8.5 anodic EOF $=2.74\times10^{-4}$.

The migration times of four inorganic anions tested showed an average decrease in migration time of -3% going from pH 2.3 to 8.5.

5.3.3. Effect of polymer concentration

The effect of PDDAC concentration in the BGE on EOF and the electrophoretic mobility of nine sample anions is shown in Fig. 6. Over the concentration range covered (0.1–1.0% or 2–20 m*M*) the change in total ionic concentration is rather small because the BGE also contains 150 m*M* lithium sulfate. Changes in EOF and the electrophoretic mobilities of sample anions 1–5 are rather small, although there are some changes in elution order. Sample anions 6–9 show much greater decreases in mobility owing to stronger interaction with the ion-exchange polymer. Electropherograms showed resolution of all nine sample peaks at PDDAC concentrations $>0.3\%$.

5.3.4. Effect of added salt

Increasing concentrations of a salt added to the BGE will decrease the ion-exchange effect and cause the sample anions to migrate more rapidly (Eq. (5)). The sample anions in Fig. 5 had migration times in the range ~1.9–2.4 min in 150 m*M* lithium sulfate but times from ~2.2 to 2.7 min in 50 m*M* lithium sulfate. But, more importantly, the separation efficiency was significantly better at the higher salt concentration. The average theoretical plate number for four anions measured was 123,000 in 150 m*M* lithium sulfate compared to 65,000 in 50 m*M* lithium sulfate. Sharper peak focusing due to electrostacking

at the higher salt concentration is a likely reason for the higher theoretical plate numbers.

The type of salt, as well as its concentration, can have a major effect on the migration of sample anions in systems containing a cationic polymer. Acetate or fluoride will have a much smaller inhibiting effect than chloride on the ion-exchange of sample anions. The divalent sulfate ion will have a still greater inhibiting effect on the ion-exchange of sample ions. For example, the migration times of bromide and iodide in 150 m*M* lithium sulfate were 5.74 and 6.88 min, respectively ($\alpha = 1.20$), but were 6.08 and 8.77 min, respectively ($\alpha = 1.44$), in 150 m*M* sodium acetate.

The counter ion of the BGE salt, as well as the particular anion, can also affect ion migration in systems containing an ionic polymer. In both sulfate and chloride salt systems longer migration times were observed when lithium rather than sodium was the counter ion [18]. Lithium is known to form weak complexes or ion pairs with carboxylates. This would tend to reduce further the fraction of sample analytes present as the more mobile free anion.

5.3.5. Effect of organic solvent

Ion-exchange selectivity with a conventional solid-phase ion exchanger appears to consist of at least two components [58]. One component comes from the attraction of sample anions for the ionic sites of opposite charge on the ion exchanger. Another component is the hydrophobic attraction of the sample anions for the organic matrix of the solid ion exchanger. The presence of an organic solvent can reduce the latter attraction substantially. It would be interesting to know the effect of an organic solvent on the present system where the ion-exchange polymer is completely soluble in the liquid phase. Comparison of sample anions in aqueous solution, 15% acetonitrile, and 30% acetonitrile showed a substantial increase in electrophoretic mobilities of larger anions such as 1- and 2-naphthalenesulfonic acid with increasing proportions of acetonitrile in the BGE. This is indicative of a reduced ion-exchange interaction.

5.3.6. Scope and reproducibility

Inorganic anions generally have significantly shorter migration times than larger organic anions. In conventional CE when conditions are established to separate inorganic anions, the slower migrating organic ions often give broad, poorly-shaped peaks. However, Fig. 7 demonstrates that a mixture containing numerous organic as well as inorganic anions can be separated with baseline resolution in a very short time [18]. All peaks are sharp except for no. 17 which may exist as a mixture of two different ionic charges at the pH employed (for 4-hydroxybenzoic acid $pK_1 = 4.6$ and $pK_2 = 9.1$). Migration times for separations of this type are very reproducible. Data from 10 consecutive analyses gave an RSD of 1.0 or 1.1% for each and every peak. It is very doubtful that separation of the 17 anions studied could be achieved by either IC or CE alone. The speed and resolution obtained in Fig. 7 were possible only by combining the two mechanisms in a single separation technique. Therefore, IC–CE seems to be a logical and simple name for this separation system.

5.4. Optimization

The addition of variation of salt type, concentration and polymer concentration to vary the mobility of analyte ions provides numerous ways in which

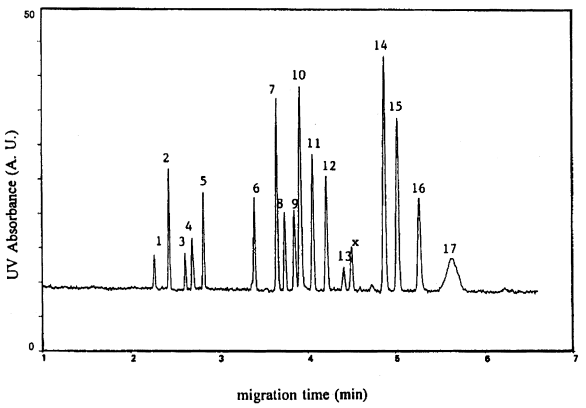

Fig. 7. Separation of 17 inorganic and organic anions. Electrolyte: 120 m*M* lithium sulfate, 20 m*M* borate, 0.3% PDDAC, pH 8.5. Peaks are 1, Br^-; 2, NO_3^-; 3, CrO_4^{2-}; 4, I^-; 5, MoO_4^{2-}; 6, phthalate; 7, 1,2,3-tricarboxylate; 8, 1,2-benzenedisulfonate; 9, terephthalate; 10, isophthalate; 11, benzoate; 12, *p*-toluenesulfonate; 13, 1,3,5-tricarboxylate; 14, 2-naphthalenesulfonate; 15, 1-naphthalenesulfonate; 16, 3,5-dihydroxybenzoate; 17, 2,4-dihydroxybenzoate; *x*, unidentified impurity. Reproduced from Ref. [18] with permission.

the separation can be changed. While this flexibility is perhaps the greatest advantage that this method offers over other packed and open tubular approaches, selection of the appropriate conditions for a desired separation can be difficult. One of the ways to aid in this is to use an optimization algorithm. This problem was addressed by Breadmore et al. [59] who used an optimization algorithm to select the best conditions for the separation of inorganic and organic ions by IC–CE. In their approach, a theoretical equation was derived from IEC and CE theory to describe the simultaneous influences of varying the concentrations of the ionic polymer and the added salt on the migration of anions. A primary data set comprising only five experimental conditions in a central composite experimental design was used to determine analyte constants in the theoretical equation, with the constants then used to predict the mobilities of 16 UV-absorbing anions over the experimental space. Excellent agreement ($r^2 > 0.98$) with experimentally measured mobilities was obtained for all analytes indicating the suitability to predict the migration of ions on a limited number of experiments. Optimization of the conditions was performed using two different resolution criteria, the normalized resolution product criterion which gives optimal conditions for peaks evenly spaced throughout the separation, or the minimum resolution criterion, which gives the conditions which maximize the resolution between the least resolved pair of peaks. This enabled the separation of ions using four different eluent anions (fluoride, acetate, chloride and sulfate) to be optimized, with improved resolution and a different selectivity from that obtainable by normal CE. This approach was later extended to the analysis of UV transparent ions where the separation of 24 anions was optimized using only five initial experiments, the separation of which is shown in Fig. 8 [60]. The separation of anions in Bayer Liquor was then optimized with the ion-exchange interaction with the polymer being used to enhance the separation resolution between sulfate and chloride.

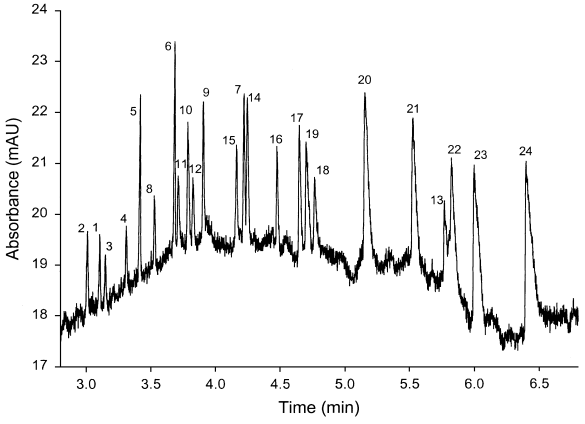

Fig. 8. Optimised separation of 24 anions using a polymeric pseudo-stationary phase and indirect UV detection with CrO_4^{2-} as the competing ion/probe. Conditions are 0.28% $PDDA^+$ and 16.25 m*M* CrO_4^{2-} buffered with 10 m*M* histidine (pH 7.70). Peaks are numbered according to their capillary zone electrophoretic migration order. 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. Reproduced from Ref. [60] with permission.

6. Conclusions

While the use of polymeric materials to introduce ion-exchange interactions into an electrophoretic system was demonstrated in the early 1990s, recent technological and scientific innovations have now begun to realize the full potential of this approach. In all cases, it offers many advantages over using surfactant-based systems, including efficiency, stability and versatility.

Packed columns have the potential to provide excellent separations and also offer an infinite migration time window along with exceptionally high capacities without significant loss in efficiency. Open-tubular columns excel with regard to column fabrication, but suffer from low efficiency due to the long diffusion paths. Continued development of chip-based separations will see the popularity of this area increase, particularly in small channels where packing is difficult and the viscous nature of soluble polymers makes them unsuitable.

The introduction of ion-exchange interactions using soluble ionic polymers such as PDDAC in the BGE is perhaps the most flexible approach to manipulating separation selectivity. Both inorganic and organic anions can be separated quickly and

efficiently. Separation of complex mixtures can be optimized in as little as five runs using polymer concentration, and the type and concentration of salt in the BGE as the major variables. The use of positively-charged polymers with different chemical structures would facilitate a greater variety of binding constants with various analytes and add greater flexibility to the technique. For example, polyelectrolytes with greater hydrophobic character would allow stronger interaction with organic anions. It is logical to assume that IC–CE methods for cations can be worked out using polymers with sulfonate or other negatively-charged groups. However, readily available polymers such as polystyrene sulfonic acid have a benzene ring that would interfere with detection in the UV spectral region.

Separation of larger bio ions such as DNA fragments presents a challenge. Use of ion-exchange interactions could be a valuable separation parameter. However, the ion-exchange interaction in this case could be so strong as to virtually prevent migration of the analytes. A polymer with only a very weak ion-exchange interaction might be the answer to this problem.

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