

# A Mechanism of Separation in Electrostatic Ion Chromatography

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**The retention mechanism of electrostatic ion chromatography (EIC) is currently under debate and is the focus of this paper. A comprehensive set of retention data has been obtained on a C18 column coated with the zwitterionic surfactant 3-(*N,N*-dimethylmyristylammonio)propane-sulfonate used with a range of mobile phases in which both the mobile-phase anion and cation have been varied systematically. Electro-osmotic flow measurements were also obtained on fused-silica capillaries coated with the zwitterion (and also some monofunctional surfactants) and were used to evaluate the nature of the surface charge on the layer of adsorbed surfactant in the presence of various background electrolytes. A new retention mechanism for EIC was developed on the basis of these data. This mechanism proposes that equilibration of the bound zwitterions with a mobile phase containing a suitable electrolyte causes the establishment of a charged layer created by the terminal sulfonate groups of the zwitterion, which acts as a Donnan membrane. The magnitude and polarity of the charge on this membrane depends on the nature of the mobile-phase ions. The Donnan membrane exerts weak electrostatic repulsion or attraction effects on analyte anions. A second component of the retention mechanism is chaotropic interaction of the analyte anion with the quaternary ammonium functional group of the zwitterion. This interaction exerts the major effect on the separation selectivity of EIC, such that analyte anions are eluted in order of increasing chaotropic interactions in accordance with the Hofmeister series.**

The technique termed electrostatic ion chromatography (EIC) utilizes a bifunctional stationary phase where cationic and anionic functional groups in close proximity occur on the stationary phase. This is a result either of chemical modification of the stationary phase or from a hydrophobic zwitterionic surfactant adsorbed onto a hydrophobic chromatographic support material.<sup>1,2</sup> In either case, the stationary phase is characterized by oppositely charged functional groups in close juxtaposition. These novel stationary

phases have been shown to have a unique selectivity, an indifference to high ionic strength matrixes,<sup>3</sup> and the ability to separate ions with a pure water mobile phase.<sup>2</sup> In the case of a sample containing multiple cations and anions, a water mobile phase results in a peak for most of the possible combinations of anion and cation in the injected sample. Thus, the same anion can appear as several peaks in the chromatogram, with each peak corresponding to that particular anion but a different cation.<sup>4</sup> The complex chromatograms that result reduce the analytical utility of water mobile phases. This can be overcome if a solution of a suitable electrolyte is used as the mobile phase, which results in a single peak appearing for each analyte.<sup>3</sup> When an electrolyte solution is used as the mobile phase, EIC has been shown to be very tolerant of high-ionic strength samples. This factor has been used for the successful separation of Br<sup>−</sup>, NO<sub>3</sub><sup>−</sup>, and I<sup>−</sup> in saline matrixes,<sup>5</sup> proving EIC to be a potentially useful analytical tool. A number of studies have been devoted to developing the analytical potential of EIC,<sup>6–10</sup> while others focus on the somewhat puzzling mechanism of separation.<sup>11–13</sup>

There have been several previous explanations offered for the EIC retention mechanism. These have involved a number of concepts, including simultaneous electrostatic attraction and repulsion of analyte ions from the two oppositely charged functional groups of the zwitterion, the formation of “ion pairs” between oppositely charged ions in solution,<sup>4</sup> and the establishment of a zwitterionic electrical double layer comprising the accumulation of oppositely charged ions (from the mobile-phase electrolyte) around the charge centers of the zwitterion.<sup>7</sup> Other possible contributors to the mechanism are ion-exclusion effects, induced ion exchange, and complex formation.

Okada and Patil<sup>12</sup> evaluated two EIC retention mechanisms (namely, ion pair and partition) based on Poisson–Boltzmann

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(1) Viklund, C.; Irgum, K. *Macromolecules* **2000**, *33*, 2539–2544.

(2) Hu, W.; Takeuchi, T.; Haraguchi, H. *Anal. Chem.* **1993**, *65*, 2204–2208.

(3) Hu, W.; Haddad, P. R. *Anal. Commun.* **1998**, *35*, 317–320.

(4) Hu, W.; Tao, H.; Haraguchi, H. *Anal. Chem.* **1994**, *66*, 2514–2520.

(5) Hu, W.; Haddad, P. R.; Hasebe, K.; Tanaka, K.; Tong, P.; Khoo, C. *Anal. Chem.* **1999**, *71*, 1617–1620.

(6) Hasebe, K.; Sakuraba, T.; Hu, W. *J. Liq. Chromatogr. Relat. Technol.* **1999**, *22*, 561–569.

(7) Hu, W.; Haddad, P. R. *Trends Anal. Chem.* **1998**, *17*, 73–79.

(8) Hu, W.; Tao, H.; Tominaga, M.; Mayazaki, A.; Haraguchi, H. *Anal. Chim. Acta* **1994**, *299*, 249–256.

(9) Hu, W.; Tanaka, K.; Haddad, P. R.; Hasebe, K. *J. Chromatogr., A* **2000**, *884*, 161–165.

(10) Jiang, W.; Irgum, K. *Anal. Chem.* **1999**, *71*, 333–344.

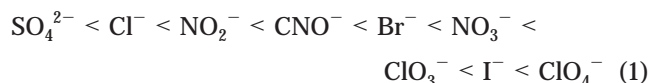
(11) Hu, W. *Langmuir* **1999**, *15*, 7168–7171.

(12) Okada, T.; Patil, J. M. *Langmuir* **1998**, *14*, 6241–6248.

(13) Patil, J. M.; Okada, T. *Anal. Commun.* **1999**, *36*, 9–11.

theory that describes the ion uptake of zwitterionic surfaces. They concluded that in water small, well-hydrated ions interact with the zwitterionic surface via a partition mechanism and large, poorly hydrated ions interact via an ion-pair mechanism. They also suggested that the retention of their probe analyte (iodide) was affected by the nature of the mobile-phase anion but not the mobile-phase cation. However, their study compared only monovalent mobile-phase anions and cations. More recently they have stated that the same stationary phase can display anion-exchange properties or cation-exchange properties depending on the nature of the mobile phase.<sup>13</sup>

The most recently proposed mechanism by Hu<sup>11</sup> is the binary electrical double-layer (EDL) mechanism. A binary-EDL is established as a result of retention of cations and anions from the mobile phase by the negative and positive functional groups on the zwitterionic stationary phase. In the case of a zwitterionic stationary phase in which the negative functional group is outermost (such as that created by adsorption of the zwitterionic surfactant 3-(*N,N*-dimethylmyristylammonio)propanesulfonate, DDAPS, used in this work), the EDL surrounding the negative functional group and comprising cations repels analyte cations but allows the partition of analyte anions. The analyte anions in turn are also repelled from the anion-EDL (surrounding the cationic functional group of the zwitterion). Retention characteristics are further explained in terms of ion-pair theory and the formation of temporary ion pairs. Those analyte anions with a high propensity for forming neutral ion pairs show strong retention, while those with a low propensity are expelled due to repulsion from the anion-EDL. Hence the following retention order for EIC is explained in terms of increasing propensity for ion-pair formation:<sup>2</sup>



Hu and Haddad<sup>3</sup> proposed that above a certain threshold concentration of electrolyte in the mobile phase the binary-EDL became saturated and this was used to explain the observed changes in retention time with increasing mobile-phase concentration.<sup>3</sup> An initial increase in retention was seen, but above 5 mM there was no further effect on retention and this behavior was evident for mobile phases comprising NaHCO<sub>3</sub>, NaCl, or Na<sub>2</sub>SO<sub>4</sub>.<sup>5</sup> However, recent work by Jiang and Irgum<sup>10</sup> showed that increasing the concentration of NaClO<sub>4</sub> in the mobile phase resulted in a decrease in the retention of analyte anions. This provides an important insight into the determination of the true mechanism of separation.

Finally, the  $\zeta$  potential existing at a zwitterionic surface has received some attention. The use of zwitterionic surfactants as electro-osmotic flow (EOF) modifiers in capillary electrophoresis (CE) has revealed that the  $\zeta$  potential arising on a zwitterionic-coated capillary could be modulated by the addition of different anions in the electrolyte.<sup>14</sup> It was suggested that different electrolyte anions became incorporated to different extents into the zwitterionic layer and this in turn altered the  $\zeta$  potential and hence the EOF. The existence of such a  $\zeta$  potential has also been suggested by the calculations of Patil and Okada<sup>13</sup> and experi-

mental data by Iso and Okada.<sup>15</sup> Such processes of anion incorporation into the zwitterion in CE are similar to those governing retention in EIC, so measurements of  $\zeta$  potential can give further information about the EIC retention mechanism.

Despite the previous studies discussed above, the lack of a systematic and comprehensive set of analyte retention data covering a wide range of mobile-phase compositions and analytes has made it difficult to fully characterize EIC and to develop a mechanism that applies under all conditions. The detailed effects of the mobile-phase cation and anion on the retention of analyte anions are still not well known, and the nature of the stationary phase has not yet been examined fully. The aim of the present study has been to address the deficiencies in the existing retention data by acquiring a systematic data set, to study in detail the  $\zeta$  potential characteristics of zwitterionic surfaces in contact with various electrolytes using EOF (and hence  $\zeta$  potential) measurements determined by CE, and to use this information to develop a new, comprehensive retention mechanism for EIC.

## EXPERIMENTAL SECTION

**Apparatus.** A Dionex (Sunnyvale, CA) DX 500 IC system was used throughout this study, configured with a GP40 pump, an AS50 autosampler with a 100- $\mu$ L injection loop, an AD20 UV-visible absorbance detector, and a Waters (Milford, MA) column heater. PeakNet 5.1 (Dionex) software was used to record the chromatograms. The column used throughout this study (L-column, 250  $\times$  4.6 mm i.d., Chemical Inspection and Testing Institute, Tokyo, Japan) was packed with ODS material which was coated with 3-(*N,N*-dimethylmyristylammonio)propanesulfonate. The procedure for coating the column with the zwitterionic surfactant has been described by Hu et al.<sup>12</sup> The surfactant loading was  $1.20 \times 10^{-3}$  mol of DDAPS/column. A total of 0.3 mM DDAPS was also included in the mobile phase to ensure a stable surface coating.

A Beckman Coulter 2050 CE system with a UV absorbance detector was used for the EOF measurements. Data acquisition was performed on a Beckman P/ACE Station Version 1. C18-coated capillaries (Supelco) with an inner diameter of 50  $\mu$ m, outer diameter of 363  $\mu$ m, and total lengths of 47 or 27 cm (40 or 20 cm to the detector) were used.

**Reagents.** 3-(*N,N*-Dimethylmyristylammonio)-propanesulfonate (Fluka), sodium dodecylsulfate (Aldrich), and myristyltrimethylammonium bromide (Aldrich) were used for column and/or capillary preparation. Inorganic salts used to prepare samples and the mobile phases were analytical grade and were obtained from Aldrich (NaBr, NaI, NaCNO, NaClO<sub>4</sub>, Mg(ClO<sub>4</sub>)<sub>2</sub>, Ce(ClO<sub>4</sub>)<sub>3</sub>, Ce<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>, CeCl<sub>3</sub>), Ajax (K<sub>4</sub>Fe(CN)<sub>6</sub>, NaNO<sub>2</sub>, NaNO<sub>3</sub>, HClO<sub>4</sub>), Hopkins and Williams (NaIO<sub>3</sub>), and BDH (Na<sub>2</sub>WO<sub>4</sub>) and were used as received. Water used throughout this study was prepared in the laboratory using a Millipore (Bedford, MA) Milli-Q water purification system.

**EOF Measurements.** The method used for the determination of the EOF was the three-injection method of Williams and Vigh.<sup>16</sup> The time length of pressure and voltage steps, and the magnitude and direction of the voltage, were varied depending on the direction and magnitude of the EOF and the capillary length. The

(14) Barylá, N. E.; Lucy, C. A. *Anal. Chem.* **2000**, *72*, 2280–2284.

(15) Iso, K.; Okada, T. *Langmuir* **2000**, *16*, 9199–9204.

(16) Williams, B. A.; Vigh, G. *Anal. Chem.* **1996**, *68*, 1174–1180.

Table 1. Electro-Osmotic Flow Measurements and Calculated  $\zeta$  Potentials Obtained with Various Background Electrolytes on a Fused-Silica Capillary Coated with a Zwitterionic, Cationic, or Anionic Surfactant

electrolyte	EOF ( $\times 10^{-9} \text{ m}^2/\text{V}\cdot\text{s}$ )	% RSD ( $n = 5$ )	$\zeta$ potential <sup>a</sup> (mV)
<b>zwitterionic surfactant</b>			
NaCl (25 mM)	23.9	0.6	-30.6
MgCl <sub>2</sub> (25 mM)	10.4	1.8	-13.3
CeCl <sub>3</sub> (25 mM)	-31.4	0.6	40.2
NaClO <sub>4</sub> (25 mM)	41.7	0.3	-53.4
Mg(ClO <sub>4</sub> ) <sub>2</sub> (25 mM)	23.9	0.7	-30.6
Ce(ClO <sub>4</sub> ) <sub>3</sub> (25 mM)	-9.1	2.6	11.7
NaCl (25 mM)	23.9	0.6	-30.6
NaCNO (25 mM)	25.9	0.4	-33.2
NaClO <sub>4</sub> (25 mM)	41.7	0.3	-53.4
CeCl <sub>3</sub> (25 mM)	-31.4	0.6	40.2
Ce(ClO <sub>4</sub> ) <sub>3</sub> (25 mM)	-9.1	2.6	11.7
<b>cationic surfactant</b>			
NaCl (25 mM)	-33.2	2.8	42.5
CeCl <sub>3</sub> (25 mM)	-25.7	4.5	32.0
trisodium citrate (5.4 mM)	-8.2	2.1	10.5
NaCl (125 mM)	-26.2	1.2	33.5
<b>anionic surfactant</b>			
NaCl (25 mM)	50.6	0.6	-64.8
NaClO <sub>4</sub> (25 mM)	51.9	1.0	-66.5

<sup>a</sup> Calculated using the Helmholtz equation.

three-injection method is very versatile in this respect and also offers highly precise and accurate measurements of EOF. New capillaries were rinsed with water for 10 min, 30 mM surfactant for 20 min at low pressure (breakthrough at 8.5 min), and 0.3 mM surfactant for 30 min at high pressure. Prior to each run, the capillary was rinsed for 2 min with 0.3 mM surfactant and then 3 min with the running electrolyte (also containing 0.3 mM surfactant).

Conditions used on the CE system were maintained as close as possible to those used on the IC system, although it was necessary to buffer the CE system to stabilize the EOF. A low-concentration buffer containing ions with low propensity for inclusion into the zwitterion (1 mM H<sub>2</sub>CO<sub>3</sub>/HCO<sub>3</sub><sup>-</sup>, pH 6.4) was used and was made up freshly each day. DDAPS (0.3 mM) was included in the buffer, except with the cerium salts as electrolytes due to precipitation effects. The neutral marker was acetone, and direct UV detection was performed at 280 nm.

## RESULTS AND DISCUSSION

**EOF Characteristics of Zwitterionic Coatings.** The EOF characteristics (and indirectly, the  $\zeta$  potentials) of an adsorbed layer of the zwitterionic surfactant DDAPS (and for comparison, a monofunctional anionic surfactant and a cationic surfactant) on a C18-functionalized capillary were studied by measuring the EOF in a series of CE experiments. A range of background electrolytes (BGEs) having different anions and cations but constant salt concentrations was used, and the results for EOF and  $\zeta$  potentials are summarized in Table 1. As expected, the cationic surfactant in combination with 25 mM NaCl as BGE gave an anodic EOF and a positive  $\zeta$  potential (42.5 mV). Replacing the BGE anion with citrate resulted in a decreased EOF, probably due to strong electrostatic interaction of citrate with the quaternary ammonium group of the surfactant. Experiments with a capillary coated with

a typical anionic surfactant resulted in the expected cathodic EOF and a negative  $\zeta$  potential (-64.8 mV) with 25 mM NaCl as BGE. Replacement of the BGE ion having the same charge as the surface (shown here with ClO<sub>4</sub><sup>-</sup>) had little effect on the EOF and  $\zeta$  potential.

Substantial EOF values and  $\zeta$  potentials were observed when the capillary was coated with the zwitterionic DDAPS and used in combination with the different BGEs. The 25 mM NaCl BGE gave a net negative  $\zeta$  potential (-30.6 mV) and a cathodic EOF of  $23.9 \times 10^{-9} \text{ m}^2/\text{V}\cdot\text{s}$  (Table 1), while the 25 mM NaClO<sub>4</sub> BGE gave a cathodic EOF of  $41.7 \times 10^{-9} \text{ m}^2/\text{V}\cdot\text{s}$  and a larger negative  $\zeta$  potential (-53.4 mV). Baryla and Lucy<sup>14</sup> reported that cathodic EOF values with potassium salts of anions increased according to the following series: SO<sub>4</sub><sup>2-</sup> < Cl<sup>-</sup> < Br<sup>-</sup> < I<sup>-</sup> < ClO<sub>4</sub><sup>-</sup>. This behavior suggested that these anions interacted more strongly with the quaternary ammonium group on the zwitterion than did the cation with the sulfonate group, resulting in an overall negative  $\zeta$  potential. The more strongly the anion interacted with the quaternary ammonium group, the more effectively it reduced the effective charge on this group and resulted in the overall  $\zeta$  potential becoming more negative.

The effect of the electrolyte cation was studied using Na<sup>+</sup>, Mg<sup>2+</sup>, and Ce<sup>3+</sup> salts of Cl<sup>-</sup> and ClO<sub>4</sub><sup>-</sup>. For both anions, the Mg<sup>2+</sup> salt resulted in a significantly lower cathodic EOF and lower negative  $\zeta$  potential than the Na<sup>+</sup> salt. This may be attributed to stronger interaction of Mg<sup>2+</sup> with the sulfonate group, leading to an effective reduction in the charge of this group and therefore a reduction in the overall negative  $\zeta$  potential. The Ce<sup>3+</sup> salt interacted even more strongly, changing the  $\zeta$  potentials from -30.6 mV for NaCl to 40.2 mV for CeCl<sub>3</sub>. For ClO<sub>4</sub><sup>-</sup> salts, the  $\zeta$  potential was altered from -53.4 mV for NaClO<sub>4</sub> to 11.7 mV for Ce(ClO<sub>4</sub>)<sub>3</sub>. Thus, both the charge on the metal cation and the chemical nature of the anion had a major effect on the  $\zeta$  potential of the zwitterionic coating.

The experimental measurements made using CE with a C18 capillary coated with a surfactant can be considered to be analogous to the situation occurring in EIC using a C18 reversed-phase material coated with a surfactant. While the actual loading of the adsorbed surfactant will differ between CE and EIC, the above results show clearly that in the CE case there is sufficient adsorbed surfactant to interact with the ions in the BGE. The CE data can therefore validly be used to assist in the interpretation of the mechanism that applies in the EIC case.

**Elution Effects in Electrostatic Ion Chromatography.** If Ce<sup>3+</sup> were to interact so strongly with the sulfonate part of the zwitterion that the negative charge was completely masked, the resultant surface would be expected to act as an anion exchanger. Therefore, plots of log  $k'$  versus log [mobile phase] should show slopes consistent with ion-exchange elution behavior. The data in Table 2 show the expected slope of -1.0 for the elution of nitrate from the anion-exchange column formed by coating reversed-phase silica with a cationic surfactant, using either sodium chloride or sodium perchlorate as the mobile phase. However, with a zwitterionic column, there was very little change in log  $k'$  as the mobile-phase concentration was increased and there was also almost no difference in behavior between Na<sup>+</sup> and Ce<sup>3+</sup> salts, except where chloride is the mobile-phase anion (this is discussed further below). Thus, there is no evidence of a conventional ion-

Table 2. Slopes of Plots of  $\log k'$  versus  $\log$  [Mobile Phase] for Various Surfactants and Mobile Phases

mobile-phase anion	slope		
	cationic surfactant (mobile-phase cation $\text{Na}^+$ )	zwitterionic surfactant (mobile-phase cation $\text{Ce}^{3+}$ )	zwitterionic surfactant (mobile-phase cation $\text{Na}^+$ )
$\text{Cl}^-$	-0.96	-0.11	0.09
$\text{ClO}_4^-$	-1.00	-0.20	-0.20

exchange mechanism operating with the zwitterionic stationary phase regardless of the type of mobile phase used. Some salt effects might be expected to occur in the  $\log k'$  versus  $\log$  [mobile phase] plots in that the amount of adsorbed surfactant might alter as [mobile phase] is changed. However, this will increase the ion-exchange capacity of the column, which will affect the linearity of the plots, rather than the slope, because the  $y$ -intercept term would vary for each [mobile phase]. The data showed excellent linearity, suggesting that the capacity of the column was essentially constant for the experiments conducted. It can also be pointed out that similar retention effects and slopes for plots of  $\log k'$  versus  $\log$  [mobile phase] were observed by Hu et al.<sup>3</sup> for mobile phases that did not contain zwitterion and for which the ion-exchange capacity of the column was therefore constant.

The elution behavior of a range of inorganic anions ( $\text{Br}^-$ ,  $\text{I}^-$ ,  $\text{Fe}(\text{CN})_6^{4-}$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ,  $\text{IO}_3^-$ , and  $\text{WO}_4^{2-}$ ) on a zwitterion-coated column was studied as a function of mobile-phase concentration for five different salts. The results are given in Figure 1 for three representative analytes,  $\text{IO}_3^-$ ,  $\text{NO}_2^-$ , and  $\text{I}^-$ , and show that an abrupt change in  $k'$  occurred when the mobile phase was changed from water alone to a low concentration of salt ( $<1$  mM). Further increases in salt concentration resulted in very little further change in  $k'$ . In some cases, the  $k'$  value decreased significantly as a result of the first addition of salt to the mobile phase (e.g., for  $\text{IO}_3^-$  as analyte with all mobile phase salts, Figure 1a), while in others,  $k'$  increased when salt was first added (e.g., for  $\text{I}^-$  as analyte and all mobile phase salts except  $\text{NaClO}_4$ ). The behavior exhibited was determined by the relative positions of the mobile phase and analyte anions in the EIC retention order shown in eq 1. When the mobile-phase anion was one that was less strongly retained in EIC than the analyte anion (e.g.,  $\text{SO}_4^{2-}$  as mobile phase,  $\text{NO}_2^-$  as analyte; see Figure 1b), then retention of the analyte increased compared to that observed in a water mobile phase. On the other hand, a mobile-phase anion retained more strongly than the analyte anion (e.g.,  $\text{ClO}_4^-$  as mobile phase,  $\text{NO}_2^-$  as analyte, see Figure 1b) caused a decrease in retention compared to a water mobile phase. The same trends were evident for all analytes studied. This behavior has not been reported earlier nor predicted to occur in EIC. Again, these unusual changes in retention are inconsistent with an ion-exchange mechanism.

The data in Figure 1 have the following implications for practical anion separations. First, analyte retention times may be increased (compared to retention obtained in water mobile phases) by using an anion in the mobile phase that shows weak retention in an EIC system (e.g.,  $\text{SO}_4^{2-}$ ) or decreased by using a more strongly retained anion such as  $\text{ClO}_4^-$ . Second, the analyte retention times are relatively insensitive to further changes in the concentration of the mobile phase. Hu et al.<sup>17</sup> overcame the latter problem by use of a column coated with a 10:1 mixture of

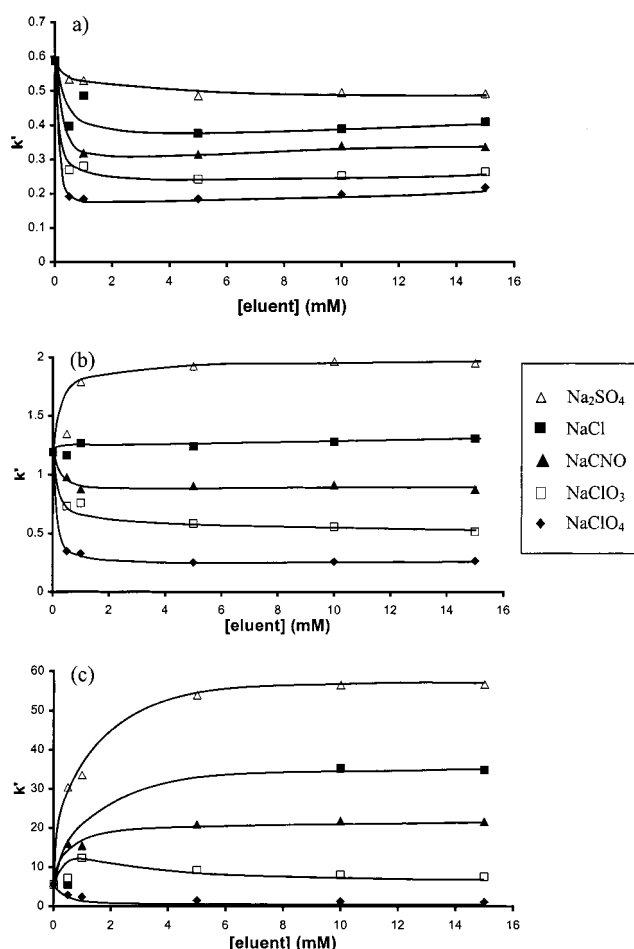


Figure 1. Effect of the concentration of mobile phases containing various sodium salt on retention times of (a) iodate; (b) nitrite, and (c) iodide. Conditions: column, ODS modified with DDAPS; flow rate, 1.0  $\text{mL min}^{-1}$ ; detection, direct UV at 214 nm; sample, 100  $\mu\text{L}$  containing 1.0 mM of each anion.

zwitterionic and cationic surfactants, thereby introducing anion-exchange effects to complement the EIC retention mechanism.

Patil and Okada<sup>13</sup> noted that EIC is primarily a method for separating anions, and they considered that the type of counter-cation in the mobile phase had almost no effect on the separation. This conclusion was based on the use of monovalent mobile-phase cations. This effect was therefore examined using divalent and trivalent cations, and the results shown in Figure 2 were obtained for mobile phases with  $\text{Cl}^-$  (Figure 2a) or  $\text{ClO}_4^-$  (Figure 2b) as the mobile-phase anion. For both mobile-phase anions, increasing the charge on the counter-cation in the mobile phase caused a

(17) Hu, W.; Haddad, P. R.; Hasebe, K.; Cook, H. A.; Fritz, J. S. *Fresenius J. Anal. Chem.* **2000**, 367, 641–644.



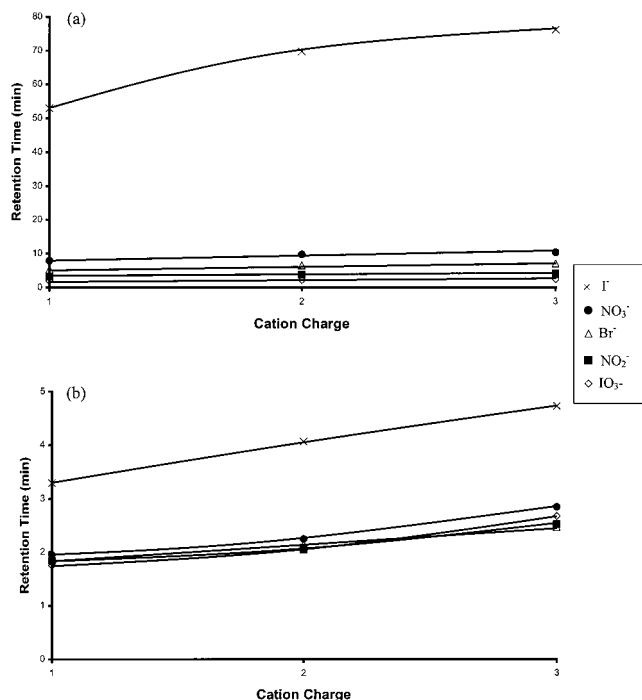


Figure 2. Effect of the mobile-phase cation charge on the retention of analytes using (a) mobile phases comprising 10 mM (with respect to the cation)  $\text{Cl}^-$  salts and (b) mobile phases comprising 10 mM (with respect to the cation)  $\text{ClO}_4^-$  salts. Other conditions as described in Figure 1.

slight increase in retention for most of the analyte anions, with  $\text{I}^-$  showing a somewhat stronger increase with the  $\text{Cl}^-$  mobile phase.

**Mechanism.** Okada and Patil<sup>12</sup> proposed that there is sufficient flexibility in adsorbed DDAPS molecules that in a pure water mobile-phase interaction between adjacent adsorbed DDAPS molecules can occur. The positive and negative charges of one DDAPS molecule are paired with the negative and positive charges, respectively, of a second. A small amount of salt in the mobile phase weakens this pairing of the zwitterion causing a relaxation (disentangling) of the surface morphology. This allows greater accessibility of analyte ions to the functional groups on the DDAPS molecule. The rapid initial change in  $k'$  values (Figure 1) in going from pure water to a 1 or 2 mM concentration of salt in the mobile phase supports this theory of an abrupt change in surface morphology.

It has been suggested<sup>15,18,19</sup> that the selectivity of a zwitterionic stationary phase for anions is a function of the chaotropic character of the anion. Ions such as  $\text{ClO}_4^-$  are chaotropic, which refers to an increase in entropy resulting from disruption of the water structure surrounding these ions in solution. Chaotropic anions are large with a low charge and have a large number of electrons spread over an appreciable volume such that the electrons are not tightly held by the nucleus. For example, the four equivalent oxygens in perchlorate can bond in all directions with quaternary ammonium groups on the zwitterionic stationary phase. The charge will be effectively distributed among all positive sites to compensate for the negative charge of the perchlorate, leading to diffuse ion-pair formation. This in turn creates a net negative

charge on the surface when the counterion of the sulfonic acid group (usually  $\text{Na}^+$ ) is not as tightly bound as the chaotropic anion. This is consistent with the large negative  $\zeta$  potential observed in CE experiments using  $\text{NaClO}_4$  as the BGE.

The chromatographic retention of smaller ions such as  $\text{F}^-$  and  $\text{SO}_4^{2-}$  is low whereas the retention increases for the series  $\text{Cl}^-$ ,  $\text{Br}^-$ , and  $\text{I}^-$  because of increased charge distribution. The increasingly negative  $\zeta$  potential across the series  $\text{SO}_4^{2-}$ ,  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{I}^-$ , and  $\text{ClO}_4^-$  shown in Table 1 and observed also by Baryla and Lucy<sup>14</sup> corresponds to increasing charge distribution and follows the Hofmeister, or chaotropic, series.<sup>20</sup> Hofmeister effects are much greater for anions than cations.<sup>21</sup>

The charge on the mobile-phase cation also has some effect on the chromatographic behavior of anions. Figure 2 shows longer retention times for sample anions as the mobile-phase cation is changed from  $\text{Na}^+$  to  $\text{Mg}^{2+}$  to  $\text{Ce}^{3+}$ . This behavior may be explained as follows. With a  $\text{Na}^+$  salt as the mobile phase, the zwitterionic stationary phase has a negative  $\zeta$  potential (as indicated by the measured EOF values) that exerts a repulsion effect on the analyte anion. Use of a 2+ or 3+ salt reduces the surface negative charge by stronger electrostatic interaction with the sulfonate functional group, making it easier for the analyte anion to approach the positively charged quaternary ammonium groups of the zwitterion.

Our proposed retention mechanism comprises two simultaneous effects, one of ion exclusion and one of chaotropic interaction. The ion-exclusion effect arises as follows. The sulfonate group on the outer part of the zwitterionic stationary phase contributes a negative charge that repels analyte anions by acting as a Donnan membrane. However, the magnitude of this negative charge (and hence the degree of repulsion created by the Donnan membrane) varies depending on how strongly the mobile-phase cations interact with the sulfonate functional groups and how strongly the mobile-phase anions interact with the quaternary ammonium groups. Strong interaction by the mobile-phase cations decreases the surface negative charge, while strong interaction of the mobile-phase anions increases the surface negative charge. In effect, the outer negative charge constitutes a barrier over which an analyte anion must pass in order to approach the quaternary ammonium functional group on the zwitterionic stationary phase. This barrier can be lowered by partial neutralization of the negative charge on the sulfonate group by a 2+ or 3+ cation, giving increased retention as observed in Figure 2.

The other part of the proposed mechanism concerns the interaction of sample anions with the quaternary ammonium functional group of the zwitterion. The experimental evidence presented here is that separation selectivity follows the order of increasing polarizability, or chaotropic character. The more chaotropic is the analyte anion, the greater will be its retention. Thus, the elution order of anions appears to be determined by "chaotropic selectivity" rather than by electrostatic effects.

These effects are illustrated schematically in Figure 3. Figure 3a shows the zwitterionic stationary phase in equilibrium with the mobile-phase cations and anions and the establishment of the Donnan membrane. The situation occurring with a  $\text{NaClO}_4$  mobile phase is depicted in Figure 3b. Here there is a weak interaction

(18) Umemura, T.; Kamiya, S.; Itoh, A.; Chiba, K.; Haraguchi, H. *Anal. Chim. Acta* **1997**, *349*, 231–238.

(19) Irgum, K. University of Umea, Sweden, personal communications, May 2000.

(20) Cacace, M. G.; Landau, E. M.; Ramsden, J. J. *Q. Rev. Biophys.* **1997**, *30*, 241–277.

(21) Collins, K. D.; Washabaugh, M. W. *Q. Rev. Biophys.* **1985**, *18*, 323–422.

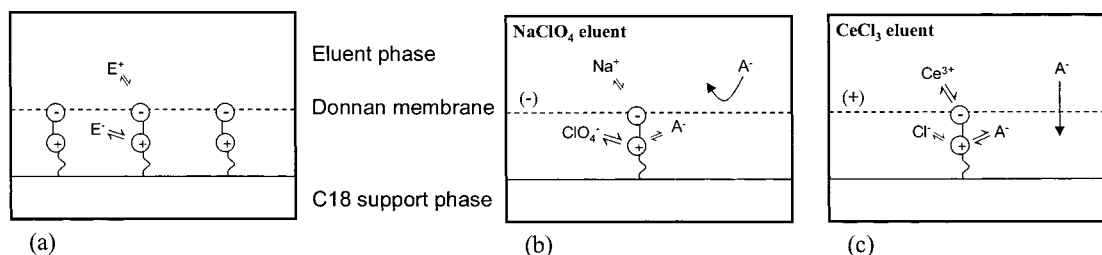


Figure 3. Schematic summary of the proposed mechanism. (a) Establishment of the Donnan membrane, (b) use of a  $NaClO_4$  mobile phase, and (c) use of a  $CeCl_3$  mobile phase. Further explanation is in the text.

between  $Na^+$  and the sulfonate functional group and a strong interaction between  $ClO_4^-$  and the quaternary ammonium functional group. These effects combine to establish a relatively strong negatively charged Donnan membrane, which exerts a strong repulsion on analyte anions, especially multiply charged anions such as  $SO_4^{2-}$ . Those analyte anions that can penetrate the Donnan membrane (and it should be remembered that the charge on this membrane will always be less than in ion-exclusion chromatography, where only sulfonate functional groups are present) can undergo chaotropic interactions with the quaternary ammonium functional groups and are therefore retained to various degrees. Figure 3c shows the situation with a  $CeCl_3$  mobile phase, with which there is a strong interaction of  $Ce^{3+}$  with the sulfonate groups and a weak interaction of  $Cl^-$  with the quaternary ammonium groups. The net result is that the Donnan membrane now becomes weakly positively charged and all analyte anions can penetrate relatively freely. Separation of these anions occurs due to chaotropic interactions with the quaternary ammonium groups.

The proposed mechanism embodies the main elements of the mechanisms suggested previously in that the mobile-phase ions are considered to interact with the functional groups in the same manner as suggested in the EDL theory of Hu<sup>11</sup> and there is a surface charge (the Donnan membrane) as suggested by Okada and Patil.<sup>12</sup> However, the proposed mechanism is the first to explain the elution order and also the effects on retention caused by changes in the nature of the mobile-phase anion and cation. This mechanism brings into question the name "electrostatic ion chromatography" that has been applied extensively, since this name suggests a predominance of electrostatic effects in the retention mechanism. It might be more appropriate to use the

name "zwitterion ion chromatography" to refer to ion chromatographic separations in which a zwitterionic stationary phase is employed.

## CONCLUSIONS

A comprehensive investigation into the effect of the mobile-phase anion and cation on the retention of analytes and the  $\zeta$  potential of the (DDAPS) EIC system has revealed characteristics not seen before. A new retention mechanism was proposed that is based on chaotropic selectivity. Retention in this system is determined by the analyte anion's ability to penetrate the repulsion effects established by a Donnan membrane and to interact chaotropically with the quaternary ammonium group of the zwitterion. Selectivity is determined chiefly by the latter consideration, so that retention follows the Hofmeister (chaotropic) series  $IO_3^- < SO_4^{2-} < Cl^- < NO_2^- < CNO^- < ClO_3^- < ClO_4^-$ . The proposed mechanism draws on the framework of existing theories of EIC but provides for the first time an explanation of the effects of the eluent cation on the retention of anionic analytes. The mechanism also offers a coherent explanation for the observation that changing the concentration of an eluent can cause the retention of some analytes to increase and others to decrease.

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