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On-Capillary Ion-Exchange Preconcentration of Inorganic Anions in Open-Tubular Capillary Electrochromatography with Elution Using Transient-Isotachophoretic Gradients. 2. Characterization of the Isotachophoretic Gradient

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Diffuse transient-isotachophoretic boundaries can be used as an elution gradient of increasing eluotropic strength to elute inorganic anions that have been preconcentrated on an open-tubular ion-exchange stationary phase prior to electrophoretic separation. The generation and characteristics of these gradients for elution after preconcentration have been investigated. The gradients are generated by placing a low-mobility, weak ion-exchange competing anion in the capillary (weak electrolyte, WE), and a high-mobility, strong ion-exchange competing anion in the electrolyte vials (strong electrolyte, SE). Application of voltage establishes a diffuse boundary with the composition changing from the weak anion to the strong anion. Comparison of elution gradients generated with different electrolyte systems was accomplished by comparing the eluotropic strength (a function of eluent concentration, ion-exchange selectivity coefficient, and charge) and the shape of the profile as it changes from WE to SE. The ion-exchange selectivity coefficient of the SE competing anion is important in establishing a sharp change in elution strength. A large difference in mobility between the WE and SE competing anions gives an SE with a higher final eluotropic strength, but the slope of the gradient is shallower. This results in a reduction in the efficiency of analyte focusing. To ensure maximum focusing efficiency, the WE and SE electrolytes should be selected such that the SE has the highest possible eluotropic strength for a given concentration of WE. The SE competing anion should also have a sufficiently low electrophoretic mobility to ensure focusing for the maximum number of analytes, and the mobility difference between the WE and SE competing anions should be as small as possible.

Capillary electrophoresis (CE) is often compared to high-performance liquid chromatography (HPLC), and while it has many advantages such as reduced analysis time, higher separation efficiency, and lower solvent consumption, one of the major disadvantages is the poor detection sensitivity obtained with the usual detection method, namely, UV absorbance detection.[1] More sensitive detection methods are available, such as electrochemical (conductivity, potentiometric, amperometric), laser-induced fluorescence, and more recently mass spectroscopy, but the UV detector is still the most commonly used.[2]

There are two main ways in which the concentration of the analytes in a sample can be increased prior to separation of the sample by CE: those based on velocity difference induced focusing (V-DIF) and those based on solid-phase extraction (SPE). V-DIF methods are based on analytes being focused as a result of differences in their velocity in two zones within the capillary.[3] Perhaps the best known example of this in CE is the phenomenon of electrostacking, where analytes are focused on the boundary between the electrolyte and the sample zone due to differences in conductivity of the two zones. Additional V-DIF preconcentration methods include isotachophoresis (using either one or two columns),[4] sweeping,[5] and various stacking methods employed to enable large sample volumes to be injected (such as large-volume sample stacking, LVSS, and field enhanced sample injection, FESI).[6] However, all of these methods suffer from the fact that the sample volume is limited at most to one capillary volume and in reality is usually somewhat lower than this in order to facilitate separation. Of course, the capillary length can be increased to enable more sample to be injected, but resolution and analysis time must be sacrificed.

In contrast, SPE methods enable multiple volumes of sample to be injected because the analytes are adsorbed onto a stationary phase. This not only results in preconcentration of the analytes but can also remove interferences. The use of SPE for CE was first reported by Guzman et al.,[7] who used a short packed capillary

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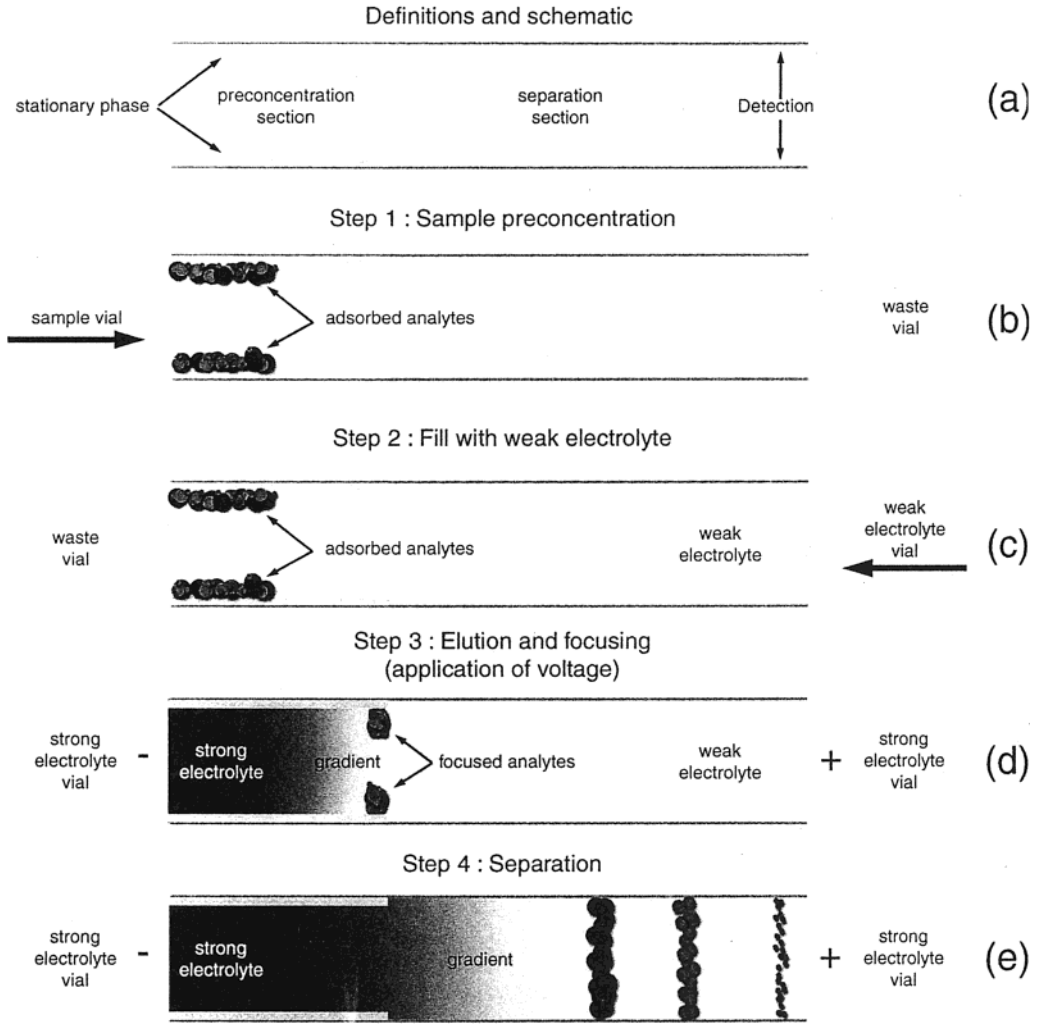

Figure 1. Schematic representation of preconcentration and separation procedures. (a) capillary schematic and definitions, (b) injection of the sample and analyte preconcentration, (c) filling of capillary with weak electrolyte from the outlet end (in order to minimize the possibility of movement of the preconcentrated analytes, (d) application of voltage resulting in the generation of a gradient that focuses and elutes the analytes from the preconcentration section of the capillary, and (e) electrophoretic separation of the focused analytes in the separation section of the capillary.

to preconcentrate methamphetamine. Since then, there have been numerous reports using open tubular columns,[8,9] small packed beds,[7,10−15] and disks,[16,17] with the later being preferred due to higher flow rates and smaller elution volumes. However, most of the work has focused on the use of reversed-phase interactions for preconcentration and inorganic species have received little attention, with only two reports in the literature. Both of these methods employ CE coupled to a flow injection system using an ion-exchange (IE) column with either the effluent being directed across the capillary inlet and injection performed via voltage[18] or the sample placed into a CE vial and injected in the normal manner.[19]

Recently, in a preliminary communication, we showed that inorganic anions can be preconcentrated by SPE using an ion-exchange open-tubular CEC (IE-OT-CEC) approach, with elution being accomplished by the use of a transient-isotachophoretic gradient.[20] Here, a short section (8.5 cm) of the capillary was coated with 70-nm-diameter cationic ion-exchange particles (functionalized with quaternary ammonium groups) to form a preconcentration section, (Figure 1a), with the remainder of the capillary being a conventional fused-silica surface. Pressure-assisted injection of several capillary volumes of the sample results in the adsorption of anionic analytes onto the ion-exchange stationary

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phase and preconcentration of the sample (Figure 1b). The capillary is then filled from the outlet end with an electrolyte containing an anion with a low ion-exchange selectivity coefficient for the stationary phase and also having a low electrophoretic mobility (called the weak electrolyte, WE) (Figure 1c). In the next step, the electrolyte vials at each end of the capillary are filled with an electrolyte that contains a competing ion having a high ion-exchange selectivity coefficient and a high electrophoretic mobility (the strong electrolyte, SE). On applying the separation voltage, some of the more mobile, stronger competing anions from SE will migrate ahead of the less mobile, weaker competing anions from WE, establishing a diffuse boundary of increasing eluotropic strength according to isotachophoretic principles (Figure 1d). This boundary constitutes a compositional gradient in which the electrolyte changes progressively from WE to SE Appropriate selection of analyte and electrolyte ions enables the analytes to be focused into an extremely sharp band prior to leaving the ion-exchange part of the capillary and then subsequently separated in the uncoated separation section of the capillary according to normal electrophoretic principles (Figure 1e). We have demonstrated this approach using one particular composition of WE and SE and two analytes (NO_3^- and Br^-), for which detection limits of 0.86 and 0.32 μM and separation efficiencies of 138 000 and 236 000 were achieved for Br^- and NO_3^-, respectively. Extension of this method to a wider range of electrolytes and analytes requires a fundamental understanding of the transient-isotachophoretic gradient, and the present paper examines the parameters that will affect the elution gradient and investigates the influence of these parameters on analyte focusing.

EXPERIMENTAL SECTION

Instrumentation. Electrophoretic separations were performed using a Hewlett-Packard ^{3D}CE (Hewlett-Packard, Waldbron, Germany). Separations were carried out using a Polymicro (Phoenix, AZ) fused-silica capillary (25-μm i.d. with a length of 64.5 cm, 56.0 cm to detector) unless otherwise noted.

Ion chromatographic separations were carried out using an Dionex (Sunnyvale, CA) DX-500 instrument. Detection was accomplished by using either suppressed conductivity detection and/or direct UV detection using a Waters (Milford, MA) 860 UV/visible spectrophotometric detector. The ions were separated on a 4 × 150 mm Ion Pac AS5A, 5-μm column (Dionex) fitted with an AS5G guard column (Dionex). The void time was measured by recording the water dip observed at 214 nm.

Reagents. AS5A latex particles with an approximate size of 75 nm were supplied as an 11% (w/v) suspension from Dionex. Analytical grade tris(hydroxymethyl)aminomethane (TRIS) was obtained from Sigma-Aldrich (Milwaukee, WI) and was used without further purification. Standards of 0.1 mM Br^-, I^-, NO_2^-, NO_3^-, SCN^-, and CrO_4^{2-} were prepared from sodium or potassium salts of analytical grade and diluted as required. Background electrolytes (BGEs) were prepared by titration of TRIS with the corresponding acid of the desired eluent competing ion ($HClO_4$ or naphthalenedisulfonic acid) to a pH of 8.05. Therefore, BGEs with a concentration of 10 mM ClO_4^- will have 20 mM TRIS. All BGEs were degassed by application of a vacuum for 1 min and filtered through a 0.45-μm filter before use. Solutions containing F^- were prepared from dissolution of the sodium salt. The carbonate eluents for IC separations were prepared from Na_2CO_3 and $NaHCO_3$ according to the desired concentration and pH, while the perchlorate eluent was prepared by titration of $HClO_4$ with TRIS to give the desired pH.

Capillary Coating Procedure. The latex was cleaned as reported previously.[21] In this study, we have used only capillaries in which the entire length had been coated with the latex particles, since the emphasis of this work is on the elution and focusing aspects of the process rather than on the sample preconcentration or separation steps. Whole capillaries were coated by flushing the capillary with a dilute suspension of particles for 20 min (repeated 3 times) before being flushed with water for 5 min, followed by equilibration with the desired BGE by flushing for 10 min.[21]

RESULTS AND DISCUSSION

Operating Principles of Preconcentration Using Isotachophoretic Gradients. The desirable characteristics of an on-capillary sample preconcentration method using a solid-phase extraction approach are that the analyte ions from a large sample volume should be adsorbed onto the preconcentration stationary phase and then eluted by the gradient as a sharp band for subsequent separation and quantification. In this way, high preconcentration factors and good separation efficiencies can be obtained. In order for the band of analyte ions to become focused on the capillary wall, the relative IE strength and mobility of the analyte, WE, and SE anions must be selected accordingly. If K_A, K_{WE}, and K_{SE} are used to denote the IE selectivity coefficients of the analyte, WE and SE anions, respectively, and μ_A, μ_{WE}, and μ_{SE} denote their electrophoretic mobilities, and then the relative magnitudes of these parameters ideally are $K_{WE} < K_A < K_{SE}$ and $\mu_A > \mu_{SE} > \mu_{WE}$. When conditions are selected to ensure that this occurs, then analytes are focused along the capillary wall by the front of the transient gradient, as illustrated schematically in Figure 2, which shows only the preconcentration part of the capillary containing the adsorbed stationary phase. In this figure, $\mu_{SE} > \mu_{WE}$, resulting in ions from the SE migrating into the WE zone, establishing a compositional gradient in which the BGE composition changes from WE to SE in a continuous manner. Alternatively, when $\mu_{WE} > \mu_{SE}$, a stepwise compositional gradient should be created. Assessment of the potential of these two types of gradients for analyte focusing requires consideration of a number of factors. While the use of a stepwise gradient should be ideal for focusing all analytes into a sharp band prior to electrophoretic separation, the continuous gradient offers the possibility of separation of some analytes during the focusing process. This could be important for analytes having similar mobilities, which would be difficult to separate by electrophoretic means if they entered the separation section of the capillary as a single, focused analyte band. In view of the wider possibilities offered by continuous gradients, the discussion in the present work will be confined to the situation where $\mu_{SE} > \mu_{WE}$. A further reason for this decision is that the alternative case ($\mu_{SE} < \mu_{WE}$) has been difficult to implement practically because of a lack of competing anions having appropriate electrophoretic mobilities and ion-exchange selectivity coefficients.

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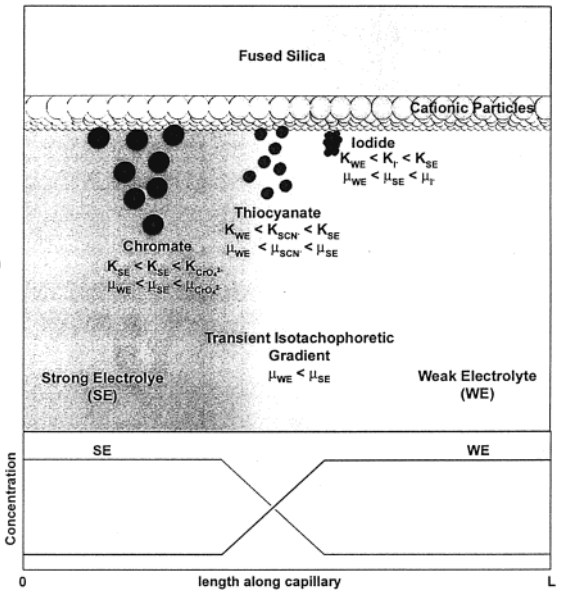

Figure 2. Schematic representation of the focusing mechanism using an IE-OT-CEC column and a transient-isotachophoretic gradient, illustrated by I^-. SCN^- is not focused because its electrophoretic mobility is lower than that of the SE, while CrO_4^{2-} is not focused because the eluotropic strength of the SE is too low.

The effects of the gradient on the movement of three different analyte ions of differing electrophoretic mobilities and ion-exchange selectivity coefficients are illustrated in Figure 2. A continuous compositional gradient has been used for illustrative purposes; however, the same principle applies when a stepwise gradient is used. In the case of I^-, when the strong competing anion from SE reaches the adsorbed analyte, immediate desorption of the analyte occurs ($K_{SE} > K_I-$). The analyte then migrates according to its electrophoretic mobility (which is higher than that of the strong competing anion) and therefore migrates ahead of the gradient front, into a zone of BGE containing WE. In this zone, the analyte has a higher interaction with the stationary phase than the competing anion ($K_I- > K_{WE}$), so I^- will therefore be readsorbed onto the wall, only to be again desorbed by the band of strong competing anion that follows. As the gradient boundary moves through the capillary, the band of I^- therefore becomes focused due to its different migration rates in the two zones and moves with the gradient boundary. If the mobility of the analyte is less than that of the SE (illustrated by SCN^-), or the elution strength of the SE is not high enough to completely desorb the analyte (illustrated by CrO_4^{2-}), then analyte focusing will not occur.

Since the nature of the elution gradient is fundamental to the achievement of focusing of analytes, methods to manipulate the gradient and to extend this focusing to as wide a range of analytes as possible are required. For this purpose, the ideal SE anion will be one with a very high affinity for the functional groups on the stationary phase (i.e., K_{SE} will be large) and a low electrophoretic mobility (i.e., μ_{SE} will be small). As will be demonstrated later in this paper, these conditions can be met by a reasonably wide range of anions.

Gradient Generation and Characterization. Generation of the elution gradient is accomplished by the use of a transient-isotachophoretic process, and therefore, the gradient will differ when different combinations of WE and SE are used. To compare different gradients, it is necessary to characterize them using a suitable parameter and we have chosen to examine how the eluotropic strength of the gradient changes with electrolyte composition.

1. Eluotropic Strength. The eluotropic strength of a particular eluent, and hence the nature of any gradient formed from this eluent, will be a function of the concentration of the eluent competing ion and the affinity of the eluent competing ion with the stationary phase. The eluotropic strength of an eluent E, denoted as S_E, will increase with the charge (y), concentration ([E]), and selectivity coefficient toward a particular univalent analyte, A ($K_{E,A}$), and can be defined by the following equation:

$$S_E = K_{E,A}[E]^y \tag{1}$$

Estimates for S_E can therefore be obtained if $K_{E,A}$ and the concentration and charge of SE are known. These parameters are discussed below.

The concentration of SE that migrates into the capillary is given by the Kohlrausch regulating function (KRF) and is dependent on the electrolyte that fills the capillary when the voltage is first applied.[22] The equilibrium concentration of SE migrating into the capillary is therefore given by

$$[SE] = \frac{Z_{WE}}{Z_{SE}} \frac{|\mu_{SE}|}{|\mu_{SE}| + \mu_{cat}} \frac{|\mu_{WE}| + \mu_{cat}}{|\mu_{WE}|}[WE] \tag{2}$$

where Z_{WE} is the charge of the WE anion, Z_{SE} is the charge of the SE anion, and cat denotes the countercation, assuming this is the same for both WE and SE. It is therefore possible to calculate the [SE] that would result for a particular type of SE competing ion if the composition of WE is known.

Values for $K_{E,A}$ can be obtained by measurement of the ion-exchange behavior of the desired ions on a chromatographic system using the same stationary phase as that employed in the IE-OT-CEC system. The latex particles used to coat the capillary are the same as those used in the latex-agglomerated stationary phase in a Dionex AS5A column. Retention data for potential eluent ions were obtained using a 50 mM carbonate eluent (pH 10.0) for the weakly retained anions and a 50 mM sodium perchlorate eluent at pH 10.0 (prepared by titration of perchloric acid with sodium hydroxide) for the more strongly retained anions. The selectivity coefficient $K_{E,A}$ for a range of anions to be used as potential eluents (and hence designated as E) can be estimated from these data using a reference analyte and nonlinear regression from the following equation:[23]

$$\log \alpha_{E,F} = \frac{1}{1} \log K_{E,F} + \frac{x-1}{1} \log \left(\frac{k'_F V_m}{w} \right) \tag{3}$$

where E is the anion, and F is the reference analyte, V_m is the volume of the mobile phase, w is the weight of the stationary

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Table 1. Electrophoretic Mobilities, Retention Factors, and Ion-Exchange Selectivity Coefficients (Relative to Fluoride) for Selected Anions

anion[a]	μ_{ep}, 10^{-9} $m^2/V\cdot s$	k', CO_3^{2-}/HCO_3^-	k' ClO_4^-	$K_{E,F}$	anion[a]	μ_{ep}, 10^{-9} $m^2/V\cdot s$	k', CO_3^{2-}/HCO_3^-	k' ClO_4^-	$K_{E,F}$
fluoride	−58.12	0.05		1.00	heptanesulfonate	−25.21	2.68		58.4
lactate	−37.79	0.06		1.00	malonate	−62.84	2.73		53.6
acetate	−44.96	0.06		1.40	2-aminobenzoate	−28.75	3.13		68.2
glycolate	−42.87	0.07		1.60	bromide	−77.42	3.50		76.4
propionate	−39.82	0.09		2.00	4-aminobenzenesulfonate	−33.12	3.79		82.6
n-butanoate	−35.28	0.11		2.40	chlorate	−67.33	3.83		83.6
iodate	−42.64	0.12		2.60	nitrate	−73.11	3.93		85.6
formate	−60.02	0.13		2.80	tartrate	−59.44	3.99		78.3
*iso*pentanoate	−32.63	0.16		3.40	phosphate	−56.74	4.02		78.8
HEPES	−21.52	0.17		3.60	benzenesulfonate	−33.02	4.27		93.0
ethanesulfonate	−43.90	0.17		3.60	oxalate	−7.05	5.20		102
methanesulfonate	−51.27	0.17		3.60	octanesulfonate	−13.82	5.44		118
MOPS	−26.24	0.21		4.60	sulfate	−76.79	6.26		123
MES	−27.99	0.21		4.60	4-toluenesulfonate	−28.62	7.20		157
HIBA	−33.00	0.21		4.60	fumarate	−60.91	8.18		160
propanesulfonate	−39.40	0.25		5.40	EDTA	−52.44	13.95		246
sulfamate	−42.17	0.26		5.60	phthalate	−51.61	17.86		350
picolinate	−33.00	0.38		8.20	molybdate	−72.83	18.28	0.56	358
hexanoate	−31.58	0.39		8.40	iminodiacetate	−29.90	18.85		369
butanesulfonate	−36.41	0.39		8.60	DTPA	−57.20	27.61		487
ascorbate	−26.83	0.45		9.80	salicylate	−28.57	29.94		587
CHES	−6.98	0.51		11.2	iodide	−75.43	32.64	0.40	711
octanoate	−27.37	0.52		11.4	4-hydroxybenzoate	−31.79	39.47		774
camphorsulfonate	−25.08	0.57		12.4	dipicolinate	−51.70	41.44	0.82	812
bromate	−61.00	0.69		15.0	2-sulfobenzoate	−48.14	43.21	1.17	847
4-aminophenylacetate	−30.75	0.70		15.2	chromate	−72.23[b]	43.73	1.25	857
pentanesulfonate	−33.75	0.72		15.6	thiosulfate	−76.86	46.03	1.08	903
nicotinate	−33.79	0.72		15.8	thiocyanate	−62.99	58.19		1270
isonicotinate	−34.37	0.83		18.2	citrate	−64.69	77.28	1.43	1360
chloride	−76.27	0.84		18.4	perchlorate	−63.66	108.0		2350
sorbate	−31.62	1.02		22.2	2,3-dihydroxybenzoate	−27.14	157[c]	1.95	2780
nitrite	−75.34	1.27		27.6	1,2-benzenedisulfonate	−47.62	157.7	1.94	3090
hexanesulfonate	−30.82	1.39		30.4	sulfosalicylate	−27.53	439.2	4.06	7750
cyanate	−47.86	1.45		31.6	5-sulfoisophthalate	−33.77	791[c]	12.61	14000
glutarate	−53.09	1.99		43.4	1,5-naphthalenedisulfonate	−57.38	860[c]	13.70	16900
benzoate	−29.57	2.09		45.6	benzenetetracarboxylate	−68.13	6387[c]	101.8	104000
adipate	−50.03	2.14		41.9	naphthalenetrisulfonate	−73.48	7592[c]	121.0	136000
succinate	−57.03	2.17		42.6	indigo carmine	−43.04	13768[c]	219.5	237000

[a] HEPES, 4-(2-hydroxyethyl)-1-piperazineethaneesulfonic acid; MOPS, 3-morpholinopropanesulfonic acid; MES, 2-morpholinoethanesulfonic acid; HIBA, 2-hydroxyisobutyric acid; CHES, 2-(cyclohexylamino)ethanesulfonic acid; EDTA, ethylenediaminetetraaectic acid; DTPA, diethelenetriamine pentaacetic acid. [b] Mobility measured in 5 mM sulfate/10 mM DEA electrolyte. [c] Retention factors in carbonate estimated from scaling the values obtained in perchlorate. Scaling factor determined by the retention factors of anions separated in both eluents.

phase, and x is the eluent anion charge. Values for the selectivity coefficient of E using fluoride as the reference ($K_{E,F}$) can be determined from eq 3 and are shown in Table 1. These values were calculated using retention factors measured in the carbonate eluent. Since the retention times of some of the more strongly retained analytes could be determined only in the perchlorate eluent, it was necessary to estimate values for their retention factor in the carbonate system. This was done by comparing how the retention factors of selected anions (I^-, 2-sulfobenzoic acid, CrO_4^{2-}, $S_2O_3^{2-}$, citrate, 1,2-benzenedisulfonic acid, sulfosalicylic acid) were related in both the carbonate and perchlorate systems and applying the same relationship to retention factors for those anions separated in only the perchlorate system. The effective charge of the eluent ion was calculated at the operating pH of 10.0 using tabulated dissociation constants.[24]

The difference in eluotropic strength between SE and WE, denoted S_G (i.e., the eluotropic range of the gradient) can be defined according to the following equation:

$$S_G = S_{SE} - S_{WE} \quad (4)$$

Combination of eqs 1, 2, and 4 shows that S_G for a particular SE/WE combination will be dependent on (i) the concentration of WE, (ii) the mobility of WE, (iii) the mobility of SE, (iv) the mobility of the countercation, and (v) the relative charges of WE and SE.

2. Gradient Profile. Because the elution gradient is formed from a transient isotachophoretic boundary, its shape can be predicted from isotachophoretic theory. Detailed descriptions and derivations of mathematical equations describing the process have been published by Foret et al.,[22] and only final equations will be repeated here. It should be noted that, for simplification, only electrolytes containing monovalent anions are considered and only for the case when $\mu_{SE} > \mu_{WE}$.

The position of the mixed zone of WE/SE along the column at time t, can be determined from x_{WE} (the position of the pure

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WE zone, where x is a distance measured from the capillary inlet) and x_{SE} (the position of the pure SE zone), which are given by[22]

$$x_{WE} = \bar{x}_{WE} t \quad (5)$$

$$x_{SE} = \bar{x}_{SE} t \quad (6)$$

where $\bar{x}_{WE}$ and $\bar{x}_{SE}$ are the linear velocities of the WE and SE zones, respectively, and are given by[22]

$$\bar{x}_{SE} = \frac{\mu_{SE} I}{SF(\mu_{WE} + |\mu_{cat}|)[WE]_{WE}} \quad (7)$$

$$\bar{x}_{WE} = \frac{\mu_{WE}^2}{SF\mu_{SE}(\mu_{WE} + |\mu_{cat}|)[WE]_{WE}} = \frac{\mu_{WE}^2}{\mu_{SE}^2} \bar{x}_{SE} \quad (8)$$

where S is the cross-sectional area of the capillary, F the Faraday constant, I the cross-sectional current, and $[WE]_{WE}$ the concentration of WE in the zone containing only WE.

The concentration of SE in the zone $[WE]_{WE}$, given by $x < x_{WE}$, is equal to 0 by definition. The concentration of SE in the zone $[SE]_{SE}$, given by $x_{SE} < x$, is given by eq 2, where SE and WE are both monovalent. The concentration of SE in the mixed zone of WE and SE, denoted by $[SE]_{SE/WE}$, where $x_{WE} < x < x_{SE}$, is given by[22]

$$[SE]_{SE/WE} = [WE]_{WE} \frac{\mu_{SE}}{\mu_{SE} - \mu_{WE}} \frac{\mu_{WE} + |\mu_{cat}|}{\mu_{SE} + |\mu_{cat}|} \left(\frac{1}{F\mu_{WE} Z(x,t)} - 1 \right) \quad (9)$$

Where $Z(x,t)$ is defined as

$$Z(x,t) = \sqrt{\frac{S[WE]_{WE}(\mu_{WE} + |\mu_{cat}|)(x-1)}{F\mu_{SE}\mu_{WE}^2 It}} \quad (10)$$

at any given time t for all $x_{WE} < x < x_{SE}$.

These equations reveal that the position of the gradient will be governed primarily by the relative mobilities of the WE and SE anions. By using defined vales of electrophoretic mobilities of the ions (from values in Table 1), and a value of -30×10^{-9} m^2/V·s for the electroosmotic flow (EOF), it is possible to construct a theoretical gradient profile representing how the electrolyte composition changes from WE to SE with time at a defined position along the column. This enables a direct comparison with experimental gradients if the change in gradient composition at the detection window can be measured.

Different gradients can be compared on the basis of their slope, which can be defined as the change in eluotropic strength, S_G divided by the time required for that change, Δt, given by

$$\text{slope} = \frac{S_G}{\Delta t} = \frac{S_{SE} - S_{WE}}{t_{SE} - t_{WE}} \quad (11)$$

Influence of WE Concentration. The concentration of WE will govern the concentration and hence eluotropic strength of

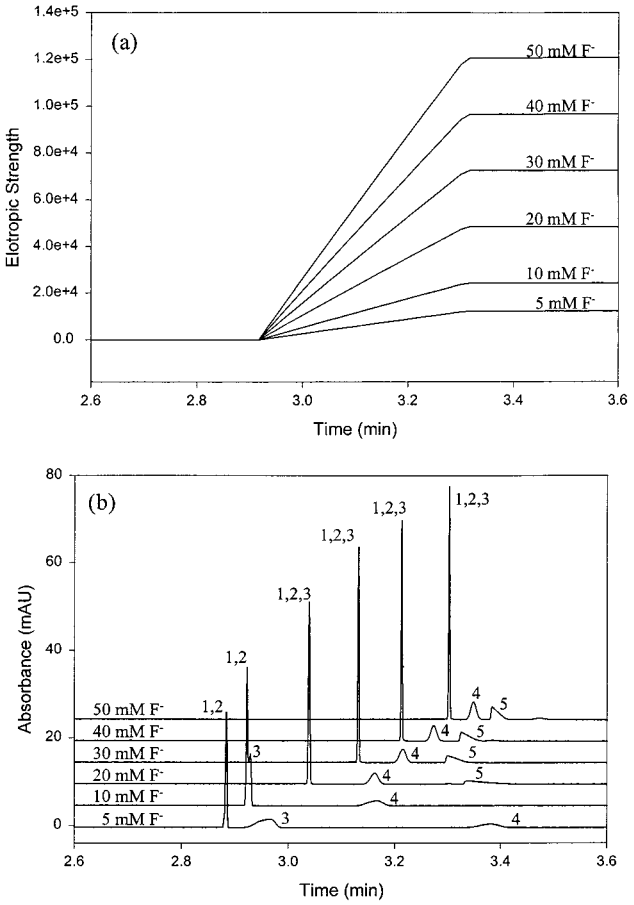

Figure 3. (a) Theoretical prediction of the influence of the concentration of the WE competing anion (in this case, F^-) on the eluotropic strength of a ClO_4^- SE entering the capillary. SE: 50 mM ClO_4^-/100 mM TRIS, pH 8.05. (b) Influence of varying the concentration of WE (NaF) on the preconcentration and elution of inorganic anions. SE: 50 mM ClO_4^-/100 mM TRIS, pH 8.05, voltage −30 kV, injection was for 4 min of 0.5 μM each anion prepared in WE. Peaks: (1) Br^-, (2) NO_3^-, (3) I^-, (4) SCN^-, and (5) CrO_4^{2-}.

SE according to eq 2. Figure 3a illustrates the theoretical influence of increasing the concentration of a F^- WE on the eluotropic strength of a ClO_4^- SE. It can be seen that as the concentration of WE increases, the eluotropic strength of the ClO_4^- SE increases, while the time over which the gradient is generated should remain unchanged. It should be noted that theoretical gradients were predicted by ignoring the influence of ionic strength, which will affect both electrophoretic mobilities and EOF. Figure 3b shows the influence of these same F^- WE concentrations on the actual focusing and separations obtained for a series of inorganic anions as analytes using a capillary that had been coated with ion-exchange particles along its entire length up to the detection window. It can be seen that, at 5 mM F^-, the Br^- and NO_3^- peaks were focused but I^- and SCN^- were eluted later as broad peaks. As the concentration of WE was increased, the I^- peak moved closer to the focused peak of Br^- and NO_3^-, until above 20 mM it comigrated with Br^- and NO_3^- due to the increase in eluotropic strength of the resultant ClO_4^- SE evident from Figure 3a. This same effect is also illustrated by the elution of SCN^- closer to the focused peak when higher concentrations of F^- were used. Increasing the WE concentration did not change the migration time of the SCN^- peak significantly due to the mobility of SCN^-

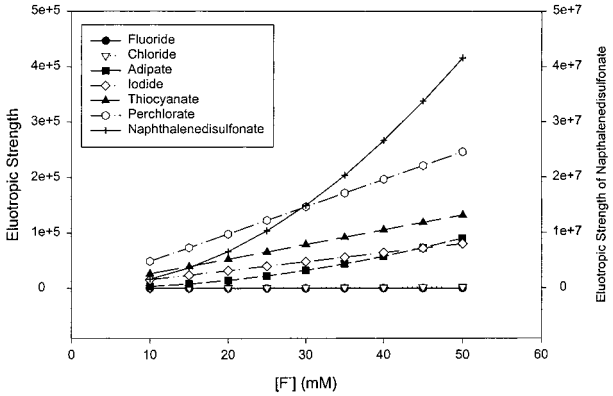

Figure 4. Influence of increasing the concentration of WE (NaF) on the eluotropic strength of an SE formed from different competing anions. In all cases, the SE countercation was Na^+. Note that naphthalenedisulfonate is displayed on a scale a factor of 100 times higher than the other anions.

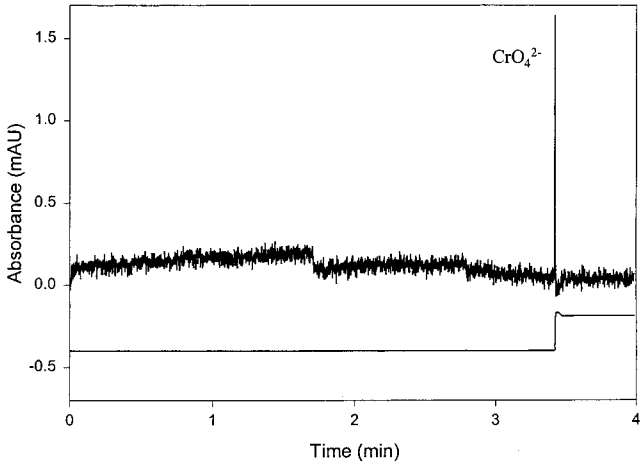

Figure 5. Focusing of CrO_4^{2-}. Top trace, detection at 370 nm; bottom trace shows gradient profile by detection at 226 nm. WE: 20 mM NaF. SE: 50 mM NDS/200 mM TRIS, pH 8.05, voltage −30 kV; injection was for 4 min of a 0.5 μM CrO_4^{2-} solution.

being lower than that of ClO_4^- and hence an inability for a focused peak to be attained. The peak for CrO_4^{2-} first appeared at 20 mM F^-, and increasing the WE concentration resulted in a decrease in retention. However, even at 50 mM F^-, the concentration of SE entering the capillary was insufficient to completely suppress interaction of CrO_4^{2-} with the stationary phase, leading to a distorted peak. While it would be possible to increase the concentration of F^- so that the resultant concentration of SE would eventually be sufficient to prevent interactions of CrO_4^{2-} with the stationary phase, the concentration of F^- necessary in the WE would cause elution of weakly retained ions (such as Br^- and NO_3^-) when the capillary was first filled with WE prior to application of the voltage (step 2 shown in Figure 1c). A further disadvantage is that the increase in ionic strength results in a lower EOF, which results in a slower separation, evident in Figure 3b as the increasing migration time of the focused peak. An alternative to increasing the concentration of WE as a means to increase the eluotropic strength of SE is to use a competing ion in the SE that has a higher selectivity coefficient than ClO_4^-.

Figure 4 shows the increase in eluotropic strength observed as the concentration of a F^- WE is increased for some of the potential SE anions shown in Table 1. It is apparent that the eluotropic strength of a particular SE is strongly dependent on the concentration of F^- in the WE, especially for multivalent eluent ions. This can be seen by comparing adipate and I^-, where the eluotropic strength of adipate increases more rapidly than I^- as the concentration of WE is increased. Thus, an SE of higher eluotropic strength will be produced using I^- (rather than adipate) as the SE competing anion if the concentration of F^- in the WE is low, but the reverse is true when high concentrations (>50 mM) of WE are used. In terms of finding a SE competing anion capable of focusing a strongly bound analyte such as CrO_4^{2-}, a high eluotropic strength is required and naphthalenedisulfonate (NDS) is a good candidate. A WE of 10 mM F^- produces a SE containing 4.96 mM NDS, giving an eluotropic strength of 1.7×10^6 (compared to an eluotropic strength of 4.9×10^5 for an SE containing 10.4 mM ClO_4^- which would be formed under identical WE conditions) Figure 5 shows the use of NDS as SE when 20 mM NaF as WE and CrO_4^{2-} as analyte are used. Detection of CrO_4^{2-} was performed at 370 nm while detection at 226 nm enabled the NDS gradient to be visualized. Detection of other inorganic anions at low-UV wavelengths was not possible due to the background absorbance of the SE. It can be seen that the peak for CrO_4^{2-} is highly focused, demonstrating that the eluotropic strength of the SE was sufficient to suppress any interaction between CrO_4^{2-} and the stationary phase, and the mobility of NDS is low enough to allow CrO_4^{2-} to be focused.

Selecting the appropriate WE and SE composition for analyte focusing is a complex procedure. The WE must ensure analyte retention, while the SE must immediately desorb the analytes. To achieve this, eluotropic strength should be calculated by using both the concentration and ion-exchange selectivity coefficient of the competing anion.

Influence of Mobility of the Countercation. Equation 2 indicates that the electrophoretic mobility of the countercation will influence the concentration of SE entering the capillary. Using eq 2 and defining the WE as 10 mM F^- and ClO_4^- as the SE competing anion, the influence of changing the mobility of the countercation can be calculated. For the countercations Li^+ (μ_{ep} = 38.66), Na^+ (μ_{ep} = 50.08), and K^+ (μ_{ep} = 73.48), the concentration of ClO_4^- produced as the SE varies from 10.36 mM for Li^+ to 10.52 mM for K^+. This suggests that while the countercation should theoretically have the highest mobility possible, in practice, the increase in SE concentration achieved by changing the nature of the countercation is not significant.

Influence of Mobility of the WE Competing Anion. Changing the mobility of the WE competing anion will affect the concentration of SE entering the capillary according to the Kohlrausch regulating function and will also affect the profile of the gradient. To examine the influence of this effect on analyte focusing, four anions with different electrophoretic mobilities and similar ion-exchange selectivity coefficients (Table 1) were selected as WE competing anions. The selected anions were isopentanoate (μ_{ep} = −32.63, $K_{E,F}$ = 3.30), ethanesulfonate (μ_{ep} = −43.90, $K_{E,F}$ = 3.59), methanesulfonate (μ_{ep} = −51.27, $K_{E,F}$ = 3.59), and formate (μ_{ep} = −60.02, $K_{E,F}$ = 2.79). Any change in the gradient profile between these anions should be related only to changes in electrophoretic mobility. To enable the gradient profile to be visualized, SCN^- was used as the SE competing anion rather

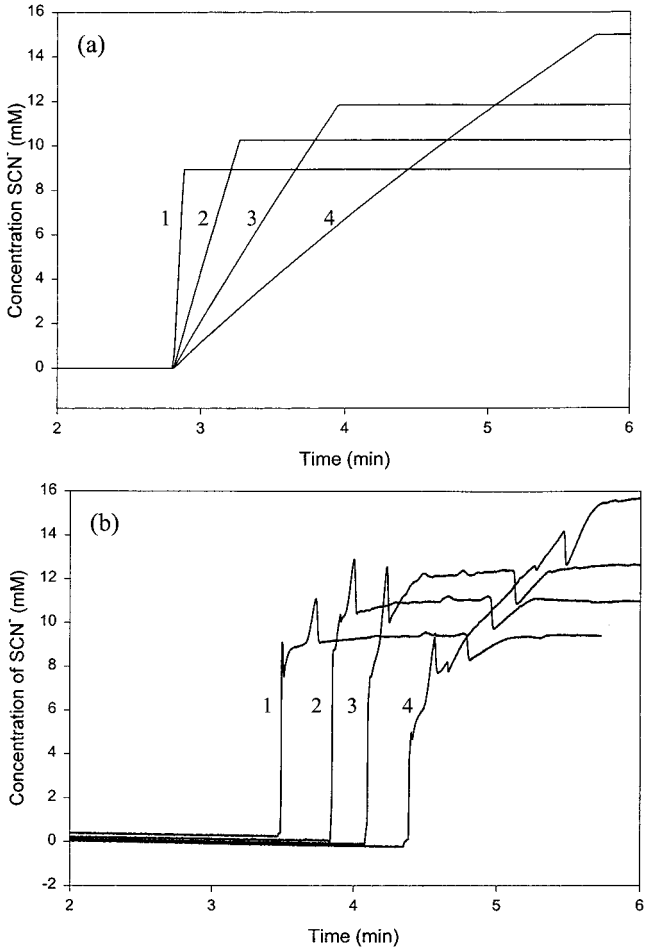

Figure 6. Theoretical (a) and experimental (b) gradients showing the influence of varying WE mobility. The WE concentration in all cases was 5 mM and NaSCN was used as the SE at a concentration of 10 mM. WE competing anions are (1) formate, (2) methanesulfonate, (3) ethanesulfonate, and (4) isopentanoate.

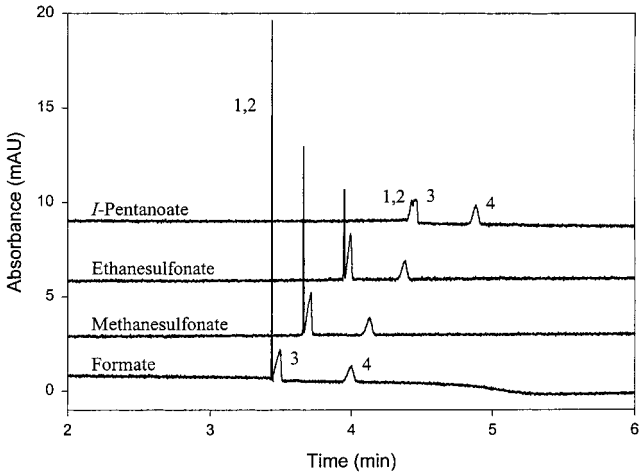

Figure 7. Influence of the mobility of the WE competing anion on focusing of selected analytes. All WE concentrations were 5 mM of the sodium salt. SE was 10 mM ClO_4^-/20 mM TRIS, pH 8.05. Other conditions as in Figure 3.

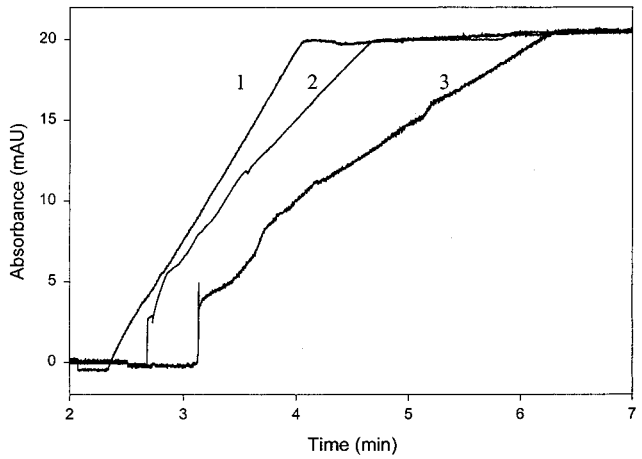

Figure 8. Influence of the ion-exchange selectivity coefficient of the SE competing anion on gradient profile. WE: 5 mM NaF. SE concentration was 10 mM of the corresponding sodium salt. Absorbance scale has been normalized to enable a relative comparison of the step. SE anions are (1) NO_2^-, (2) I^-, and (3) NTS.

than ClO_4^- since both have similar mobilities and ion-exchange selectivity coefficients, but SCN^- is UV-absorbing. Figure 6a shows the transient gradient profiles predicted from theory for the four different WE competing anions and Figure 6b shows the gradient profiles obtained experimentally.

There is general agreement between Figure 6a and 6b with regard to the slope of the gradient and the overall increase of [SCN^-] in SE as the mobility of the WE competing anion is decreased. Small differences between theory and experiment may be explained by differences in EOF (an average EOF was used in the theoretical case) and as a result of some uncertainty in the mobilities used in the theoretical predictions (due to the fact that there will be some interaction between the anions and the stationary phase, so the mobilities in the present system could differ from those determined from CE experiments). However, there is a major difference between the two figures, in that the experimental gradient profiles show an initial stepwise increase in [SCN^-] (shown by the vertical rise in the plots in Figure 6b), followed by the expected gradient profile. This step is due to adsorption of the SE anions on the stationary phase at the front of the gradient due to their high ion-exchange selectivity coefficient, so that the gradient becomes compressed until the stationary phase becomes saturated with the SE anions. The influence of this step on analyte focusing can be seen in Figure 7, which shows that as the mobility of the WE competing anion decreases, the peak of Br^- and NO_3^- becomes less focused. This is due to a reduction in the size of the step, as seen in Figure 6b.

To ensure that the efficiency of analyte focusing is as high as possible, the above results indicate that the mobility of the WE and SE competing anions need to be as close together as possible to give the maximum gradient slope.

Influence of Ion-Exchange Selectivity Coefficient of the SE Competing Anion. To confirm the origin of the step at the front of the gradient, the ion-exchange selectivity coefficient of the SE competing anion was varied by using NO_2^-, I^-, and naphthalenetrisulfonate (NTS), all of which are UV-absorbing (allowing the gradient profile to be visualized; see Figure 8), have similar electrophoretic mobilities (−75.33, −75.43, and −73.47, respectively, Table 1), but have different ion-exchange interactions with the stationary phase ($K_{E,F}$ = 27.6, 712, and 16 700, respectively, Table 1). It can be seen that the step is virtually nonexistent in the NO_2^- system and is the greatest in the NTS system,

suggesting that the front of the gradient is compressed due to retention of the SE competing anion on the stationary phase. It should be noted that the size of this step will be limited by the capacity of the stationary phase and this is apparent when comparing I^- and NTS, which have similar step sizes although NTS has a much higher ion-exchange selectivity coefficient. This suggests that both I^- and NTS are adsorbed sufficiently strongly that they both saturate the stationary phase in a short time. It can also be noted that the front of the gradient occurs later for the more strongly interacting SE competing anions, and this is due to two reasons. First, the interaction of the SE competing anion with the wall results in this anion having a lower effective mobility than expected, and second, the stronger the interaction between the SE competing anion and the stationary phase on the wall, the lower will be the EOF.[25]

CONCLUSIONS

The generation of diffuse transient-isotachophoretic gradients and their use for elution of inorganic anions after ion-exchange preconcentration has been investigated. The gradients were characterized using eluotropic strength and concentration profile. The eluotropic strength of SE is a function of the charge, ion-exchange selectivity coefficient, and concentration of the SE competing anion. The concentration is governed by the Kohlrausch regulating function and is dependent strongly on the difference in mobility between the WE and SE competing anions. The concentration profile of the isotachophoretic gradient is likewise strongly dependent on the difference in mobility between the WE and SE competing anions and depends also on the ion-exchange selectivity coefficients of these ions. A larger difference in mobility results in a higher final eluotropic strength of SE, but a longer gradient of reduced slope is produced. A high ion-exchange selectivity coefficient of the SE competing anion causes compression of the front of the gradient, resulting in a sharp initial change in eluotropic strength. This sharp change is reduced as the gradient slope decreases, resulting in less efficient focusing. The SE and WE should be selected so that (i) the SE has the highest possible eluotropic strength for a given concentration of WE, (ii) the competing anion in the SE has a mobility as low as possible to ensure that the maximum number of analyte anions can be focused, and (iii) the difference in electrophoretic mobility between the WE and SE competing anions is as small as possible.

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