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Pressurized-flow anion-exchange capillary electrochromatography using a polymeric ion-exchange stationary phase

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Abstract

The feasibility of using capillary columns equipped with silica frits and packed with a polymer-based anion exchanger (Dionex AS9-HC) for CEC separations of inorganic anions has been investigated. Experiments using a conventional 25 cm packed bed, and mobile phase flow that is a combination of hydrodynamic and electroosmotic flow were used to demonstrate that by varying the applied voltage (electrophoresis component) or the concentration of the competing ion in the mobile phase (ion-exchange component), considerable changes in the separation selectivity could be obtained. Using an artificial neural network, this separation system was modelled and the results obtained used to determine the optimum conditions (9 m*M* perchlorate and −10 kV) for the separation of eight inorganic anions. When a short (8 cm) packed bed was used, with detection immediately following the packed section, the separation of eight test analytes in under 2.2 min was possible using pressure-driven flow and a simple step voltage gradient. A more rapid separation of these analytes was obtained by only applying high voltage (−30 kV), where many of the same analytes were separated in less than 20 s and with a different separation selectivity to that obtained in conventional ion-exchange or capillary electrophoresis separations.

Keywords: Electrochromatography; Pressurized flow; Anion exchange; Stationary phase, CEC; Artificial neural network

1. Introduction

Capillary electrochromatography (CEC) has recently received interest as a complementary technique to established electroseparation methods such as capillary zone electrophoresis (CE) and micellar electrokinetic chromatography (MEKC). Major benefits of this new technique are its separation efficiency, which is often superior to that of other liquid chromatographic methods which employ mechanical pumping, and the possibility to combine electrophoretic and chromatographic separation mechanisms. A survey of the literature published so far indicates that CEC has been used primarily for the separation of neutral analytes using reversed-phase (RP) stationary phases, with the aim of achieving high separation efficiencies as a result of the plug-like profile of the electroosmotic flow (EOF). In this case the electrophoretic portion of the separation mechanism is restricted to the use of the EOF instead of a mechanical pump to drive the mobile phase and the analytes towards the detection end of the capillary [1–3]. Recently, there have been some reports describing CEC separations of charged

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analytes using polar stationary phases such as ion-exchangers [4–10], mixed-mode phases [11–19] or bare silica [20,21]. Since the analytes used in these separations are charged, they will exhibit some electrophoretic mobility and, therefore, a mixed-mode separation mechanism applies in which electrophoretic as well as chromatographic mechanisms contribute to the separation. This approach results not only in increased separation efficiency but also changes in separation selectivity compared to CE or chromatographic methods based on laminar flow.

To date most CEC separations have been performed either using capillary columns packed with silica-based stationary phases (to the best of our knowledge there is only a single manuscript describing the use of polymer particles [22]) since the packed bed can be conveniently retained in the column by sintering the packing material to form porous frits [23–25]. Alternatively, monolithic columns prepared from either silica based materials [26,27] or organic polymers [28,29] have been used. Focusing on ion-exchange CEC (IE–CEC) applications, silica-based packings have some significant drawbacks including their restricted working pH range, the poorly defined characteristics of the silica surface and especially in the case of anion-exchangers their limited commercial availability. In this work we demonstrate the suitability of a polymer based anion-exchanger (Dionex AS9-HC) for IE–CEC. Control of the separation selectivity in IE–CEC can be accomplished by changing the relative contributions of electrophoretic and chromatographic separation mechanisms to the overall separation. This can be achieved in two ways. First, the ion-exchange (IE) interactions between the analytes and the stationary phase can be controlled by using competing ions with different eluting strength [8]. The second approach involves varying the separation voltage to control the electrophoretic movement of the analytes. Since the separation voltage also controls the EOF, pressure-induced flow is often required to provide an appropriate mobile phase flow-rate. This second approach has been described previously in both RP–CEC [30–32] and IE–CEC [5], but in these cases purpose-built CEC instrumentation was required in which a mechanical pump was added to a CE system. Another disadvantage of this approach is that the use of significant pressurized flow together with electrodriven flow results in reduced separation efficiency [30–32].

This paper presents systematic investigations on the combination of both of the above-mentioned methodologies using an artificial neural network (ANN) for optimization, allowing the CEC separation of eight inorganic anions employing an anion-exchange stationary phase in less than 2.5 min. Furthermore, because of the nature of the polymeric packing used, this work also demonstrates the effective combination of hydrodynamic and electroosmotic flow for IE–CEC using a commercial CE instrument without modification.

2. Experimental

2.1. Instrumentation

Experiments were performed using a HP^{3D} CE system (Hewlett-Packard, Waldbronn, Germany), equipped with a diode array detector and connected to a HP 3D-CE Chemstation (Hewlett-Packard) for data processing. Unless stated otherwise, a pressure of 10 bar was applied to the inlet end of the column using helium gas and the column was thermostatted at 25°C during all separations. Samples were injected hydrodynamically at 10 bar for 3 s.

2.2. Materials and reagents

Fused silica capillaries (75 μm I.D.×360 μm O.D.) obtained from Polymicro Technologies Inc. (Phoenix, AZ, USA) were used throughout this work. Water was purified using a Milli-Q water (Millipore, Bedford, MA, USA) system. The columns were packed with IonPac AS9-HC (latex agglomerated, alkyl quaternary ammonium, 9 μm, 200 nm pore size) stationary phase, supplied by Dionex Corporation (Sunnyvale, CA, USA). Frits were prepared from 5 μm silica (10 nm pore size, Develosil, Nomura Chemical Co., Japan). All chemicals used were of analytical reagent grade.

Chloride, sulfate and perchlorate mobile phases were prepared from tris[hydroxymethylamino]methane (Tris) and titrated to the appropriate pH using hydrochloric, sulfuric or perchloric acid, respectively. Carbonate mobile phase was prepared from

sodium carbonate and sodium hydrogen carbonate. All mobile phases were filtered through a 0.45 μm membrane filter of Type HA (Millipore, Bedford, MA, USA) and degassed before use.

2.3. Column preparation

Untreated fused-silica capillaries were packed using a slurry packing technique similar to that published previously [8]. Firstly, approximately 5 cm of silica was packed into the column at 6000 p.s.i. and this was sintered to form an outlet frit whilst flushing the column with water at 4500 p.s.i. The excess silica was then removed from either side of the frit by flushing and the polymer packing material was then packed into the column at 3000 p.s.i. A further plug of silica was then packed into the column and sintered to form an inlet frit. Finally the excess silica was removed and the column cut to length. The columns used in this work were total length 34.5 cm and either 25 cm packed length (26 cm to detector) or 8.0 cm packed length (8.5 cm to detector).

Acetone was used as a flow marker for all separations and retention factors were calculated using:

$$k = \frac{t_R - t_0}{t_0}$$

where t_R is the elution time of the analyte and t_0 the elution time of the flow marker.

3. Results and discussion

Previous IE–CEC systems have used silica-based stationary phases, typically of 3–5 μm particle size. With these stationary phases the maximum 12 bar pressure which can be applied with the HP^{3D}CE instrument results in a very low flow-rate, such that about 30 min is required to flush one capillary volume of mobile phase through a 25 cm packed bed. For the same packed length, this time was reduced to approximately 5 min when the polymer packing material (9 μm particle size) was used. This proved to be a great advantage since conditioning procedures could be performed within the CEC instrument and there was no more need to remove the fragile capillary column from the cartridge to flush it using an external pump. Additionally, the reduced pressure resistance of the polymer columns allowed the use of pressure injection instead of the commonly used electrokinetic injection method and enabled the difficulties connected with this technique to be avoided [33].

3.1. Choice of competing ion

In order to select an appropriate mobile phase for IE–CEC separations, a set of test analytes was chosen so as to cover as wide a range of ion-exchange selectivity coefficients as possible. Only UV absorbing anions were used so that direct UV detection could be employed. The test analytes comprised (in order of increasing ion-exchange selectivity coefficient), iodate, bromate, nitrite, bromide, nitrate, iodide, thiocyanate and chromate. Previous IE–CEC studies have shown that peak shape is governed mainly by the chromatographic contributions to the separation [8,34], so the system was operated in the chromatographic mode using only pressure-driven flow to determine the retention of these analytes with different competing ions in the mobile phase (Fig. 1). Whilst good separations were obtained using the sulfate and carbonate mobile phases for those analytes which showed only moderate retention, peak shapes were poor for the more strongly retained analytes. When a stronger competing ion (e.g. perchlorate) was used, peak shapes for all analytes were improved greatly because of the reduced chromatographic interactions. Therefore, perchlorate was chosen as the most suitable competing ion throughout this study.

3.2. Varying the CE component of the separation

Whilst good peak shapes were obtained for all the test analytes when the perchlorate mobile phase was employed, resolution was poor in the pressure-driven system due to the high eluotropic strength of perchlorate. Therefore, to facilitate resolution of the analytes and also to effect selectivity changes by adding a CE component to the separation mechanism, a separation voltage was applied. When a separation voltage was used in the absence of

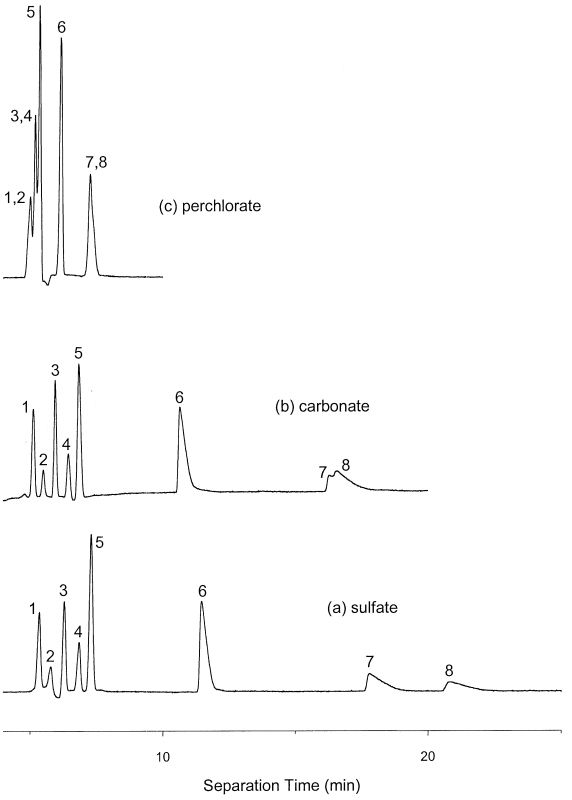

Fig. 1. Comparison of different mobile phases using pressure-driven flow only (10 bar). Capillary: 75 μm I.D.×25 cm packed with Dionex AS9-HC (26 cm to detector, 34.5 cm total). Eluent: (a) 5 m*M* H_2SO_4 (titrated to pH 8.05 with Tris), (b) 2.5 m*M* Na_2CO_3/2.5 m*M* $NaHCO_3$ (pH 10.33), (c) 5 m*M* $HClO_4$ (titrated to pH 8.05 with Tris). 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-}.

additional pressure-driven flow, the system became unstable even with 10 bar pressure overpressure applied to both buffer reservoirs. Although the surface charge on this stationary phase should be quite substantial due to the functional groups present, and it would be expected that the stationary phase should support intrapore flow (200 nm pore size), the EOF observed was generally quite low ($1–5\times10^{-9}$ $m^2\ V^{-1}\ s^{-1}$) and this led to the instability of the system [35]. To overcome this problem an inlet pressure of 10 bar was applied to stabilize the system and to increase the overall flow-rate by employing a mixture of pressure-driven and electrodriven flow.

The CE component of the separation was altered by varying the applied voltage and thereby affecting the electrophoretic mobility/retention factor ratio, with resulting selectivity changes as shown in Fig. 2. As can be seen from this figure, quite significant changes could be obtained for a number of analytes by increasing the applied voltage from 0 to −20 kV. These are most pronounced for those analytes of relatively low mobility (e.g. iodate, bromate) or high mobility (e.g. chromate) as these are most affected by voltage changes. Voltages above −20 kV could not be used due to unacceptably high currents.

3.3. Varying the IE component of the separation

The IE component of the separation can be varied by either changing the eluotropic strength of the

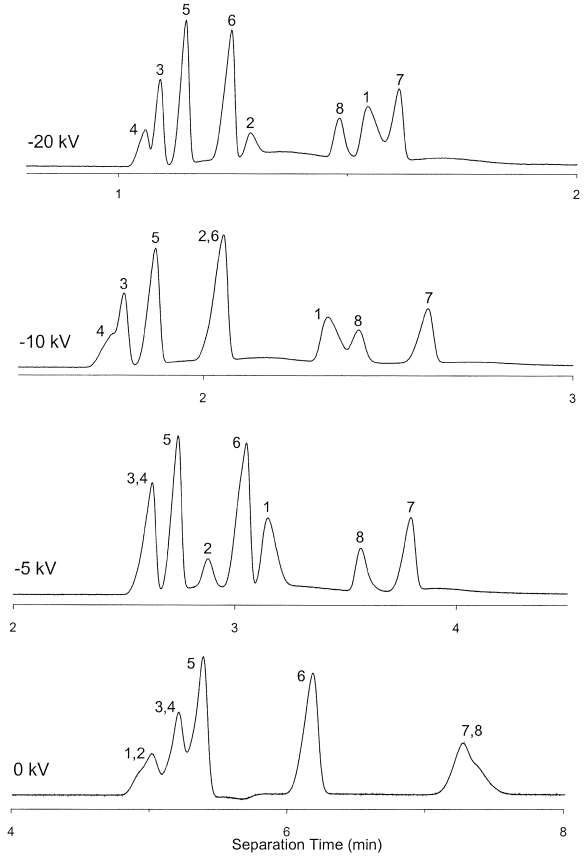

Fig. 2. Effect of the separation voltage on separation selectivity. Flow is a combination of 10 bar pressure and EOF (voltage as indicated in the figure). Mobile phase: 5 m*M* $HClO_4$ (titrated to pH 8.05 with Tris). All other conditions are as given in Fig. 1.

competing ion or by changing the concentration of the competing ion in the mobile phase. Since the perchlorate mobile phase had already been selected on the basis of the IC separation, the second approach was taken and the perchlorate concentration varied over the range 2.5–10 m*M* (at a constant applied voltage of −10 kV). The results are illustrated in Fig. 3, from which it can be seen that significant changes in separation selectivity were again observed, especially for analytes such as iodate, iodide and chromate.

3.4. Optimization of the separation for the test analytes

As the separation selectivity varied considerably when changing both the IC and CE components, developing a retention model for the system should allow the conditions for optimum separation of the test analytes to be determined. However, as the mobile phase flow in this system was a mixture of both hydrodynamic and electroosmotic flow, developing a mathematical model to describe the migration of the analytes was difficult. For this reason, we chose to model the system using an artificial neural network (ANN) since this approach did not require a detailed description of the separation mechanism. ANNs have already been used for modeling chromatographic and electrophoretic systems [36–39]. The modeling was performed using Trajan Neural Network Simulator version 3.0 [40] running on a PC-based desktop computer. The experimental space investigated was restricted to an area bounded by 0 to −20 kV and 2.5 to 12 m*M* perchlorate. Below 2.5 m*M* perchlorate peak shapes were poor for several of the test analytes, and when the concentration of perchlorate was increased above 12 m*M* there was negligible ion-exchange interaction for almost all the analytes. Based on this experimental space, a star composite design was used as the training set for the neural network consisting of 13 experimental conditions as illustrated in Fig. 4. A 3-layer neural network was used, with 2 inputs (applied voltage and [perchlorate]), 7 hidden nodes and 8 output values (retention factors for the eight test analytes). A Delta Bar Delta routine was used,

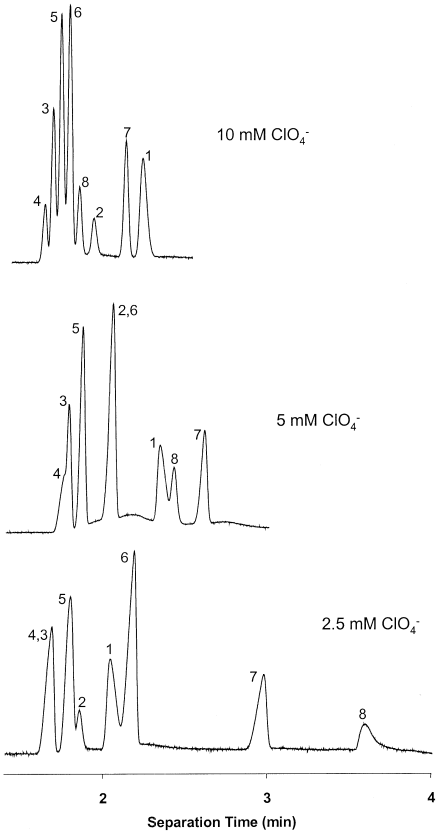

Fig. 3. Effect of mobile phase concentration on separation selectivity. Mobile phase: 2.5–10 m*M* $HClO_4$ (titrated to pH 8.05 with Tris). Flow is a combination of 10 bar pressure and EOF (−10 kV). All other conditions are as given in Fig. 1.

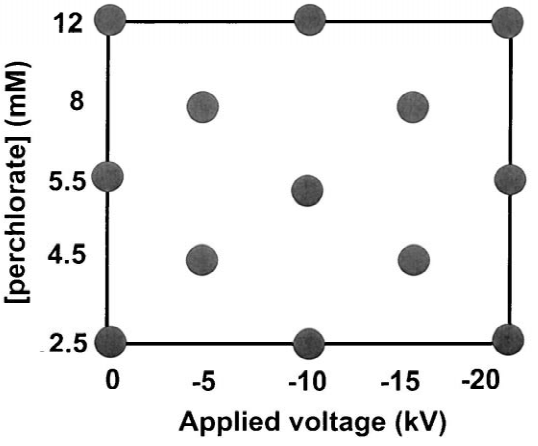

Fig. 4. Schematic illustration of the experimental data set used for training the ANN.

with 50 000 epochs. The correlation between the actual and calculated retention factors was excellent with $r^2 = 0.9952$ ($n = 520$). Using the data generated by the ANN, response surfaces for each analyte over the experimental space were generated, with an example illustrating response surfaces for iodide, iodate and thiocyanate being shown in Fig. 5. From this it can be seen the changes in separation selectivity for these analytes which over the experimental space, and this in turn can be used to optimize the separation. Optimization of the separation of all 8 test analytes was undertaken using the minimum resolution criterion in which the resolution value for the pair of adjacent peaks having the worst separation was calculated as follows:

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

The resolution surface generated in this way is shown in Fig. 6 and indicates that the best separation occurred at 9 m*M* perchlorate and −10 kV. The separation obtained under these conditions is shown in Fig. 7. Unfortunately, baseline resolution of all the analytes was not obtained which may be due to restricted peak capacity resulting from the high flow-rate caused by the combination of hydrodynamic and electroosmotic flows. Use of inlet pressures less than 10 bar was not possible in this system due to instability in the current so the flow-rate could not be reduced easily whilst maintaining the separation voltage at −10 kV. We believe that the need to apply 10 bar pressure was due to the presence of discontinuities in the packed bed and further inves-

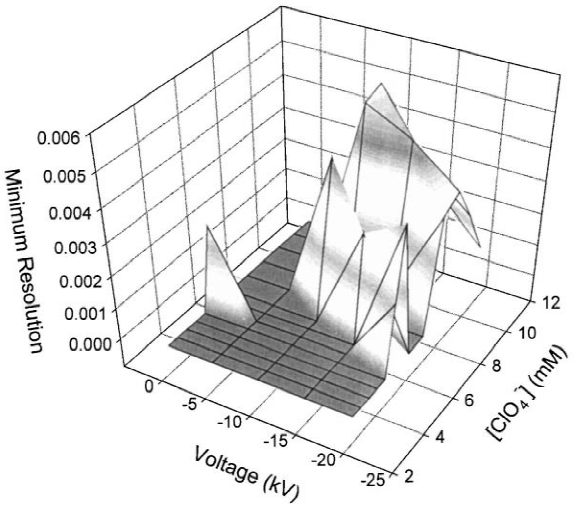

Fig. 6. Optimization response surface calculated using the minimum resolution criterion. Optimum conditions are 9 m*M* perchlorate, −10 kV.

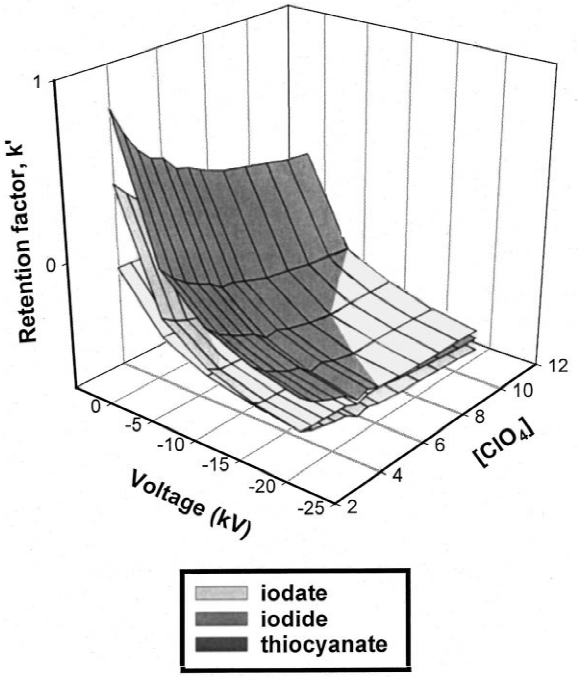

Fig. 5. Response surfaces for iodate, iodide and thiocyanate calculated using the ANN.

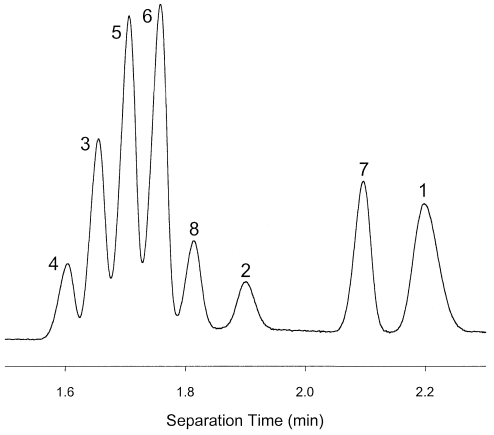

Fig. 7. Separation of the test mixture under the optimum conditions. Mobile phase: 9 m*M* perchloric acid (titrated to pH 8.05 with Tris). Flow is a combination of 10 bar pressure and EOF (−10 kV). All other conditions as given in Fig. 1.

tigations are currently under way to develop a more homogeneous column structure.

3.5. Rapid separations with short packed beds

The feasibility of using a shorter packed length was investigated to see if some of the problems encountered with a 25 cm packed polymer column could be avoided. In this case a packed bed of only 8 cm was used, with the detection window being placed immediately after the end of the outlet frit, 8.5 cm from the injection end. This approach has already been used for CEC applications and in some cases has offered considerable advantages, especially in terms of the speed of analysis [41,42]. When the short packed bed was used there were noticeable improvements in the stability of the system. Specifically, 10 bar inlet pressure was no longer required for stable operation of the column and the separation voltage could be increased to −30 kV. In addition, the shorter packed length further reduced the pressure resistance of the column. However, with the shorter separation length, the perchlorate mobile phase was found to be much too strong, so weaker competing ions such as chloride or sulfate were used.

When the short packed column was operated in a pressure-driven mode (applying 10 bar), the linear flow-rate was 2.18×10^{-3} ms^{-1} compared with 8.67×10^{-4} ms^{-1} for the 25 cm packed bed. This increase in flow-rate not only resulted in faster separations, but also in improved separation efficiency with a reduction in peak width of 71–83% being observed for all the analytes. Using a mobile phase containing 10 m*M* chloride as the competing ion, separation of all eight test analytes with IE selectivity was possible, but the more strongly interacting analytes (iodide, thiocyanate and chromate) suffered from significantly tailed peaks. Furthermore, chromate, the most strongly retained analyte, showed retention times longer than 15 min. Therefore, in order to improve both the peak shapes for the more strongly retained species and to decrease the separation time, a voltage gradient was introduced. Although several gradients were investigated, the most effective approach for these analytes was the addition of a simple step gradient of −30 kV at 1.3 min, as illustrated in Fig. 8. In this way

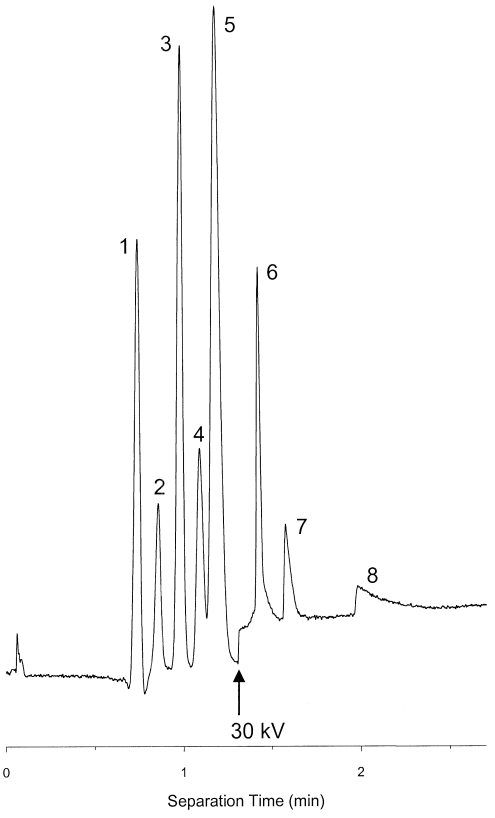

Fig. 8. Separation of the test mixture using a step voltage gradient and a short packed column. Capillary: 75 mm I.D., 8 cm packed with Dionex AS9-HC (8.5 cm to detector, 34.5 cm total). Mobile phase: 2.5 m*M* hydrochloric acid (titrated to pH 8.05 with Tris). Flow is a combination of 10 bar pressure and EOF with −30 kV added at 1.3 min. All other conditions as given in Fig. 1.

separation of all eight analytes was possible within 2.2 min, with excellent peak shapes for all analytes, except for chromate which still was affected by the strong chromatographic interactions with the stationary phase.

In both CE and CEC, very fast separations (in the order of 20–30 s) have been demonstrated for a range of analytes when a short capillary length to the detector is employed [41–43]. In the case of CE separations, this has been restricted to the separation of analytes of significantly different mobilities, leading to some limitations in the application of this approach. Fig. 9a shows the CE separation of seven of the test analytes obtained using a short, open capillary and illustrates that very short analysis times

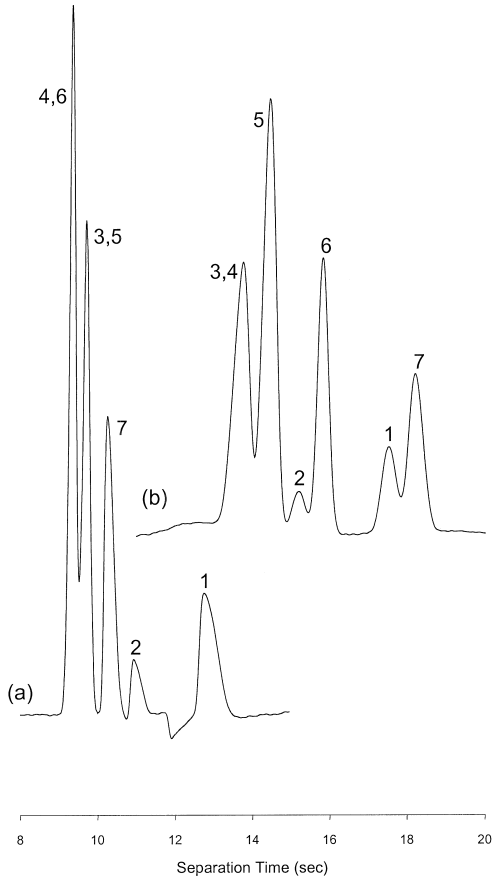

Fig. 9. Comparison of (a) CE and (b) IE–CEC separations of the test mixture using short columns. Capillary: 75 mm I.D., (a) 34.5 cm total, 8.5 cm to detector, (b) 8 cm packed with Dionex AS9-HC (8.5 cm to detector, 34.5 cm total). Flow is EOF only (−30 KV). Mobile phase: 2.5 m*M* sulfuric acid (titrated to pH 8.05 with Tris). All other conditions as given in Fig. 1.

could be achieved but the separation was quite poor with co-migration of bromide/iodide and nitrite/nitrate. Using a short packed column (Fig. 9b), some improvements were obtained, due primarily to changes in separation selectivity brought about by IE interactions. In this case, sulfate was used as the competing ion as this resulted in the best peak shapes for all the analytes. Although baseline separation of all the analytes was not achieved, Fig. 9b clearly demonstrates that short packed columns are a useful alternative for fast separations of inorganic anions.

4. Conclusions

This work has demonstrated the feasibility of pressurized anion-exchange CEC for the separation of inorganic anions with unique separation selectivity, and has shown that polymeric stationary phases are suitable for this purpose. In addition, use of an ANN to determine optimum separation conditions has been shown to be particularly useful in this case where the system has a mixed-mode separation mechanism, together with a mixture of voltage-induced and pressure-induced flow. This approach led to optimized conditions without the need for extensive experimental work (13 experiments required). The suitability of short packed columns for high-speed separations of the selected analytes has also been investigated, with promising results obtained both in pressure-driven and/or electrodriven mode. Although additional experimental work is required involving the use of further polymer-based stationary phases, together with an improved column architecture, this approach provides a number of benefits compared with traditional IE or CE methods.

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