ELSEVIER

Journal of Chromatography B, 695 (1997) 169–174

JOURNAL OF CHROMATOGRAPHY B

Short communication

# Capillary zone electrophoresis and ion-exchange capillary electrochromatography: analytical tools for probing the Hanford nuclear site environment

Daming Li, Hugo H. Knobel, Vincent T. Remcho*

*Department of Chemistry, West Virginia University, Morgantown, WV 26506-6045, USA*

**Abstract**

Ion-exchange capillary electrochromatography (IE-CEC) is a relatively new separation technique based on the combination of ion-exchange chromatographic and electrophoretic separation mechanisms. IE-CEC offers both the efficiency of capillary electrophoresis and the selectivity and sample capacity of ion-exchange chromatography. The utility of the method was examined with $I^-$ and $IO_3^-$, which are common constituents of nuclear wastes at Hanford, Washington and other U.S. Department of Energy (DoE) sites, and $ReO_4^-$, a surrogate for $TcO_4^-$. The advantages and limitations of IE-CEC relative to capillary zone electrophoresis (CZE) are explored. The chief advantages are increased loading capacity and an alternative selectivity to that of CZE, in addition to increased efficiency (relative to conventional ion-exchange chromatography). The run-to-run reproducibility of IE-CEC, however, was found to be a limitation of the technique.

*Keywords:* Electrochromatography; Iodide; Iodate; Perrhenate; Anions

## 1. Introduction

Capillary electroseparation methods (CE methods) have captured the interest of analytical chemists over the last few years. These techniques use 25 to 200 μm I.D. capillaries, 0.5 to 1.5 m long, across which high voltages are applied. These methods include capillary zone electrophoresis (CZE) [1,10], micellar electrokinetic capillary chromatography (MECC) [2], capillary gel electrophoresis (CGE) [3] and capillary electrochromatography (CEC) [4,8]. All of these techniques offer high efficiency (>100 000 plates/m).

Of all these CE methods, capillary electrochromatography (CEC) is the least developed. In CEC, silica-based reversed-phase particles are packed into fused-silica capillaries with inner diameters of 25–100 μm. In generally used buffer systems, the silica particles have a negative surface charge due to ionized silanol groups, which is compensated for by counterions in the eluent solution. By applying a potential across the capillary, a surface originated flow will be generated, i.e., the electroosmotic flow (EOF). The flow profile of the EOF in electrochromatography is essentially flat, compared to the parabolic flow profile of pressure-driven HPLC. This flat flow profile is the primary contributing factor to the high efficiencies observed in CEC. The highest reported efficiencies to date were obtained by Smith and Evans [5] who obtained 387 000 plates per meter ($h$=0.86) for a retained solute on a 50-μm I.D. 3 μm Spherisorb ODS-1 packed capillary, and >300 000 plates per meter for a retained solute on a 50-μm I.D. 1.8 μm Zorbax SBC8 packed capillary.

*Corresponding author.


*PII* S0378-4347(97)00071-6

In this paper, ion-exchange capillary electrochromatography (IE-CEC) is discussed. In IE-CEC, silica-based ion exchangers, rather than silica-based reversed-phase sorbents, are packed into fused-silica capillaries. The columns produced were evaluated with a mixture of iodide, iodate and perrhenate, and compared to conventional open tubular capillaries used in the CZE mode.

## 2. Experimental

### 2.1. Preparation of columns

Fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) with inner diameters of 75 μm were cut to a length of 60 cm. Frits were installed in the outlet end of the column as follows:

- A 0.01-g amount of silica packing material (Macherey-Nagel, Düren, Germany) was mixed with 100 μl of water glass (The PQ Corporation, Valley Forge, PA, USA) and 10 μl of formamide (Aldrich, Milwaukee, WI, USA), producing a homogeneous mixture;
- the capillary was tapped end downward into the mixture, thus forcing some of the mixture into the end of the capillary;
- once the capillary was filled to about 1 mm, the mixture was sintered into place by aligning the non-sintered frit with the electrodes of an arc fusion splicer (Alcoa-Fujikura, Tokyo, Japan) and heating the mixture for 1 s.
- after sintering, the capillary was baked in an oven for 2 h at 120°C.

The columns were slurry packed using a procedure similar to that described by Borra et al. [6] and Remcho et al. [9] for preparing capillary columns of larger inner diameter (100–300 μm). A slurry was prepared in a ratio of 80:1 (ml:g) slurry liquid–packing material. The slurry liquid was a sodium phosphate buffer (5 m*M*, pH 2.6). Nucleosil (Macherey-Nagel) 5 μm SB packing material, a strong anion exchanger, was used in producing the packed capillaries. The slurry was ultrasonicated for 15 min and transferred to a reservoir. The reservoir was then connected to a high pressure syringe pump (ISCO, Lincoln, NE, USA) which was operated in the constant pressure mode at 24 MPa. While packing took place, the slurry reservoir was placed in an ultrasonic bath to minimize settling out of the slurry (Fig. 1). The particles going into the column were observed periodically throughout the packing process with a zoom stereomicroscope. Once the desired length of the column was filled, the pressure was maintained for 30 min and an outlet frit was made using the fiber optic fusion splicer to sinter a portion of the packing material. Following this, the pump was turned off and the pressure was allowed to slowly decrease for another 30 min. The column was then removed from the packing apparatus and flushed with sodium phosphate mobile phase (5 m*M*, pH 2.6) at 24 MPa and a narrow (0.5 mm) detection window was made immediately adjacent to the outlet frit, again using the fusion splicer.

### 2.2. Chromatographic system

An ATI-Unicam (now Thermo-CE, Franklin, MA, USA) Model 300 CE system was used to carry out the chromatographic and electrophoretic runs. Detection was performed on column by use of a Linear Instruments (Fremont, CA, USA) Model 200 UV absorbance detector at 190 nm. In order to prevent

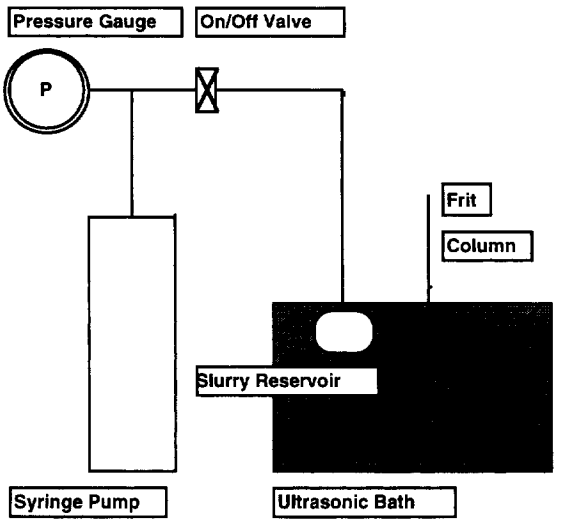


Fig. 1. Apparatus used for slurry packing fused-silica capillaries.

the nucleation of gas bubbles, the instrument was modified in-house to allow the entire system (buffer reservoirs and capillary) to be operated at up to 689 Pa applied pressure; longitudinal $\Delta P$ for the capillary was 0 Pa. Samples were injected electrokinetically into the packed capillary by placing the sample vial at the cathodic end of the capillary, as $\mu_{ep}$ for each of the analytes exceeded $\mu_{eo}$. The mobile phase vial was then replaced at the cathodic end and the operating voltage was applied. Mobile phases were degassed before use by ultrasonication for 20 min.

### 2.3. Reagents

Sodium iodide, sodium iodate and sodium perrhenate were obtained from Aldrich. Sodium phosphate was obtained from Sigma (St. Louis, MO, USA). The 5 m*M* mobile phase, pH 2.6, was prepared by dissolving sodium phosphate in distilled deionized water. Solutions of the test probes were prepared in distilled deionized water.

## 3. Results and discussion

### 3.1. Selectivity

Both CZE and IE-CEC were used to separate the mixture of iodide, iodate and perrhenate. Representative examples of the chromatograms obtained with IE-CEC and the electropherograms obtained with CZE are shown in Fig. 2a,b. The three components are easily separated using both techniques. There is, however, a striking difference in selectivity. With CZE, the iodate is eluted after perrhenate, while with IE-CEC, the iodate is eluted before perrhenate. This selectivity difference is for the most part predictable, based on the retention mechanism for ion-exchange chromatography [7]. For a sample set of ions of similar charge, ions with lesser hydration needs will interact more strongly with the stationary phase and will therefore elute later. Based on this argument, iodide, being the smallest of the three analyte ions and having the strongest interaction with water in the mobile phase, should elute first; perrhenate would elute last, spending the most time associated with the stationary phase.

### 3.2. Efficiency

The high efficiency of IE-CEC is demonstrated by the reduced plate heights ($h$) listed in Table 1. These reduced plate heights are within the range of values for $h$ calculated for other electrochromatographic separations of compounds and are smaller than the reduced plate heights obtained in HPLC, supporting the conclusion that the efficiency of electrically driven chromatography is higher than that of pressure-driven chromatography. That the efficiency obtained in IE-CEC was so much greater than that for CZE came as a surprise. It is likely that a chromatofocusing effect, in which the analytes are highly retained in a fine band at the head of the column, is responsible for boosting the efficiency to such a high value. When compared to the previously mentioned results of Smith and Evans [5], who reported a reduced plate height of 0.86 for a retained solute in a reversed-phase CEC separation, the value reported below for IE-CEC appears to be within reason. The hypothesis that chromatofocusing is partly responsible for the high efficiencies measured for IE-CEC is supported by the data in Fig. 3, which indicate a decrease in efficiency for high mass loads of analyte. As the maximum loading capacity of the packing material is approached, the efficiency enhancement due to zone focusing is decreased.

### 3.3. Limit of detection

In order to obtain the limit of detection for IE-CEC and CZE, analytical calibration curves for iodide were constructed. Very dilute solutions of sodium iodide ($1\cdot10^{-7}$ *M*) were employed. Chromatograms and electropherograms were obtained for various injection times. The calibration curve is a plot of signal (area), vs. amount of analyte injected (Fig. 4). The limit of detection of IE-CEC for iodide is about $2.5\times10^{-16}$ mol. The limit of detection of CZE for iodide is about $4.5\times10^{-15}$ mol, which is about 20 times higher than that of IE-CEC.

### 3.4. Qualitative reproducibility ($t_R$)

To study the reproducibility of a single IE-CEC packed capillary column, ten consecutive runs were performed for the analysis of sodium iodide and,

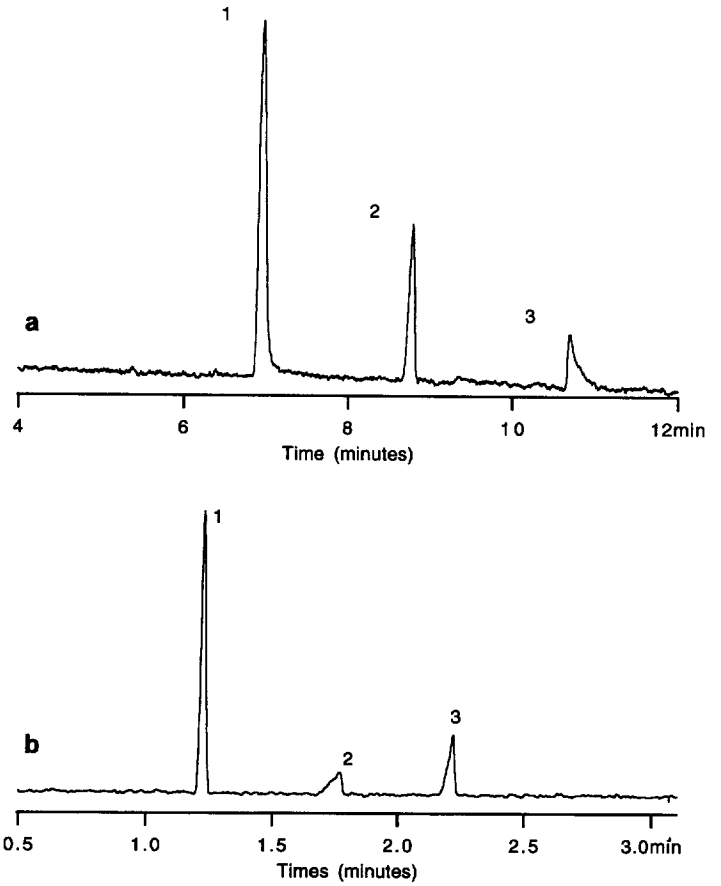


Fig. 2. (a) Separation of mixture of iodide, iodate and perrhenate on an IE-CEC column containing Nucleosil 5 μm SB. $L_{Tot}$=60 cm, $L_{Bed}$=40 cm, $L_{Det}$=40 cm, I.D.=75 μm. Applied potential: −30 kV. UV absorption at 190 nm. Mobile phase: 5 m*M* phosphate buffer (pH 2.6). Solutes: (1) iodide; (2) iodate and (3) perrhenate. (b) Separation of mixture of iodide, iodate and perrhenate on an open tubular capillary. $L_{Tot}$=60 cm, $L_{Det}$=40 cm, I.D.=75 μm. Applied potential: −30 kV. UV absorption at 190 nm. Mobile phase: 5 m*M* phosphate buffer (pH 2.6). Solutes: (1) iodide; (2) perrhenate and (3) iodate.

independently, acetone, using a capillary of the following dimensions: $L_{Tot}$=60 cm, $L_{Bed}$=40 cm, $L_{Det}$=40 cm, I.D.=75 μm. Fig. 5a,b illustrates the run-to-run qualitative reproducibility for iodide, and reproducibility in electroosmotic flow velocity using acetone, a neutral marker.

Table 1
Column efficiency (measured using iodide), plate height and reduced plate height for each of the two techniques studied

| Method | *N* | *N*/m | *H* (μm) | *h* |
|---|---|---|---|---|
| IE-CEC | 115000 | 287500 | 3.48 | 0.70 |
| CZE | 18500 | 46250 | 21.62 | 0.29 |

## 4. Conclusions

We have demonstrated the feasibility of IE-CEC, an emerging new technique for use in capillary separation methods. The method can be used routinely in the laboratory to achieve reproducible sepa-

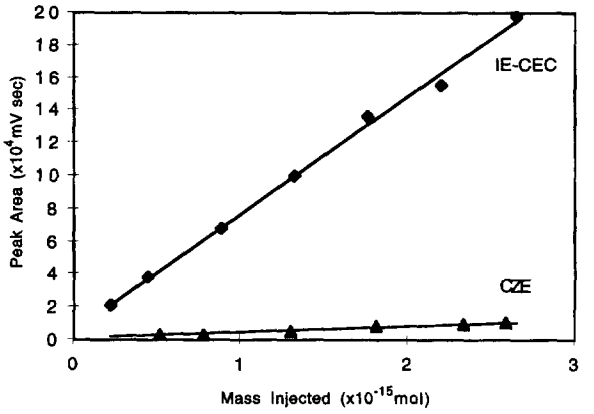


Fig. 3. Effect of analyte mass load on efficiency for IE-CEC and CZE. Each point represents the mean of three runs.

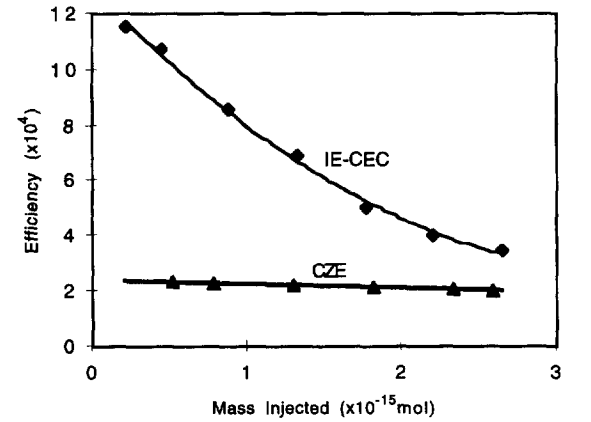


Fig. 4. Calibration curves for iodide quantitation by CZE and IE-CEC. Each data point represents the mean of three runs. $R^2$ for the IE-CEC line is 0.996 and for the CZE line is 0.994.

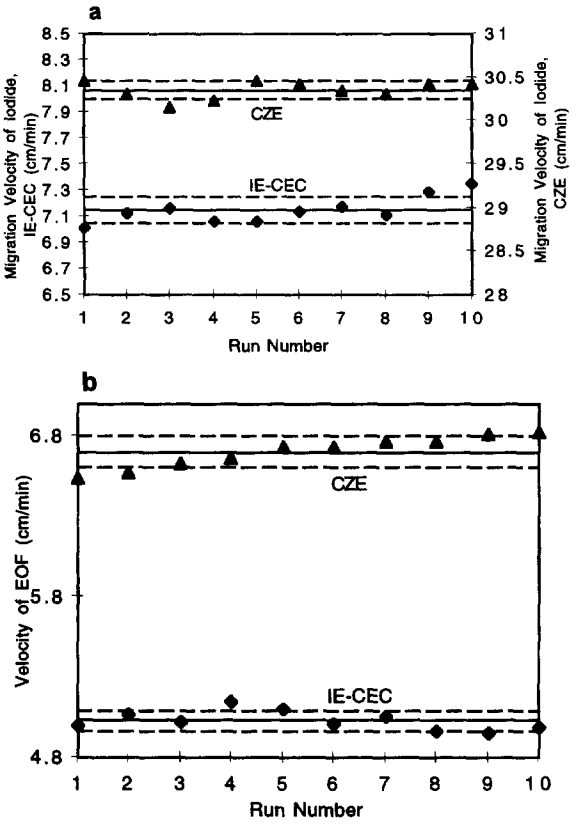


Fig. 5. (a) Migration velocity reproducibility for iodide in IE-CEC and CZE. Mean $V_{IE\text{-}CEC}$=7.147 cm/min, s=±0.101 cm/min. Mean $V_{CZE}$=30.349 cm/min, s=±0.103 cm/min. (b) Electroosmotic flow velocity reproducibility for IE-CEC and CZE, measured using acetone as a neutral marker. Mean $V_{IE\text{-}CEC}$=5.029 cm/min, s=±0.062 cm/min. Mean $V_{CZE}$=6.704 cm/min, s=±0.100 cm/min.

rations of ionic compounds. IE-CEC is capable of providing higher efficiency than CZE in separations where chromatofocusing is possible. The high loading capacity of IE-CEC columns makes it a favorable candidate for analyses of species of low concentration.

## Acknowledgments

The authors thank Don Feathers and Allen Burns of the WVU chemistry mechanics shop for their efforts in modifying the instruments, and acknowledge the Society for Analytical Chemists of Pittsburgh (SACP) and the US DoE (contract no. DE-FC21-92MC29467) for financial support of this research.

## References

[1] J.W. Jorgenson and K.D. Lukacs, J. Chromatogr., 218 (1981) 209.

[2] S. Terabe, K. Otsuka, and T. Ando, Anal. Chem., 57 (1985) 834.
[3] A. Guttman, A. Paulus, A.S. Cohen, N. Grinberg and B.L. Karger, J. Chromatogr., 448 (1988) 41.
[4] J.H. Knox and I.H. Grant, Chromatographia, 24 (1987) 135.
[5] N.W. Smith and M.B. Evans, Chromatographia, 38 (1994) 649.
[6] C. Borra, M.H. Soon and M. Novotny, J. Chromatogr., 385 (1987) 75.
[7] H. Small, Ion Chromatography, Plenum Press, New York, 1989.
[8] Z.J. Tan and V.T. Remcho, Anal. Chem., 69 (1997) 581.
[9] V.T. Remcho, W.H. Wilson and H.M. McNair, J. High Resolut. Chromatogr., 13 (1990) 442.
[10] V.T. Remcho, N.E. Ballou, G.R. Ducatte and C. Quang, J. High Resolut. Chromatogr., 19 (1996) 183.