Gabriela Chirica
Vincent T. Remcho

Department of Chemistry,
Oregon State University,
Corvallis, OR, USA

Silicate entrapped columns – new columns designed for capillary electrochromatography

Designed especially for capillary electrochromatography (CEC), silicate-entrapped columns are made by trapping particles of chromatographic packing material in a network of silica. Once entrapped, the capillary no longer requires frits. This renders a more homogeneous and stable packed bed. Accidental breakage of the fragile frits is not an issue with these robust columns. Columns packed with reverse-phase material subjected to silicate entrapment demonstrated faster separations of retained analytes and increased efficiencies compared with nonentrapped columns. The method was also used to prepare chiral CEC columns by entrapping a molecular imprinted polymeric (MIP) packing having minimal surface charge density, thus being unable alone to support sufficient electroosmotic flow for CEC.

Keywords: Electrochromatography / Stationary phases / Molecular imprint polymer / Fritless packed capillary
EL 3300

1 Introduction

Recently, the performance characteristics of various capillary electromigration separation methods have been intensively investigated in several research laboratories. Aside from the advantages provided by the microscale liquid phase separation format (small sample volume, high mass detection sensitivity, low solvent consumption, suitability to mass-spectrometry coupling), electromigration methods offer increased efficiencies compared with both conventional and micro HPLC. The improved characteristics are largely due to the uniform radial velocity profile specific to ideal electroosmotic flow *versus* the laminar profile generated by pressure-driven flow. In 1974 Pretorius, Hopkins and Schieke applied an electric field across a packed column and obtained the first capillary electrochromatography (CEC) separation [1]. In this new method, the subject of several recent review articles [2–5], the transport of mobile phase through the column was achieved by replacing the high-pressure pump of HPLC with electroosmotic flow (EOF). Solute partitioning between the mobile phase and the stationary phase is responsible for separation. In addition to partitioning, differential electromigration contributes to the separation of charged compounds.

Correspondence: Professor Vincent T. Remcho, Department of Chemistry, 153 Gilbert Hall, Oregon State University, Corvallis, OR 97331-4003, USA
E-mail: remchov@chem.orst.edu
Fax: +541-737-2062

Abbreviations: CEC, capillary electrochromatography; **MIP**, molecular imprinted polymer; **PAH**, polyaromatic hydrocarbons

Flow velocities in CEC are determined by the properties of the mobile phase as well as of the stationary phase. In open tubular CEC the fused-silica tubing, having ionizable silanol groups at the surface of the capillary, can provide the electrical double layer that sustains EOF when a high potential is applied across the column. However, in packed column CEC, the packing material itself must provide ionizable groups at the surface of the particles to sustain EOF in larger ID capillaries. Another important feature of EOF is that the flow velocity is independent of the particle size or the porosity of the packing material over a wide range, encompassing the most popular particle and pore sizes of HPLC packings [6]. This feature makes possible the use of submicrometer-sized particles that could provide for liquid phase separations the efficiencies attainable in capillary gas chromatography [7]. It also makes possible the use of very long columns since the back pressure build-up problem encountered in HPLC is eliminated.

A recent panel published in LC-GC [8] indicated that major instrumentation shortcomings include sample introduction, gradient elution, and detection. CEC columns introduce further limitations: air bubbles, column fragility, column-to-column reproducibility, and a limited selection of suitable packing materials. These limitations must be overcome if CEC is to become a successful niche, if not a routine separation technique. In attempts to improve CEC column design, numerous studies have proposed the use of fritless columns in the monolithic format [9–13]. Continuous rods of porous polymers with various functional groups able to provide some degree of selectivity have been prepared inside capillary columns; however, it is difficult to synthesize *in situ* a sorbent offering the range of particle/pore size distribution, variety of surface

 0173-0835/99/0101-0050 $17.50+.50/0

chemistries, and surface homogeneities typical of that obtained in the more controllable environment of a beaker.

This study addresses column fragility by proposing another type of fritless column, a silicate entrapped column, which employs commercially available packing material packed in a fused-silica capillary and subsequently entrapped in a silicate matrix. This approach intends, to the extent that is possible in CEC, to begin to decouple bulk transport from separative transport, allowing further optimization of each component of the chromatographic experiment.

2 Materials and methods

2.1 Reagents

The mobile phase used throughout this study was a solution of 80% acetonitrile (HPLC grade, Aldrich, Milwaukee, WI, USA) and 20% 0.1 M acetate buffer, pH 3.0. The analytes to be separated were polyaromatic hydrocarbons (PAH): naphthalene, phenanthrene, fluorene and anthracene, all from Sigma (St. Louis, MO, USA), and acetone (HPLC grade; Aldrich) as an EOF velocity marker. The concentration of each of the PAHs was about 0.1 mg/mL of mobile phase. The solution of the D- and L-isomers of dansyl phenylalanine (0.2 mg/mL of each isomer) was prepared using reagents purchased from Sigma. Kasil 2130 (PQ Corporation, Valley Forge, PA, USA) potassium silicate (wt. ratio SiO_2/K_2O = 2.10, solids 30%) and Kasil 1 potassium silicate were used for the preparation of the entrapment mixture and inlet frit, respectively. The cation exchange resin, AG 50WX4 (H form), was purchased from Bio-Rad (Hercules, CA, USA).

2.2 Materials

The polyimide-coated fused-silica tubing of 75 μm ID and 350 μm OD was obtained from Polymicro Technologies (Phoenix, AZ, USA). Macherey-Nagel Nucleosil silica-based particles with 100 Å pore size and 10 μm diameter, procured from MetaChem Technologies (Torrance, CA, USA) were used to prepare the frit. In this study, silica-based reverse-phase packing material of 5 μm diameter (Nucleosil C18) purchased from MetaChem was used to produce packed beds of 25 cm effective length. Dr. N. Snow and Mr. T. O'Brien (Seton Hall University, South Orange, NJ, USA) kindly provided the molecular imprinted polymeric (MIP) packing designed for the HPLC separation of D- and L-dansyl phenylalanine.

2.3 Instrumentation

The apparatus used to perform the separations included a Hewlett-Packard HP^{3D} CE (Waldbronn, Germany) instrument modified such that pressures of up to 12 bar can be applied to the inlet and outlet vials to avoid air bubble formation during runs. The temperature of the cassette was held at 20°C. After packing and/or entrapping, the columns were conditioned in the instrument for 60 min by applying 9 bar at both vials. The capillaries were subsequently held at 30 kV and 9 bar were applied at both ends of the column for about 30 min until the current became stable. Electrokinetic injection (10 kV for 10 s) was used for sampling the analytes. On-column UV detection at 254 nm was performed on the open tubular part of the column, which was about 9 cm long for all the columns used (entrapped and nonentrapped); the detection window was situated next to the end of the packed bed.

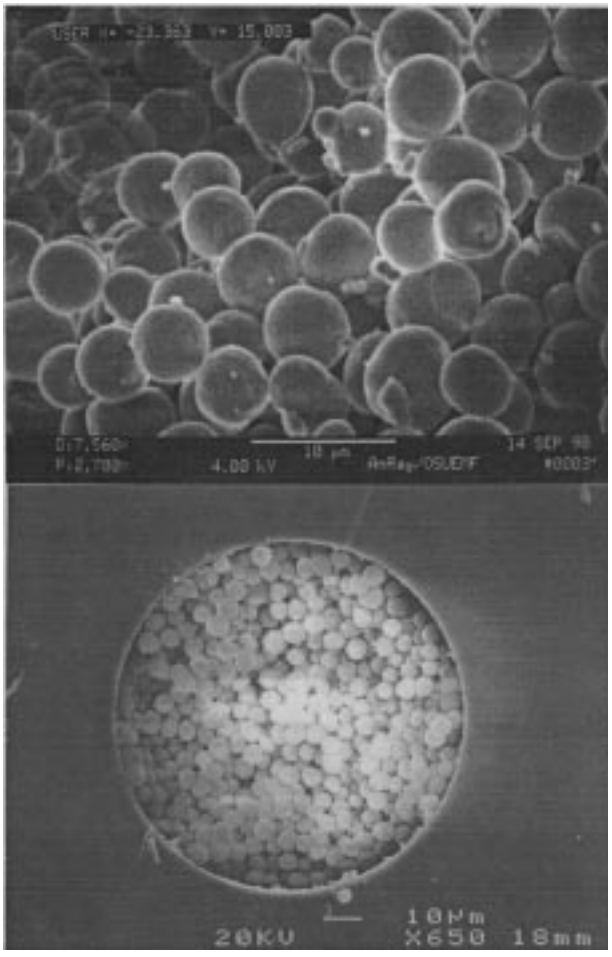

Figure 1. Scanning electron micrographs of a reverse-phase silicate entrapped column of 75 μm ID packed with particles of 5 μm diameter. A small amount of silica (irregularly shaped fragments in this image) deposited in the entrapment procedure is responsible for the immobilization of the Nucleosil C18 particles. Under typical working conditions (30 kV; 9 bar applied at both vials) the entrapped column behaves essentially like a monolith.

2.4 Columns

The first step in the manufacture of packed capillaries is the production of a retaining frit. Several recipes found in the literature were evaluated in the early stages of this study [14, 15]. In many instances these methods require a long time for mixing and drying of the frit material. It is also often difficult to obtain a homogeneous paste inside the capillary and, most importantly, the resulting frit often generates high back pressures that preclude good packing. The paste ultimately used to prepare the inlet frits was obtained by mixing 20 μL Kasil 1 and 0.03 g Nucleosil Si packing (1000 Å pore size, 10 μm diameter). Column blanks cut to the desired length were then introduced as a bundle about 5 mm into the viscous mixture. Due to capillary action the liquid rose inside the tubing. Using an arc fusion splicer (Fujikura FSM 05S, Alcoa Fujikura, Duncan, SC, USA) the frits were sintered and subsequently cut to about 2 mm length and the columns were ready to be packed. For capillary packing a slurry-packing procedure, described elsewhere, was used [16]. Since the frits are resistant to high pressures (400 psi), yet highly porous, the packing of a 10 cm long bed requires less than 2 min. Further sintering of the packing (if it is silica-based) with the fusion splicer in the vicinity of the initial frit with the subsequent removal of the original frit, yields a new stable frit. The resulting column has similar packing density throughout its entire length. Once the packed bed grew to a length of ~ 30 cm, an outlet frit was made using the fusion splicer at a point about 25 cm from the inlet frit. The capillary was then flushed with solvent until the excess packing adjacent to the new outlet frit was removed. The polyimide coating was removed close to the outlet frit (again using the fusion splicer) to obtain a detection window. To make entrapped reverse-phase columns, 2 mL Kasil 2130 diluted with 4 mL distilled, deionized water were passed through a bed of cation exchange resin in the hydrogen form in order to adjust the pH to about 6.0. Another solution consisting of 1 mL Kasil 2130 and 2 mL water was used to entrap the MIP columns. The apparatus previously used for packing was then used for flushing the entrapment mixture through the column, which consisted of a capillary containing an inlet frit made as described above and a packed bed of reverse-phase sorbent. About 1 mL of the entrapment mixture was introduced into a slurry reservoir connected to a syringe pump and the packed capillary. The column was subsequently heated gradually from 40 to 160°C over a period of several days. The inlet frit retained the packing material during the entrapment procedure and was subsequently removed. In the final step the column was cured by flushing it with dilute ammonium hydroxide (0.1 M) for 2 h followed by drying in an oven at 160°C. Figure 1 is a scanning electron microscopy (SEM) image of an entrapped reverse-phase column.

3 Results

The chromatograms in Fig. 2 present the best separations achieved with a nonentrapped column (out of 7 columns, about 25 runs total) *versus* a typicial entrapped column.

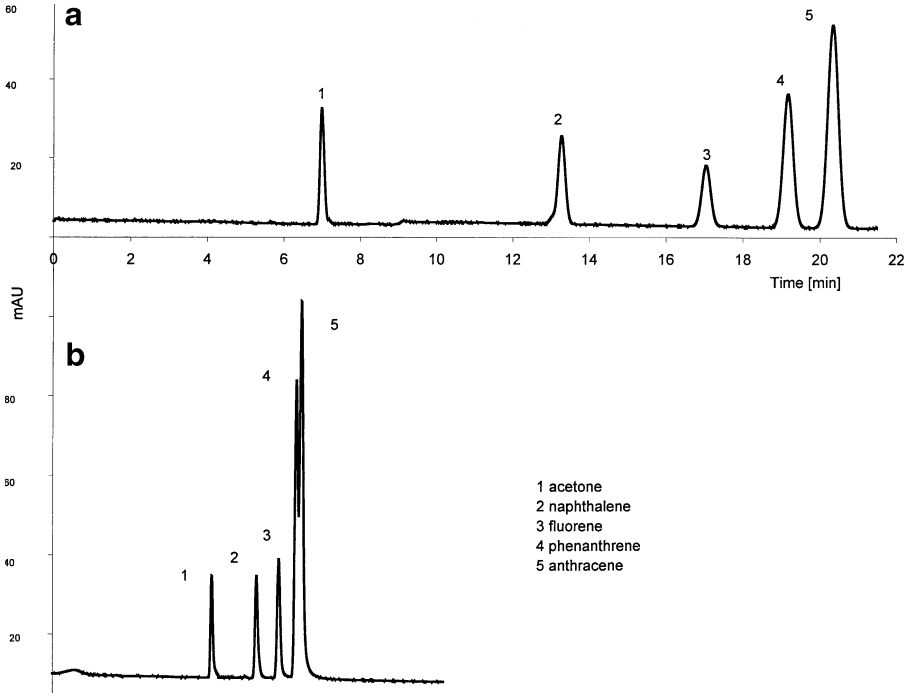

Figure 2. Separations of a mixture of PAHs by CEC on reverse-phase capillaries: (a) without and (b) with silicate entrapment. The capillaries are 75 μm ID fused-silica packed with 5 μm Nucleosil ODS particles. Column effective lengths are 25 cm for the nonentrapped column and 17 cm for the entrapped column. Both chromatograms were obtained under the same conditions: mobile phase, 80% acetonitrile, 20% 0.1 M acetate buffer (pH 3.0); applied voltage of 30 kV; UV detection at 254 nm; *T*, 20°C; 9 bar applied at both vials; electrokinetic injection 10 kV for 10 s.

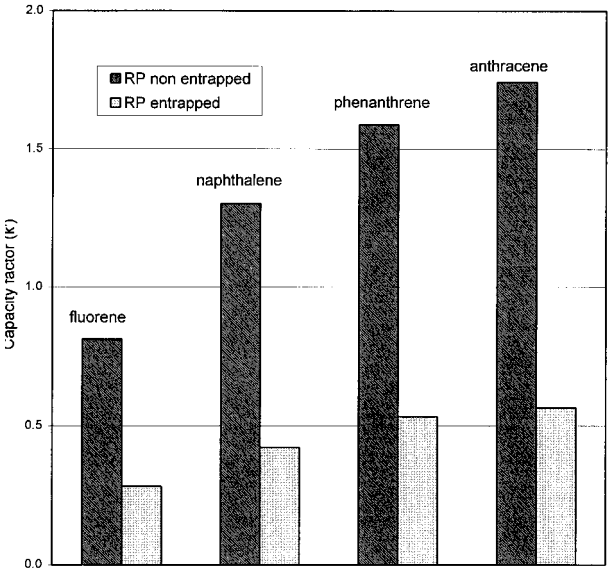

Figure 3. Comparison of capacity factors for (1) naphthalene, (2) fluorene, (3) phenanthrene and (4) anthracene in the entrapped *versus* nonentrapped reverse-phase packed capillaries.

The analytes and their corresponding reduced plate heights are indicated. Both separations were performed at 30 kV, 20°C using the same mobile phase (80% acetonitrile, 20% 0.1 M acetate buffer, pH 3.0). Aside from the improvement in efficiency, the elution of the last retained peak is twice as fast; however, phenanthrene and anthracene, baseline-separated on the nonentrapped column, are no longer resolved by the entrapped column. A run performed at a lower applied potential still did not produce a separation of anthracene and phenanthrene, and the efficiency was reduced. A certain degree of occlusion of the pores of the packing material with the entrapment solution, and hydrolysis or masking of the chemically bonded stationary phase during the entrapment procedure (which includes the ammonium hydroxide rinse), is likely to be responsible for the observed loss in selectivity. These side effects also contribute to the decrease in capacity factor observed for the entrapped column, as indicated in Fig. 3. The decrease in capacity factors is responsible for the reduced analysis time. The use of silica as entrapment matrix seems also to cause the analytes to exhibit slight peak tailing as might be expected in the presence of free silanol groups. However, the tailing was not considered to be significant; efficiency (N) was therefore calculated using the equation:

$$N = 5.54\left(\frac{t_r}{w_{1/2}}\right)^2 \quad (1)$$

where t_r is the retention time of each analyte and $w_{1/2}$ is the peak width at half the peak height.

Continuing the comparison of column performance, data were gathered to assess the variation of the reduced plate height with increasing flow velocity. It was decided to maintain a constant thickness of the electrical double layer; hence the mobile phase composition was held constant. Flow velocity was changed only by varying the applied potential. A set of four runs was performed at each of the applied potentials (10, 15, 20, 25 and 30 kV); the reduced plate heights achieved for acetone, naphthalene and phenanthrene, with the corresponding error bars (± standard deviation), are represented in Fig. 4.

A major limitation of CEC when compared to HPLC is the reduced number of stationary phases suitable for CEC *versus* the overwhelming variety of packing materials already available for conventional liquid-phase separations. Silicate entrapment offers an alternative, enabling the use in CEC of packing materials which might ordinarily be more appropriate to HPLC, since the ionizable groups required to sustain electroosmotic flow are provided by the silicate matrix. Certainly, this solution can be applied only to analytes that do not interact adversely with silica and whose elution is not affected by the smaller available interstitial/interparticle space.

Recently, much effort toward the synthesis and use of stationary phases based on MIP in the open-tubular, packed and monolithic formats has been documented [17, 18]. Using a packing material produced at Seton Hall University by bulk polymerization of an L-dansyl phenylalanine imprinted polymer, followed by grinding and sieving, we have packed and entrapped a column as

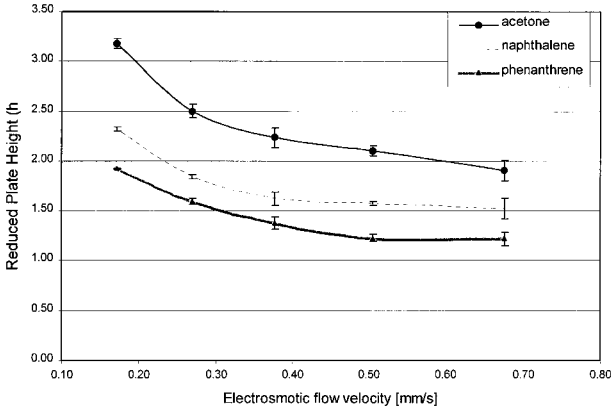

Figure 4. Variation of reduced plate height with EOF velocity in the reverse-phase silicate entrapped columns for acetone, naphthalene and phenanthrene. Data was obtained by applying 10, 15, 20, 25 and 30 kV (four runs each), which resulted in a corresponding increase of the EOF velocity. The error bars represent ± 1 standard deviation of the reduced plate height calculated at corresponding applied potentials. Column: 75 μm ID, L_{eff} 17 cm, d_p 5 μm.

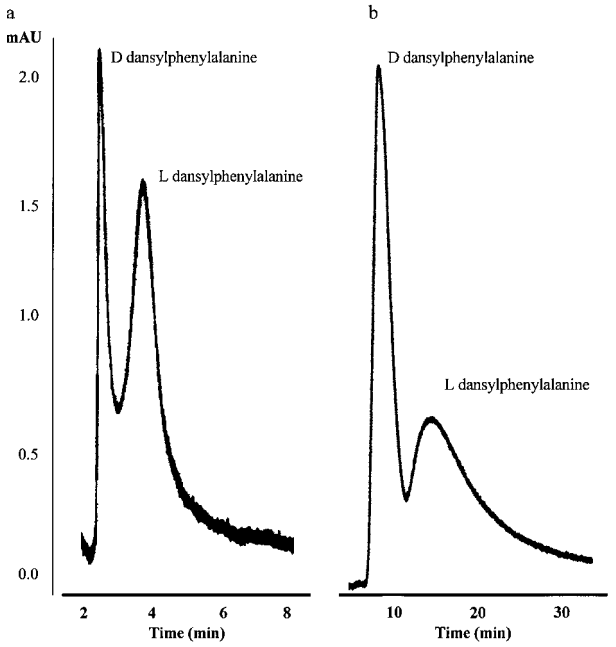

Figure 5. Chiral separation of D- and L-dansylphenylalanine on a L-dansylphenylalanine imprinted polymer stationary phase. (a) Silicate entrapped CEC column: ID, 75 μm; L_{tot}, 25 cm; L_{eff}, 17 cm; applied potential, 30 kV; electrokinetic injection, 10 kV for 10 s; UV detection at 280 nm, mobile phase 80/20 acetonitrile/100 mM acetate buffer (pH 3.0). (b) HPLC column: 15 cm × 0.46 cm, 1.0 mL/min flow rate, 2% acetic acid in acetonitrile, 30 μL injection, UV detection at 280 nm (Courtesy of Mr. T. O'Brien and Dr. N. Snow, Seton Hall University).

described previously. The CEC separation is both faster and more efficient than the corresponding HPLC analysis (Fig. 5). The entrapped columns do not require frits; thus, accidental or deliberate cutting of the capillary does not result in destruction of the column and, in fact, can be beneficial in extending the useful lifetime of a column used with "dirty" samples. The "extended path length" flow cells recently commercialized by Hewlett Packard can be used with these columns, and columns may also be coupled with plastic tubing sleeves to open tubular capillary segments. Another interesting observation is that the back pressure build-up (during pressurized flushing) due to the presence of the silicate matrix is not significant. Both entrapped and nonentrapped dry columns require the same amount of time to be completely flushed with mobile phase using an external pump. This might be due to the extremely small amount of silica that is required to hold the particles in place given that the matrix is extended over the entire length of the column.

4 Discussion

At this point results are presented and possible causes for the improvements in efficiency and analysis time noted for the entrapped columns are assessed. Further studies will be oriented towards detailed analysis of the effect of entrapment on each term of the height equivalent to a theoretical plate (HETP) equation in an attempt to quantify contributions to zone broadening in silicate-entrapped columns.

4.1 Improvements in efficiency and analysis time

The equation describing EOF velocity in CEC (μ_{eof}) indicates direct proportionality with the zeta potential (ξ), hence direct proportionality with the charge density at the

$$u_{eof} = \frac{\varepsilon_o \varepsilon_r \xi E}{\eta} = \frac{\sigma E}{\kappa \eta} \tag{2}$$

surface of shear (σ).

Here η is the viscosity of the eluent and $1/\kappa = \delta$, the thickness of the electrical double layer (κ is the Debye-Hückel parameter), constant in our experiments since the temperature and electrolyte composition were not changed. Thus, the ratio of the EOF velocity in the entrapped column *versus* the nonentrapped column

$$\frac{u_{eof\ entrapped}}{u_{eof\ nonentrapped}} = \frac{\xi_{entrapped}}{\xi_{nonentrapped}} = \frac{\sigma_{entrapped}}{\sigma_{nonentrapped}} \tag{3}$$

equals the ratio of mean ξ potentials of the entrapped column *versus* the nonentrapped column.

In order to evaluate the change in surface charge density (σ) arising from column entrapment, both entrapped and nonentrapped reverse-phase columns were run at the same applied potential using the same mobile phase. The data obtained somewhat unexpectedly indicated that the surface charge density in the entrapped column is almost equal to that of the nonentrapped column ($\sigma_{entrapped}$ = 1.08 $\sigma_{nonentrapped}$). The observations of entrapped columns achieved via scanning electron microscopy revealed tiny disparate pieces of silicate which adhere to and retain the porous packing material, rather than a highly porous silicate monolith as might have been expected. This in turn suggests that only a modest contribution to surface charge density arises directly from the silicate entrapped matrix for the reverse-phase entrapped columns. A similar comparison could not be made for the MIP columns since without entrapment there are an insufficient number of ionizable groups to sustain appreciable EOF. In addition, the entrapment mixture used for MIP columns was far more concentrated than that used for the reverse-phase columns.

Table 1. Mean of reduced plate height (h) for compounds separated at the same flow velocity (0.68 mm/s) using the entrapped and nonentrapped reverse-phase columns.

Analyte	$h_{\text{nonentrapped column}}$	$h_{\text{entrapped column}}$	Plate height enhancement (%)
Acetone	3.3	1.9	42%
Naphthalene	2.3	1.5	35%
Fluorene	2.0	1.6	20%
Phenanthrene	1.9	1.2	37%
Anthracene	2.0	1.5	25%

4.2 Zone broadening

The HETP equation for liquid chromatography proposed by Horváth [19, 20] and adapted for CEC by Dittmann and Rozing [21] assumes independent contributions of each of the following terms to zone broadening:

$$H = H_{\text{a,diff.}} + H_{\text{eddy,diff.}} + H_{\text{e,diff.}} + H_{\text{i,diff.}} + H_{\text{t,diff.}} + H_{\text{kin}} \quad (4)$$

where $H_{\text{a,diff}}$ is the plate height contribution arising from static diffusion in the axial direction; $H_{\text{eddy,diff}}$ accounts for the eddy diffusion contribution to plate height; $H_{\text{e,diff}}$ describes the contribution to plate height of the resistance of analyte diffusion in the stationary phase; $H_{\text{i,diff}}$ accounts for intraparticle diffusion; $H_{\text{t,diff}}$ describes the contribution of trans-channel mass transfer; and H_{kin} is the plate height contribution of the kinetics of solute-stationary phase interaction. For a fixed flow velocity the approximate values for the reduced plate height are given in Table 1.

The largest improvement in plate height (entrapped *versus* nonentrapped) is realized for the nonretained flow marker acetone ($\Delta h = 1.4$; a 42% reduction), while for the retained analytes the reduced plate height improvement is not as significant. This could indicate that the major decrease in plate height is due to minimization of dispersive terms in the rate equation. A possible explanation considers the fact that during entrapment the interparticle channel diameters become smaller than in the nonentrapped column; hence the eddy diffusion and trans-channel diffusion terms are reduced for the entrapped column. Another aspect that should be considered is that the different EOF velocities in the packed beds render inconsistencies in the amount of sample injected electrokinetically in the entrapped *versus* nonentrapped columns, hence variable peak widths. The unretained compound, *i.e.* acetone, is most sensitive to such effects, thus the use of acetone as a comparison factor might introduce additional errors. It is unlikely, however, that these features would result in the marked efficiency enhancement achieved in these studies. Another characteristic of the entrapped column is the fact that a more homogeneous surface charge distribution drives the radial variability in the electric field to zero. This should diminish the term accounting for static diffusion in the radial direction, further decreasing the plate height. As stated earlier, it is also possible that the silicate entrapment process denies analytes access to a fraction of the pores, decreasing the intraparticle diffusion. A concomitant reduction in the capacity factors of the retained analytes (illustrated in Fig. 3), perhaps arising from masking or hydrolysis of the stationary phase, further decreases the plate height contributions of stationary-phase film resistance to diffusion ($H_{\text{e,diff}}$) and intraparticle diffusion ($H_{\text{i,diff}}$). Primarily, however, the reduction in k' decreases to an appreciable extent the contribution of the kinetics of the analyte-stationary phase interaction (H_{kin}), given that H_{kin} is proportional to $k^2/(1 + k)$. We suspect there are two mechanisms through which the mass transfer contribution to plate height is diminished in silicate entrapped columns: first, a reduced mass of stationary phase is made available to the analytes; second, enhanced in mass transfer is due to the presence of free silanols in the silica matrix. However, at present no quantitative data are available to support these hypotheses.

4.3 Conclusion

Silicate entrapment results in a new type of CEC column that takes better advantage of the opportunities offered by this separation technique. The analysis time is halved, the efficiencies are slightly increased, and the mechanical robustness of the capillary is significantly improved. Further research is oriented towards preserving the selectivity of the packing material and tailoring the pore sizes of the silica network by varying the composition of the entrapment mixture.

The authors thank Mr. Tom O'Brien and Dr. Nicholas Snow (Seton Hall University, South Orange, NJ, USA) for

providing us with the molecular imprinted polymer. Special thanks to Ms. Diane Berry of NIOSH (Centers for Disease Control, Morgantown, WV, USA) and Dr. Al Soeldner at OSU for their help in obtaining the SEM images. Part of this work was presented at the 19th International Symposium on Capillary Chromatography and Electrophoresis, Wintergreen, Virginia, May 18–22, 1997.

Received May 10, 1998

5 References

[1] Pretorius, V., Hopkins, B. J., Schieke, J. D., *J. Chromatogr.* 1974, *99*, 23–30.

[2] Robson, M. M., Cikalo, M. G., Myers, P., Euerby, M. R., Bartle, K. D., *J. Microcol. Sep.* 1997, *9*, 357–372.

[3] Colon, L. A., Guo, Y., Fermier, A., *Anal. Chem.* 1997, *69*, 461A–467A.

[4] Crego, A. L., Gonzalez, A., Marina, M. M., *Crit. Rev. Anal. Chem.* 1996, *26*, 261–304.

[5] Remcho, V. T., *Chem. Educator* 1997, *2*.

[6] Remcho, V. T., Li, D., *J. Microcol. Sep.* 1997, *9*, 389–397.

[7] Knox, J. H., Grant, I. H., *Chromatographia* 1991, *32*, 317–328.

[8] Majors, R. E., *LC-GC* 1998, *16*, No. 1, 12–14; No. 2 96–110.

[9] Fujimoto, C., Fujise, Y., Matsuzawa, E., *Anal. Chem.* 1996, *68*, 2753–2757.

[10] Liao, J.-L., Chen, N., Ericson, C., Hjertén, S., *Anal. Chem.* 1996, *68*, 3468–3472.

[11] Peters, E. C., Petro, M., Svec, F., Fréchet, J. M. J., *Anal. Chem.* 1997, *69*, 3646–3649.

[12] Palm, A., Novotny, M. V., *Anal. Chem.* 1997, *69*, 4499–4509.

[13] Minakuchi, H., Nakanishi, K., Soga, N., Ishizuka, N., Tanaka, N., *Anal. Chem.* 1996, *68*, 3498–3501.

[14] Behnke, B., Grom E., Bayer, E., *J. Chromatogr.* 1995, *716*, 207–213.

[15] Boughtflower, R. J., Underwood, T., Paterson, C. J., *Chromatographia* 1995, *40*, 329–335.

[16] Borra, C., Soon, M. H., Novotny, M., *J. Chromatogr.* 1987, *385*, 75–85.

[17] Tan, J. Z., Remcho, V. T., *Electrophoresis* 1998, *19*, 2055–2060.

[18] Schweitz, L., Andersson, L. I., Nilsson, S., *Anal. Chem.* 1997, *69*, 1179–1183.

[19] Horváth, C., Lin, H.-J., *J. Chromatogr.* 1976, *126*, 401–420.

[20] Horváth, C., Lin, H.-J., *J. Chromatogr.* 1978, *149*, 43–70.

[21] Dittmann, M. M., Wienand, K., Bek, F., Rozing, G. P., *LC-GC* 1995, *13*, 800–814.