Gabriela S. Chirica
Vincent T. Remcho

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

A simple procedure for the preparation of fritless columns by entrapping conventional high performance liquid chromatography sorbents

A rapid and direct method for immobilizing conventional high performance liquid chromatography (HPLC) packing material inside fritless capillaries has been developed. Due to the simple composition of the entrapment matrix (tetraethoxysilane, alkyltriethoxysilane, ethanol and water), straightforward manufacturing procedure and modest equipment requirement, the method can readily be transferred to any laboratory and easily automated. The entrapment procedure has minimal influence on the structure and chromatographic properties of the original reverse-phase sorbent. Various immobilization solutions have been tested, and a comparison between columns entrapped with different immobilization mixtures and conventional packed capillaries is presented. High effficiency separations were obtained using *tert*-butyl-triethoxysilane entrapped columns in both capillary electrochromatography (reduced plate heights of 1.1–1.4 were measured) and microliquid chromatography (reduced plate heights of 2.2–2.6 were observed) formats. Elimination of frits, stabilization of the packed bed and on-the-fly customization of column length render mechanically robust columns that are remarkably stable over time, from which manufacturing imperfections can be removed easily.*

Keywords: Capillary electrochromatography / Micro-liquid chromatography / Packed capillary columns / Fritless capillaries / Monolithic columns / Silica nanoglue EL 4101

1 Introduction

With the goal of achieving high efficiency separations of complex samples in the liquid phase, researchers explored the advantages of employing an electric field in transporting analytes through chromatographic columns. In 1981 Jorgensen und Lukacs [1] recognized that electrophoretic and electroosmotic transport affords a spectacular increase in separation efficiency in the capillary format. Since then, capillary electrophoresis (CE) has become the method of choice for numerous bioanalytical separations, while capillary electrochromatography (CEC), a hybrid of CE and HPLC, is evolving toward suitability for more routine use. In CEC the broad distribution of flow velocities typical of pressure-driven separations is replaced by the narrow distribution of velocities typical of electroosmotic flow (EOF), which leads to reduced band broadening, hence higher efficiencies. The main features that require improvement in CEC include column stability, simplicity of column production, delivery of solvent composition gradients, and enhancement of detection sensitivity.

Aside from the technical challenges brought about by miniaturization, properties intrinsic to CEC open doors to innovation. One of the attributes that makes CEC a unique chromatographic technique is the independence of flow velocity on the particle size for a given applied field strength, which circumvents the pressure limit constraints of HPLC. This feature allows for use of longer columns and smaller-diameter packing materials, while the lack of backpressure translates into increased packed bed stability. As a result, more fragile sorbents can be used and the task of manufacturing fritless columns is made easier in CEC than micro-LC.

Column fragility and fabrication complexity are obstacles that prevent routine use of CEC. The typical CEC column is a packed capillary in which the chromatographic bed, packed as a slurry, is confined between two frits. These retaining frits are made of porous silica-based sorbents and/or silica gel fixed onto the walls of the capillary by sin-

Correspondence: Dr. Vincent T. Remcho, Department of Chemistry, Oregon State University, Corvallis, OR 97331 4003, USA
E-mail: vincent.remcho@orst.edu
Fax: +541-737-2062

Abbreviations: ODS, octadecyl-silica; **SEM**, scanning electron microscope; **TEOS**, tetraethoxysilane

* Portions of this work were presented at the 12th International Symposium on High Performance Capillary Electrophoresis and Related Microscale Techniques, January 1999, Palm Springs, CA, USA.

 0173-0835/00/1515-3093 $17.50+.50/0

tering. The heat generated during this process removes the protective polyimide coating of the fused-silica column blank rendering fragile columns, and evaporates the packing solvent, often generating voids in the packed bed neighboring the frits. In addition, the decomposition of the stationary phase during sintering of the outlet frit extends the mechanical nonuniformity of the packed bed to surface chemistry nonuniformity. Frit fabrication is not a reproducible process, which in turn delivers columns with nonreproducible performance characteristics. A problem often encountered in CEC with conventional packed capillaries is the occurrence of gas bubbles, which disrupt EOF. To avoid disruption of EOF, gas pressure is applied at both ends of the column. However, prolonged pressurization can generate voids in the packed bed.

A viable alternative to packed capillary CEC has been offered by "fritless" columns-monolithic sorbent rods, retained within the walls of fused-silica capillaries. Two main approaches have been employed in the synthesis of such chromatographic beds that do not require frits. The first approach employs the *in situ* synthesis in a chromatographic column of silica-[2–4] or organic-[5–11] based polymeric media. Both types of chromatographic media were first employed in HPLC separations [2, 3, 5, 6] and the technology was transferred to the capillary format [4, 7–11] for micro-LC and CEC. Peters *et al.* [11] designed methacrylate-based monoliths and conducted a thorough study on the effect of porosity and surface chemistry on their remarkable chromatographic performance. However, these polymers need further development in order to approach the wide spectrum of chemistries offered by currently available silica-based sorbents. Another type of porous monolith was prepared *in situ* by hydrolytic polycondensation of tetramethoxysilane in the presence of water-soluble organic polymers [3, 4]. Subsequent octadecylation of the silica network provided a stationary phase well suited for reversed-phase LC. However, stepping from the well-known manufacturing technology of the silica beads to the synthesis of silica rods requires more than just an adjustment in scale. At the moment, silica-based monoliths that are free of cracks and homogeneous throughout the length of the column cannot be obtained reproducibly.

Recently, a new generation of fritless columns that incorporate conventional HPLC packing materials immobilized within fused-silica capillaries has been developed. The first conventional sorbent-based monoliths were realized by immobilizing a tightly packed bed inside a silicate matrix produced by the polycondensation of a solution of water-glass (potassium silicate) [12]. Performance similar to that achievable with "classic" packed capillary columns was achieved, but column-to-column reproducibility suffered. Another approach for immobilizing the packed bed involves sintering octadecyl-silica (ODS) sorbents using high temperature [13] or the intrinsic property of surface silanol reactions which form agglomerates if the external conditions (temperature, pH) are favorable [14]. Particle sintering is a rather harsh treatment and regeneration of the stationary phase is required. However, the micro-LC separation achieved with a sintered column [14] demonstrated that column permeability similar to that of a conventional packed column could be attained. Fritless columns were manufactured through a one-step procedure [15] by packing a slurry of sorbent material and silica sol. The presence of particles in the gel during the aging and drying process reduces, to a certain extent, cracking and shrinking of the glass matrix, the main problems encountered with earlier methods employed in production of porous glass chromatographic media from silica-sols. Tang *et al.* [16] perfected the characteristics of the porous silica matrix by employing the supercritical CO_2 drying step of the sol-gel. The resulting matrix is a crack-free, 90% porous network, which delivers columns with performance characteristics similar to those of conventional packed capillaries and improved column-to-column reproducibility.

In this study we employ a different facet of sol-gel chemistry: the use of silica-sol as a "nanoglue" [17]. The result is a simple procedure that requires a pump, an oven, and a solvent rinsing kit to produce fritless columns with excellent chromatographic performance. A comparison between separation performance of entrapped and nonentrapped columns, as well as columns entrapped with different compositions, is presented.

2 Materials and methods

2.1 Reagents and materials

Tetraethoxysilane (TEOS), *n*-octyl-triethoxysilane, *tert*-butyl triethoxysilane and ethanol were purchased from Aldrich (Milwaukee, WI, USA). The analytes (methyl-paraben, propyl-paraben, butyl-paraben, naphthalene, phenanthrene, fluorene, anthracene, and chrysene), acetone, thiourea, and tris(hydroxymethyl)aminomethane (Tris) were purchased from Sigma (St. Louis, MO, USA). The buffers used in this study, Tris and sodium acetate, were prepared using deionized water; the pH was adjusted with acetic acid and sodium hydroxide, respectively, and measured in the aqueous phase before mixing. Mobile phases were prepared by diluting the specified volume of buffer with acetonitrile (HPLC-grade) purchased from Mallinckrodt (St. Louis, MO, USA). Solutions of analytes were prepared in the mobile phase at a concentration of 0.1 mg/mL. Nucleosil C18 (Macherey-Nagel,

Düren, Germany), a silica-based reversed-phase packing material of 5 μm diameter, was packed in fused-silica tubing of 75 μm inner diameter (ID) and 350 μm outer diameter (OD) as purchased from Polymicro Technologies (Phoenix, AZ, USA).

2.2 Instrumentation

Electrochromatographic and microliquid chromatographic separations were conducted using a Hewlett-Packard HP^{3D} CE instrument (Waldbronn, Germany) provided with external pressurization capabilities. HP ChemStation software (Hewlett-Packard) was employed in data acquisition and processing. An Isco 100 DX syringe pump (Isco, Lincoln, NE, USA) was employed in column manufacture. The inline filter and the cartridge guard column holder used for column packing were purchased from Upchurch Scientific (Oak Harbor, WA, USA). An arc fusion splicer (Fujikura FSM 05S; Alcoa Fujikura, Duncan, SC, USA) was used to obtain the frits and burn the polyimide coating to render the detection window. The entrapment setup, consisting of a typical solvent rinsing kit and a glass sleeve vessel, is described elsewhere [18].

2.3 Packing the columns

Capillaries of 75 μm ID were packed using a method slightly modified from that described by Boughtflower *et al.* [19]. A schematic of the capillary packing procedure is presented in Fig. 1. A 40 cm length of fused-silica capillary was mounted at one end onto an inline filter provided with a metal frit, and at the other end onto a cartridge guard column holder that served as a packing reservoir. A slurry of 15 mg ODS Nucleosil particles and 1 mL of a 50% acetonitrile/50% water solution was sonicated for 5 min and subsequently transferred into the column holder. To this a small magnetic bar was added and the packing reservoir was placed on a magnetic stirrer to ensure uniform packing. The slurry was introduced into the column using 50% acetonitrile and 50% water as a displacement liquid at an initial applied pressure of 500 psi. The column packing process was observed under a microscope and the pressure was gradually increased to ensure an essentially constant packing velocity. When the packed bed was 5 cm long (applied pressure around 3000 psi) a frit, which would serve as a temporary outlet frit, was produced by sintering the silica-based particles with the fusion splicer. During packing, the capillary was continuously sonicated to ensure a tight, homogeneous packed bed while the pressure was further increased to 8000 psi. When the packed portion of the capillary was about 25 cm long the second temporary frit (the inlet frit) was prepared in the same manner. After preparing both

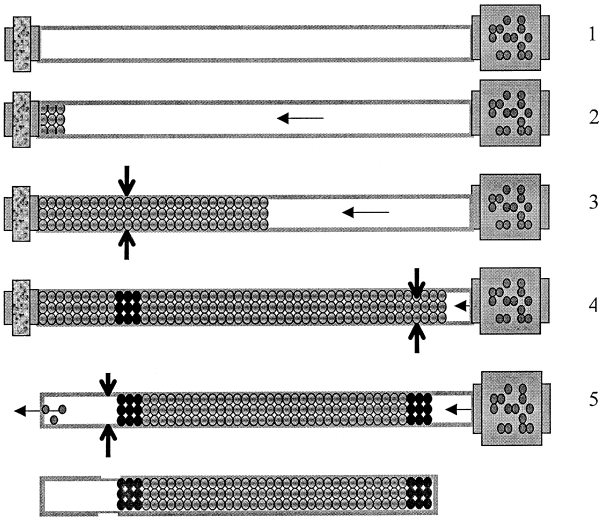

Figure 1. Schematic representation of the packing procedure used in this study. (1) Mount the capillary in the guard column cartridge holder filled with slurry of packing material and solvent at one end and the inline filter at the other end; (2) force the slurry into the column upon application of pressure; (3) prepare the outlet frit, while continuing packing; (4) prepare the inlet frit, while the packed bed is still pressurized; and (5) remove the inline filter and wait until excess packing is flushed out to prepare the detection window.

frits, the inline filter was removed and the excess packing was subsequently flushed out. Next, the pressure was allowed to bleed to zero; this required approximately 15 min. To this point, both entrapped and nonentrapped columns were prepared identically. On the nonentrapped columns, used as a reference, an on-column detection window was then prepared by removing 2 mm of polyimide coating close to the outlet frit using an exacto knife or the fusion splicer. For columns that were subsequently entrapped (using the procedure described in Section 2.4 below) frits were needed merely to retain the particles during the packing and immobilization procedure. Once the nanoglue cured, the frits were removed and the monolith, *i.e.* the column, was cut to the desired length. Consequently, the major drawback of frits — ireproducible chromatographic behavior — is obviated. A short piece of fused-silica tubing (9–12 cm) with the polyimide coating removed for 2 mm was coupled to the monolith using a Teflon sleeve union to provide the detection window.

2.4 Column entrapment

Once packed, the sorbent was immobilized by simply flushing the dried packed capillary with the nanoglue solution and subsequently curing the column in an oven.

Table 1. Description of entrapment mixtures employed in packed bed immobilization

Entrapment mixture	Alkyl radical R	(TEOS + RTEOS) (mL)	TEOS (mL)	EtOH (mL)	AcOH (0.01 N) (mL)
A	–	0.30	0.30	1.0	0.30
B	–	0.25	0.25	0.75	0.50
C	–	0.12	0.12	0.50	0.25
D	–	0.50	0.50	1.30	0.73
E	–	0.50	0.50	1.30	0.55
G	**–**	**0.25**	**0.25**	**1.00**	**0.25**
H	*n*-Octyl	0.15	0.1	0.70	0.13
I	*n*-Octyl	0.48	0.28	0.85	0.30
J	***n*-Octyl**	**0.42**	**0.20**	**0.70**	**0.30**
K	*tert*-Butyl	0.45	0.30	0.60	0.13
L	*tert*-Butyl	0.28	0.20	1.40	0.25
M	*tert*-Butyl	0.28	0.20	0.80	0.25
N	*tert*-Butyl	0.42	0.25	0.86	0.30
O	***tert*-Butyl**	**0.44**	**0.28**	**0.84**	**0.27**

Entrapment compositions that provided the best chromatographic performance for a given combination (TEOS + RTEOS) are shown in bold letters

A mixture of TEOS, *tert*-butyl-triethoxysilane or *n*-octyl-triethoxysilane, ethanol, and acetic acid (see Table 1 for details) was transferred into the 4 mm diameter vial, which was subsequently introduced into the solvent rinsing kit. The capillary was inserted into the entrapment solution and upon application of gas pressure (approximately 200 psi) the silica-sol advanced in the capillary. In dried columns it was very easy to observe, with the naked eye, the advancement of the liquid inside the capillary. Dry columns ensured that no dilution of the entrapment mixture occurred. Gentle drying (at room temperature) before entrapment was found to prevent packing movement. Before placing the column filled with entrapment mixture in the oven, low-pressure nitrogen was passed through the column for 2–5 min. Finally, the columns were cured at 80°C for 24 h and 100°C for another 48 h. Polymerization rendered an immobilized chromatograpic bed, such that the frits could be removed and the column cut to any desired length.

2.5 CEC operation

After packing and/or particle immobilization, the columns were flushed with mobile phase for about 1 h and further conditioned by applying 10 kV and 8 bar (pressure applied equally at both vials) until a stable current was recorded. The sample was introduced using an electrokinetic injection (3 kV for 3 s) and detection was performed at 254 nm. Separations were obtained by applying voltages in the range of 5–30 kV. To avoid bubble formation while running conventional packed columns, pressure was applied at both the inlet and outlet vials. No pressurization was needed during the operation of entrapped columns. The cartridge temperature was set at 25°C.

3 Results and discussion

A perusal of articles describing immobilization or "entrapment" procedures reveals the critical issues in reproducible manufacturing of high quality columns: (i) homogeneity of the initial chromatographic bed, (ii) the amount and structure of the silica entrapment matrix, including porosity and surface chemistry, and (iii) the interaction between the sorbent and immobilization solution. The entrapment procedure described here commences from a high-pressure-packed bed stabilized by two frits, and therefore the distribution of packing material throughout the entire length of the bed is comparatively uniform. What remains is to ensure that the immobilization matrix is also uniformly distributed, and free of cracks and voids. Entrapped columns were cut at various lengths and the chromatographic bed was observed using a scanning electron microscope (SEM). The images show a uniform and stable bed (no particle movement during sputter-coating or upon application of high voltage during imaging) throughout the entire column length. In the process of gelling and drying the immobilization matrix, the surface tension localizes the silica-sol at the interparticle boundaries. Upon drying, the silica acts essentially like a "glue" linking two or three adjacent particles together. As can be observed in Fig. 2, only a small fraction of the sorbent surface is in contact with the immobilizing silica and the

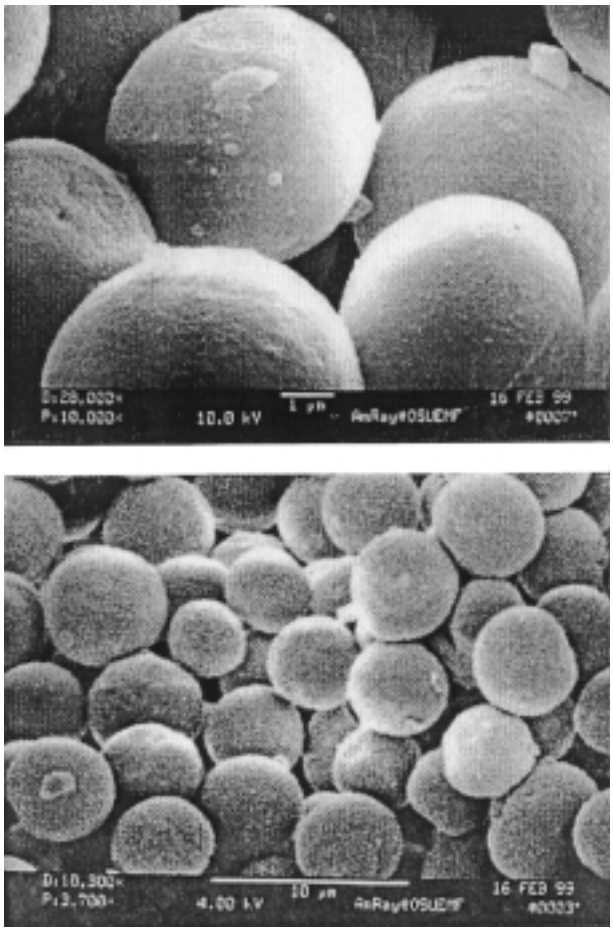

Figure 2. Scanning electron micrographs of a cross-section of a column packed with Nucleosil ODS particles of 5 μm diameter, entrapped with mixture O (see Table 1 for detailed description). A small amount of silica (irregularly shaped fragments in this image) deposited in the entrapment procedure is responsible for the immobilization of the packed bed.

remaining surface appears to be unaffected by the entrapment process. This is a result of the fact that only a small amount of silica is used in this procedure and the interaction time between the silica-sol and the sorbent before gelation and aging is minimized [17]. As mentioned earlier, an important problem encountered during operation of packed capillary columns in CEC is the occurrence of gas bubbles. Although a clear explanation of the origin of the bubbles has not been given, the problem disappears when fritless columns are employed. This was also true for the entrapped capillaries we developed earlier [20].

The goal of this project was to develop an entrapment method by which the sorbent particles would be immobilized with minimal disruption of the structure and chromatographic behavior of the packed bed. Therefore, various mixtures of TEOS, ethanol, water, and acid catalyst were prepared (compositions noted A through G described in Table 1). The mechanical stability of the immobilized bed was tested after removal of the temporary frits by applying a pressure of 300 psi. If the bed was stable, the solution was further diluted with water and/or ethanol to minimize the amount of silica to be added, and a new column was prepared. Entrapment with mixture G (described in detail in Table 1) provided a stable bed while demonstrating the best chromatographic performance. Representative chromatograms of aromatic hydrocarbons obtained with the same column prior to the entrapment and entrapped with solution G are depicted in Fig. 3a and b, respectively. The separation on the entrapped column is achieved in a slightly reduced period of time ostensibly due to the excess of silanol groups introduced by the matrix. More notably, the selectivity of the stationary phase is diminished, probably due to the occlusion of pores in the packing material with nanoglue and/or partial hydrolysis or coverage of the bonded phase. Also, the variation of plate number with EOF velocity obtained with this type of entrapment composition indicates separation efficiencies that are half the typical value for conventional packed capillaries.

Alkyl groups can readily be incorporated into a silica matrix [21] by adding alkyl-triethoxysilane to the original mixture of TEOS, ethanol, and water. In an attempt to improve on the initial effort, various mixtures that included octyl-triethoxysilane (mixtures noted H through J in Table 1) were tested in the fashion described earlier. The tests converged towards composition J; Fig. 3d depicts a typical separation obtained with one of these columns. A comparison of chromatographic performance of columns entrapped with TEOS (Fig. 3b) and octyl-triethoxysilane (Fig. 3d) indicates that the separation of phenanthrene and anthracene is improved. In addition, the separation achieved on the entrapped column is twofold faster than the corresponding separation completed on a non-entrapped column of equal effective length (Fig. 3c). This particular column was further used to demonstrate the advantage of length customization of fritless columns. The capillary was gradually cut and the reduced plate height was recorded as the shorter column allowed for higher field strength (at fixed applied potential), hence a greater EOF velocity (Fig. 4). These columns can be cut to the optimum length for a given application, thus providing baseline separation of the analytes of interest in the shortest possible amount of time.

At this point, however, it was not clear whether the improvement in selectivity and overall column performance might be due to the added presence of the octyl function-

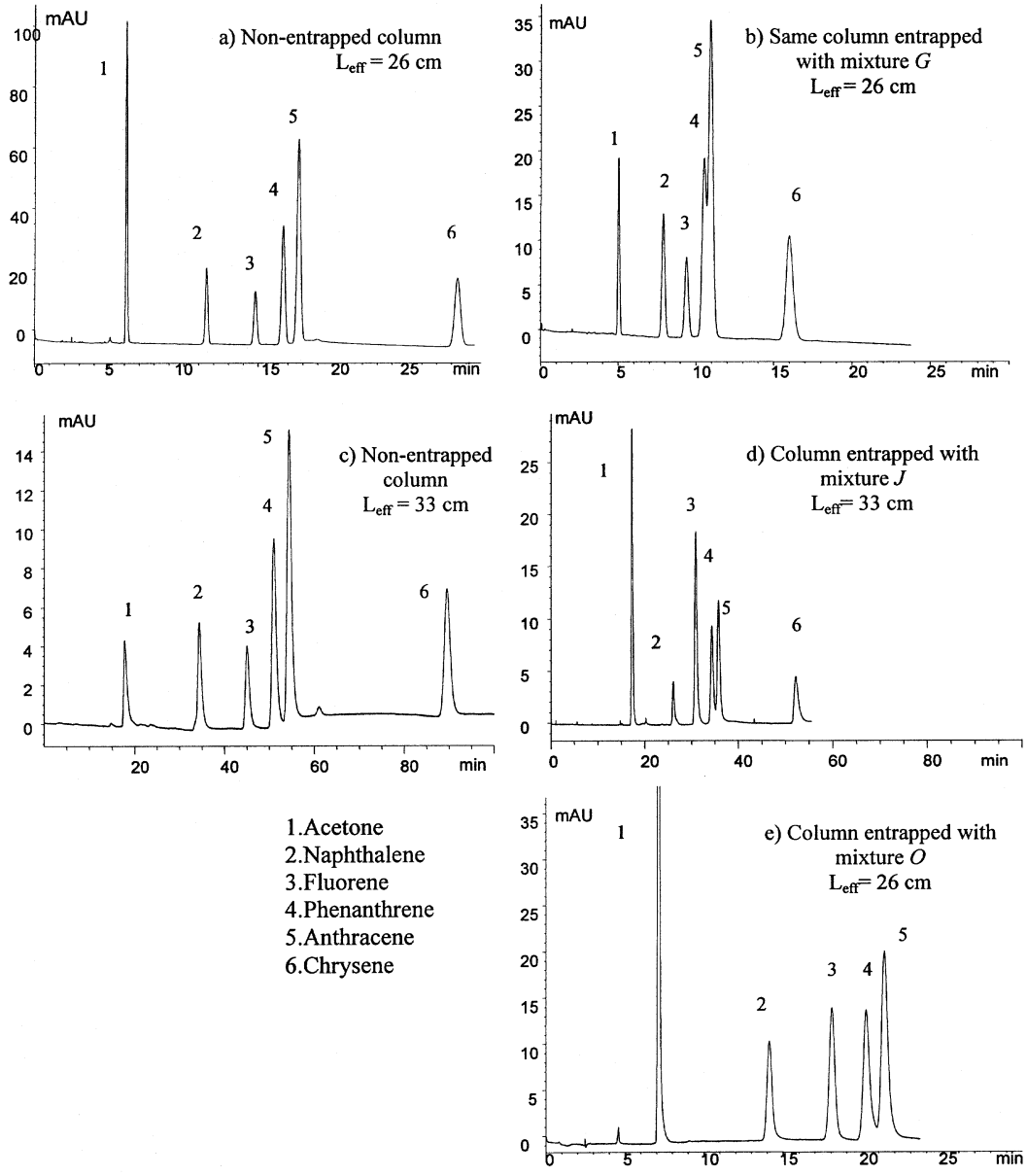

Figure 3. CEC separations of a mixture of organic analytes by CEC on packed columns: (a) nonentrapped column of 26 cm effective length; (b) the same column entrapped with mixture G; (c) nonentrapped column of 33 cm effective length; (d) column entrapped with mixture J (L_{eff} = 33 cm); and (e) column entrapped with mixture O (L_{eff} = 26 cm). Conditions: mobile phase, 80% aceton-itrile/20% acetate buffer, 10 mM, pH 3.0.

ality (which could presumably participate in reversed-phase interactions with the analytes), rather than to an improvement in the nanoglue in terms of surface inertness and overall structure. Unfortunately, direct analysis of the structure of the silica glue was not possible. In the absence of this option, it was decided to replace the octyl group with a shorter, bulkier *tert*-butyl functionality (mixtures noted K through O in Table 1).

The overall performance of the columns entrapped with the mixture noted O (see Table 1 for detailed description) was superior to that of the previous entrapment solutions as demonstrated in Fig. 3e. In order to achieve elevated EOF velocities, an eluent consisting of 80% acetonitrile and 20% of a 25 mM Tris buffer, pH 8.0, was employed to promote the ionization of silanol groups. A significant improvement in separation efficiency was recorded for the

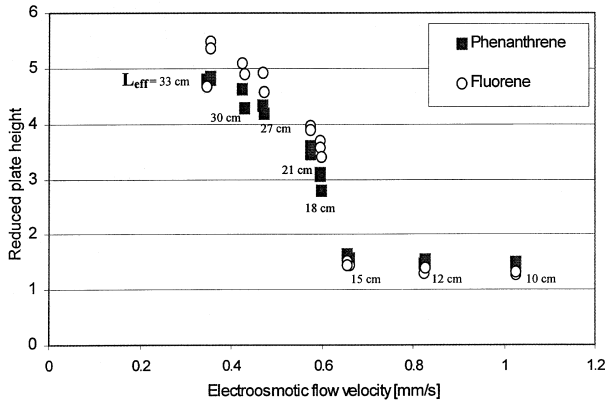

Figure 4. Variation of reduced plate height with flow velocity in a column entrapped with mixture J (see Table 1 for detailed description). Different flow velocities were obtained by cutting the column to shorter lengths. The applied voltage was 30 kV in all runs, demonstrating highest efficiency for a given length of entrapped bed. Mobile phase, 80% acetonitrile/20% acetate buffer, 10 mM, pH 3.0.

columns entrapped with solution O. Reduced plate heights from 1.6 to 2.1 were obtained for the optimum flow velocity (Fig. 5). Baseline resolution of phenanthrene and anthracene (the critical pair in earlier entrapment compositions) was achieved, while peak symmetries varied from 0.93 to 1.0 (Fig. 6a). Cutting the column shorter, combined with application of a higher voltage (30 kV) allowed for rapid separation of the nine analytes; all (other than phenanthrene and anthracene) were baseline resolved in less than 5 min. Columns entrapped with mixture O were used for more than one month, during which period retention times varied by less than 5% relative standard deviation.

In order to compare the performance of the different nanoglues employed in this study, analyte retention factors (*k*) were employed as descriptors of the extent to which entrapment affects separation performance and packed-bed structure of a conventional packed capillary. Therefore, retention factors for various analytes on conventional packed capillary and silica sol immobilized columns (under identical operating conditions) are presented in Table 2. A significant improvement relative to the water-glass "glue" described in a previous paper [20] is demonstrated. Values of *k* in the entrapped *versus* the nonentrapped columns differ increasingly as the analyte has more time to sample the chromatographic media. For entrapment mixture O the retention factor of naphthalene is 0.76 *versus* 0.90 in the nonentrapped column, while for chrysene *k* is 2.50 *versus* 3.71 in the nonentrapped column.

The silica glue O provided an improvement in the retention factors in terms of their proximity to the value characteristic of the original packing, and also an improvement in the separation efficiency and peak symmetry. The best separations provided reduced plate heights of 1.1–1.4. Symmetry factors of 0.90–1.03 were obtained in the CEC and the microliquid chromatographic separations on the same column entrapped with mixture O (Fig. 6a and b, respectively). The fact that high efficiency micro-LC separations (reduced plate heights of 2.2–2.6 for the peaks separated in Fig. 6b could be obtained with an entrapped column was surprising. A closer look at the immobilized bed provided by the SEM images indicates that column permeability is not affected by the presence of the nanoglue.

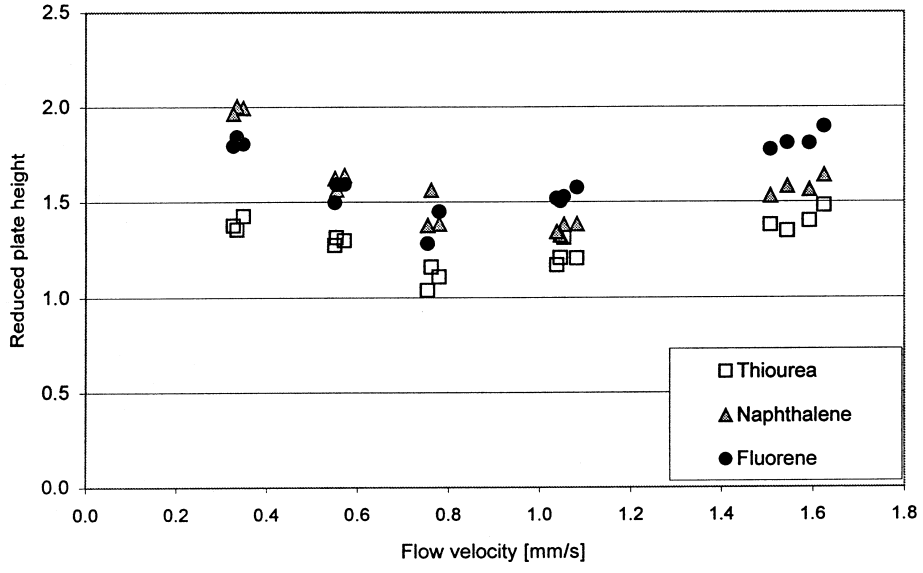

Figure 5. Plot of reduced plate height as a function of flow velocity for CEC separation of thiourea, naphthalene and fluorene. Data were obtained using a column entrapped with mixture O (see Table 1 for detailed de-scription). Conditions: mobile phase, 80% acetonitrile/20% Tris buffer, 25 mM, pH 8.0; effective length, 26 cm.

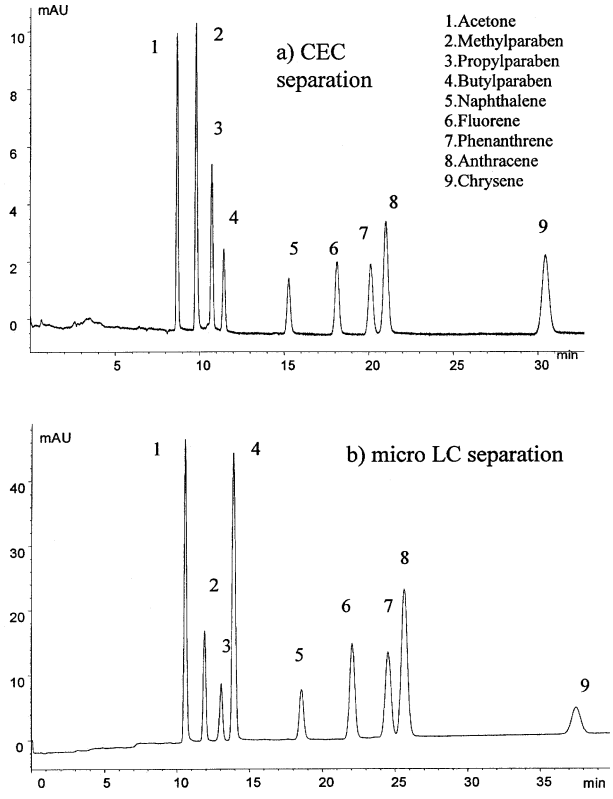

Figure 6. CEC and micro-LC separations of nine analytes achieved on the same column entrapped with composition O (see Table 1 for detailed description). (a) The CEC separation was achieved by applying 10 kV on 26 cm long packed bed. (b) The micro-LC separation was performed on the same instrument ($HP^{3D}CE$) by applying 9 bar gas pressure on the inlet vial (L_{eff} = 24 cm). Conditions: mobile phase, 80% acetonitrile/20% Tris buffer, 25 mM, pH 8.0.

Factors that may have contributed to the observed variation in chromatographic performance of the entrapped columns when compared to nonentrapped columns were considered. For example, it was possible that some coverage of the stationary phase might result in lower retention factors, though the presence of the *tert*-butyl moiety in the immobilization matrix might be expected to augment the reversed-phase characteristics of the entrapped bed. Likewise, if the entrapment matrix entered the pores of the sorbent, upon polymerization the solidified glue could possibly have obstructed (partially or completely) access of the mobile phase and analytes to the intraporous stationary phase. Further studies may provide a more definitive explanation of the effects that entrapment has on the chromatographic bed.

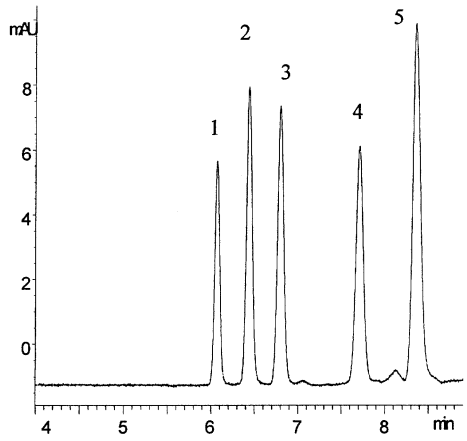

Figure 7. High efficiency separation of five compounds obtained on a column entrapped with mixture O (see Table 1 for detailed description). The injected sample contains, in order of elution: methylparaben, propylparaben, butylparaben, fluorene, and naphthalene. Peak symmetries varied from 0.90 to 0.95 and reduced plate heights of 1.1–1.4 were obtained. Conditions: mobile phase, 80% acetonitrile/20% Tris buffer, 25 mM, pH 8.0. Effective length, 26 cm.

4 Concluding remarks

The advantages reported for other particle entrapped columns, namely the lack of frits, length customization, and cessation of bubble formation during runs, were realized here as well. In addition, the immobilization approach presented in this paper is easy to implement in any laboratory and delivers a chromatographic medium very similar to the original "conventional" packed capillary. As a result, these fritless columns offer excellent chromatographic performance in both the CEC and micro-LC formats, and column-to-column reproducibility is significant improved. Most notably, these fritless columns show remarkable stability over time, which, in addition to the acquired mechanical robustness, affords columns with significantly longer lifetimes.

The authors thank Dr. Al Soeldner (OSU EM facility) for his assistance in obtaining the SEM images. We gratefully acknowledge the financial support of our research by the National Science Foundation and the US Department of Energy.

Received April 3, 2000

Table 2 Retention factors (k) obtained from electrochromatograhic separation of five PAHs

Entrapment composition	$k_{naphthalene}$	$k_{fluorene}$	$k_{phenanthrene}$	$k_{anthracene}$	$k_{chrysene}$
Nonentrapped column	0.90	1.44	1.75	1.93	3.71
Mixture O	0.76	1.09	1.31	1.42	2.50
Mixture G	0.57	0.87	1.10	1.18	2.18
Mixture J	0.52	0.82	1.02	1.11	2.13
Water-glass [20]	0.28	0.42	0.53	0.56	NA

Separation on nonentapped column and columns entrapped with compositions O, G, J and water-glass (the latter from [20]). Conditions: mobile phase, 80% acetonitrile/20% acetate buffer, 10 mM, pH 3.0; 75 μm ID capillaries packed with ODS Nucleosil particles of 5 μm diameter immobilized with the corresponding mixtures.

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