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Aspects of column fabrication for packed capillary electrochromatography

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Abstract

Various parameters have been evaluated to develop a process for optimization of column manufacture for packed capillary electrochromatography (CEC). Spherisorb ODS-1 was packed into 75 μm I.D. capillaries to establish a standard set of packing conditions to afford high-performance columns free of voids. Numerous silica-based packing materials including porous and non-porous reversed-phase and ion-exchange phases were employed to evaluate the applicability of the standard conditions. Success of column manufacture and performance demonstrate a relationship to the colligative properties of the packing materials under the applied conditions. Frequently encountered difficulties arising from inadequate column conditioning and void formation in the packed bed are identified and discussed.

Keywords: Column fabrication; Electrochromatography; Stationary phases, CEC

1. Introduction

In 1974, Pretorius [1] was the first to show improvement in column efficiency resulting from employment of electroendosmotic flow (EOF) instead of hydrodynamic flow to pump mobile phase through a column packed with silica-based particles. This idea was later extended to fused-silica capillaries packed with silica-based materials by Lukacs and Jorgenson [2], with further theoretical and technical groundwork laid by Knox and Grant [3–6]. This initial work formed the basis of what has become known as packed capillary electrochromatography (CEC).

The column serves as the single most important component in a CEC system. Electroendosmotic flow is generated from the interaction between charges on the particles of packing, column walls, and in the mobile phase when an axial electrical potential is applied. A plug-like flow velocity profile is generated in the interstitial spaces of the packed bed. This profile results in an improvement in column efficiency through minimization of band dispersion due to flow inequalities.

Neutral compounds are usually injected electrokinetically and detection is performed on the column after exiting from the packed bed. In the most basic form of CEC, the column acts as the pump, injector, separation medium, and detection cell. Thus, it is evident that uniform and simple manufacture of reproducible and durable columns is essential if CEC is to become a dependable and rugged separation technique that gains acceptance in the scientific community.

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Numerous ways to pack capillaries for CEC have been reported. These include slurry packing [5–8], centrifugal packing [9], draw packing [5], supercritical fluid (SFC) packing [10], and electrokinetic packing [11–13]. In most cases, methods similar to those previously found to be effective for the preparation of capillaries used for pressure driven liquid chromatography (LC) [14–35] have been employed with only slight modification. As of yet, there is no standard process for the manufacture of CEC columns. Typically, each laboratory has its own unique method, and each procedure varies depending upon the type and polarity of the stationary phase used.

The major difference in the manufacture of CEC columns relative to those for capillary HPLC is the fabrication of in-column frits. Frits have been made employing a wide range of approaches [7,8,36–41]. In the most common method, silica or the packing material is heated in order to ''glue'' the particles together to form a permeable and stable frit. Smith and Evans [7] found that manufacture of frits while conditioning the column under pressure with water proved satisfactory.

The frits are necessary to prevent packing material from migrating out of the capillary by electrophoresis, EOF, or pressure. One benefit of having in-column frits is the elimination of unions and connective tubing between the column and injector, and between the column and detection cell. This results in minimization of extra-column band dispersion and contributes to improved peak efficiency along with the plug-like velocity profile generated by EOF. Conversely, in-column frits contribute to column band spreading, bubble formation, and column fragility [41–43]. Therefore, some researchers have investigated the feasibility of manufacturing frit-less columns [5,6,44–55].

As in HPLC, it would be beneficial to explore and compare the various column making methods using a defined process for doing so. Currently, a procedure to determine if a CEC column is a ''good'' column does not exist. Published reports have focused upon efficiency, k, and t_r, while data on quantitative reproducibility has been meager. There are currently no well established parameters, such as column permeability, separation impedance, and column resistance factor as used in HPLC to evaluate a well packed high-performance column. Such guidelines for column assessment would aid in development of methods for uniform and consistent column manufacture.

In this report, we describe successes and failures in attempting to manufacture CEC columns. The primary criterion for success was the lack of void development over the lifetime of the column. Users may have a tendency to disregard the possible impact of voids since columns with and without voids are capable of generating up to 260 000+ plates per meter for neutral analytes on 3 μm particles; however, since no reports admit to formation of voids in the columns, it is not clear how voids may affect separation, especially of ionic compounds. Finally, it can be stated that voids may have a significant influence on the efficiency and resolution of samples containing neutral and ionic compounds as can be inferred from the work of Rathore and Horváth [56,57].

The aim of the present research is to provide users with points to consider when making and using packed CEC columns. To this end, apparent trends in column packing have been identified and when possible rationalized and related to the colligative properties of the various stationary phases being packed. The overall goal of this research is the establishment of a process for preparing CEC columns. Once achieved, the consistent manufacture of columns, free of voids, should then facilitate the development of standard protocols for the evaluation of column performance, durability, and reproducibility.

2. Materials

2.1. Chemicals

Fused silica capillary (75 μm I.D., 375 μm O.D.) was purchased from Polymicro Technologies, Inc. (Phoenix, AZ). Potassium dihydrogen phosphate, thiourea, naphthalene, biphenyl, anthracene, fluoranthene, and fluorene were purchased from Aldrich (Milwaukee, WI). Acetonitrile, 2-propanol, hexane, methanol, tetrahydrofuran, and acetone were of analytical grade and purchased from Burdick and Jackson (Muskegon, MI). Glacial acetic acid was purchased from Mallinckrodt Baker (Paris, KY).

Tris(hydroxymethyl)aminomethane (Tris) was purchased from Sigma (St. Louis, MO). Water was Millipore Super Q (Milford, CT). Compounds of pharmaceutical interest were the property of G.D. Searle (Skokie, IL). Porous silicas were purchased or were gifts from Phase Separations (Spherisorb), Hypersil (Runcorn, UK), Alltech (Deerfield, IL), YMC, Inc. (Wilmington, NC), Bischoff (Leonberg, Germany), ES Industries (West Berlin, NJ), and Phenomenex (Torrance, CA). Non-porous silica (NPS) stationary phases were a gift from Micra Scientific (Northbrook, IL).

Mobile phases were prepared by mixing appropriate volumes of acetonitrile and aqueous buffer. The pH of the buffer was adjusted using concentrated phosphoric acid prior to mixing with the organic component. Mobile phases were neither filtered nor degassed prior to use in CEC. Samples were prepared at a concentration of 0.5–1 mg/ml in mobile phase unless otherwise noted.

2.2. Instrumentation

A Hewlett-Packard HP^{3D}CE (Waldbronn, Germany), with the capability to apply up to 12 bar pressure to both ends of the capillary, was employed for these experiments. Data was gathered and analyzed using HP Chemstation software (version 4.02 and 5.02) or Turbochrom (PE Nelson). Capillaries were operated at 5–30 kV, 25–35°C, and 8–10 bar pressure applied to both ends. Injections were 2 kV for 12 s. Detection was UV diode array at 200, 210, or 254 nm.

2.3. Column preparation

All columns used in this report were packed in-house using one of two methods. The first method, from here on referred to as method A, was adopted from the work of Kennedy and Jorgenson [27,30]. As shown in Fig. 1a, a stainless steel reservoir was constructed with an internal volume of approximately 3 ml. One port of the reservoir was attached to an HPLC pump (Shimadzu 5A LC, or Beckman 114M Solvent delivery module); the other port allowed for the insertion of the raw fused-silica capillary to be inserted for packing. Capillary stock was cut to lengths of 50 or 70 cm and fitted with a retaining frit as mentioned by Kennedy and Jorgenson [27,30], or with a stainless steel mesh frit (2 μm pores) in a Valco zero dead volume union to which the capillary was connected using a stainless steel nut, vespel ferrule, and PTFE sleeve between the end of the ferrule and the frit. The reservoir (3 ml) could be placed on a magnetic stir plate and a small stir bar could be placed inside of the reservoir to provide for a well mixed slurry. Slurries were made at a concentration of 300 mg packing per 3 ml slurry solvent (10% w/v). Pressure was ramped from 0 to 6000 p.s.i. with packing solvent over 3–5 min at a flow-

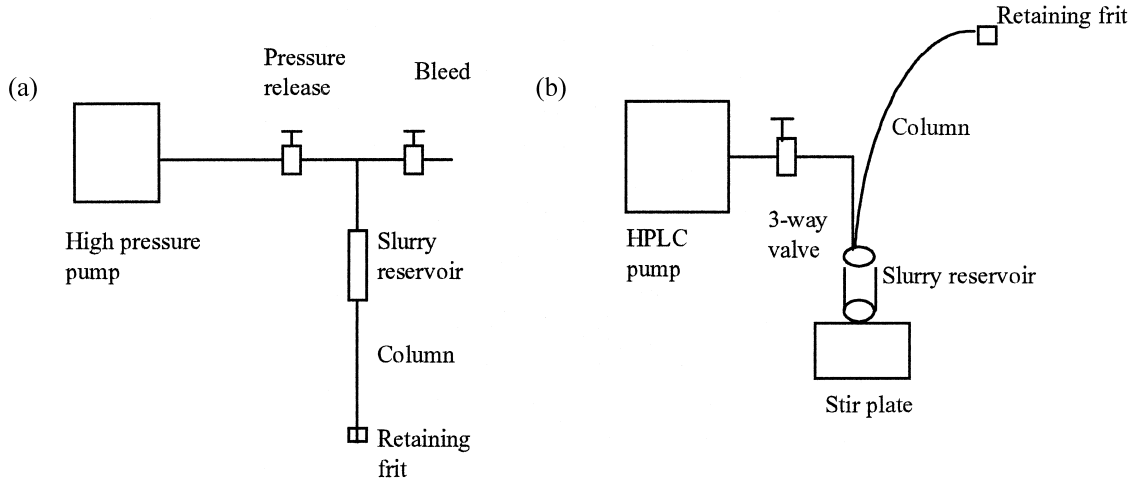

Fig. 1. (a) Packing apparatus for method A. (b) Packing apparatus for method B.

rate of 1.3 ml/min. Packing time varied depending upon the particle size and type of stationary phase, bore of the capillary, and solvents used for packing. Packing continued until the full length of the capillary was packed, then pressure was released slowly (>1 h). Capillaries were then washed with water for varying lengths of time, pressures, and flow directions. Frits were subsequently fabricated while the capillary was still under pressure.

In the second method, method B, a high pressure (8000–9500 p.s.i.) syringe pump (100DM, ISCO, Lincoln, NE), or pump as mentioned above, and small volume reservoir (1/4″ O.D.×4.6 mm I.D.×4 cm; volume=0.66 ml) were used as described in our previous reports [58,59]. Fig. lb provides a schematic of the packing arrangement. A given length of fused-silica capillary (50 cm) was fitted with a stainless steel mesh retaining frit as described above, and the other end was connected to the slurry reservoir in such a manner as to place the end of the capillary just even with the bottom of the reservoir cone. Slurries were prepared (50–60 mg material per 150–200 μl slurry solvent; 25–40% w/v) and sonicated for 5–10 min or agitated until the slurry appeared uniform by visual inspection. The slurry was transferred via pipet into the slurry reservoir followed by the addition of sufficient packing solvent to fill the slurry container. While the slurry reservoir was being filled pressure was built-up in the pump against a two-way valve. Once the reservoir and capillary were attached securely to the pump, the valve was released quickly to allow a high pressure burst to pack the capillary in the downward direction. Using this approach, the entire length of capillary filled nearly instantaneously if appropriate solvents had been chosen for the packing process. Occasionally the capillary would not fill completely and required brief agitation with an engraving tool to break-up any aggregates and thus allow the column to finish packing. After the initial packing process, the capillary was kept at the packing pressure for a minimum of 2 h. Subsequently the pressure was then released slowly (>1 h) and the packed columns conditioned with water as mentioned previously with frit formation being accomplished while the capillary was still bathed with water under pressure unless otherwise specified.

Frits were made with a heated coil apparatus (Fig. 2a) as described by Smith [7] and Boughtflower [8], or with a fiber optic splicer (Fig. 2b) (PFS500 Fusion Splicer, Power Technologies, Little Rock, AK). Column lengths of were 25 cm packed with an overall length of 33.5 cm. The detection window was made immediately after the terminal frit with either the heated coil, hot sulfuric acid, or with a blade.

3. Results and discussion

3.1. Evaluation of packing methods

Initial studies were geared toward establishing which packing method provided the most uniformly packed and efficient columns for use in CEC. A 70 cm length of 75 μm I.D. fused-silica capillary was packed with 3 μm CEC Hypersil C_{18} by method A. The phase was slurried in hexane and packed into the capillary with 2-propanol at 6000 p.s.i.. The column was conditioned with water at 3000 p.s.i. for 1 h. Both on-column frits were made using the fiber optic splicer. The inlet frit (on-column) was made first, about 5 cm from the off-column retaining frit, then the outlet frit (on-column) was fabricated 25 cm up-column from this new frit. The retaining frit was removed, the column depressurized, then reversed and flushed in the opposite direction to remove the extra packing that existed past the outlet frit. A detection window was made by heating the capillary with the coil apparatus to remove the polyimide coating about 1 mm from the outlet frit. A second column of the same material and dimensions was packed downward at 8000 p.s.i. by method B. The particles were slurried in acetone (50 mg/ml) and agitated manually until they appeared well dispersed. Water was employed as the packing solvent. The column remained pressurized overnight to accomplish column conditioning. Frits were made with the splicer while the column was under pressure with the region furthest from the pump connection becoming the outlet frit again, extraneous packing eluting following removal of the retaining frit. The inlet frit was fabricated 25 cm up-column from the outlet frit. The finished column was then slowly depressurized.

Results from these initial experiments are shown in Fig. 3. Columns packed by method B demonstrated plate numbers twice that of the column

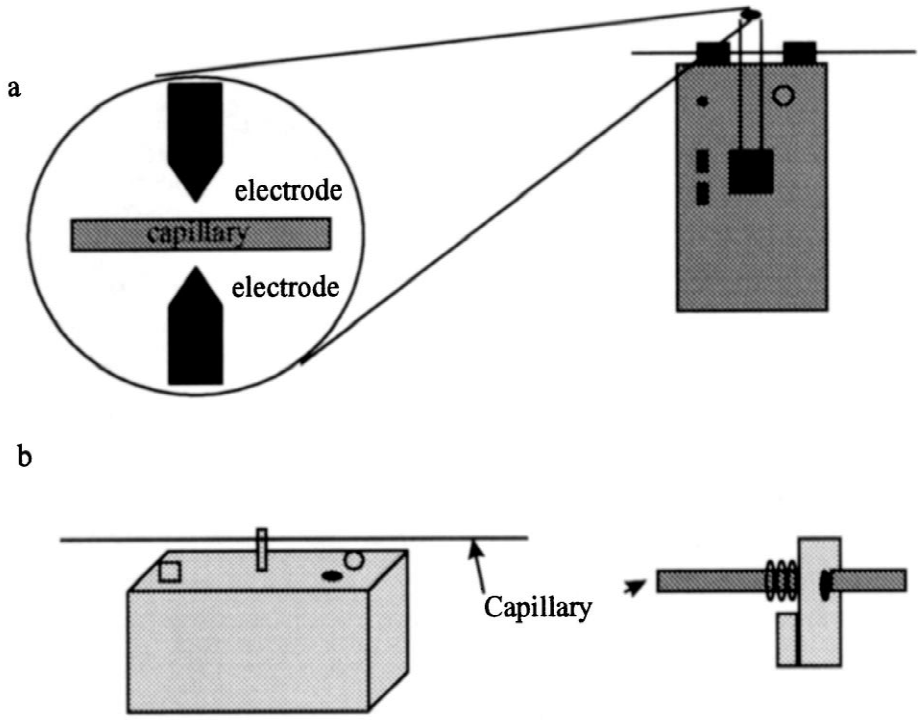

Fig. 2. (a) Fiber optic splicer used in frit manufacture. (b) Heated coil with time and temperature control used to make frits.

packed by method A. Both columns showed very good peak shape. It should be noted that the column packed by method A had a higher EOF as calculated from the elution of thiourea. This may be due to a more open bed structure produced in this method of packing. As an additional comparison, a column was packed with 3 μm Spherisorb ODS-1 by method B. This column displayed efficiency similar to the column packed with ODS-Hypersil, however the elution time was reduced by about 3 min. This is likely due to differences in the base silica, method of manufacture, and/or the carbon load of the materials. From these results, it was decided to employ the Spherisorb material in further column packing optimization experiments.

3.2. Packing parameters

Numerous parameters have to be considered when attempting to optimize packing of small diameter capillary columns. One must keep in mind the type of pump and pressure to use, the length of capillary to be packed, the material from which it is made, the inner bore, the solvents to use for slurries and for packing, and the size and chemical composition of the packing material. Additionally, and especially in the manufacture of columns for CEC, consideration must be given to the method and materials used to fabricate frits once the column is packed.

3.2.1. Pumps

Selection of a pump to use in slurry packing of capillaries can play an important role in how uniform and stable the packed bed will be. Pumps that provide a high flow velocity that is uniform at constant pressure offer the best advantage to achieving this goal. Knox suggested that employment of a pump capable of sustaining a pulse-less flow would accommodate this requirement [60]. This can be accomplished through use of large volume air driven intensifier pumps (e.g. Haskel DSTV 122) or a syringe pump. In our experience, employing method B in combination with a high pressure syringe pump has provided the most stable and efficiently packed

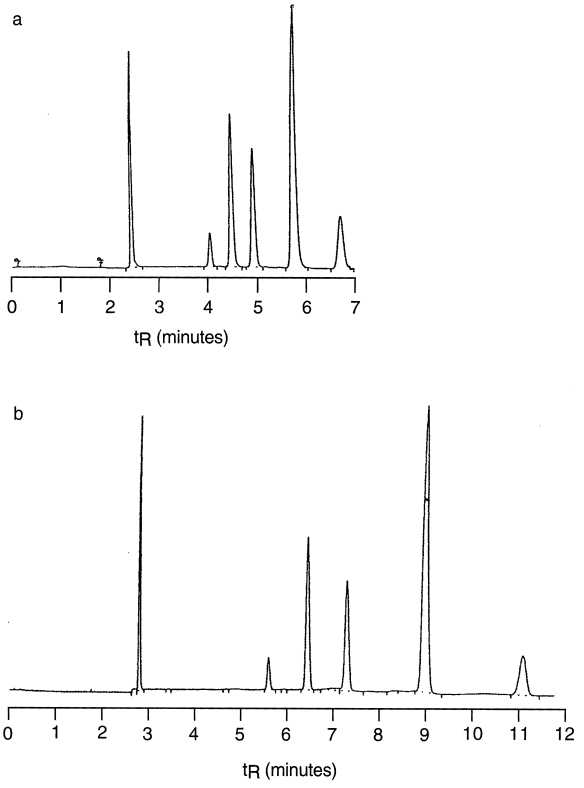

Fig. 3. Comparison of columns packed by different methods. (A) Method A, naphthalene $N=22\,000$/column. (B) Method B, naphthalene. $N=47\,000$/column. Columns: 75 μm×25(33.5) cm packed with CEC Hypersil C_{18}, 3 μm. Mobile phase: 75:25 ACN:50 m*M* Tris (pH 8.0). Conditions: 35°C, 10 bar, 25 kV. Detection: UV DAD at 254 nm. Injection: 2 kV for 12 s. Sample: 0.5 mg/ml in mobile phase.

columns. In situations where a low volume reciprocating pump (e.g. an HPLC pump) has been used, reciprocation of the pump has resulted in poorly packed beds, particularly if this occurs just as the inlet value is opened to start the packing process. In the case of method A, a normal HPLC pump can be used because the large volume of the slurry reservoir serves as a pulse dampener.

3.2.2. Slurry reservoir

Only two types of slurry reservoirs have been used in our investigations. These are shown in Fig. 1. While the reservoir in method A is relatively straightforward in design, the reservoir in method B was observed to depend upon the design of the cone funneling the particles into the capillary. Menet et al. have described the importance of the half-angle of the cone at the bottom of the slurry chamber [18]. In our experience, experiments with varying cone angles agree in general with this discussion.

It was also mentioned that there should be no leaks in the packing system [18] and any leak may damage the integrity of the packed bed. Our experience has served to verify assertion. When leaks occurred in the packing system, usually in the connections between the pump and the reservoir, columns took longer to pack, were less efficient, and many times failed due to difficulty in manufacturing frits or development of voids after little use. The leak is believed to have an adverse effect upon the impact speed of the particles and prevents stable packing of the bed. Furthermore, once the pump is stopped the column depressurizes too quickly, thus likely disturbing the uniformity of the bed.

3.2.3. Packing pressure

In choosing what pressure to pack capillaries, many options must be considered. First is the availability of a pressure source. Capillaries can be packed using conventional HPLC pumps, but are limited to about 6000 p.s.i. in most cases without modification to the pump. High pressure syringe pumps are available commercially, but are expensive. Many types of air intensifier pumps are available, which can have large displacement volumes and can be constructed to provide pressures of 7000 p.s.i. and much higher [34].

Next, the strength of the material that the column blank is constructed from must be considered. Fused silica can be operated at very high pressure, but its strength is dependent upon its inner and outer diameter and may become brittle after packing [61]. Smaller bore capillaries may require higher packing pressures due to their reduced cross-sectional area and associated higher resistance to fluid flow. The length of the column blank also influences the pressure and time required to pack a uniform and stable bed with the desired material [17,24].

The size, porosity, and strength of the packing is also crucially important. Smaller particles require higher pressures to maintain adequate kinetic energy and impact velocity [25]. Highly porous materials may be crushed at high pressures, yet non-porous

materials may be subjected to very high pressure without concern for mechanical deformation.

Most accounts of packing capillaries report using pressures between 200 and 1000 bar (2900–14 500 p.s.i.). There are virtually no reports on how packing pressure affects column performance in CEC. However, from initial results described above, the higher pressure appears to provide for more efficient columns. Most columns used hereinafter were packed at pressures of 500–630 bar (8000–9500 p.s.i.) and demonstrated quite acceptable performance.

3.2.4. Capillary length

The length of the column blank to be packed can affect the impact velocity of the particles when forming the packed bed. In the present investigation lengths from 0.5 to 1.5 m were prepared with the general observation that columns of 0.5 m or less provided better performance and longevity without a propensity for void formation. When 1.5 m lengths were used in an effort to manufacture three columns at one time the efficiencies attained were highly variable from column to column. Based on these results the decision was made to use 50 cm as the standard column blank length for all further efforts.

3.3. Conditions for packing capillaries

Following the assembly of components necessary to pack capillaries, the next step is to determine a process for a systematic approach to assess packing conditions for manufacture of columns with uniform stable beds, durability, and high-performance. This process is comprised of a series of steps as follows: (a) determination of appropriate slurry and packing solvents, (b) determination of packing pressure, (c) conditions to wash the packed bed for preparation to make frits (assuming frits will be made in-column), and (d) frit fabrication (assuming frits will be made in-column).

The initial conditions are usually determined through systematic trial and error and may require significant time and patience for optimization. Each step of this process must be considered for separate packing materials, particle sizes, and column diameters. Proposed guidelines for selection of the ideal conditions in each of these steps for silica-based materials as well as a systematic approach employed in our laboratory for packing CEC columns with 3 μm Spherisorb ODS-1 are described below.

3.3.1. Packing and slurry solvents

Selection of packing and slurry solvents can have a significant influence upon the resulting efficiency of columns used in CEC. It is well known in the process of packing microbore and capillary columns for HPLC that the best beds are achieved with use of solvents providing for good flow properties of the slurry and the resulting bed [19,23–25]. This can be accomplished by the use of low viscosity solvents when packing fused-silica capillaries [23,25]. When high pressure is applied to a low viscosity solvent (assuming Newtonian behavior), particles experience a high velocity and kinetic energy. If this energy is high enough, interparticle friction and/or electrostatic repulsion can be overcome allowing particles to forcibly displace others already packed. This results in the development of a dense and stable bed structure. However, others have presented arguments supporting use of a slower, ramped pressure method of packing to make uniform and dense beds [24,35].

A good slurry solvent results in a well dispersed suspension of non-aggregated particles. The surface tension and effect of the solvent upon the electrostatic and van der Waals forces between particles should thus be considered to achieve this goal [19]. Although balanced density and high viscosity solvents prevent the particles from settling, Verzele and Dewaele [25] suggest use of low viscosity solvents. This prevents long packing times and settling is avoided if the slurry is packed rapidly [62] as in method B. Sonication of the slurry for 5–15 min helps to break up aggregates. Additionally, sonication of porous materials replaces some of the air in the pores with solvent, increasing the apparent density of the particles and thus their kinetic energy during the packing process. It is unclear if flocculated or deflocculated slurries give superior performance.

Table 1 provides a list of slurry and packing solvents found through practical application to provide high-performance CEC columns for various hydrophobic silica-based phases. In this work, packing solvents of low viscosity worked the best, especially ACN, acetone, and methanol. Solvents such as water were also used to pack capillaries,

Table 1
Selected list of solvents employed to slurry various packing materials

Packing material	Slurry solvent	Packing solvent
Alltech 1.5 μm silica	Water	Water
Alltech 1.5 μm ODS AB	Hexane, THF	Acetone
Alltech 5 μm SCX/C_{18}	ACN, THF	Acetone
Astec 5 μm Cyclobond I	ACN, MeOH, THF	Acetone
Hypersil 3 μm ODS	Hexane, 2-propanol, THF	Acetone, hexane
Hypersil 3 μm MOS	ACN, hexane, THF	Acetone
Hypersil 3 μm Phenyl	Hexane, MeOH, THF	Acetone
Micra Scientific 1.5 μmNPS ODS I	Hexane, THF	ACN, acetone
Micra Scientific 1.5 μm NPS ODS II	Hexane, THF	ACN, acetone
Micra Scientific 1.5 μm NPS C_{18}	Hexane, THF	ACN, acetone
Micra Scientific 1.5 μm NPS TAS-1	THF	ACN, acetone
Micra Scientific 3 μm NPS	ACN, water	Acetone, water
Spherisorb 3 μm ODS-1	Hexane, THF	Acetone
Spherisorb 3 μm silica	ACN, water	Acetone
Spherisorb 5 μm SCX	THF	Acetone

although in general the performance of these columns was substantially lower. While perhaps obvious to many, it is worth noting that miscibility of the packing and slurry solvents is also important for the efficient packing of capillaries. When the solvents are not compatible, the slurry may aggregate (as in the case of water used to pack ODS) or a bilayer of solvent forms (such as ACN and hexane) causing the formation of a loose bed structure.

Selection of slurry solvents was based on addition of approximately 500 μl solvent to 100 mg of stationary phase. The vial containing the packing and solvent were manually agitated, then subjected to sonication for 5–10 min. Slurries were inspected visually for aggregates and allowed to stand to observe the rate of settling. Solvents were considered desirable if the stationary phase slurried quickly with manual shaking and/or with initiation of sonication. In most cases, THF was found to slurry particles well and keep them suspended long enough to pack.

Various slurry concentrations were also investigated. Concentrations of slurries were 10% w/v for method A and proved adequate for packing capillaries of 25–100 μm I.D. with particles of 1.5–7 μm d_p. Slurries of similar concentration were used by method B and in general, resulted in longer packing times and columns with lower efficiency as compared to those packed with higher slurry concentrations. Concentrations in the range of 25–40% w/v resulted in the best column performance and stability. Capillaries usually filled instantaneously upon application of pressure depending upon the size, porosity and surface modification of particles. Porous materials of 3–8 μm d_p packed the fastest as did non-porous particles of 3 μm d_p. Both porous and non-porous particles in the range of 0.5– 1.5 μm d_p took longer to pack. These observations agree with those of Gluckman et al. [17] who found columns packed with more concentrated slurries (3–26% w/v) resulted in shorter packing times, better performance, and beds less likely to compact with use in LC.

Selection of the amount of pressure to employ depends upon the particle size and porosity, column bore, column length desired, and available pressure source. Higher pressures result in densely packed beds and shorter packing times. Some researchers suggest employing pressures as high as the packing materials can withstand [25]. Method B uses pressures up to 630 bar (9500 p.s.i.), which is sufficient to manufacture high-performance CEC columns packed with 1.5–7 μm porous and non-porous silica materials. Pressure should be maintained long enough to allow the bed to fully condense and settle in the solvent system employed. Columns are packed in a downward direction by Method B, however, there is insufficient evidence to support an ideal packing direction to use for capillaries as discussed by Verzele and Dewaele [25]. Furthermore, allowing the capillaries to depressurize

slowly, over 1 h or more, maintains the stability of the bed.

Prior to making in-column frits, packing and slurry solvents should be washed out of the column. This is usually done with water when making columns for CEC. Rozing and Unger have rationalized the use of ACN mixed with aqueous buffers at high pH to flush columns after packing and enhance the frit making process [63]. At high pH, dissolution of silica is enhanced and upon application of heat, more silica is available as ''glue'' to make frits. Attempts to make frits in solvents other than water or aqueous solutions have never been successful in our experience with porous silica. However, ACN proved to be very good in columns packed with 1.5 μm NPS materials.

There have been no detailed investigations into the effect of washing duration upon the stability of CEC columns. Washing time depends upon the pressure used and size of the particles, with lower flows occurring with smaller particles. Shelly and co-workers [19] have noted that washing with water for 8 h or more resulted in avoidance of bed settling for ODS materials and increased bed stability. Others have verified improved bed stability with water conditioning [22,64]. This was attributed to water causing the silica particles to swell and enhance attractive forces between hydrophobic chains, thus promoting bed stabilization through strong particle cohesion. In the manufacture of CEC columns, this may contribute to consistent frit fabrication.

Selection of conditions for frit making are currently determined through trial and error. Many variables come into play that may influence the consistency of the frit making process. The column inner and outer diameter, capillary coating thickness, packing material size and porosity, and device to make the frits are a few parameters to consider. Solvents in the column, degree of particle aggregation, and amount of pressure applied to the column also may become very important. Various methods for making in-column frits have been described in the literature, but little detailed information can be found dealing with any of the variables mentioned above.

In this work, two separate devices have been employed to make frits in CEC columns. Both have proven to be effective. Use of a portable fiber optic splicer allows for the control of arc time and current generated between two electrodes as shown in Fig. 2a. When a capillary column is placed between the electrodes the electric arc heats the packing, making the frit. This device is very easy to use and can be operated by battery power. Results are reproducible if the electrodes are kept clean and the battery retains sufficient charge. Heated coils with time and temperature control, as described by Smith [7] and Boughtflower [8] (Fig. 2b), also have been used. Various coils can be used with this apparatus, but caution must be taken to monitor coil lifetime [65]. The heated coil has proven to be slightly more flexible to use, but the splicer is more consistent. Both can make frits of various sizes, which may affect the performance of the column [41].

3.4. Application of the process

Spherisorb 3 μm ODS-1 was chosen to develop a systematic process to manufacture columns for use in CEC. In each case, 50 cm lengths of 75 μm I.D. fused-silica capillary were packed by method B at 8000–9500 p.s.i. and remained under packing pressure for a minimum of 1 h. Results indicate that solvent selection and washing under aqueous conditions at 6000 p.s.i., have a role in the outcome and performance of the column. Columns were considered successfully made if they were uniform and free of voids after conditioning, use, and storage. Table 2 lists the conditions and outcome for 14 separate columns.

Results of this experiment may be rationalized in view of investigations into the importance of slurry and packing solvents upon the colligative properties of reversed-phase packing materials used to pack preparative columns and capillaries for microcolumn LC [19,24,32,66]. Findings from these studies indicated that packing and/or conditioning solvents that caused particles not to aggregate afforded the best columns in terms of peak shape, efficiency, separation impedance, permeability, and flow resistance. Slurry solvents did not appear to influence packed capillary performance significantly [32], while slurry concentrations and conditioning methods for preparative columns did impact column performance [66].

With this in mind, the trial stationary phase was slurried and packed in various combinations of organic, aqueous, or organic/aqueous solvent mix-

Table 2
Conditions used to compare packing procedures for manufacture of 75 μm×25 cm columns packed with 3 μm Spherisorb ODS-1

Column	Solvents		Pressure		Wash			Outcome[g]
	Slurry	Packing[a]	p.s.i.	Time	Solvent[a,b]	Time	Direction	
1	THF	Water	9500	1	Water	[c]	[d]	Voids
2	THF	ACN	9500	>8	Water	2	[d]	Voids
3	THF	Acetone	9500	1.5	0.5% GAA	1	[d]	Uniform
4	THF	Acetone	9500	1.5	0.5% GAA	3	[d]	Uniform
5	THF	Acetone	9500	1.5	0.5% GAA	16	[d]	Uniform
6	THF	Acetone	9400	4	Water	>8 1	[e] [f]	Uniform
7	THF	Acetone	9200	2	Water	2 >8	[e] [f]	Uniform
8	THF	Acetone	8000	>8	Water	4	[d]	Uniform
9	THF+GAA	ACN	9500	1	Water	6	[d]	Voids
10	THF+GAA	Acetone	9500	1.5	0.5% GAA	1 1	[e] [f]	Voids
11	THF+GAA	0.5% GAA	9500	1	0.5% GAA	1 1.5	[e] [f]	Voids
12	THF+GAA	0.5% GAA	9500	1.5	0.5% GAA	[c]	[d]	Voids
13	THF+GAA	0.5% GAA	9500	2	0.5% GAA	[c]	[d]	Voids
14	Hexane	Acetone	9400	>8	Water	3.5 1	[e] [f]	Uniform

[a] 0.5% GAA=0.5% glacial acetic acid in water (pH 2.78).
[b] Wash at 6000 p.s.i.
[c] Packing solvent and packing time served as wash.
[d] Both inlet and outlet frits made while column flushed in packing direction.
[e] Outlet frit made 10–15 cm from pressure source while flushing in packing direction.
[f] Inlet frit made 25 cm closer to pressure source from midfrit after reversing and flushing column in direction opposite to packing direction.
[g] Condition of packed bed following conditioning with 70:30 ACN:5 m*M* $(NH_4)_2HPO_4$ (pH 3.0) at 3000 p.s.i., conditioning with voltage, and five injections of test mix. Columns with uniform beds never formed voids even after months of storage and intermittent use.

tures. Hexane and THF were used as slurry solvents because they were determined to provide very good, well dispersed slurries for Spherisorb ODS-1 as indicated in Table 1. Acetone and ACN were also determined to be non-aggregating solvents as confirmed by Vissers et al. [32].

From Table 2, trends indicate that use of both non-aggregating slurry and packing solvents resulted in the best columns for CEC. In each case where water or an aqueous solution of acetic acid was used as the packing solvent, column manufacture was unsuccessful and performance was poor. Since both of these solvents were aggregating, this argues against use of these solvents to pack CEC columns. Acetone, which is non-aggregating, proved to be the best packing solvent in this investigation.

Both THF and hexane resulted in the manufacture of good columns when used as slurry solvents. Slurries prepared in THF and acetic acid (150 and 50 μl, respectively) also appeared to be uniform and well dispersed, however, these slurries usually required longer packing times and sonication to complete packing. Slurries prepared in this solvent mixture always resulted in column failure regardless of the packing solvent used. From this observation, it appears as if the packing material may have been aggregated in these slurries. Since the zeta potential of Spherisorb ODS-1, thus electrostatic repulsion, has been demonstrated to enhance its slurry stability [32], addition of acetic acid likely neutralizes charge on the particle surface, thus allowing for aggregation driven by the van der Waals forces between the C_{18}

chains. It appears, therefore, as if solvents preventing aggregation should be used for both slurries and packing.

Pressure did not appear to influence the results in this study. The column packed at 530 bar (8000 p.s.i.) demonstrated equivalent performance to those packed at 630 bar (9500 p.s.i.). However, it was observed that columns remaining under packing pressure less than 1 h resulted in failure.

Trends also indicate that column washing with aqueous solvents following packing have a significant effect upon the tendency of the column to form voids and the ability to make uniform frits. When water was employed, the duration of the water wash appeared to influence the stability of the bed. In general, columns conditioned with water overnight provided the most stable beds and best results with frit making. Columns in which the total water wash time was less than 4 h usually formed voids after some period of use, even though the frits and bed initially appeared homogenous. This stability is most likely caused by aggregation of the bed through swelling of the silica and hydrophobic interaction between bonded ligands as mentioned by Shelly [19]. Void formation is averted as is damage done to the bed upon making frits because the extra void volume has been minimized.

In case where a column failure occurred, even after using a 6 h wash time, the direction of solvent flow during frit manufacture may have been of influence. In general, the best columns were flushed with water in both directions before manufacture of the last frit. This serves to flush out extra packing to allow preparation of a window, tests outlet frit strength, and compacts the bed. When voids formed in columns, parabolic profiles were observed at the end of the bed. Direction of the profile often corresponded to the direction in which the column was packed, especially in columns washed only in the packing direction. This evidence suggests that washing the column in both directions may, in effect, help to flatten the parabolic profile of the bed caused by packing. This in turn reduces the likelihood of void formation.

Columns washed in acidic aqueous solution following use of THF and acetone as slurry and packing solvents demonstrated very good efficiency and stability. In contrast to observations made above with water washes, columns washed in acid did not appear to depend upon duration or direction of the wash. As explained above, addition of acetic acid may expedite aggregation of the particles through minimization of electrostatic interaction and promotion of van der Waals attraction. However, this wash procedure did not prevent void formation if columns were packed with solvents promoting aggregation. It is assumed that this may be due to production of a non-homogenous bed upon packing, which cannot be effectively rearranged during washing with water or acid.

Both the fiber optic splicer and heating coil approaches were employed to make frits in these columns. There was no significant difference in performance observed between columns made with either technique. The fiber optic splicer was found to be very useful in deciding when a packed bed was ready for frit manufacture and determining the amount of heat required to make frits in various materials. A test frit was made at the distal end of the bed with the fiber optic splicer, which afforded assessment of bed damage and recovery through the attached microscope as the packing was heated. A column was judged to be ready to frit if the bed showed no cracks, voids or shock areas, as indicated by dark and light bands that disappeared quickly following application of heat. In almost all cases where there was low solvent flow through the frit and slow recovery of the bed, column performance was poor and voids would form shortly after use. Furthermore, if the bed was fragmented or damaged, manufacture of other frits in the column would also be complicated or lead to bed damage and voids with use. However, visual observation of uniform frits and packed bed structure was not reliable for the determination of a well made column. Many times columns would void with use despite appearing homogenous following manufacture.

3.5. Application of the process to other packing materials

3.5.1. Hydrophobic

Once a process for manufacture of good columns had been established with Spherisorb ODS-1, it was employed as a reference point for determination of conditions to pack other phases. Hypersil phenyl (3

μm), Hypersil MOS (3 μm), and CEC Hypersil C_{18} (3 μm), were packed under the conditions found to be the best for Spherisorb ODS-1. The results attained are provided in Table 3.

As can be observed from Table 3, the optimized packing conditions for Spherisorb ODS-1 were not applicable to these other phases. Use of ACN and methanol as slurry solvents for MOS and phenyl, respectively, gave more uniform and stable beds. Washing the CEC C_{18} material with water in both directions was better than using dilute acetic acid.

Following conditioning and testing, three Hypersil phenyl, one CEC Hypersil C_{18}, and three Spherisorb ODS-1 columns were allowed to remain undisturbed in a vertical position for 2 weeks with the inlet frit in water at the downward end. Each column was manufactured with THF, acetone, and acetic acid solution as the reference conditions for slurry solvent, packing solvent, and conditioning solvent, respectively. All columns were stored in 70:30 ACN:aqueous buffer (5 m*M* $(NH_4)_2HPO_4$, pH 3.0). Observations made at the end of the 2-week period indicated that the Hypersil phenyl and CEC Hypersil C_{18} phases had settled, allowing 1–2 cm voids to appear between the end of the packed bed and the outlet frit. No settling occurred in the Spherisorb ODS-1 columns. Settling was more severe in the Hypersil phenyl columns and indicated that packing density was not adequate prior to frit fabrication.

Sedimentation rates and sedimentation quotients for hydrophobic C_{18} reversed-phase materials in organic packing solvents have been investigated [19,24,32,66]. All of the tested materials were deflocculated in both acetone and THF. Columns

Table 3
Conditions used to compare packing procedures for manufacture of 75 μm×25 cm columns packed with Spherisorb ODS-l (3 μm), CEC Hypersil C_{18} (3 μm), Hypersil MOS (3 μm), and Hypersil phenyl phases (3 μm)

Column[a]	Solvents		Wash			Voids		
	Slurry	Packing	Solvent[b,c]	Time (h)	Direction	Initial[g]	Condition/ voltage[h]	Storage
ODS-1	THF	Acetone	0.5% GAA	1	[d]	None	None	None
ODS-1	THF	Acetone	0.5% GAA	3	[d]	None	None	None[i]
ODS-1	THF	Acetone	0.5% GAA	16	[d]	None	None	None
CEC C_{18}	THF	Acetone	0.5% GAA	1	[d]	None	1–2 mm	>1 cm[h]
CEC C_{18}	THF	Acetone	Water	>8	[e]	None	None	None[j]
				1	[f]			
MOS	THF	Acetone	0.5% GAA	15	[d]	None	Lost frits	n/a
MOS	ACN	Acetone	Water	2	[e]	None	None	None[j]
				>8	[f]			
Phenyl	THF	Acetone	0.5% GAA	2	[d]	None	1–2 mm	2 cm[i]
Phenyl	THF	Acetone	0.5% GAA	1.5	[d]	None	1–2 mm	2 cm[i]
Phenyl	THF	Acetone	0.5% GAA	1	[d]	None	1–2 mm	2 cm[i]
Phenyl	MeOH	Acetone	Water	2	[e]	None	None	1–2 mm[j]
				1	[f]			

[a] Packing materials as mentioned in text.
[b] 0.5% GAA=0.5% glacial acetic acid in water (pH 2.78).
[c] Wash at 400 bar (6000 p.s.i.).
[d] Both inlet and outlet frits made while column flushed in packing direction.
[e] Outlet frit made 10–15 cm from pressure source while flushing in packing direction.
[f] Inlet frit made 25 cm closer to pressure source from midfrit after reversing and flushing column in direction opposite to packing direction.
[g] Presence of voids in the packed bed following manufacture.
[h] Voids in packed bed following conditioning with 70:30 ACN:5 m*M* $(NH_4)_2HPO_4$ (pH 3.0) at 200 bar (3000 p.s.i.), conditioning with voltage, and minimum of five injections of a test mix.
[i] Presence and size of voids after 2 weeks of vertical storage in 70:30 ACN:5 m*M* $(NH_4)_2HPO_4$ (pH 3.0).
[j] Presence of voids and size after storage in ACN:water or MeOH:water for>1 year.
n/a=Not applicable.

slurried and packed in acetone gave contradicting results between groups [19,32]. However, poor performance was attained for columns that were slurried and packed with THF. Previously it has been argued that electrostatic repulsion between particles caused the bed to pack loosely [32]. Nevertheless, if columns packed in THF/THF (slurry solvent/packing solvent) were allowed to settle prior to conditioning, column performance improved dramatically [19]. This can be explained in part by the fact that sedimentation of a well dispersed suspension eventually results in a densely packed cake where electrostatic repulsion forces are overcome by van der Waals attraction [67]. By not allowing the bed to settle over adequate time (at a minimum corresponding to the time necessary to complete thorough sedimentation in the solvent) once the bed is conditioned with a coagulating mobile phase, the bed structure is reorganized causing it to be disordered and result in poor performance [30,31].

Extending these arguments to the case at hand, the rationale for the observed settling of the CEC Hypersil C_{18} and Hypersil phenyl phases is apparently multi-faceted. First, THF may not have been an adequate slurry solvent, particularly for the phenyl phase and thus the particles may have aggregated prior to packing. Tables 3 and 4 show that a better phenyl column was produced when methanol was the slurry solvent. Furthermore, an apparent relationship is noted between solvent strength of slurry solvents used and the various chemistries used to modify the base silica (such as chain length, and percent carbon load on the packing material) as listed in Table 4. As the surface of the packing material becomes more polar and/or surface silanols become more accessible, use of organic solvents displaying a strong polar solvent strength appears to create a more uniform slurry. Aggregation is prevented through ''solvation'' of the packing material in the polar surroundings, reducing dipolar interactions, hydrogen bonding, and van der Waals interaction between particles.

Table 4
Empirically determined best slurry solvents for various supports

Packing material[a]	Best slurry solvent	% Carbon[b]
Hypersil phenyl	Methanol	5
Hypersil MOS (C_8)	ACN	6.5–7
CEC Hypersil C_{18}	THF	8.5
ODS-1	THF	6.2–7

[a] All materials were 3 μm d_p.
[b] Taken from information provided by Hypersil and Keystone Scientific, Inc. (1998–1999).

Second, the degree and rate of settling appear to increase as the bonded chain length decreases. This may arise from the accessibility of silanol groups, which in turn cause the packed bed to be dispersed due to electrostatic repulsion, especially as the column is washed with aqueous solvent prior to frit making. Upon subsequent conditioning with an aggregating mobile phase, the suspension of particles in the bed collapses, resulting in voids. With application of voltage, the aggregates are once again dissociated, as observed by Inagaki et al. [12], allowing the bed to be resuspended and rearranged. Aggregates reform and settle quickly creating voids as the column is allowed to stand undisturbed. Alternatively, aggregation of particles in the slurry solvent may result in an initially loose bed that remains aggregated during conditioning, then disperses, rearranges, and settles following exposure to voltage.

It is evident from the points discussed above that solvents must be chosen carefully for each packing material to affect the desired properties of colloidal suspensions at the appropriate steps in the column manufacturing process. Differences in chain length and percent carbon between various hydrophobic reversed-phase materials have a strong influence upon this decision. Silica derivatized with short chain hydrophobic groups and low carbon load may require a period of time to allow for settling and compaction of the bed prior to an aqueous wash period or aqueous conditioning in both directions prior to frit fabrication. Further investigation into relationships between chain length, void size, and rate of settling in C_{18}, C_8 and phenyl phases is required to delineate these observed trends.

3.5.2. Base-deactivated, non-porous, and polar

The standard conditions for making ODS columns above were applied to numerous other silica-based packing materials. Non-porous reversed-phase, ultrapure base-deactivated reversed-phase, mixed mode, cation-exchange, and bare silica (both porous and non-porous) have all been packed with variations to the standard conditions. In almost every case, the

conditions had to be specific to each packing material.

Non-porous particles tend to pack more quickly and form very uniform beds. The advantage to packing non-porous materials, especially 1.5 μm materials, lies in the higher density of each particle as compared to porous material of the same size. This density improves the kinetic energy and impact velocity of the particles upon the forming bed, making it very compact.

Most of the reversed-phase non-porous materials slurried well in THF. Though other solvents such as hexane were also used, THF allowed the particles to remain suspended longer. When using non-porous particles, it is especially important to apply pressure immediately following placement into the slurry chamber to avoid rapid settling.

Non-porous materials were not as dependent on the aqueous washing duration. However, care had to be taken in frit manufacture with 1.5 μm particles due to very low flow-rates through the beds. As a result, columns were often conditioned in a low viscosity solvent such as acetonitrile from which frits could be made without any detriment to the column. The frit making technique did show some influence upon the performance of non-porous columns as reported by Angus et al. [59].

Ultrapure silica with very high ligand density and endcapping proved difficult if not impossible to pack and retain in capillaries using the presently described methods. Lack of charge on the base-deactivated silica may prevent formation of well suspended, deaggregated slurries. In almost every case, particles appeared to slurry well by visual observation. However, packing took longer and often required sonication of the slurry reservoir to complete packing, indicating possible aggregation under the employed conditions. Beds often compacted with washing and flow were highly variable, lending further evidence to support this argument.

The most difficult aspect of manufacturing columns with these materials lies in the fabrication of the frit. Since these materials are densely bonded, endcapped, pH stable over a larger range, and have less metal contamination, they are less likely to experience the same degree of silica dissolution when subject to frit heat. This means that an inadequate amount of silica is available to form a ''glue'' allowing particles to stick together. Attempts were made with both porous and non-porous materials as seen in Table 5. Capillaries were packed and washed with water, 0.5% GAA, or with 20 m*M* sodium phosphate (pH 3.0). Attempts to make frits with both the heated coil and splicer under these conditions failed, although use of higher frit temperatures and longer times showed promise. Aggregation of the slurry may also contribute to poor ability to frit the material due to large amounts of void space between particles resulting from non-homogenous packing.

Table 5
List of base-deactivated high purity silica phases employed to pack CEC columns

Manufacturer	Phase	Particle size (μm)
Bischoff	ProntoSIL 120-3-C_{18} H	3
ES Industries	AquaSep C_8	5
Micra Scientific	NPS ODS II	3
Micra Scientific	NPS ODS II EC	3
Phenomenex	Luna C_8 (2)	3
Phenomenex	Luna C_{18} (2)	3
YMC	YMC basic	3

The long sought after attributes of these supports, pH stability, purity, and reduced silanol activity, that spurred development of these phases for modern HPLC use, are in fact detrimental to the manufacture and use of CEC columns. However, these materials may become useful in the development of pressurized-flow electrochromatography (PEC) [63,68,69], thus advances in the fabrication of capillary columns using these materials is potentially beneficial for the future. Frit fabrication from these materials is not currently feasible by the presently employed techniques. Apparently, frits will have to be external to the column, or made from other grades of silica. Efforts to make glass frits for CEC columns by UOP mat/sen may hold promise in these cases.

The success rate for fabrication of uniform columns packed with mixed mode (SCX/C_{18}), strong cation-exchange (SCX), and bare silica was also minimal. Optimized slurry solvents for these phases are under evaluation currently. However, due to the density of charged groups and strength of the anion, particularly the sulfonate groups on the mixed mode and SCX phases, it appears likely that packed beds

remain loose as a result of electrostatic repulsion. This was evidenced in both the mixed mode and SCX materials that voided after conditioning, use, and storage. In almost every column packed with these phases, beds and frits appeared uniform initially, then consistently formed voids equaling 10% of the packed bed length. As mentioned previously, a well dispersed suspension of particles can settle to form a dense cake by overcoming electrostatic forces. This property of colloidal suspensions may aid in understanding the manufacture of stable columns with charged materials. Further investigation into settling and its affect upon stability and performance in CEC columns is ongoing.

3.6. Column conditioning

Following manufacture of a column for use in CEC, the next step is to condition it properly to afford reproducible performance. While columns may be conditioned directly on the instrument with voltage, the best results have come after initial conditioning the column with run mobile phase by an HPLC pump. This is then followed by conditioning on the instrument. A typical conditioning cycle employed in this laboratory is initial treatment with 70:30 acetonitrile:water or run mobile phase at 65–200 bar (1000–3000 p.s.i.) overnight or until no bubbles elute from the outlet frit. Column conditioning is continued on the instrument for 30 min at 15 kV, then 30 min at 30 kV while both ends of the capillary were pressurized to 10 bar. This conditioning procedure was found to give very stable EOF and prevented the stationary phase from drying out with most packing materials. Slight modifications are required for non-porous packings as mentioned previously [59].

Effects of inadequate conditioning can be difficult to ascertain. A column thought to be well conditioned may show a well defined baseline and stable

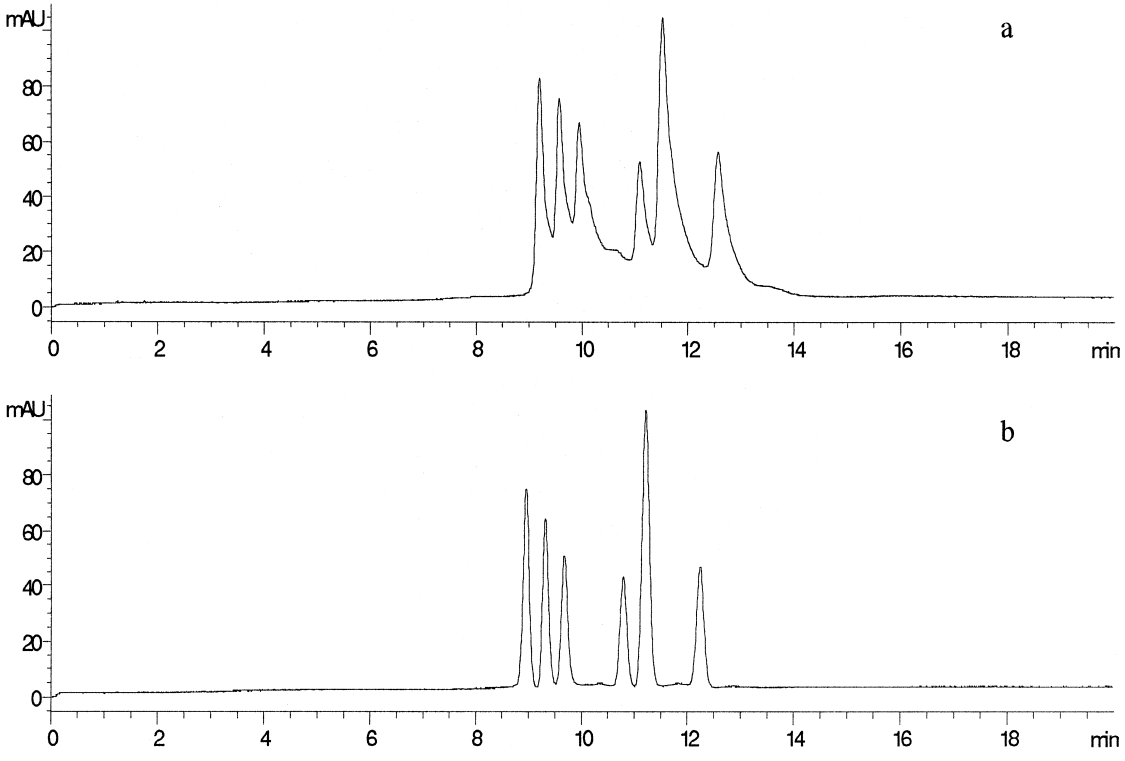

Fig. 4. Effect of inadequate column conditioning. (a) Column is not fully conditioned. (b) Column is conditioned. Column: Micra Scientific NPS C_{18}, 1.5 μm, 75 μm×25(33.5) cm. Mobile phase: 60:40 MeOH:2 m*M* KH_2PO_4 (pH 3.0). Conditions: 60°C, 10 bar, 30 kV. Detection: UV DAD at 210 nm. Injection: 2 kV for 12 s. Sample: 0.5 mg/ml in mobile phase.

current. It may even appear wetted under a microscope. However, columns with dry areas may also demonstrate these characteristics. A poorly conditioned column will show inconsistent elution times and efficiencies, broad and/or tailing peaks, and inconsistent day-to-day reproducibility. Fig. 4 demonstrates the effect of conditioning upon the peak shape and performance in a CEC column. It behooves the user to have patience in conditioning columns before use in CEC.

Following use, columns were rinsed with ACN:water and stored with the ends of the capillaries submerged in water. This was to prevent dissolution of the polyimide. Columns stored in this way retained efficient performance with intermittent use over more than 1 year.

3.7. Troubleshooting

A challenging aspect of CEC is how to identify differences between conditioning effects and physical defects in the packed column. This can be very difficult to decipher without direct inspection of the column under a microscope. However, characteristics of voids or fractures in the bed or frit can be recognized. Fig. 5 demonstrates the effect of voids upon the performance and peak shape. Peak splitting, swells in the baseline, and tailing are usual tell tale signs of voids or fractures. This may also indicate damage to the inlet end of the capillary as shown by Dittmann et al. [44]. Loose areas of packing material may also show similar results as indicated in Fig. 6. A segment of loose packing material was observed by microscope at the end of the outlet frit. After application of voltage to pull the particles away in the reverse direction, performance was drastically improved.

Columns can demonstrate very good performance and qualitative reproducibility of retention times, efficiency, and retention factor even though voids

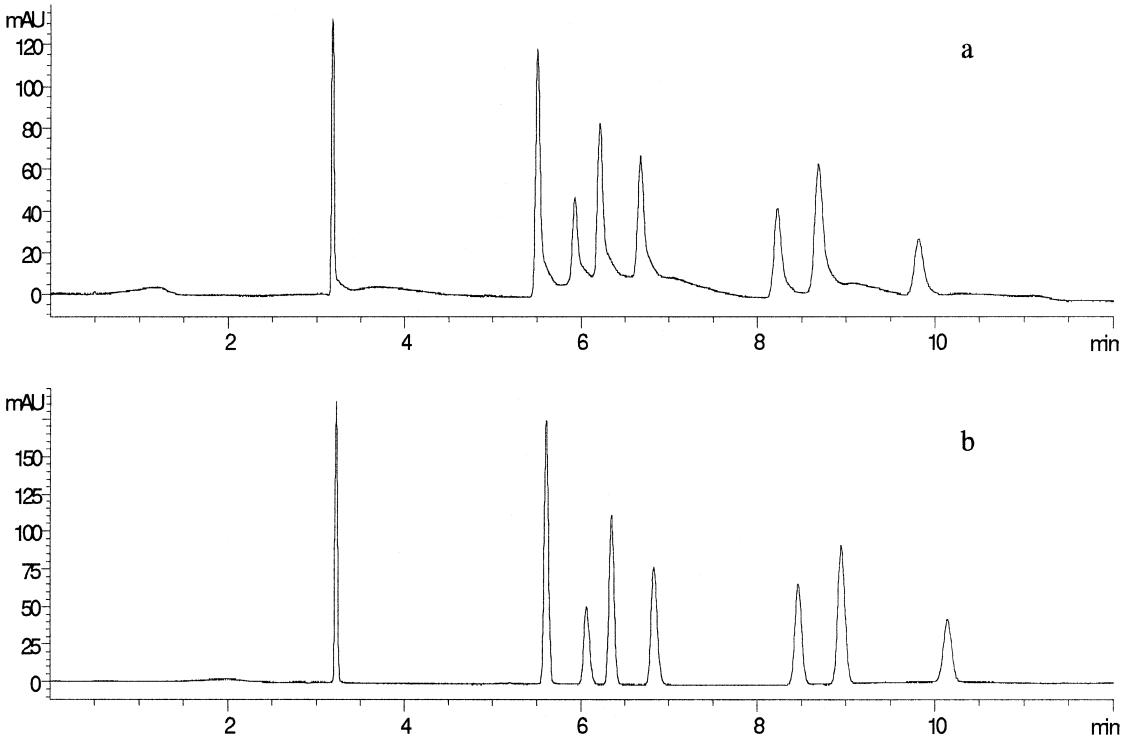

Fig. 5. (a) Formation of a void near the outlet frit results in poor peak shape. (b) Peak shape is improved once bed has consolidated under voltage. Column: Spherisorb ODS-l, 3 μm, 75 μm×25(33.5) cm. Mobile phase: 60:40 ACN:2 m*M* KH_2PO_4 (pH 3.0). Conditions: 30 kV, 10 bar, 35°C. Detection: UV DAD at 200 nm. Injection: 2 kV for 12 s. Sample: 1 mg/ml in mobile phase.

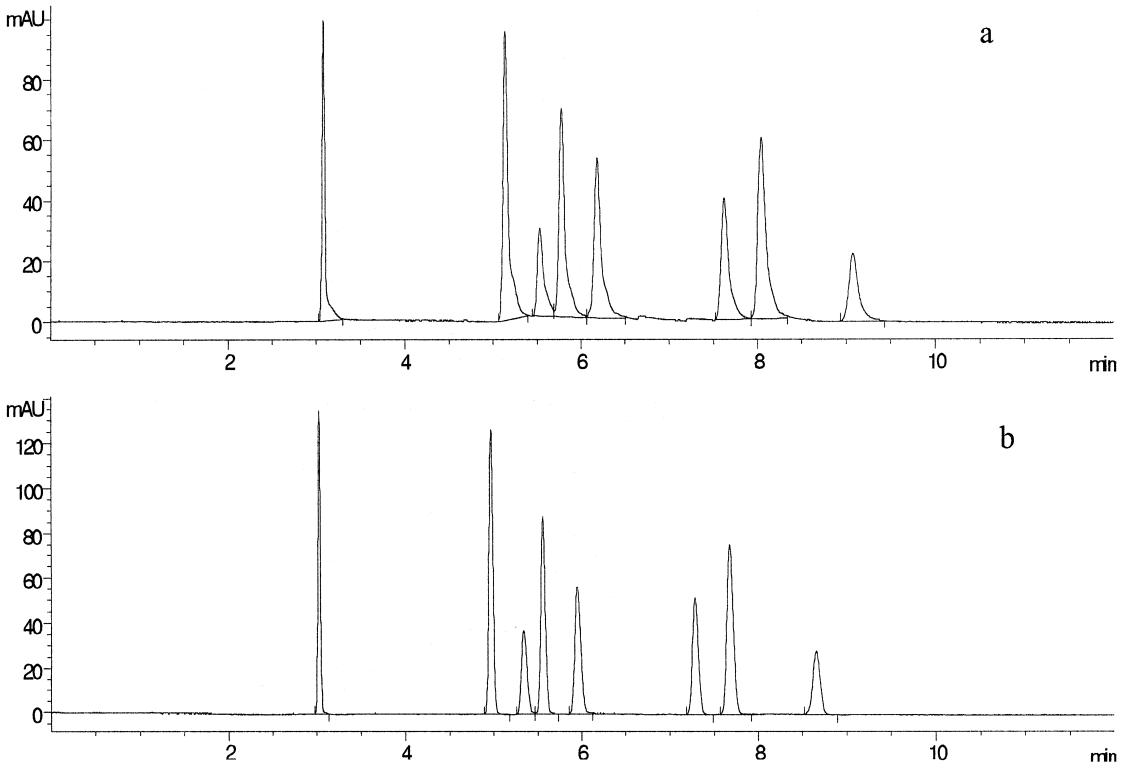

Fig. 6. (a) Peak tailing caused by a loose fragment between the outlet frit and window. (b) Peak shape is improved after removal of the loose material by applying −10 kV for 10 min. Column: Spherisorb ODS-1, 3 μm, 75 μm×25(33.5) cm. Mobile phase: 60:40 ACN:2 m*M* KH_2PO_4 (pH 3.0). Conditions: 30 kV, 10 kV for 10 min. Column: Spherisorb 3 μm ODS-1, cm. Mobile phase: 60:40 ACN:2 m*M* KH_2PO_4 (pH 3.0). Conditions: 30 kV, 10 bar, 35°C. Detection: UV DAD at 210 nm. Injection: 2 kV for 12 s. Sample: 1 mg/ml in mobile phase.

exist in the packed bed. Usually, problems as noted above are diminished as the bed is exposed to voltage over time. This is likely due to rearrangement and consolidation of the bed while under the applied potential. However, once voltage is stopped and the column becomes inactive, the bed may again change, requiring an extensive conditioning period to reproduce results once the column is employed at a later date.

4. Conclusions

When the user first initiates the preparation of packed bed capillaries, he or she must become fully familiar with the packing material and its behavior in various solvents. Observations made in this laboratory regarding the colloidal behavior of silica-based packing materials agree with results compiled from other research groups. It is hoped that these findings will stimulate more detailed investigations into packing capillaries for CEC and making the process less of an art and more of a science for all users.

Development of a theoretical understanding of the complex interaction between electrophoresis and chromatographic retention in CEC is ongoing. Currently, there are no defined methods for determination of a well packed column in CEC as there are in HPLC. It may be possible that the flow characteristics due to EOF can serve to mask some of the untoward performance characteristics of a poorly packed bed, minimizing dependence upon bed structure. Although columns reported here were considered unsuccessful when void formation was observed, the performance and efficiency were still very high. However, the existence of voids in the columns may affect the quantitative reproducibility and true performance of a packed bed. Manufacture

of well packed and void free columns is one step in the process of attaining a more complete understanding of the influence of packed bed structure upon the flow characteristics and performance in CEC.

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