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Review

Packing columns for capillary electrochromatography

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Abstract

Considering the current interest in capillary electrochromatography (CEC), performed in packed columns, we present the different methods used to pack capillary columns for use in CEC. General considerations on column packing are given and the column fabrication process is discussed in sufficient detail to allow instruction to those who are not experienced in the field. Five different packing methods are discussed to deliver packing material into the capillary column from a practical view point: slurry pressure packing, packing with supercritical CO_2, electrokinetic packing, using centripetal forces, and packing by gravity. Entrapment of particulate material by sintering and sol–gel technology is also mentioned. Although slurry pressure packing procedures are most common, higher separation efficiencies are obtained using other packing approaches. Electrokinetic packing seems to be the simplest technique to deliver the packing material into the capillary columns. Nevertheless, as with the other packing techniques, skill and experience are required to complete all the steps involved in the fabrication of packed columns for CEC.

Keywords: Reviews; Stationary phases, CEC; Electrochromatography; Packed columns; Packing procedures

Contents

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1. Introduction

Capillary electrochromatography (CEC) is a relatively new technique in the arsenal of electroseparation technology for chemical analysis. CEC can be performed in open tubes or packed structures. In the open tubular format the stationary phase is attached at the inner surface of a capillary column. For best performance, capillary tubes with an inner diameter (I.D.) of <20 μm are recommended [1]. Columns with a packed structure consist of a capillary tube in which chromatographic material is contained. We classify such packed structures into three categories: (1) columns packed with particulate material [2–30], (2) columns filled with a separation material that has been polymerized in situ, creating a ''rod-like'' structure (also called monolithic structures) [31–36], and (3) columns entrapping particulate material [37–40]. This third category is the result of combining the first two categories. By far, however, the most used columns in CEC are contained within the first category – columns packed with suitable chromatographic particulate. This can change in the future as new CEC column technology develops, particularly with the emerging approach of columns of the second category mentioned above. The chromatographic packing material that has been utilized the most for CEC is that typically used in high-performance liquid chromatography (HPLC), with reversed-phase on spherical particles (1.5–10 μm diameters) being the most popular one.

Several techniques have been used to pack the columns under our first category. Herein, we will discuss the fabrication of CEC capillary columns with packed structures of this first category, including the packing techniques used. We also discuss the third category (entrapped chromatographic material) because essentially particles are packed inside the column. Although briefly introduced in the discussion of entrapped chromatographic materials, the second category (monolithic structures) will not be discussed here, since the subject is discussed in detail in a separate article in this issue. We concentrate on the procedures that deliver particulate material into the capillary column. We do not intend to be exhaustive or inclusive. Rather, we offer an overview of the different packing procedures reported in the literature and of the column fabrication process as performed in our laboratory. We will also provide our own experiences and views regarding packing procedures as used in our laboratory when appropriate.

2. General considerations

Packing columns for HPLC has been referred to as a ''black magic technique,'' requiring a trial-and-error approach [41]. Packing columns for CEC is not an exception. At present, this is an elaborated process that requires practice and skills. Perhaps the statement of Yost et al. [42] in 1980 for HPLC applies today for CEC.

> *Packing of the column tubing with very small diameter particles requires high skill and specialized equipment. For this reason, it is generally recommended that all but the most experienced chromatographers purchase prepacked columns, since it is difficult to match the high-performance of professionally packed columns without a large investment in time and equipment.*

The integrity of the chromatographic packed bed dictates the column performance, which is ultimately controlled by the packing process. As in HPLC [43], variables affecting column packing include quality of packing material, slurry composition, packing procedure, impact velocity (i.e., velocity at which particles arrive to the accumulating bed), and the characteristics of the tube to be packed.

Several protocols have been reported for the fabrication of packed capillary columns for CEC [14,17,20,27,30,44,45]. Despite such detailed protocols, one can still consider column fabrication in CEC as an *art*. A reliable and reproducible column performance depends on the column fabrication. Columns that have been packed poorly can lead to low efficiency, poor resolution and asymmetric peak shapes. Today, the columns typically used in CEC are fused-silica capillaries with inner diameters of 100 μm or less, with 50 and 75 μm I.D. being the most popular. The small inner diameter is necessary to dissipate the heat generated by the applied electric field. As mentioned before, packing such columns is a skill that requires experience.

2.1. Packing material

Because of capillary dimensions in CEC of the column, it is important to have a narrow size distribution of the particles. This is mostly to alleviate the problems encountered while packing the column. Different packing materials of the same size can yield different efficiencies in CEC based on their packing chemistries. The effect of particle size distribution on efficiency in CEC is expected to be similar than in HPLC. Cikalo et al. [46] pointed out that different efficiencies have been reported for the separation of PAHs (''isocratic'' CEC) using different packing materials of the same sizes. These differences can in part be due to the size distribution of each material [46].

In CEC the packing material should be able to support the electroosmotic flow (EOF) not only for the neutral compounds but also for charged compounds since it is the EOF that is responsible for the bulk transport of analytes [7,16]. In the absence of EOF, the resistance offered by the packed bed will only allow the appropriate charged compounds that have really high electrophoretic mobilities to reach the detector. Several HPLC packing materials have been used to pack columns for CEC. A comprehensive list, including their applications, can be found in the review by Robson et al. [47]. The most popular materials used are the CEC Hypersil C_{18} and Spherisorb ODS I, which among the reversed-phase materials, these two seem to support the fastest EOF. These materials are HPLC supports that have not been end-capped. Consequently, a relatively large amount of silanol groups are left on the surface, which can generate EOF. Other silica-based [48–50] and non-silica based [51] materials have also been used.

2.2. The column

Most typically, a packed column in CEC consists of two segments – a packed and an unpacked (or open) section. Fig. 1 illustrates a typical CEC column with a packed and an open segment. Each segment supports different EOF velocities, as reported by Rathore and Horváth [52], resulting in a net EOF that is a combination from both segments. In most cases, an optical window is fabricated on the open segment by removing the polyimide coating on the capillary tube to facilitate detection by spectroscopic means. This can be achieved by any of the methods already in use for capillary electrophoresis (CE) [53]. Optical detection through the packing material has been reported to decrease detectability due to light scattering by the particles [18,24,27]. The packing material is kept in place by means of retaining frits (see below). Total packing of the column is possible; however, connecting tubing to a detection system is required if detection through the packed bed is not used. The potential of introducing band broadening to the system through connecting tubing exists. Nevertheless, this is an option that can be used to connect detection schemes that offer higher detectabilities (e.g., Z-cell for UV detection) or to mass spectrometric methods. In such instances, the loss in efficiency is not critical and the gain in detectability and/or structural information is far more important.

A slightly different column configuration has been used by Yang and El Rassi [54]. A segmented

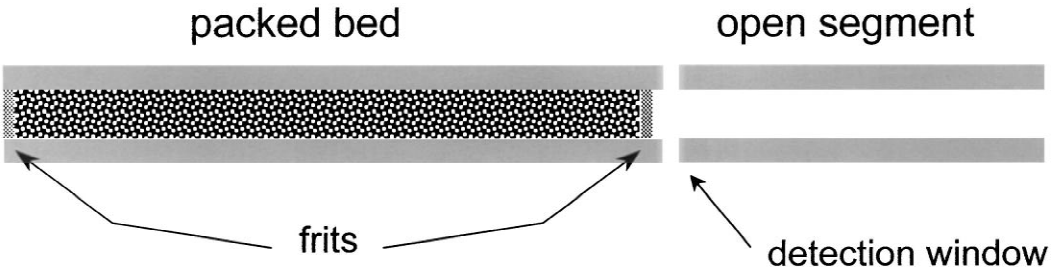

Fig. 1. Schematic representation of a typical packed-capillary column for CEC.

capillary column, possessing one segment with the stationary phase and a second segment containing bare silica, was fabricated to control the EOF. The column was totally packed. The typical open section of a CEC column was packed with bare silica, which accelerates and provides a steadier EOF. The first segment functioned as the separation column. Such a configuration allowed an increased EOF that translated into shorter analysis times.

Some of the initial work on CEC at the beginning of this decade was performed on drawn-packed capillaries [11], a procedure originally introduced by Tsuda et al. [55]. Columns of large bore (thick walled Pyrex tubing) were packed with underivatized packing material. Using a glass drawing machine, the columns were pulled at high temperatures to a desired diameter. After the columns were drawn, the stationary phase was then attached to the packing material by silane chemistry. This procedure is not commonly used since there is a low success rate in fabricating the columns.

2.3. Retaining frits

The retaining frits used to restrict the packing material to the inside of the capillary column seem to be the ''Achilles heel'' of the column fabrication process in CEC. Poppe and co-workers [56,57], for example, emphasized that the major problem in column packing seems to be the frits, mentioning the following three inconveniences. Frit fabrication causes removal of the protective column coating, making the column fragile at the frit. There is a lack of reproducibility in the manufacture of the frit. The heating applied to produce the frits changes the characteristics of the packing material at the frit position, creating non-homogeneous packing at the frit. This last problem can contribute to the non-uniformity in EOF, which can lead to bubble formation at the boundary between the frit and the unpacked segment of the capillary [52,58]. It has also been reported that placement of two frits in a capillary column (without chromatographic packing material) are flow restrictive points, reducing EOF by 35% compare to a tube without frits [54]. It was further suggested that such restriction could contaminate the EOF with a viscous flow component. High permeability, therefore, is a very desirable characteristic and the heating conditions and method used to prepare the frits will affect such a characteristic.

The retaining frits must be mechanically strong to contain the packing material and resist the pressures used to pack and/or flush the column. At the same time, the frits must posses high permeability to solvent flow. The most common approach to frit construction is by sintering silica material. Behnke et al. [27] studied the performance of columns fabricated under similar conditions but using three different frit fabrication procedures. One procedure used the method by Cortes et al. [59] where the frits were formed by polymerization of a potassium silicate solution containing formamide. Frits fabricated by this approach were the most mechanically stable. Under CEC conditions, however, the columns having these frits showed baseline and electrical current stability problems. In a second approach, the frits were constructed by sintering (using heat) a plug of silica gel wetted with potassium silicate. The columns fabricated using these frits displayed similar behavior than the previous one. In a third method, a plug of silica gel wetted with water was sintered. Columns containing frits fabricated by this method showed a stable baseline and current. However, they lacked mechanical stability with relatively large column diameters (150 μm I.D.); decreasing the column diameter to 50 μm I.D., increased the stability of the frit.

Another approach to fabricate frits is sintering the actual chromatographic material in place (if silica-based). Nowadays, this seems to be the most used approach to frit fabrication. Care must be taken, however, in order to minimize degradation of the alkylated silica that will not be part of the frit [24]. The time used for sintering, which can be reflected in frit quality, depends on column I.D., particle size and type of stationary phase material to be fritted [60]. As an alternative to frit formation, tapers have been fabricated on the fused-silica column to retain the particulate material. There are two types of tapered capillary columns that can be prepared for CEC and have been shown to have utility in coupling CEC with mass spectrometry: externally [61] and internally [62] tapered. The externally tapered columns are made using a laser-based micropipette puller, while the internally tapered columns are fabricated by sealing the end of the capillary using a high tempera-

ture flame, and then the sealed end is carefully ground to produce as small opening. The externally tapered columns are inherently fragile. The tapers are weak points on the capillary, which are prone to breakage. On the other hand, the internally tapered columns are not fragile, since only the inner diameter is reduced in dimension. The outer diameter retains its dimensions. The internally tapered capillary columns can also obviate the need for having a temporary retaining frit, facilitating column packing.

Another approach to retain the packing material in the capillary column is to use a fritless column, which posses a tapered end at the entrance and no outlet frit at all [60]. Columns fabricated using this approach, however, can only be used if the electrophoretic mobility of the packing material is larger than the EOF generated. Even so, there are problems in keeping the material inside the column. The tapered entrance is also prone to breakage.

3. Column fabrication

The fabrication of a typical packed column for CEC is schematically represented in Fig. 2 and is as follows. An appropriate piece of fused-silica capillary is selected, typically about 10 cm longer than the desired column length. Our practice is to rinse the column with a sodium hydroxide solution (~0.1 *M*) and water before used. A provisional retaining frit is fabricated at one end of the column. This can be accomplished by tapping onto a pile of wet silica material until a small section of the column is packed; then the material is sintered by heat. The amount of heat applied to form the frits depends on the column diameter and particle diameter [60] and the heating element used. Heating elements used vary from optical splicers [28,63], thermal wire strippers [28], microtorch [18], burners [54], to more sophisticated assemblies with heating elements [14].

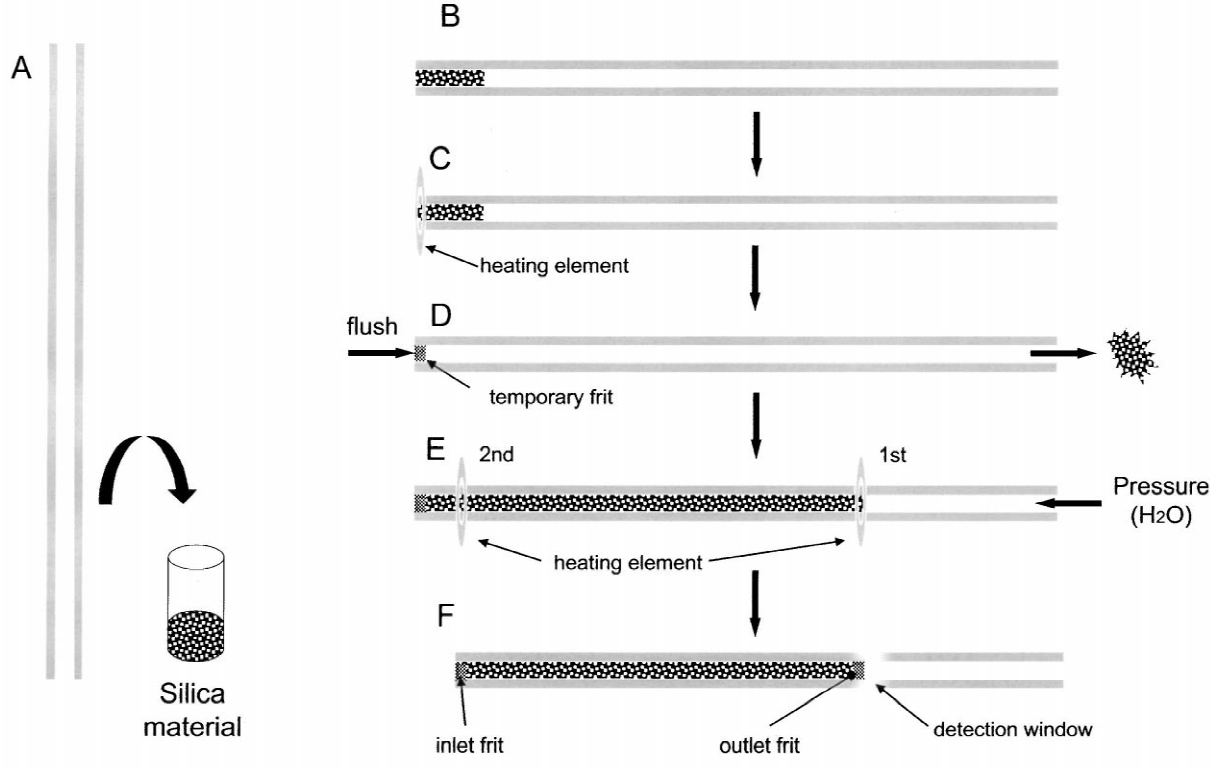

Fig. 2. Representation of the steps involved in the column fabrication processes: (A) tapping empty column into a container with wet silica material, (B) the silica material in place ready to fabricate a temporary frit, (C) formation of the temporary frit with a heating element, (D) flushing out the excess of silica material in the column after temporary frit is formed, (E) packed capillary pressurized with water to form the retaining frits with a heating element, (F) a fabricated column with frits and detection window in place.

In our laboratory, we use a relatively inexpensive soldering gun fitted with a Nichrome ribbon (1 mm thick) heating element. A small hole (~0.5 mm) is punched through the ribbon to accommodate the column diameter, allowing the column to go through the hole, which facilitates heating of a small spot at any desired point of the capillary.

After the temporary frit is formed, the excess of the material inside the column is removed by flushing. Applying pressure with an HPLC pump can test the integrity of the temporary frit. This will test if the frit is mechanically stable to resist the packing conditions; yet, it should be permeable enough to allow solvent flow. This frit will be removed eventually. Alternatively, the column can be connected to a union containing a metallic frit, which will retain the packing material inside the column during the packing process. The column is then packed (see below) to a desired length. Once packed, the column is flushed with water and while still under pressure and water is flowing, the frits are fabricated with the heating element, one at the time. At a predetermined distance from the temporary frit, heat is applied to form the outlet retaining frit. A second frit is fabricated at a desired length (typically 20–30 cm) from the outlet frit. Fabrication of frits while flowing any organic packing solvent is not recommended since carbonaceous material, presumably from the solvent, has been observed to remain at the frit [64]. The temporary frit is cut off and the excess particles inside the column are removed by flushing using a pump. Usually, the heat applied to form the outlet frit removes a portion of the protective polyimide coating of the capillary column that extends towards the open segment of the capillary, exposing a portion of the fused-silica which can serve as the optical window for detection. The column is then rinsed with well-degassed mobile phase using an HPLC pump and connected to the CEC system. In our experience, the column should be allowed to equilibrate with the mobile phase by applying voltage across the column in a stepwise approach. We condition our columns at 5kV with mobile phase, then the voltage is increased in 5 kV steps until 25–30 kV is reached. At each step, the column is allowed to equilibrate until a stable electrical current is observed.

4. Packing methods

There are several methods that can be used to deliver the particles into the capillary column for CEC. These packing methods include the use of pressure packing using slurries in HPLC solvents [14,17,20] or using supercritical CO_2 [30], using electrokinetic packing [45], using centripetal forces [65–67], and packing by gravity [68]. Capillary columns packed by slurry pressure packing and electrokinetically are commercially available.

4.1. Pressure packing using slurry

The most popular approach to pack capillary columns for CEC is using the pressure packing methods typically used in HPLC – slurry packing. The column to be packed is connected to a packing reservoir, such as a short 2 mm I.D. HPLC column or another suitable system, which is connected to a high-pressure solvent delivery pump. The column must have a means to retain the packing material, as explained above. The slurry containing the packing material (50–100 mg/ml) is prepared in a suitable solvent; acetone and methanol have been used the most, and placed in the packing reservoir. The slurry should be sonicated before packing. The packing material is transported into the capillary column by pressure, typically between 5000 and 10 000 p.s.i. (~350 to ~700 bar). Ultrasonication can be used to aid the packing [20]. Once the column is packed, the pump is turned off and the column is allowed to ''bleed'' until depressurized. This ensures that there is no disturbance to the packed bed by backpressure when disconnecting the column from the packing reservoir. After packing, the frits are formed as mentioned above. The column is rinsed with the mobile phase by pressure and further equilibrated with mobile phase while applying the electric field.

4.2. Using supercritical CO_2 as the carrier

Supercritical CO_2 has been used to pack capillary columns for HPLC and supercritical fluid chromatography (SFC) [69,70]. Robson et al. [30], evaluated the method to pack columns for CEC. To pack a capillary column, one end of the capillary is attached

to a reservoir (e.g., a short 2 mm I.D. HPLC column) containing an appropriate amount of dry-packing material to be used. At the other end of the capillary, a connecting union containing a metallic frit (2 μm) is attached. The metallic frit retains the packing material during packing. Alternatively, a temporary frit can be fabricated at the end of the column. To the other side of the connecting union, a piece of small fused-silica capillary (~10 μm I.D.) is attached as a restrictor to maintain the pressures required for supercritical conditions. The column is immersed in an ultrasonic bath and sonicated during packing. The temperature of the bath is maintained above the critical temperature of CO_2 and the column is packed to a desired length at a constant pressure above the CO_2 critical pressure. For example, packing can be performed at temperatures of 60–70°C and pressures of 200–300 bar (~3000–4500 p.s.i.). The column is depressurized over a period of time (e.g., 4–5 h) to avoid disturbances to the packed bed. The capillary column is then flushed with water and the frits formed as described above. As with a slurry packing, the column is rinsed with the mobile phase by pressure and then equilibrated with mobile phase while applying the electric field.

4.3. Electrokinetic packing

An alternative to pressure packing is to pack the columns by an electrically driven flow [44,45]. A method to pack capillary columns by this approach has been developed and patented by Yan [44]. In this approach, it is claimed that the particles are driven into the capillary column by the EOF, while the column and packing reservoirs are vibrated. A slurry is prepared in a methanol–water mixture containing an electrolyte and sonicated before packing. The slurry of particles is placed in a vial maintained in a vertical upside down position. By means of a septum in the cap of the vial, the end of the capillary to be packed is inserted into the vial. An electrode is also inserted in the vial and serves as the anode. The outlet end of the capillary, with a frit in place, is inserted through a septum cap on a second vial, which also contains an electrode serving as the cathode, and is located below the first vial. Each vial is mechanically shaken to aid the packing process. This can be accomplished very simply by attaching each vial to a speaker connected to a music system. Instead, the speakers can be connected to a frequency generator. The electric field is applied gradually until reaching 30 kV and kept constant until a desired length of the column is packed. After packing, the system is turned off, the column removed and pressurized with water. Then the retaining frits are formed and the column equilibrated as stated above. One of the advantages of electrokinetic packing is that high-pressure pumps and special fittings are not required. It can be carried out as a low cost operation and allows multiple columns to be packed simultaneously.

4.4. Packing by centripetal forces

Our laboratory has introduced the use of centripetal forces to pack capillary columns [65–67]. Packing is based on the centripetal acceleration of the particles through the capillary column. The velocity of the particles is given by the sedimentation velocity [66,67]. Slurry of particles in a suitable solvent (~10–50 mg/ml) is placed in the slurry reservoir. Low viscosity solvents are preferred (e.g., acetone) since the velocity of the particles is higher using such solvents. Two columns containing temporary end-frits are attached to the reservoir. To avoid wrapping of the flexible fused-silica tubes around the central reservoir during rotation, extending arms support the columns (i.e., columns are inside the extending arms, which are made of 1 mm I.D. stainless steel tubing). Rotation of the apparatus forces the particles to move outward. The particles are carried into the column and sediment at the fritted end. The packing apparatus allows rotation speeds up to 3000 rpm using a 1.0 horsepower electric motor. Packing time is about 5 to 15 min, depending on the solvent used to prepare the slurry. For example, 25 to 30 cm length of a 50 μm I.D. capillary column is packed in 5 min at 2000 rpm using a slurry of reversed-phase packing material in acetone (10 mg/ml). Once packed, the column is submitted to the same procedure of rinsing and frit fabrication as mentioned above. This approach as well as electrokinetic packing allows packing of multiple capillaries at once.

4.5. Packing by gravity

Based on sedimentation, particles can also be introduced into the capillary column by gravitational forces [68]. This method uses gravity to transport the packing material into the column using a simple device. The packing device is a 1-ml syringe, which connects to the open end of the capillary to be packed via plastic tubing attached to the syringe needle. About 100 μl of a slurry (~10 mg/ml in acetone) previously sonicated is placed in the syringe cylinder. The syringe plunger is attached and secured in place by means of a screw top and tape to avoid slurry solvent evaporation. Capillary columns with a temporary frit at one end are filled with acetone (sedimentation is faster in low viscosity solvents) and connected to the packing apparatus. Sedimentation is allowed to proceed for a period of at least 10–12 h, replacing the slurry with a freshly sonicated one about every 4 h. After the column is packed, it is submitted to the rinses and retaining frits fabrication procedures mentioned above. Columns with inner diameter of 50 μm and length of 20–28 cm have been packed by this approach [68]. We have used this method to pack columns with packing materials from 200 nm to 3 μm. Although this packing procedure is relatively simple; it most be pointed out that relatively long times are required to fill the capillary column with the packing material. Depending on the particle size, our experience has been that it could take 12 to 48 h to pack the capillary column.

4.6. Entrapped chromatographic material

The concept of monolithic columns [31–40,71–77] is very attractive since, for one, it eliminates the problems associated with frits. One approach to monolithic columns is to polymerize certain precursors inside the capillary column. The precursors are introduced into the capillary in the liquid-like stage and polymerization is allowed to proceed to form a rod-like structure inside the capillary. The structures are porous in nature and can be of two types: rigid structures [33–36,76] or soft gels [31,32,71–75]. A detailed discussion on the fabrication of such columns is beyond the scope of this article; the subject is treated in details in a separate article of this journal issue.

An approach to monolithic columns, which we discuss here, is the one that we categorize as ''entrapment'' of chromatographic material. We discuss this approach here because in essence chromatographic particulate material is packed inside the column. However, the particles are maintained in place by entrapment, instead of using retaining frits. The packing material is retained or entrapped inside the column by means of sol–gel processing [38–40] or sintering the particles by heat [37]. One advantage of entrapped chromatographic material is that the longevity of the column improves. Thus, the problem of emptying of the column due to loss of frit, either at the inlet or the outlet end, is avoided. In addition, the possible formation of gaps within packed capillary columns can be prevented.

In the sintering method [37], a column is packed with silica particles containing the stationary phase (e.g., C_{18}) and submitted to washings (with 0.1 $NaHCO_3$ and acetone), drying (with nitrogen gas), and a heating treatment (two steps, 120°C and 360°C). In situ re-silanization is required to introduce the stationary phase that may have been damage during the process. Columns fabricated by this approach have produced theoretical plates of about 125 000 plates/m, reproducible separations, and was reported to be very stable [37]. A drawback of this approach is the multistep procedure involved in the fabrication process, which can be time-consuming, particularly with the re-attachment of the stationary phase.

Entrapment of the material has also been accomplished using silicates or sol–gel technology [38–40]. Sol–gel processing has also been used to fabricate stationary phases for open tubular CEC [77]. The entrapment of the particles via sol–gel processing has been accomplished in two ways. One procedure is to prepare a sol–gel solution (typically a mixture containing alkoxysilanes, ethanol and hydrochloric acid) to which particulate material containing the stationary phase is added, forming a suspension containing the particles [38]. This solution is introduced inside the column by vacuum. After drying, the packing material is embedded in the sol–gel matrix. As shown in the literature, columns prepared by this approach do not show the typical efficiencies seen in CEC (plates/m of 80 000 and below were reported). In addition, reproducibil-

ity in column preparation and homogeneity of the packing material inside the column seem to be a problem.

The second approach to prepare the entrapment of chromatographic material is by introducing the sol–gel solution after the column has been packed. Chirica and Remcho [39] packed the columns pressurizing a slurry of particles, while Tang et al. [40] used supercritical CO_2 to pack the columns. After packing, the sol–gel solution is forced inside the column and the column is dried. Once the entrapping solution is dried, the temporary retaining frit (if used to pack the column) is eliminated. This approach is more attractive than the previous one since higher efficiencies have been reported; efficiencies are above 125 000 plates/m. In addition, the amount of entrapment matrix is much less than the previous method and since the columns are packed before entrapment, reproducibility in packing and homogeneity of the packing material seem to be higher.

5. Conclusion

The efficiency of columns packed by the different procedures mentioned above, as reported in the literature, vary considerably. There are several factors that can contribute to this. Some of these are: the use of different packing materials (although with the same nominal particle size), experience of the analyst in making the frits, the use of different separations systems (e.g., different sizes of the illumination spot on the optical window, 200 μm vs. 2 mm), where detection is performed (after or before the end frit), and different separation conditions. These factors make it difficult to assess which packing procedure offers the best-packed column from the values reported in the literature.

In order to identify which method produces CEC columns with the best performance, the above-mentioned factors must be avoided or minimized. To this end, columns must be fabricated using the same packing material under similar conditions, with the exception of the packing procedure per se. The same analyst should perform the frits fabrication, packing and testing, using the same CEC apparatus. We have initiated a study taking in consideration all of these factors. We have acquired data (retention factors and efficiencies) from columns packed by electrokinetic packing, pressure packing (slurry and supercritical CO_2 packing), and using centripetal forces; column fabrication, packing and testing were performed by the same individual. Although preliminary, the data indicate that slurry pressure packing renders column with the lowest efficiency (Table 1). Columns packed by centripetal and supercritical CO_2 packing offered similar efficiencies, being superior to electrokinetic and slurry pressure packing. From the four methods utilized, electrokinetic packing is the simplest and easiest method to implement and use.

Thus far, it is clear that column fabrication in CEC requires skill and experience, particularly constructing the retaining frits. All the methods discussed above seem suitable to pack columns for CEC. The use of slurry pressure packing has been most common because of the established knowledge in packing columns for HPLC. However, columns packed by the other methods discussed here offer higher efficiencies; at least this has been our experience. Obviously, the use of current slurry packing procedures will have limitations for small packing materials (<1 μm). Our experience has been that such materials can be handled easier by the electrokinetic, centripetal and gravity-packing procedures, although with each technique the packing parameters must be optimized experimentally.

In general, the preference of which method to use

Table 1
Retention factors and theoretical plates for an unretained and a retained compound in columns packed by different methods[a]

Packing method	Component	Retention factor	Plates/m
Slurry pressure	Thiourea	–	86 600
	Amylbenzene	2.4	104 100
Supercritical CO_2	Thiourea	–	143 200
	Amylbenzene	2.1	179 400
Centripetal forces	Thiourea	–	181 800
	Amylbenzene	2.2	181 800
Electrokinetic	Thiourea	–	98 800
	Amylbenzene	2.3	136 700

[a] The values reported were obtained under identical separation conditions and each value is the average of six injections performed in two different columns (three injections per column) packed by each method. Data collected at optimum linear velocities (~1 mm/s). All columns were fabricated and tested by the same individual.

seems to depend on the familiarity of a particular procedure in a given laboratory. Further improvement in frit fabrication is necessary. As column technology in CEC advances, problems associated with frit fabrication will be overcome. The monolithic and entrapped structures are clear alternatives, which without a doubt, will develop further.

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