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Journal of Chromatography A, 1044 (2004) 23–52
JOURNAL OF CHROMATOGRAPHY A
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Review

Sol–gel stationary phases for capillary electrochromatography

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Abstract

A review is presented on the current state of the art and future trends in the development of sol–gel stationary phases for capillary electrochromatography (CEC). The design and synthesis of stationary phases with prescribed chromatographic and surface charge properties represent challenging tasks in contemporary CEC research. Further developments in CEC as a high-efficiency liquid-phase separation technique will greatly depend on new breakthroughs in the area of stationary phase development. The requirements imposed on CEC stationary phase performance are significantly more demanding compared with those for HPLC. The design of CEC stationary phase must take into consideration the structural characteristics that will provide not only the selective solute/stationary phase interactions leading to chromatographic separations but also the surface charge properties that determine the magnitude and direction of the electroosmotic flow responsible for the mobile phase movement through the CEC column. Therefore, the stationary phase technology in CEC presents a more complex problem than in conventional chromatographic techniques. Different approaches to stationary phase development have been reported in contemporary CEC literature. The sol–gel approach represents a promising direction in this important research. It is applicable to the preparation of CEC stationary phases in different formats: surface coatings, micro/submicro particles, and monolithic beds. Besides, in the sol–gel approach, appropriate sol–gel precursors and other building blocks can be selected to create a stationary phase with desired structural and surface properties. One remarkable advantage of the sol–gel approach is the mild thermal conditions under which the stationary phase synthesis can be carried out (typically at room temperature). It also provides an effective pathway to integrating the advantageous properties of organic and inorganic material systems, and thereby enhancing and fine-tuning chromatographic selectivity of the created hybrid organic-inorganic stationary phases. This review focuses on recent developments in the design, synthesis, characterization, properties, and applications of sol–gel stationary phases in CEC.
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Keywords: Reviews; Sol–gel; Stationary phases, electrochromatography; Electrochromatography

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doi:10.1016/j.chroma.2004.04.079

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1. Introduction

Capillary electrochromatography (CEC) is a rapidly growing area [1] in separation science. The prevailing extraordinary level of theoretical and practical interests in CEC is explained by the fact that CEC effectively combines inherent advantages of two major separation techniques: capillary zone electrophoresis (CZE) providing high separation efficiency and HPLC (providing tunable selectivity and remarkable versatility in separation), and may potentially become a viable alternative to HPLC, micro HPLC and CZE [2]. While CZE separation is based on differential electrophoretic migration rates of charged analytes (CZE is not applicable to the separation of uncharged analytes) in an electric field, and HPLC separation relies on differential strengths of molecular level interactions offered by the stationary and mobile phase systems toward the analytes, CEC effectively combines both of these separation mechanisms and thereby provides efficient separations for both charged and uncharged solutes. The surfactant-free nature of CEC mobile phases, combined with low mobile phase flow rates used in CEC makes the technique ideally suited for hyphenation with mass spectrometry [3]. In this respect, CEC enjoys a significant advantage over micellar electrokinetic chromatography (MEKC) [4] that uses surfactant-based micellar solutions as a pseudo-stationary phase in an electrically driven separation mode to achieve high-efficiency liquid-phase separation of uncharged analytes. The presence of surfactants in the eluting liquid phase causes compatibility problems, and presents a serious hurdle to the hyphenation of MEKC with mass spectrometry.

Unlike HPLC, CEC does not require high-pressure pumping systems for mobile phase delivery. The typical high-voltage operation in CEC is associated with electroosmotic pumping mechanism that serves as the driving force for the mobile phase flow through the CEC column. The possibility of pressure-free operation in CEC provides the technique with some significant advantages over HPLC, and makes CEC operation practically free from particle size-, column length-, and available maximum pressure limitations inherent in HPLC. Thus, CEC opens up real possibilities to achieve extremely high separation efficiencies and column performances in liquid-phase separation. However, materialization of this great potential of CEC will require effective solution of a number of problems – mostly in the area of stationary phase development and column technology. In CEC, the role of the stationary phase is two-fold: (a) like in HPLC, it selectively interacts with various types of solute molecules causing them to acquire differential rates of migration through the column, and (b) the CEC stationary phase also facilitates the generation of electroosmotic flow (EOF) the driving force for mobile phase movement through the column. The magnitude and direction of EOF within the CEC column is determined by net amount and sign of the electric charge on the stationary phase surface. As a mobile phase propulsion mechanism in CEC, EOF finds its origin in the electrical double layer formed at the interface between the stationary and the mobile phase systems. Therefore, the design, synthesis, and evaluation of stationary phase in CEC presents a more complex problem than in conventional chromatographic separation techniques like GC or HPLC. Because of its high separation efficiency, CEC has been termed by Knox [5] as “the high-resolution liquid-phase analog of capillary gas chromatography”. It provides a unique separation methodology [6–11] that has been successfully used to separate a wide variety of analytes [12–16] including polycyclic aromatic hydrocarbons, [17–21] carbonyl compounds, [22,23] phenols, [24,25] acidic [26,27] and basic analytes, [28–30] chiral compounds, [31,32] environmental pollutants, [33–35] explosives, [36,37] pesticides, [38–39] herbicides, [40–42] natural products, [43] lipids, [44,45] vitamins, [46,47] illicit drugs, [48–50] pharmaceuticals, [51–56] amino acids, [57–59] peptides and proteins, [60–62] carbohydrates, [63–64] nucleic acids, [62,65] and a host of other important samples [66–69]. In addition to the applications in separation, CEC columns have also been used as a means for on-line preconcentration of various samples [70–74].

A number of factors may affect the performance in CEC. These include the composition, surface charge, and structural characteristics of the stationary phase, chemical make-up and concentration of the running buffer (mobile phase), the magnitude and direction of the applied electric field, nature of the solutes and the sample matrix, capillary temperature, and the detector characteristics. In CEC, like in all other chromatographic techniques, stationary phase is perhaps the most important element directly responsible for the physical separation of analytes. This is explained by the two important functions carried out by the stationary phase in CEC. First, it provides suitable medium for the distribution of analytes between itself and the mobile phase. Second, the stationary phase provides the surface charge

facilitating the formation of the electrical double layer—the physical basis for electroosmotic pumping of the mobile phase through the column under high-voltage operation. Since stationary phase is the key to achieving enhanced performance in CEC, scientific research involving development of superior stationary phases has been drawing a lot of attention worldwide.

In separation science, stationary phase and column are two closely related concepts, the column being the tubular separation chamber in which a chromatographically active material system, the stationary phase, may be secured in different formats: as a particle-packed bed, a porous monolithic separation medium encapsulated within the tubular chamber, or a thin coating on the inner surface of the tube used to prepare the column. Accordingly, the columns used in CEC can be simply classified into three categories (1) packed columns, (2) monolithic columns, and (3) open-tubular columns. To avoid excessive Joule heating during high-voltage operation CEC columns are prepared using small-diameter tubing (typically 25–100 μm i.d. capillaries).

A number of review articles have been published on the subject of column technology and stationary phases in CEC [75–83]. Various techniques have been developed to prepare the stationary phases in which the chromatographically active ligands are either physically or chemically attached to the substrate (capillary wall or the support material). Perhaps, the simplest of these approaches is the dynamic coating procedure which is used for unmodified packings [84]. By this method, the stationary phase is attached to the surface of capillary or packing materials merely by the physical forces of adsorption. Although simple to prepare, such stationary phases often suffer from insufficient stability. A significant improvement in stationary phase stability can be achieved by using methods involving chemical bonding of chromatographic ligands to the support material. Stationary phases prepared by chemical methods can withstand harsher experimental conditions such as temperature, pH, and organo-aqueous solvent systems that are common in CEC. A number of procedures have been employed to prepare chemically bonded stationary phases. These include methods based on surface derivatization using silane chemistry, organic polymerization-based techniques, and the sol–gel approach providing organic–inorganic hybrid sol–gel stationary phases. A careful look at the published papers devoted to the fabrication of CEC stationary phases over the last decade reveals that sol–gel technology is a rapidly growing direction in CEC stationary phase research and development. Over the years, the number of publication on sol–gel based stationary phases in CEC has steadily increased. This is especially obvious since the beginning of the new century.

In this review, we summarize the advances made in the area of scientific research devoted to the design, synthesis, characterization, and application of sol–gel stationary phases for various CEC column formats, including packed columns, monolithic columns, and open-tubular columns. The advantages of sol–gel stationary phases are pointed out. Although silica-based sol–gel CEC stationary phases constitute the primary emphasis of this review, other non-silica material-based sol–gel stationary phases are also covered to some extent, considering the infancy of those areas.

2. Sol–gel technology

2.1. Historical background

Sol–gel technology provides a versatile approach to the synthesis of inorganic polymers and organic–inorganic hybrid materials. Its existence can be traced back to mid 1800s [85]. Almost one century later, this technology was used in the glass industry by the Schott glass company in Germany [85]. Since sol–gel processes can occur under extraordinarily mild conditions (often at room temperature), and can be used to obtain products of various shapes, sizes, and formats (e.g., monoliths, films, fibers, and monosized particles) sol–gel technology has found ever increasing applications in a diverse range of scientific and engineering fields, such as ceramic industry [85], nuclear-fuel industry [85], and electronic industry [85]. The inherent advantages of sol–gel process are summarized in Table 1 [86]. It can be noticed that some of these advantages bring significant promise to further development chromatographic stationary phases.

The use of sol–gel technology for the creation of chromatographic stationary phases started only very recently. In 1987, Cortes et al. [87] reported the sol–gel technology to create monolithic ceramic beds within small-diameter capillaries and used such capillaries as separation columns in liquid chromatography (LC). In 1993, Crego et al. [88] described a method for the in situ preparation of a sol–gel stationary phase in the form of surface-bonded coating for open tubular liquid chromatography. Using a similar method, Guo

Table 1
Some advantages of the sol–gel method that can be utilized in preparing stationary phases for CEC

	Advantage of the sol–gel process
1	Better homogeneity-from raw materials
2	Better purity-from raw materials
3	Lower temperature of preparation
4	Good mixing for multi-component systems
5	Control of particle size, shape and properties
6	Better products from special properties of gel
7	Special products such as films
8	New non-crystalline solids outside the range of normal glass formation
9	Possibility of creating hybrid organic–inorganic materials, and thereby fine-tuning chromatographic selectivity
10	Possibility to design the material structure and property through proper selection of sol–gel precursor and other building blocks
11	Possibility to achieve enhanced stationary phase stability and performance in chromatographic separations

Adapted from [86] and complemented.

and Colón prepared sol–gel based open-tubular columns for CEC in 1995 [89,90], in which C_8-TEOS/TEOS precursors were employed to prepare C_8-bonded sol–gel stationary phase coatings for open tubular electrochromatography (OTEC). The authors also used these sol–gel coated capillaries in open tubular liquid chromatography [89]. The sol–gel process was acid-catalyzed, and the sol–gel approach to column technology provided an effective means to chemically bind chromatographic stationary phases to the column inner surface. The sol–gel approach brought new promise to provide high stationary phase stability and column efficiency in liquid-phase separations. These early works stimulated further developments in the area of sol–gel stationary phases in chromatographic, [91–97] and electrophoretic separations [98–105] and sample preparation technologies. [106–109] Meanwhile, the choice of sol–gel matrix is being further extended to non-silica-based materials. Primary focus of the present review is on silica-based sol–gel stationary phases in CEC. A brief description will also be provided on the current status of transition metal oxide-based sol–gel stationary phases in CEC.

2.2. Fundamental chemical reactions in sol–gel process

Understanding the general chemical reactions involved in sol–gel process is important for proper design and production of stationary phases, since it allows the analyst to control the whole process from starting materials to the end products. Chemical reagents for the preparation of sol–gel stationary phases normally include (1) at least one precursor, which is usually a metal alkoxide $M(OR)_x$, [110] (2) a solvent to disperse the precursor(s), (3) a catalyst, which can be an acid, [111,112] a base, [113] or a fluoride [114,115] depending on the type of end products desired, and (4) water. Sol–gel processes can also be initiated by irradiation [59,105,116]. In this case, the precursors, such as methacryloxypropyltrimethoxysilane (MPTMS) are initiated by the application of UV light (e.g., 350 nm wavelength) [116]. Generally, the chemical reactions inherent in the production of sol–gel stationary phase include (1) hydrolysis of the precursor(s); (2) alcohol- or water condensation of the sol–gel-active species present in the sol solution. The sol–gel-active species may include the alkoxysilane-based precursors, partial or complete hydrolysis products of these precursors, and any other chemical species reactive to alkoxysilane, silanol, and analogous non-silica species. Fig. 1 illustrates hydrolysis and condensation of tetramethoxysilane (TMOS) as an example. The condensation process can continue leading to the formation of a three-dimensional sol–gel network that can be utilized as CEC stationary phase.

Depending on the CEC column format, these reactions are carried out under different sets of conditions using reaction vessels of vastly different dimensions. In the case of packed columns, these reactions are carried out in traditional laboratory glassware (e.g., 200 mL beaker) and the conditions

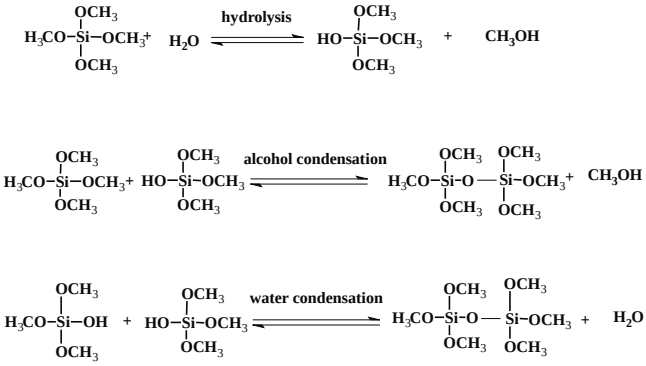


Fig. 1. Typical chemical reactions in sol–gel process.

are adjusted to create micrometer or sub-micrometer size sol–gel particles. The prepared sol–gel particles are subsequently packed inside a small-diameter capillary to prepare the CEC column. To prepare a surface-coated sol–gel open tubular column or a sol–gel monolithic column for CEC, the sol–gel reactions are carried out inside a small-diameter fused silica (quartz) capillary to in situ create the sol–gel stationary phase in the form of a surface coating or a monolithic bed. In this case, the sol–gel reactions are carried out in the confinement of a small-diameter capillary (with microliter range volume) that serves as the reaction vessel. The inner surface of the fused silica capillary, like any silica surface, contains silanol groups, participation of hydroxyl groups on capillary walls in condensation reaction with the sol–gel-active species, leads to the formation of chemical bonding between the in situ created sol–gel stationary phase and the fused silica capillary inner surface.

Performance characteristics of the sol–gel stationary phase are greatly affected by the identity and relative proportions of the components in sol–gel system as well as the reaction conditions, such as type of catalysts, temperature and reagent concentrations. It is generally agreed that acid-catalyzed sol–gel processes are more likely to produce linear branched polymers [117], while base-catalyzed processes produce highly condensed particulate structure [118]. This is because under acidic condition, the hydrolysis of alkoxide precursors undergo faster than the condensation process. On the other hand, when nucleophilic catalysts (e.g. bases) are used, condensation reaction is faster and the rate of the overall sol–gel process is determined by the relatively slow hydrolysis step. All these features enable researchers to manipulate experimental conditions to facilitate the formation of the end products with desired characteristics.

3. General procedures involved in the preparation of CEC columns with sol–gel stationary phases

As is clear from the discussion presented in the previous section, the preparation of CEC stationary phases is closely related to the preparation of CEC columns and vice versa.

Therefore, these two topics should be discussed in conjunction with each other.

Several steps are involved in the preparation of CEC columns with sol–gel stationary phases. The preparation procedures vary depending on the types of the columns and the intended applications. They include pretreatment of the capillary, fabrication of the sol–gel stationary phases, and the post-gelation treatment of the CEC stationary phases.

3.1. Pretreatment of the capillary

The purpose of capillary pretreatment is to increase the concentration of surface silanol groups. Since silanol groups on the capillary surface represent the principal binding sites for in situ created sol–gel stationary phases, higher concentration of these binding sites on the capillary surface would facilitate the formation of highly secured sol–gel stationary phases through chemical bonding with the capillary inner walls. Alkali solutions are used to clean the capillary surface in addition of some organic solvents [89,90]. In the reported one-step synthesis of monolithic silica column by Freitag's group [119], the pretreatment of the bare fused silica was accomplished by flushing with 1 M NaOH, then, with 0.1 M HCl, and followed by rinsing with purified water. The similar pretreatment method was used in other research groups to prepare sol–gel open-tubular [120] and monolithic columns [121–123]. Toyo'oka and co-workers [102,103,124] performed capillary pretreatment using methacryloxypropyltrimethoxysilane (MPTMS) to form an anchor onto the silicate matrix and prevent the gel from being leached out of the capillary. In Zare's group [104,105], the pretreatment procedure was simplified by mere flushing the capillary with a filling solution to wet the wall surface. No special pretreatment was necessary for the bonding of photopolymerized sol–gel monoliths on the wall. Hayes and Malik [100,101] reported the use of hydrothermal treatment of the inner surface of the fused silica capillary for the preparation of both sol–gel monolithic [100] and sol–gel open tubular [101] columns. The purpose of hydrothermal pretreatment was explained to be two-folds: cleaning the capillary inner surface and increasing the surface concentration of silanol groups to effectively anchor the in situ created sol–gel stationary phases, and it is also being used by other researchers [125,126].

3.2. Sol solution ingredients for the fabrication of the sol–gel stationary phases

The typical major components in the sol solution include precursor(s), a solvent system, a catalyst and water. However, the actual operations for creating sol–gel stationary phases involve the use of various additives to provide the desired end products. In the sol solution, a porogen is often used, especially in creating a porous monolithic bed. Porogens play a dual role: they serve (a) as a thorough-pore template and (b) as a solubilizer of silane reagent. A porogen is used to create desired morphologies with intended permeabilities and surface areas in the construction of monolithic columns. Toluene was found to be a suitable porogen for photopolymerized sol–gel monoliths for CEC [105,116]. A water-soluble organic polymer, poly(ethylene oxide) (PEO) was used as a porogen by Breadmore et al. [127] and Tanaka and co-workers [128]. Fig. 2 shows the structural differences in sol–gel silica monoliths prepared by using PEO of different molecular weights as the porogens. The use of M_r 10 kDa PEO resulted in a much more closed gel structure with a smaller percentage of pores in the μm size (Fig. 2A) than gels created using M_r 100 kDa PEO (Fig. 2B).

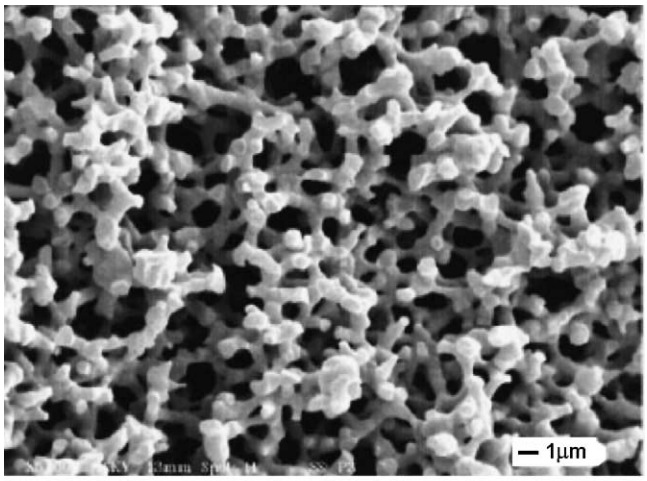


Fig. 2. SEM of silica monoliths created using: (A) M_r 10 kDa PEO; and (B) M_r 100 kDa PEO. Adapted from [127].

A structurally related polymer, polyethylene glycol (PEG), was used as a porogen by Norris and co-workers [129] and Schmidt and co-workers [130] to adjust the size of through-pores in the sol–gel monolithic stationary phases. In order to get a fine-tuned porosity of the mono-

liths, the water amount in sol system should be carefully controlled. Constantin and Freitag [119] found that there is an optimum content of water (approximately 200%) that facilitates the formation of uniform porous monoliths. These authors observed no significant microstructure developments (pores) in the monoliths when the content of water was much less than 200%. On the other hand, with water content larger than 300%, sol–gel beads with broad distribution and blocks of non-macroporous structures were formed. Denser monolithic beds are less permeable and higher pressures are needed to drive liquid flow through. Superficially, column permeability may seem irrelevant in CEC separation since EOF serves as the driving force in a CEC to propel the mobile phase through the column without requiring mechanical pressure. However, columns with high permeability provide some significant advantages especially in pressure-assisted CEC operation and in sample injection or quick flushing of the capillary during column regeneration or equilibration [131,132]. The macroporous monolithic structures facilitate the mobile phase flow through the pores, and thereby, promotes effective solute/stationary phase interaction by bringing them together. Effective solute transport mechanism operating within this monolithic structure due to mobile phase flow through the macropores together with the flat flow profile of EOF leads to high speed and separation efficiency in CEC.

Deactivation reagents represent another important type of sol solution additives used to derivatize residual silanol groups on the stationary phase, and thereby reduce harmful adsorptive effects of the latter on CEC separation. Hayes and Malik [100–101] reported the use of phenyldimethylsilane (PheDMS) as a deactivation reagent for both open-tubular and monolithic sol–gel columns. The deactivation reagent reacts with the residual silanol groups on the stationary phase resulting in the reduction of chromatographically harmful adsorption sites on the stationary phase. The effect of the deactivation was evaluated by the comparing the column performance obtained on columns prepared with and without the addition of deactivation agent.

3.3. Post-gelation treatment of CEC stationary phases

The purpose of post-gelation treatment is to minimize or eliminate the volume shrinkage during the fabrication of sol–gel stationary phase, especially for sol–gel monoliths. Post-gelation treatments in the construction of a sol–gel stationary phase include aging, drying, conditioning, and cleaning. Various techniques have been developed to accomplish these tasks. In the case of open-tubular columns, organic solvents were used to flush the sol–gel stationary, followed by equilibrating the columns with running buffers [89,90,120]. Zare and co-workers [105,116,133] reported their post-gelation treatment protocol for the production of PSG monolithic columns. The PSG capillary was first washed with ethanol using pressure, and then conditioned with the running buffer for 5 min using a syringe, followed by further conditioning with the separation buffer using pressure or electroosmotic flow. Zou et al. [134] found aging under moist conditions at lower temperatures benefits the encapsulation of biological macromolecules. While an accelerated rate of aging can be achieved under higher temperature, accelerated aging process may lead to cracks on the dried gel.

3.4. Characterization of sol–gel stationary phase

Various techniques have been used to investigate the properties of the organic–inorganic hybrid materials created through sol–gel process. These include scanning electron microscopy (SEM), atomic force microscopy (AFM), transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FT-IR), fluorescence spectroscopy, nuclear magnetic resonance (NMR), attenuated total reflectance (ATR), X-ray photoelectron spectroscopy (XPS), etc. These and other techniques were employed to elucidate the morphology of the created sol–gel stationary phases and the presence of characteristic chemical bonds between various atoms within these phases.

3.4.1. The morphology of sol–gel stationary phases

Scanning electron microscopy (SEM) is a powerful tool to study surface characteristics and fine structural details of a wide range of micro-objects that serve as SEM samples. In SEM a very fine electron incident beam is scanned across the sample surface producing an image with great depth of field and an almost three-dimensional appearance. With this feature, SEM is the most widely used technique to evaluate the morphology of a sol–gel stationary phase. Usually, SEM images of cross sectional view of the prepared sol–gel capillary column are used to illustrate the structural characteristics of the fabricated sol–gel stationary phase, its adherence to the capillary surface, integrity of the sol–gel structure, the porosity of the sol–gel material, and the distribution of the pores in the stationary phase structure. [59,100,101,104,105,116,119,121–123,127,128,135,136]. In the case of sol–gel surface-coated open tubular columns, SEM can reveal the uniformity of coating thickness and structural defects therein. It can also be used to study effects of various experimental parameters on the structure of the created sol–gel stationary phase. For example, the SEM micrographs published by Zare's group [116] clearly showed the effect of catalyst concentration on the formation of the sol–gel matrix as well as the durability of the PSG monolithic column. In order to show the structural information for the whole skeleton of the sol–gel material, Tanaka used both cross sectional and longitudinal SEM images [137].

In addition, atomic force microscopy (AFM) is also used to investigate the topographical imaging of the sol–gel modified capillary [134]. Meanwhile, Almeida et al. [138] used extended X-ray absorption to study fine structure and near-edge structure of silica–titania sol–gel films. Trans-

mission electron microscopy (TEM) was used by Yan et al. [139] to characterize the magnesium silicate thin films obtained through sol–gel technique.

3.4.2. Study of the chemical bonds within sol–gel structure

To study the chemical bonds in sol–gel structure, various techniques, such as Fourier transform infrared (FT-IR) [139–141], fluorescence [140] and nuclear magnetic resonance (NMR) [140,142–144] have been used. FT-IR is one of the commonly used spectroscopic methods for studying polymers. Since spectra can be scanned, recorded, and transformed in an extremely rapid pace, this technique enables the study of sol–gel process in its progression with time. Toyo'oka and co-workers [145] used attenuated total reflectance (ATR) FT-IR hybrid technique to monitor the content of residual silanol groups in sol–gel material as the gelation process progressed. Zuo et al. [134] used the FT-IR technique to confirm the encapsulation of bovine serum albumin (BSA) in the sol–gel matrix. IR technique was used by Zeng and co-workers [146] to characterize the capillary modified with macrocylic dioxopolyamine stationary phase. The typical IR absorptions of NH stretching (3303 cm^{-1}), NH bending (1560 cm^{-1}), C=O stretching (1652 cm^{-1}), and CH stretching (2866–2933 cm^{-1}) obtained from the modified capillaries provided the evidence for successful preparation of dioxo[13]aneN4—modified capillary for open-tubular capillary electrochromatography.

To investigate the esterification reaction between stearic acid and the epoxy groups of glycidoxypropyltrimethoxysilane, Zhao et al. [120] used X-ray photoelectron spectroscopy (XPS). The obtained XPS spectrum provided evidence on existence of carbon in the reaction product indicating to the success of the on-column octadecyl silylation reaction. Consequently, the C_{18} group from the stearic acid, intended to act as the stationary phase, was confirmed to be chemically bonded to the sol–gel matrix.

Nuclear magnetic resonance (NMR), another powerful analytical technique, was used by Rodriguez and Colón [147] to investigate the species present in the sol–gel solution used to modify the inner surface of an open tubular CEC column. It is established that in a sol–gel solution containing more than one precursor, a homogenous hybrid system is usually formed if the monomeric precursors undergo hydrolysis reactions at similar rates. However, if one of the precursors has much faster rate for the hydrolysis reaction leading to pronounced self-condensation [148], a heterogeneous composite will be produced. Since the properties of the final sol–gel columns can be indicated by the species present in the sol–gel solutions prior to the coating process, it is very important to understand the characteristics of the sol–gel solution in details. The C_{18}-TEOS/TEOS sol–gel system was studied by ^{29}Si NMR [147]. The acquired spectra indicated the reactions of C_{18}-TEOS were increased when reacting in the C_{18}-TEOS/TEOS hybrid system, and the maximum degree condensation was achieved within 2 h.

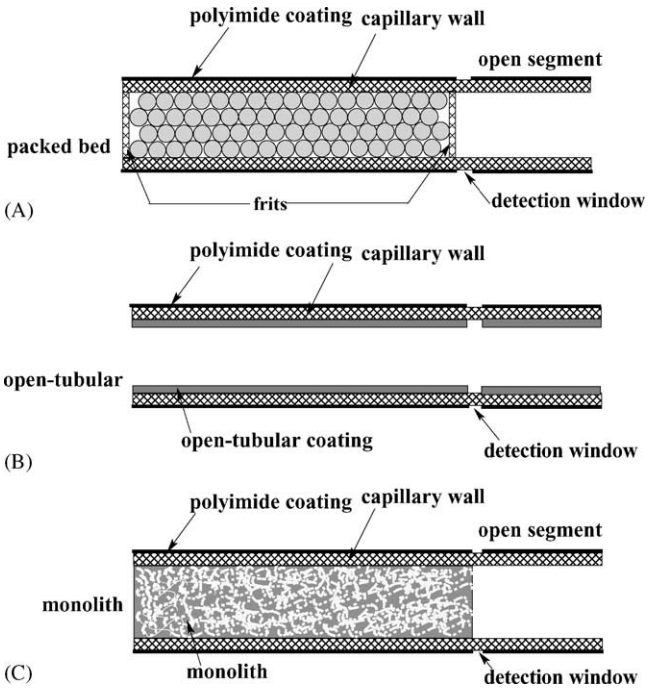


Fig. 3. Schematic representations of three different types of columns used in CEC: (A) a typical packed-capillary column for CEC (adapted from [77]); (B) an open-tubular capillary column for CEC; and (C) a monolithic capillary column.

4. Sol–gel technology for silica-based packed columns in CEC

CEC is characterized by outstanding efficiency in liquid-phase separations, and can be operated in packed [59,97,149–173], open-tubular [89,90,101,105,125,131,146,174–187], and monolithic columns [58,70,100,104,116,121–124,126,128,133,145,188–198]. Fig. 3 shows the structural detail of these three kinds of columns used in CEC.

Analogous to HPLC, columns packed with stationary phase are widely used in CEC. There are several techniques being used to pack the columns. As summarized by Colón et al. [199], these techniques include: (1) pressure packing using slurry; (2) slurry packing using supercritical CO_2 as the carrier; (3) electrokinetic packing; (4) packing by centripetal forces; (5) packing by gravity; and (6) entrapped chromatographic material. Fig. 4 shows the steps involved in the packed capillary column fabrication. In packed columns technology, the most problematic step is the preparation of the frits, which retain the packing materials in place within the column. Desirable properties for good frits include adequate mechanical strength of the used material, batch-to-batch consistency in porosity and permeability.

In packed column technology for CEC, there are three distinct areas, where sol–gel technique has been used. These are: (1) preparation of micrometer and submicrometer size sol–gel particles to be used subsequently for creating the stationary phase bed within the capillary, (2) creation of

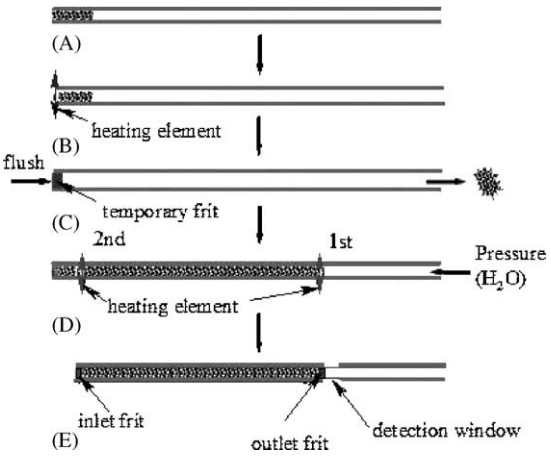


Fig. 4. Representation of the steps involved in the packed column fabrication processes: (A) the silica material in place ready to fabricate a temporary frit; (B) formation of the temporary frit with a heating element; (C) flushing out the excess of silica material in the column after temporary frit is formed; (D) packed capillary pressurized with water to form the retaining frits with a heating element; and (E) a fabricated column with frits and detection window in place. Adapted from [77].

sol–gel frits in packed columns and (3) preparation of packed columns with sol–gel entrapped chromatographic packing material. The purpose of sol–gel technique in the packing process is to "entrap" the packing material and avoid the use of frits. CEC columns filled with spherical particles or particle aggregates resulting from sol–gel processing of a sol solution are included in monolithic columns category, and will be discussed in a later section.

4.1. Sol–gel frits for packed columns

Retaining end frits commonly used in CEC and HPLC packed columns are meant for keeping the particulate packing material inside the capillary. To minimize the frit contribution to peak dispersion, on-column frits are more commonly used in CEC packed columns. Traditionally, on-column frits are produced by sintering silica-based packing materials by heating a short segment of the packed bed with a flame or applying low-voltage resistive heating for a short period of time. Consequently, the particles of the packing material in this segment become connected with neighboring particles and/or the capillary wall at their contact points to form a permeable barrier and retain the stationary phase. However, this procedure puts a high thermal stress on the protective polyimide coating of the fused silica capillary external surface and the stationary phase; it may lead to some problems like fragility, variable permeability, and destruction of the chemical bonds in the frit region, etc. [156–158,200]. To solve this problem, alternative methods have been developed to either avoid the use of frits or by other techniques that are available under relatively mild conditions. Several methods have been used to avoid frit making, these include the use of in-line filters [155], restrictors [201], or drawing the capillary out to a fine taper [201,202]. Additionally, silica-entrapped columns are used to avoid the use of frits as well.

Since sol–gel process can occur under mild conditions, it has been used in producing frits for CEC by some research groups. Thus, polydimethoxysiloxane (PDMOS) sol–gel frits have been produced and investigated by Schmid et al. [150]. Those authors used a 20% PDMOS sol solution in methanol to treat about 0.2–1 mm long segment of the packing bed repeatedly. Participation of the PDMOS molecules and their hydrolysis products in the condensation reactions with the available silanol groups on the neighboring packing particles and/or the capillary inner surface led to the creation of sol–gel frits due to the formation of chemical links between (a) the neighboring particles and (b) capillary wall and the packing particles adjacent to it. Optimum manufacturing conditions were determined by comparing the performances of the frits made under different conditions. Meanwhile, specific permeability and mechanical stability of the frits were measured and tested. The test results showed this method is capable of producing frits with high permeability and good mechanical stability. Finally, the packed capillary columns with sol–gel PDMOS frits were used to separate mixtures nitroaromatic compounds and aminonitrobenzenes. The resulting electrochromatograms indicated a negligible influence of sol–gel frits on the detector performance and the column efficiency. Tetraethoxysilane (TEOS) is another precursor molecule that has been used to create sol–gel frits [97]. The TEOS sol–gel frit preparation method used by Channer et al. was based on a protocol introduced by Zare and co-workers [159] for the preparation of porous monolithic capillary columns loaded with chromatographic particles. According to this protocol, a sol–gel monolithic bed was produced from a sol solution containing TEOS, ethanol and hydrochloric acid. This sol solution was further mixed with the chromatographic packing material to fabricate the particle loaded monolithic bed. A modified version of this method was used by Channer to prepare the retaining end frits [97]. Here, instead of mixing the sol solution with the chromatographic packing material, the sol solution was repeatedly applied to a 1–2 mm end segment of the packed capillary. For this, the capillary was first filled with the packing material and then the packed end segment was repeatedly dipped into the prepared sol solution that was allowed to be drawn into the packed end segment due to the capillary action. This process was carried out at room temperature. After hydrolysis and polycondensation, a stable retaining frit was formed due to the creation of sol–gel connecting bridge between the neighboring particles as well as the particles and the capillary wall. A series of experiments were designed to make a comparison between the sol–gel frits and conventional frits prepared by hydrothermal treatment. Table 2 shows the comparison of these two types of retaining end frits.

From Table 2, we can see the influence of sol–gel frits on mobile phase linear velocity (and hence on EOF), retention factor and selectivity of the capillaries was ignorable. How-

Table 2
Comparison of standard hydrothermal frit technology with that of TEOS sol–gel/coupled capillary approach using the 3 μm Hypersil CEC C_{18} material

CEC peak characteristics	Frit type ($n = 3$)	
	Hydrothermal	TEOS
Biphenyl efficiency (mean plates m^{-1})	194600	181000
Selectivity (mean $\alpha_{anisole/benzamide}$)	2.95	3.02
Linear velocity (mean mm s^{-1})	1.02	0.96
Biphenyl symmetry (mean)	0.98	0.87

Adapted from [97].

ever, there was approximately 7% loss in efficiency with the sol–gel frit. It is noticed that the peak symmetry obtained on CEC capillaries with sol–gel glued frits was less than that obtained on conventional ones prepared by hydrothermal treatment. This loss of peak symmetry was attributed to the peak dispersion effect due to the introduction of the coupling in the detection methodology rather than to the use of TEOS frits.

Zhang and Huang [151] developed a method for the preparation of end frits for packed CEC columns using methyltriethoxysilane (METS) in conjunction with an on-column detection window. The sol solution included METS as the precursor, trifluoroacetic acid (TFA) as the catalyst, methylene chloride as the solvent, and water. To the uniform sol solution, silica gel particles were added to form a suspension. A plug of this suspension (several millimeters in length) was drawn into a piece of empty pretreated capillary. This formed the outlet frit after gelation reaction. The capillary was then packed with ODS-bonded particles. This step was followed by the creation of a second frit at the capillary inlet using the repeat dipping technology. An on-column detection window was made by removing a segment of the protective polyimide coating from the outer surface of the capillary. The sol–gel frits were proved to possess good mechanical strength, and high pH and solvent stabilities.

As mentioned in previous sections, sol–gel process can be either catalyst-initiated or photo-initiated. The preparation method for packed columns with photopolymerized sol–gel frits was reported by Kato et al. [59]. A sol solution comprising 3-(trimethoxysilyl)propylmethacrylate, hydrochloric acid, water, toluene and Irgacure 1800 was injected into a piece of fused silica capillary. A UV light was applied through a 3 mm segment for 5 min, where the polyimide coating was removed before the injection of sol solution. After an opaque and porous frit was formed, a 15 cm segment of the column was packed using silica particles with a bonded chiral stationary phase. Finally, a second photopolymerized sol–gel frit was made at the capillary inlet using the same method as for the outlet frit. Since the sol–gel process occurs only upon the application of UV light, the reaction can be well controlled. However, the necessity to remove the protective polyimide coating from the outer surface of capillary at the fritted segments is likely to make these segments vulnerable to mechanical breakage. SEM image of the formed sol–gel frits showed a porous structure of the outlet frit, which allowed the passage of analytes and liquid but not the particles of the chiral stationary phases. The chiral stationary phase packed column was used to perform enantiomeric separations. The experimental results obtained by these researchers suggested that chiral packed column provided greatly improved separations for the studied enantiomers compared with those obtained on a particle-loaded monolithic column. The improved separation efficiency and resolution of amino acids in the packed column over particle-loaded monolithic column was explained based on the availability of more interaction sites between amino acids and chiral stationary phase particles. In the particle-loaded monolithic column, some of those active sites were likely to be shielded by the sol–gel matrix. Another possible explanation was as follows: during the sol–gel process, some small gaps or cracks could be formed when the solvent evaporated from pores of the sol–gel material during the drying process conducted by the application of heat.

Reproducibility and repeatability are two important concepts for newly developed analytical techniques. In the area of column technology, these concepts are employed to characterize both the method of column preparation as well as column performance. Generally, column-to-column reproducibility is evaluated from the experimentally determined values of one or more characteristic column parameters (e.g., retention factor, column efficiency, resolution between a particular pair of analytes, etc.) obtained on a number of columns prepared under identical conditions using the newly developed method. Relative standard deviation of the measured values for any of these parameters can be used as a measure of column-to-column reproducibility. Run-to-run repeatability is used to characterize the performance consistency of the same column over a number of chromatographic runs carried out under identical conditions. Using the same column, replicate measurements are carried out to determine one or more analytical parameters (e.g., retention time) and the relative standard deviation of these measurements is usually used to characterize run-to-run repeatability. Hayes and Malik reported the run-to-run repeatability of sol–gel monolithic columns (<0.3% R.S.D. for retention time) [100] and sol–gel open tubular columns (<0.7% R.S.D. for retention time) [101].

Recently, Piraino and Dorsey's [153] reported their research results on the performance of several types of frits, including sintered frits, photopolymerized frits, and frits made by sol–gel process. According to their findings, capillaries with sol–gel frits showed the greatest electroosmotic mobility which was 1.29×10^{-3} cm^2 V^{-1} s^{-1}, compared to 1.12×10^{-3} cm^2 V^{-1} s^{-1} obtained from sintered frit and 1.00×10^{-3} cm^2 V^{-1} s^{-1} obtained from photopolymerized frit. In addition, the capillary with the sol–gel frit exhibited the best day-to-day repeatability. Over 3 days, its R.S.D. of electroosmotic mobility was <2%. The best column-to-column reproducibility was obtained from the columns with

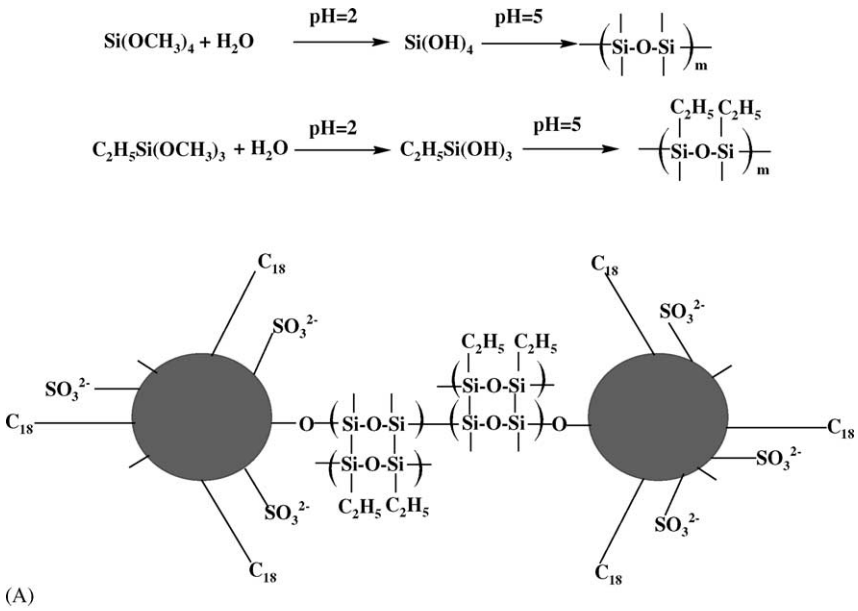
photopolymerized frits. The R.S.D. for all runs was <3%. However, its electroosmotic mobility was the slowest. The sintered frits contributed to the least amount of band broadening. Euerby and co-workers [97] studied the repeatability and reproducibility of three different sol–gel related approaches for frit production in terms of migration speed, retention time, retention factor, column efficiency and selectivity.

4.2. Packed columns with sol–gel entrapped stationary phase particles

Since the use of frits is associated with a number of problems, including bubble formation during CEC operation [154], column fragility [155], variable permeability and related shortcomings [156–158], attempts have been made to find methods to prepare fritless packed columns. Packed columns with entrapped chromatographic materials make it possible to avoid the use of frits. Sol–gel technology has been used to entrap the bonded stationary phase particles inside the capillary. The methodology developed by Zare's research group [159] involved the preparation of a sol–gel solution containing TEOS, ethanol, and hydrochloric acid followed by addition of ODS particles to create a suspension. This suspension was then introduced into the capillary by pressure. A microscope was used to ensure that a relatively uniform distribution of ODS particles occurred throughout the column. Polymerization of the sol–gel matrix due to condensation reaction led to effective entrapment of the particles within the sol–gel matrix. And the whole structure was tightly fixed onto the wall of the capillary. Good electroosmotic flow was obtained through the packed columns with sol–gel-entrapped chromatographic stationary phase. To evaluate the performance of the packed column, a mixture of aromatic and non-aromatic compounds were used. The sol–gel-entrapped packed column provided baseline separation for all the test solutes. Efficiencies of up to 80 000 plates/m were achieved in columns packed with 3 μm ODS particles. This technique was used to prepare a sol–gel entrapped particle-loaded column for the enantiomeric separation of protein- and non-protein amino acids [203]. Silica particles (5 μm) were modified with chiral selectors (*S*)-*N*-3,5-dinitrobenzoyl-1-naphthylglycine or (*S*)-*N*-3,5-dinitrophenylaminocarbonyl-valine, respectively. Enantiomeric separation was achieved using columns obtained by entrapping those particles within a fused silica capillary using a sol solution. A similar strategy has been successfully used by Ratnayake et al. [160,204] to prepare particle loaded sol–gel monolithic columns for CEC. It was further demonstrated that this method to entrap chromatographic material inside the capillary without the use of frits was suitable for CEC.

Another approach to produce packed columns with entrapped chromatographic materials by sol–gel technology was introduced by Tang et al. [161], who described a method that differed from that developed by Zare [159] in that the capillary was first packed with the chromatographic particles prior to the application of sol solution. The packing was carried out using a supercritical fluid based slurry packing technique [161]. A sol solution containing tetramethoxysilane (TMOS), ethyltrimethoxysilane (ETMOS), methanol, trifluoroacetic acid, water and formamide was then introduced into the ODS packed capillary using a syringe. After the conversion of the sol to a gel, supercritical CO_2 was used to dry the whole sol–gel bonded ODS packed column. The SEM image of a cross-section of the column showed that the ODS particles were bonded together by the sol–gel matrix and attached onto the wall of the capillary. The obtained column was tested for mechanical strength and stability. Additionally, the effects of applied electric field strength, buffer pH, buffer concentration, and organic solvent content in the buffer were also investigated. Finally, the chromatographic performance of the sol–gel bonded ODS packed column was evaluated using aromatic compounds and PAHs as test solutes. Efficiencies of up to 130,000 plates/m were obtained on 9% sol–gel bonded packed column (with 5 μm ODS particles) prepared by the described method. The asymmetry factors calculated based on these figures are less than 1.1, which means the sol–gel matrix was practically inert and no post deactivation or functionalization of the column bed was necessary. These authors also used the same method to prepare continuous-bed columns containing silica particles with mixed-mode octadecyl and propylsulfonic acid functional groups (ODS/SCX) [162–164]. Fig. 5A presents the chemical reactions involved in the formation of sol–gel "glue" and schematically illustrates how this sol–gel "glue" can establish chemical links between two neighboring ODS/SCX particles. SEM image in Fig. 5B shows two particles that are bonded to each other and to the capillary inner wall through the sol–gel matrix. Related performance tests of the sol–gel bonded ODS/SCX columns have shown suitability of the columns for CEC applications. Additionally, Roed and co-workers [165–167] used a similar method to construct continuous bed columns for retinyl esters. The packing material they used was 7 μm Nucleosil 4000 Å C_{18} and 5 μm Nucleosil 4000 A C_{30}. The prepared columns showed excellent chromatographic properties, and no bubble formations were observed during the evaluation tests. Honda and co-workers [168] filled a capillary with silica gel particles, and introduced chromatographically active stationary phase by in-column derivatization using bis[3-(trimethoxysilyl)propyl]ethylenediamine.

Another important sol–gel application in packed columns for CEC is reported by Colón and co-workers [169,205]. They applied sol–gel technique to synthesize sub-micrometer sized organo-silica spheres for CEC. In their one-step procedure, the uniform packing particles with chromatographically active C_8 stationary phase was produced by copolymerization between alkyltriethoxy-silane and tetraethoxysilane. Fig. 6 shows two different fractions of sol–gel synthesized organo-silica particles. Thanks to the judicious choice of the sol–gel precursors, the prepared



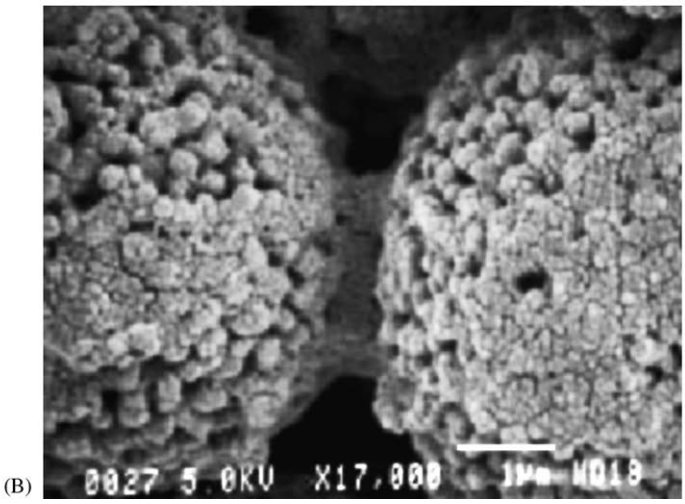


Fig. 5. (A) Synthetic scheme for the sol–gel and sol–gel bonded ODS/SCX particles. Adapted from [162]; and (B) scanning electron micrograph of continuous-bed columns containing sol–gel bonded large-pore ODS. Adapted from [163].

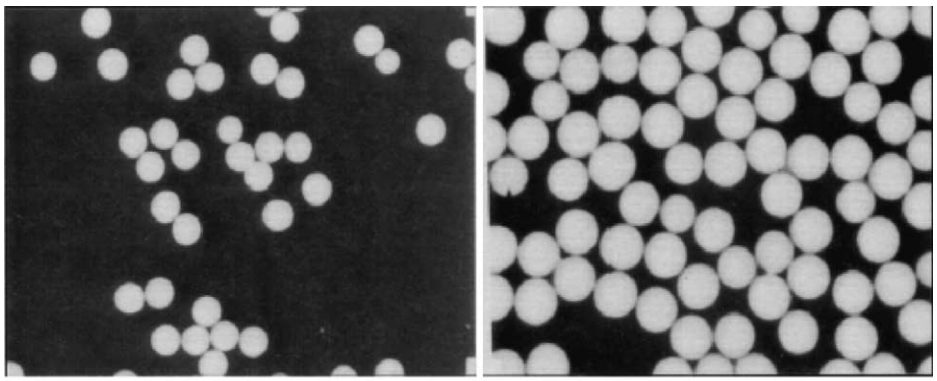


Fig. 6. TEM micrographs of two different organo-silica particles fabrication: (A) 440 nm particles magnified 10 000 times; (B) 340 nm particles magnified 20 000 times. Adapted from [169].

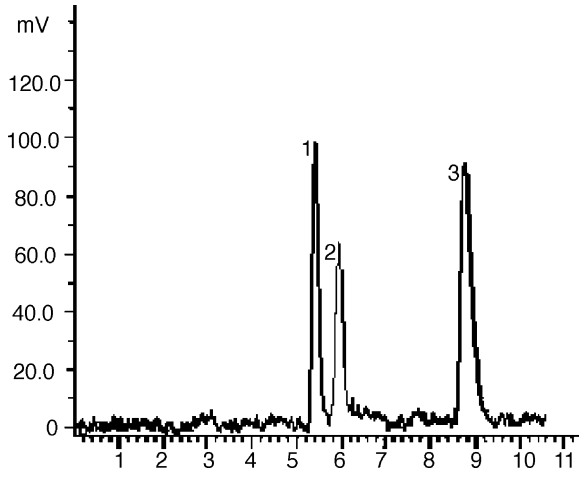


Fig. 7. Separation of some acidic pharmaceutical compounds. Stationary phase: poly(styrene-divinylbenzene)-encapsulated hybrid silica packing (350 nm). Column: 48 cm (16.5 cm effective length) × 250 μm. Mobile phase: acetonitrite (ACN)-buffer [1 mM $HClO_4$ + 5 mM 2-(*N*-morpholino) ethanesulfonic acid (MES), pH 4.06] (70:30, v/v). Applied voltage: 15 kV. Detection: 254 nm. Peak identification: (1) aloe-emodin; (2) emodin; and (3) chrysophanol. Adapted from [206].

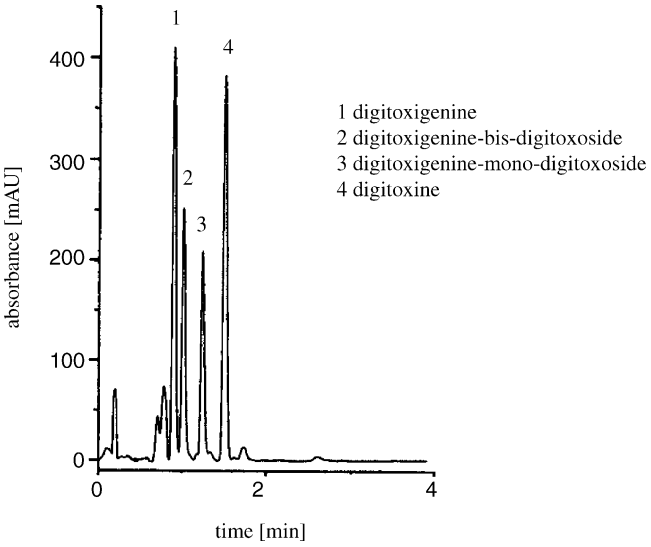


Fig. 8. CEC separation of four cardioactive substances. Capillary, 8.5 (38) cm × 10 μm, packed with 0.5 μm C_8; mobile phase, acetonitrile–12 mM Tris–HCl, pH 6 (60:40), 30 °C, −790 V/cm; detection, 254 nm UV. Adapted from [76].

sol–gel particles inherently possessed chromatographically active C_8 ligands chemically bonded to the particles. The obtained packed columns filled with these particles were ready to perform separation without requiring any additional surface derivatization step to chemically attach the alkyl ligand to the particle surface.

Xu and co-workers [206] also applied sol–gel technology to produce sub-micrometer sized organic–inorganic hybrid silica packing particles. The hybrid silica particles were prepared using TEOS and vinyltriethoxysilane (VTEOS) as precursors. The obtained packing particles were encapsulated with a layer of polymerization product between styrene and divinylbenzene (DVB). The formed stationary phase was used in CEC and the chromatographic behaviors were studied. Fig. 7 shows the electrochromatogram of a mixture of some acidic pharmaceutical compounds.

The sol–gel approach was also used by Unger and coworkers [76] to synthesize micrometer- and sub-micrometer size silica particles, considering the fact that classical technology for the synthesis of silica particles has practically reached its limit with respect to ultimate minimum particle size of about 2 μm. The obtained silica particles showed significantly higher hydrothermal stability than silica xerogels or MCM-41 type silica. Fig. 8 shows an electrochromatogram of digitoxin and related cardiotonic compounds obtained on a packed capillary column prepared by using 0.5 μm sol–gel C_8-bonded silica particles. It also illustrates the speed of CEC separation that can be achieved by using sol–gel nanoparticle-based stationary phases. The analysis was completed in less than two minutes.

Tang et al. [164] compared the efficiency characteristics for packed and continuous columns. Table 3 lists the summarized experimental results of sol–gel bonded small-pore ODS column, the packed large-pore ODS column, and the sol–gel bonded large-pore ODS column. Table 3 shows the advantages of sol–gel bonded columns

Table 3
Efficiency characteristics for packed and continuous bed columns[a]

Parameter	Packed column (7 μm, 4000 Å)	Continuous bed column (7 μm, 4000 Å)	Continuous bed column (5 μm, 90 Å) from [161]
H_{min} (μm)[b]	7.92	4.56	7.71
h_r[b]	1.14	0.65	1.54
N (plates m^{-1})[b]	1.26×10^5	2.40×10^5	1.30×10^5
V_{opt} (mm s^{-1})	0.61	1.20	1.00
A (μm)	1.63	0.091	4.72
B ($\times 10^3$ μm^2 s^{-1})	1.70	2.69	1.49
C (10^3 s)	6.56	1.86	1.50

Adapted from [164].

[a] Chromatographic conditions: 60% (v/v) acetonitrile in water mobile phase containing 2.5 mM Tris buffer at pH 8.0, 5 kV, 2 s electrokinetic injection; 254 nm UV detection. Packed column, 41 cm (effective length 32 cm) × 75 μm i.d. column containing large-pore ODS (7 μm, 4000 Å) and continuous bed column, 41 cm (effective length 32 cm) × 75 μm i.d. column containing 9% sol–gel bonded large-pore ODS (7 μm, 4000 Å).

[b] Measured with unretained thiorurea, $k = 0$.

over packed column in terms of efficiency, EOF, and mass transfer.

5. Open-tubular CEC columns with silica-based sol–gel coatings

Open-tubular columns, in which the stationary phase is bonded or spread as a coating on the inner surface of the capillary, constitute an important category of CEC columns. They represent a potential alternative to packed columns in CEC. Open-tubular columns are free from the problems caused by the frits in traditional packed columns. Fig. 9 shows the typical steps to make sol–gel open-tubular columns. In general, open-tubular columns should have thick stationary phase coating in order to provide sufficient retentive properties and sample capacity, which are usually difficult to achieve using conventional fabrication methods. Research work devoted to solving these problems include four main directions [79]: (1) using thick, immobilized organic polymer coatings to improve the column phase ratio, (2) creating etched inner surface of the capillary with bonded organic ligands to provide higher surface area and enhanced solute/stationary phase interactions, (3) using dynamic nanoparticles as pseudostationary phases, and (4) coating the capillary with sol–gel technology. Crego et al. [88], Guo and Colón [89–90] used the sol–gel approach to prepare organic–inorganic hybrid sol–gel coatings for open-tubular columns for liquid-phase separations. The CEC open tubular columns with sol–gel stationary phase coatings reported by Guo and Colón [89–90] showed enhanced surface area, improved hydrolytic stability, increased retentive characteristics. The sol–gel approach provided a much simpler column preparation procedure than traditional methods.

In open-tubular column technology, several strategies have been developed to incorporate the organic stationary phase components onto the sol–gel coating. Chemically bonded alkyl groups, such as C_6-, C_8,- C_{16}-, and C_{18}- [89,90,131,174–177] are the most commonly used organic stationary phase components suitable for the reversed-phase separation of uncharged polycyclic aromatic hydrocarbons, aromatic ketones and alcohols. Accumulated research results show that in the case of alkyl bonded stationary phase, the separation quality generally improves with increasing alkyl chain length [131]. Since some precursors with different alkyl groups are commercially available, e.g. hexyltriethoxysilane for C_6, hexadecyltrimethoxysilane for C_{16}, octadecyltrimethoxysilane for C_{18}, and so on. The easiest way to create a sol–gel stationary phase with a desired organic ligand is to use an appropriate sol–gel precursor that carries the same organic ligand as the substituted side group. In this case, another alkoxysilane monomer acting as a co-precursor is usually chosen to facilitate the formation of organic–inorganic hybrid sol–gel network. The steps used for the preparation of sol–gel stationary phases for open tubular CEC columns are described in Section 3.

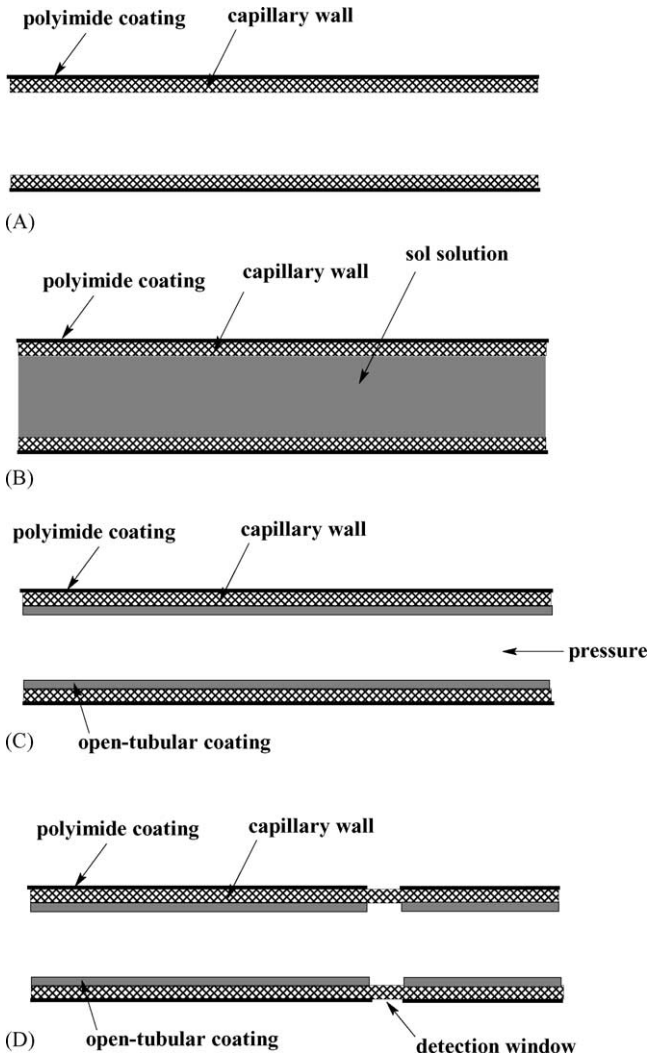


Fig. 9. Typical steps in making sol–gel open-tubular columns: (A) the inner walls of a piece of capillary is cleaned and pretreated; (B) the capillary is filled with the prepared sol solution that stays inside the capillary for a certain time to allow the formation of a surface-bonded sol–gel coating on the capillary inner walls; (C) pressure is applied to remove unbonded components of the sol solution followed by post-gelation treatments; (D) the protective polyimide coating is removed from a small segment (e.g., 5 mm) of the coated capillary near the exit end to create an optical detection window.

Since it is the alkyl groups that act as the chromatographically active component of the stationary phase, it can be expected that the amount of organic moiety existing in the sol system will affect the chromatographic performance of the obtained sol–gel open tubular columns. This aspect of sol–gel column technology was experimentally studied by Freitag and Constantin [174], who prepared sol–gel C_8-bonded stationary phase coatings for open tubular CEC columns using TEOS and C_8-TEOS as sol–gel co-precursors. Fig. 10 shows the influence of the content percentage of C_8-TEOS in relation to TEOS on the retention time and the theoretical plate heights for the prepared columns in CEC operation. As can be seen in Fig. 10, no

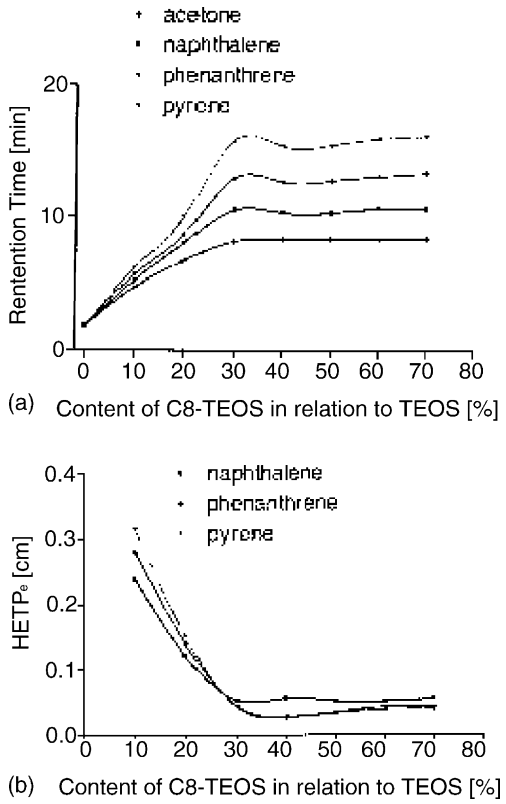


Fig. 10. Influence of the C_8-TEOS content of the reaction mixture during sol formation on (a) the retention time of the various test components (including acetone as inert tracer), (b) the theoretical plate height. Conditions: electric field: 212.8 V cm^{-1}, detection wavelength: 208 nm, mobile phase: ACN–water (1:1, v/v) containing 2 mM NaCl, capillary length: 67 cm (60 cm from the injection point to the detection point), capillary inner diameter: 50 μm, injection: hydrodynamically (1 s, 1.36 bar), preparation of the stationary phase: standard, save for the ratio TEOS: C_8-TEOS, which was varied as indicated in the plot. Samples containing naphthalene, phenanthrene, and pyrene (10 mM each) were prepared in ACN–water (7:3, v/v). Adapted from [174].

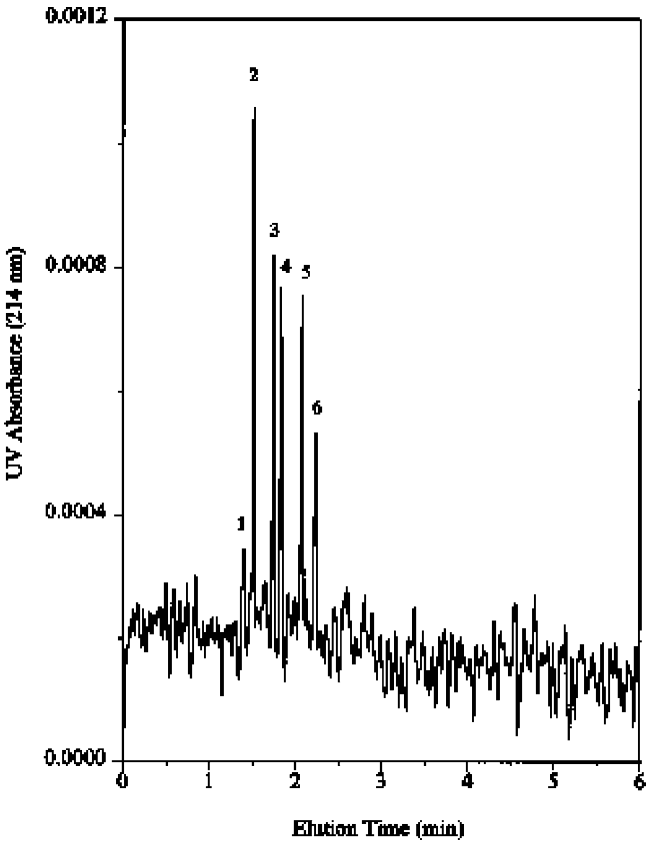


Fig. 11. OTC CEC separation of a six-peptide mixture using a column with APS coating. Separation conditions are column length, 30 cm (25 cm to detector); separation voltage, −12 kV; injection, −2000 V, 3 s; sample concentration, 1×10^{-5} M; UV detection at 214 nm. Six peptides are: (1) methionine enkephalin; (2) bradykinin; (3) angiotensin III; (4) methionine enkephalin–Arg–Phe; (5) substance P; and (6), neurotensin. Adapted from [179].

appreciable retention and separation of the test compounds were obtained on a sol–gel open tubular column that contained no C_8-moieties in the stationary phase coating (no C_8-TEOS was used in the sol solution to prepare this column). There is an almost linear increase in both retention times and separation efficiencies with the increase of C_8-moieties when the C_8-TEOS content in the sol solution varied in the range of 0–30%. Above 30%, the retention times and the theoretical plate heights leveled off. To attach a C_{18} moiety to the silica layer, Dube and Smith [178] used a different method. They first coated the capillary with a porous silica layer by using a sol solution containing TEOS. After this silica layer stabilized and dried, the C_{18} moiety was introduced to the existing silica layer by filling the capillary with a 10% (w/v) of octadecyltrichlorosilane in xylene. The high separation efficiency of 101 533 plates/m obtained from the coated column also showed an improved phase ratio on this open-tubular column.

Wu et al. [179] developed a reversed-phase open-tubular column coated with a sol–gel stationary phase containing an amine moiety. An enhanced electroosmotic flow in an acidic buffer and reduced adsorption of peptides on the capillary wall were achieved. These columns provided fast separation for peptide samples: six peptides were baseline resolved within 3 min. Fig. 11 demonstrates high separation efficiency of this type of open-tubular columns in CEC.

Hayes and Malik [101] developed a positively charged sol–gel ODS stationary phase for open-tubular CEC providing reversed electroosmotic flow. A key precursor used by these researchers to fabricate the open-tubular column for CEC was (*N*-octadecyldimethyl[3-(trimethoxysilyl)propyl]-ammonium chloride). This molecule contains a long straight hydrocarbon chain C_{18}, three methoxysilyl groups as well as a quaternary amine group. The use of this precursor in the sol solution brought advantageous features to sol–gel stationary phase created for open-tubular columns. The methoxysilyl groups enabled the creation of sol–gel network structure and attachment of the created sol–gel stationary phase onto the capillary wall. The presence of the octadecyl group

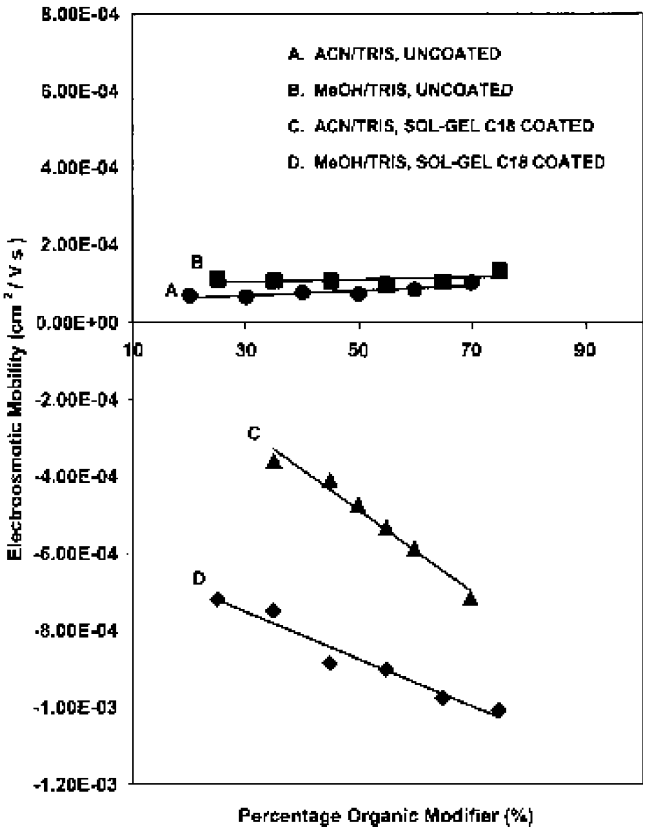


Fig. 12. Evaluation of the effects of organic modifier on the EOF generated within both uncoated (A and B) and sol–gel ODS coated (C and D) OT-CEC columns. Experimental conditions: fused-silica capillary (70 cm × 25 μm i.d.); injection for 0.04 min at 100 mbar; UV detection at 254 nm. Mobile phase ACN–Tris–HCl (pH 2.34) (65:35, v/v): (A) run +30 kV, 0.9 μA; (B) run +15 kV, 0.5 μA; (C) run −30 kV, −0.7 μA. (D) run −15 kV, −0.5 μA. Adapted from [101].

reinforced the chromatographic interactions between organic analytes and the newly designed sol–gel stationary phase. In addition, the quaternary amine group chemically incorporated in the stationary phase structure provided a positively charged capillary surface which is in contrast with the electrical properties of bare fused silica capillary surface that carries a negative charge. Over a wide pH range, the quaternary amine group can maintain its positive charge. Therefore, the direction of EOF for this sol–gel column is reversed compared with that obtained on an untreated fused silica capillary column. Fig. 12 indicates that this sol–gel ODS coated column is not only characterized by a reversed EOF but also a significantly stronger EOF compared the magnitudes of EOF for the uncoated and coated columns.

With the reversed EOF, mixtures of PAHs, aromatic aldehydes and ketones, benzene derivatives were successfully separated. An efficiency values of over 400 000 theoretical plates per meter was achieved in this sol–gel open-tubular column in CEC operation.

Constantin and Freitag [131] introduced ion exchange groups into open-tubular column by sol–gel process to separate amino acids. The ion exchange groups were the results from the addition of dimethyloctadecyl[3-(trimethoxysilyl)-propyl]-ammonium chloride and (pentafluorophenyl)dimethylchlorosilane, respectively into sol solution. However, the dimethyloctadecyl[3-(trimethoxysilyl)propyl]ammonium column grafted with octyltriethoxysilane molecules gave an inferior separation of the amino acid mixture. Only the (pentafluorophenyl)dimethylsilane columns were able to separate the amino acids.

Narang and Colón [180] demonstrated the use of fluorinated stationary phase for the separation of fluorinated organic compounds and halogenated organic compounds. These compounds are often detrimental to conventional stationary phases and can only be separated on chemically inert stationary phases. The open-tubular column bearing sol–gel-derived fluorinated stationary phase were prepared by using a sol solution containing tridecafluoro-1,1,2,2-tetrahydrooctyl-triethoxysilane (F_{13}-TEOS), TEOS, ethanol, hydrochloric acid and water. To facilitate the formation of the organic–inorganic hybrid stationary phase through sol–gel reactions, and chemical bonding of the created stationary phase to the capillary inner walls, the sol solution was allowed to stay inside the capillary for 15–20 min. Such a treatment provided an open-tubular column with a surface-bonded thin film of sol–gel-derived fluorinated stationary phase. After removing the excess sol solution, the column was dried and equilibrated prior to use. Precursor F_{13}-TEOS bears the functional group serving as a chromatographically active stationary phase while co-precursor TEOS facilitates the formation of the sol–gel network. A mixture of fluorinated organic compounds was used to evaluate the selectivity of the fluorinated sol–gel stationary phase in the CEC column. Research results indicated that chromatographic retention is primarily due to the fluorine-fluorine interactions between the solute and the fluorinated sol–gel stationary phase. This column was used to separate a mixture of halogenated organic compounds containing flurobenzene, bromobenzene, 1,2-dichlorobenzene, 1,2-difluorobenzene and 1,2,4-trifluorobenzene for which a baseline separation was obtained.Sometimes, the stationary phase is required to bear specific structural features to have special selectivity for a particular group of analytes. In the absence of a suitable sol–gel precursor, an alternative approach to introduce a certain organic moiety into the sol–gel matrix is through chemical reaction of the sol–gel matrix with a suitable reagent that carries the desired organic moiety. In order to graft special molecules into the sol–gel matrix, Wang and Zeng [146,181] used 3-(2-cyclooxypropoxyl)propyl-tri-methoxy silane (KH-560) as a bridge to connect 1,4,7,10-tetraazacyclotridecane-11,13-dione (dioxo[13]aneN_4) and 2,6-dibutyl-β-cyclodextrin (DB-β-CD) to TEOS. By doing this, macrocyclic polyamine derived- and β-cyclodextrin derived stationary phases were attached onto the sol–gel matrix. Fig. 13 illustrates the structures of the precursors containing these macrocyclic organic moieties with characteristic molecular cavities. The obtained open-tubular columns containing macrocyclic

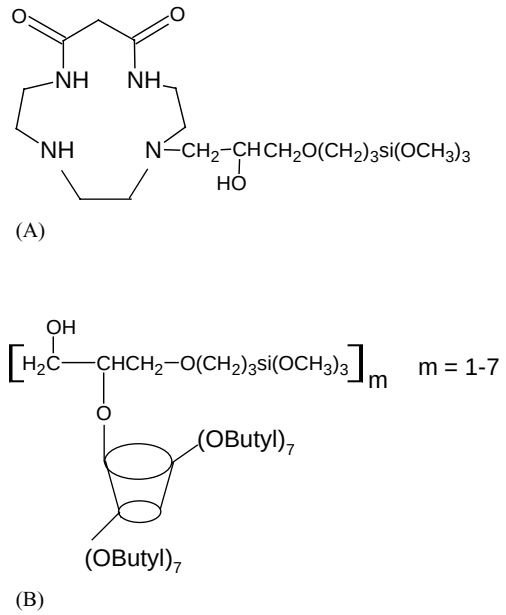


Fig. 13. Chemical structures of sol–gel precursors containing macrocyclic cavities. Structure of sol–gel precursor-containing (A) macrocyclic dioxopolyamine (adapted from [146]); (B) DB-β-cyclodextrin (adapted from [181]).

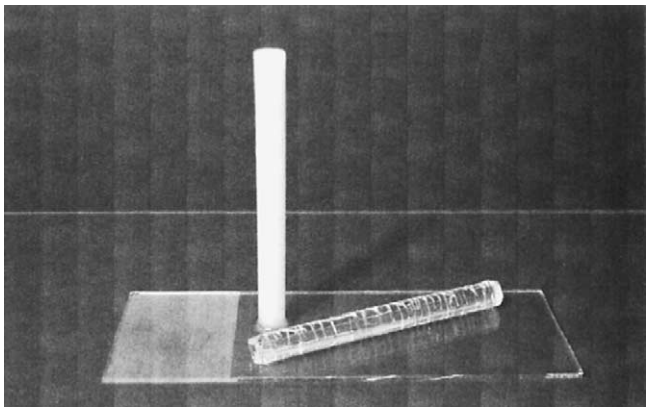


Fig. 14. Monoliths obtained after gelation induced by addition of diethylamine. The transparent monolith was made with Hr = 100%, the white one with Hr = 200%. (Hr: amount of water theoretically required for complete hydrolysis of all precursors). Cracks appeared in both monoliths, in the transparent one this occurred as soon as the gel was removed from the syringe. Note common microscope slide for scale. Adapted from [119].

polyamines and β-cyclodextrin were used to separate isomeric nitrophenols and benzenediols, isomeric aminophenols, diaminobenzenes, dihydroxybenzenes, and biogenic monoamine neurotransmitters. An open tubular sol–gel column coated with a macrocyclic dioxopolyamine provided very high separation efficiency (340,000 plates/m) in CEC.

6. Silica based sol–gel monolithic columns

According to Brinker and Scherer [85], "monoliths are defined as bulk gels (smallest dimension, ≥1 mm) cast to shape and processed without cracking." Fig. 14 shows two kinds monoliths obtained after gelation under different process conditions.

In chromatographic science, monolithic columns are sometimes referred to as continuous bed columns, fritless columns or rod columns [188]. An important aspect of monolithic columns is that it overcomes the drawbacks caused by the use of the end frits in packed columns [207]. Monolithic columns can be broadly divided into two categories: (a) organic polymer-based and (b) silica-based. Organic polymer-based monolithic CEC columns were first introduced by Hjertén et al. [189]. This technology was further advanced by Svec and Fréchet [190].

Cortes et al. [87] used sol–gel technology to create silica-based monolithic beds inside fused capillary and used it as a liquid chromatographic separation column. In 1996, Minakuchi et al. [95] prepared the sol–gel monolithic columns for reversed-phase liquid chromatography. With the development of CEC, the monolithic columns are becoming widely used in this electroosmotically driven mode of liquid-phase separation. Compared to the traditional packed columns, monolithic columns have many advantages such as ease in construction, higher surface area and porosity, improved mass transfer, absence of retaining end frits, and elimination or significant reduction of certain operational problems inherent in packed columns due to the presence of the retaining end frits. Compared to open-tubular columns, the solute molecules do not have to diffuse a long distance through the liquid mobile phase to reach the stationary phase in monolithic columns. This has led to the growing interest in the research of monolithic columns for CEC.

Preparation of monolithic columns by using sol–gel technology offers a number of important advantages over other methods. The fact that sol–gel reactions can take place under extraordinarily mild thermal conditions (often room temperature) is especially important in the context of CEC column technology where reactions need to be carried out inside small diameter capillaries. In such confined environments, it will be difficult to achieve intended results, if we are to use a chemical process that involves high-temperatures. Since sol–gel monolithic columns possess significant advantages over traditional packed columns and open-tubular columns, a lot of research activities can be observed in this area.

Analogous to the development of sol–gel monolithic stationary phases in HPLC columns [95,208–213], alkyl groups were commonly used as the functional stationary phase in the early developmental stage of sol–gel monolithic CEC column technology. In 1999, Fujimoto published a detailed procedure for the preparation of sol–gel monolithic columns for CEC [188]. The sol solution he used contained acetic acid, poly(ethylene glycol), and TMOS. This solution was allowed to stay inside the capillary for 20 h at 40 °C. The formed sol–gel silica material was then washed with water and treated with ammonium hydroxide solution for 24 h

at 40 °C. After thermal conditioning, the C_{18} moiety was chemically bonded to the sol–gel matrix by using a 10% solution of dimethyloctadecylchlorosilane in dry toluene. For this, the capillary was first filled this solution and then heated at 90 °C for 10 h. The SEM image of the cross-section of the sol–gel monolithic column showed a morphology resembling aggregates created from spherical silica particles. Column characteristics such as the permeability and EOF were evaluated. The results showed that a sol–gel monolithic column prepared in this way was suitable for use in CEC. Mixtures of acetophenone and valerophenone was separated providing 21,400 and 23,300 total theoretical plates for acetophenone and valerophenone, respectively (The effective length of the columns is 25.0 cm). Tanaka and co-workers [128,191] also fabricated sol–gel monolithic columns suitable for both HPLC and CEC. These authors used a two-step procedure: first a sol–gel silica monolithic bed was created inside the capillary, and then used a surface-derivatization reaction to chemically bind the desired chromatographic ligand to the surface of the porous silica monolith. The silica monolith was created by using a sol solution containing TMOS (precursor), PEO (porogenic agent, MW = 10,000), and acetic acid (catalyst). After stirring for 45 min, the sol solution was introduced into a NaOH-pretreated (3 h, 40 °C) fused silica capillary (100 μm i.d.) and allowed to react overnight at 40 °C. The created monolithic silica bed was further treated with aqueous ammonium hydroxide solution (0.01 M) at 120 °C for 3 h. This was followed by wash with ethanol and heat treatment at 330 °C for 24 h. Surface derivatization was accomplished by treating the created sol–gel silica monolithic bed with a solution of octadecyldimethyl-*N*,*N*-diethylaminosilane in toluene at 50 °C for 2 h. The C_{18} moiety was thus chemically attached onto the silica skeleton.

Hayes and Malik [100] used a single-step procedure to prepare sol–gel monolithic stationary phase for CEC. These researchers also used octadecyl group as the organic component of the stationary phase facilitating the solute/stationary interactions during CEC separations. In this method, the introduction of the C_{18} group was accomplished in one step with the formation of the sol–gel matrix. For this a novel precursor, *N*-octadecyldimethyl[3-(trimethoxysilyl)propyl]ammonium chloride (C_{18}-TMS) was added to the sol solution, that contained a co-precursor (TMOS), a deactivation reagent [phenyldimethylsilane (PheDMS)], catalyst trifluoroacetic acid (TFA), and water. Here, all the process including the formation of sol–gel monolithic matrix, the introduction of the organic moiety C_{18} and the deactivation of the unreacted silanol groups were accomplished virtually in one step. This provided a much simpler and faster method for the preparation of sol–gel ODS monolithic CEC column. Fig. 15 illustrates the steps involved in the preparation of sol–gel monolithic columns according to Hayes and Malik [100]. By using this method, a wall-bonded monolithic bed can be produced inside the fused silica capillary leaving an undisturbed (empty) end section of the capillary to be used for creating an optical detection window.

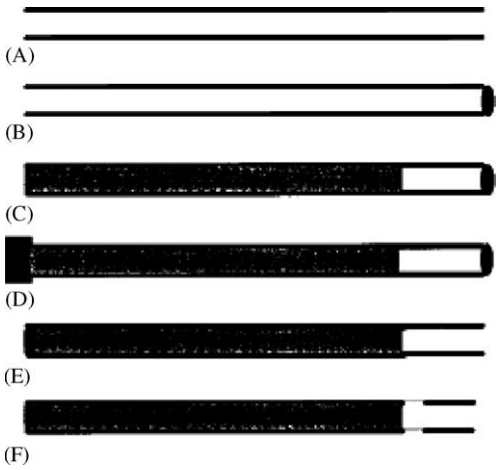
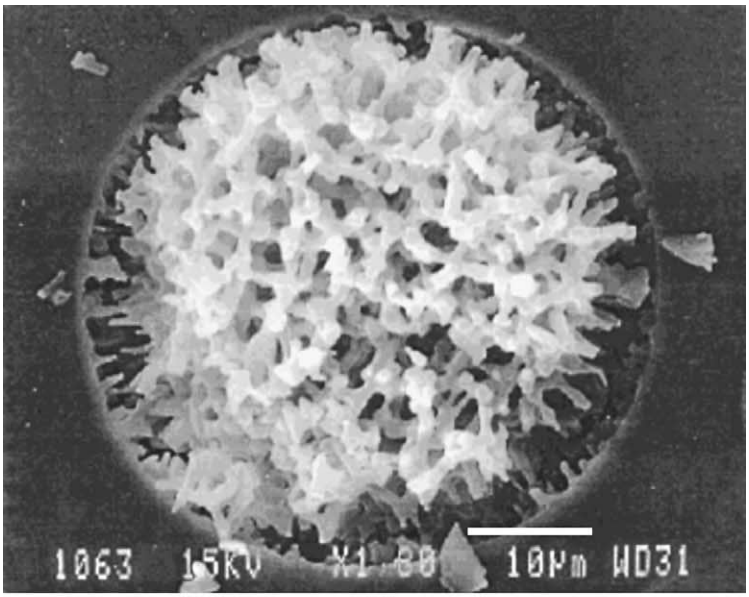
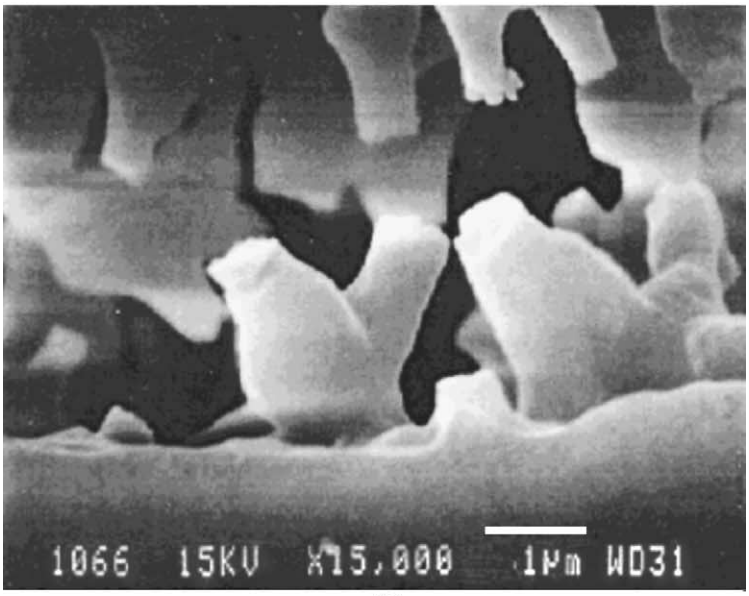


Fig. 15. Preparation of a sol–gel mediated monolithic CEC capillary: (A) 60 cm of hydrothermally pretreated 50 μm i.d. fusedsilica capillary; (B) closure of the distal capillary end via an oxyacetylene torch; (C) filling 50 cm of the capillary with the sol–gel solution using 100 psi helium pressurization; (D) closure of the proximal capillary end via a 60 s epoxy glue, followed by thermal conditioning; (E) opening of both capillary ends via an alumina wafer following the completion of thermal conditioning; (F) preparation of an UV detection window just adjacent to the termination of the monolithic matrix. Adapted from [100].

SEM investigations of the formed monolithic column revealed the porous structure of the created sol–gel monolithic bed with an average pore diameter of ∼1.5 μm. These flow through pore allowed sufficient column permeability for the mobile phase. The cross-sectional view and the surface view of the monolithic column shown in Fig. 16 reveal that the entire cross section of the capillary contains the monolithic matrix (Fig. 16A) and that the sol–gel monolithic bed is chemically bonded to the inner capillary wall (Fig. 16B). As mentioned in the previous section, since the key precursor (C_{18}-TMS), not only contains the chromatographically active C_{18} ligand but also a positively charged quaternary amine moiety, the EOF generated in such a sol–gel monolithic column has a direction which is opposite to that obtained on an untreated fused silica capillary that inherently possesses a negatively charged inner surface. The chromatographic performance of the sol–gel monolithic CEC column was evaluated by the separation of a mixture of polycyclic aromatic hydrocarbons (PAHs), a mixture of benzene derivatives, and a mixture of aldehydes and ketones. The high separation efficiencies obtained on these sol–gel monolithic columns provided impetus for the further study in this area. In a recent study, Allen and El Rassi [126], adapted the above-mentioned sol–gel approach developed by Hayes and Malik [100] to create positively charged sol–gel monolithic columns. In their two-step preparation method, the sol–gel silica backbone was first prepared, and followed by the introduction of organic moieties, which contributed to the positive charge on the obtained columns. The organic



(A)



(B)

Fig. 16. Scanning electron micrographs of a sol–gel C_{18} monolithic column: (A) cross-sectional view, magnification, 1800×; (B) surface view, magnification, 15 000×. Adapted from [100].

stationary phase ligands were chemically attached to the sol–gel silica monolithic bed by reacting with the following reagents: [3-(trimethoxysilyl)propyl]octadecyldimethyl ammonium chloride (TODAC), *N*,*N*-dimethyloctadecylamine (DMODA) or octadecylamine. Sol–gel monolithic columns with cyano or cyano/hydroxy stationary phases were produced using a similar pathway reported by these authors recently [192]. Fig. 17 shows the two reaction pathways for the producing of the cyano or cyano/hydroxy stationary phases on the sol–gel silica backbone. Because of high polarity imparted by the polar CN and OH groups, the created monolith provided normal-phase CEC separation for various polar compounds such as phenols, chloro-substituted phenols, nucleic acid bases, nucleosides, and nitrophenyl derivatives of mono- and oligosaccharides.

In order to understand the chromatographic behavior of separated solutes on the sol–gel monolithic columns, Allen and El Rassi [125] did systematic studies focused on how to improve the surface modification and control the pore structure by adjusting the experimental conditions. For this, a series of experiments were designed and carried out. To maximize the incorporation of the organic component in the prepared sol–gel stationary phase, these authors used 2,6-lutidine to enhance silanization reaction. The result

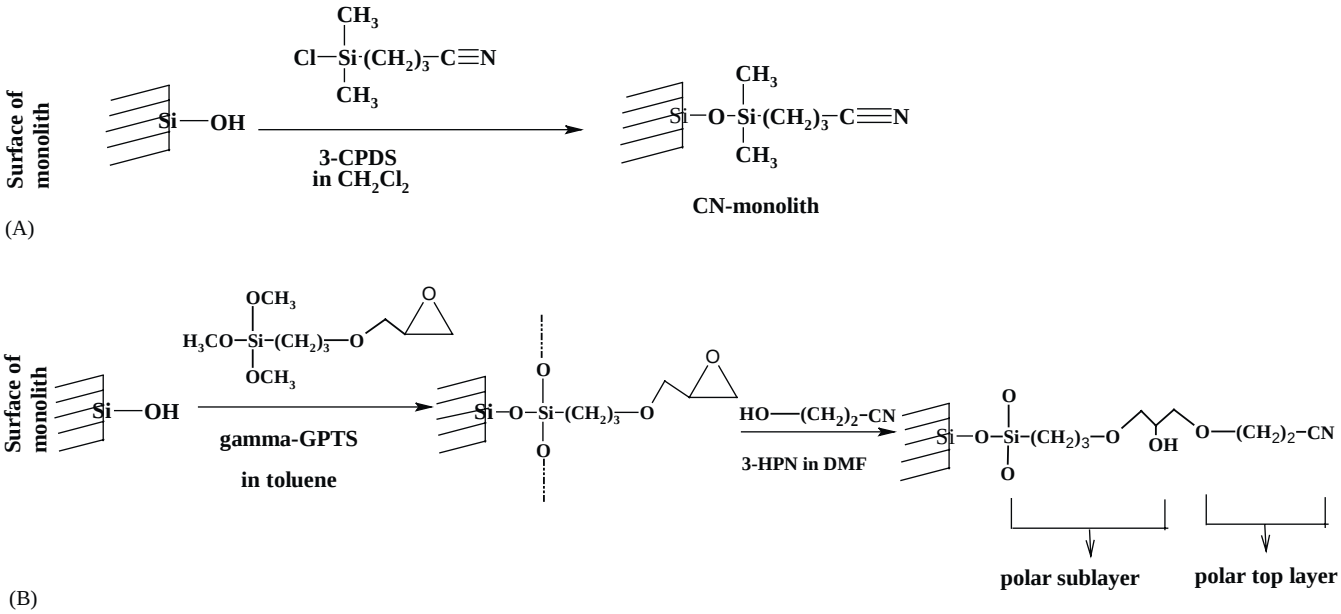


Fig. 17. Schematic of the two reaction pathways investigated. (A) Reaction of 3-CPDCS catalyzed by 2,6-lutidine in methylene chloride at 50 °C. (B) Reaction of γ-GPTS in toluene at 110 °C followed by reaction of 3-HPN in DMF catalyzed by BF_3 at room temperature. Adapted from [192].

showed that higher density of surface-bound C_{18} moieties was obtained in the presence of 2,6-lutidine. The optimum reaction time was determined by a second series of experiments. To manipulate the pore structure of the sol–gel monolithic column, an aqueous solution of ammonium hydroxide was used. Fig. 18 shows the electrochromatograms of alkyl benzenes (ABs) obtained on monolith treated with NH_4OH with different times. The optimum treatment time and conditions were determined to achieve the best pore sizes distribution and phase ratio. These research results provided important information for understanding the retentive chromatographic properties of sol–gel monolithic stationary phases towards the test analytes.

Zare and co-workers [105,116,133] developed a method to construct sol–gel monoliths by photopolymerization. The advantages of the photochemical route to prepare photopolymerized sol–gel (PSG) monoliths include: (a) the ability to control the pore size, (b) higher control over the placement and length of the PSG segment, (c) possibility to prepare sol–gel monoliths avoiding the use of high temperatures which might lead to cracking, and (d) high mechanical strength of the obtained monolithic beds. The sol solution was prepared by mixing methacryloxypropyltrimethoxysilane (MPTMS), hydrochloric acid, toluene (porogen), and a photoinitiator Irgacure 1800. The polyimide coating outside the segment where the monolith is supposed to be fabricated was removed for the irradiation light entering the capillary to initiate the polymerization. After irradiation, the capillary was washed, conditioned and equilibrated prior to use. MPTMS serves as the key precursor containing the chromatographically active organic ligand in the one-step fabrication of sol–gel monolithic column. The column was used to analyze PAHs, alkyl benzenes, and alkyl phenyl ketones to examine the chromatographic performance. For all the mix-

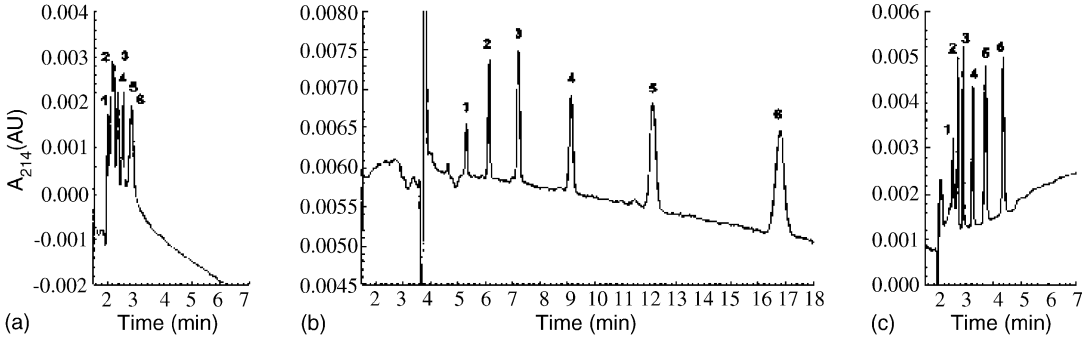


Fig. 18. Comparison of electrochromatograms of ABs obtained on monolithic C_{18}-silica columns previously treated with NH_4OH for: (a) 0 min; (b) 180 min; (c) 360 min. Conditions: capillary column, 27 cm (effective length 20 cm) × 100 μm i.d.; hydro-organic mobile phase, 10 mM Tris (pH 8) at 60% (v/v) acetonitrile; voltage, 20 kV; wavelength, 214 nm; column temperature, 30 °C. Solutes: (1) benzene; (2) toluene; (3) ethylbenzene; (4) propylbenzene; (5) butylbenzene; (6) amylbenzene. Adapted from [125].

Table 4
Retention factors (k) for 3 PAHs in different column morphologies[a]

Toluene/monomer ratio	Analytes	k (average)	R.S.D. (%)
90/10 ($n = 4$)	Naphthalene	0.17	10
	Phenanthrene	0.34	8.2
	Naphthalene	0.52	8.8
80/20 ($n = 3$)	Naphthalene	0.39	0.0
	Phenanthrene	0.69	0.86
	Naphthalene	0.96	0.62
73/27 ($n = 4$)	Naphthalene	0.53	1.8
	Phenanthrene	0.97	1.5
	Naphthalene	1.35	1.9

Adapted from [105].
[a] Sample solution and separation solution. 50 mM ammonium acetate–water–acetonitrile (1:3:6): 0.5 psi pressure injection (psi = 6894.76 Pa), 3 s; applied voltage, 10 kV; temp, 20 °C; detection, 214 nm.

tures examined, baseline separations were achieved. The authors established that an increase in the volume of monomer in the sol solution led to an increased formation of the photopolymer. Therefore, a higher retention of the analytes on the sol–gel photopolymer was observed. Table 4 shows that as the volume of MPTMS increases, the k values increase, which is an indication of stronger retention of the analytes.

The same research group [104] further developed the PSG columns to bonded-phase PSG monoliths to alter the hydrophobicity of the columns without degrading their chromatographic performance. Since the hydroxyl groups on the PSG surface can be easily derivatized with organochlorosilane or organoalkoxysilane coupling reagents, the bonded-phases of pentafluorophenylpropyldimethyl, pentafluorophenyl, 3,3,3-trifluoropropyl, n-octadimethyl, perfluorohexyl, and aminopropyl were obtained with appropriate coupling reagents. Compared to the underivatized PSG monoliths, these bonded-phase PSG monoliths have higher stability at pH values below 4. The comparison of the separation of thiourea and alkyl phenyl ketone on a PSG parent column (underivatized) and five bonded-phase PSG columns revealed the enhanced resolution of the test analytes on the bonded-phase PSG columns. The organic ligands in the prepared bonded-phase PSG columns included PSG-C_3F_3, PSG-CF_{13}, PSG-PFP, PSG-C_8, PSG-PFPDM. The structures of those silane coupling reagents are shown in Fig. 19.

The separations of nucleosides (Fig. 20A) and positively charged peptides (Fig. 20B) obtained on the prepared bonded-phase PSG columns look promising for these columns to be applied in biological and pharmaceutical areas. The same research group also introduced the preparation of a photopolymerized sol–gel monolithic column modified with dimethyloctadecylchlorosilane (DMOS), followed by chlorotrimethylsilane to end-cap the residual silanol groups [133]. The end-capping reaction was carried out to deactivate the residual silanol groups, and therefore, to prevent adsorption of polar analytes by the silanol groups. The obtained PSG monolithic columns were used to separate amino acids derivatized with 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F). Real biological samples such as glutamine and serine in rat cerebrospinal fluid (CSF) were also determined by using such a column (Fig. 21).

Besides the application in the separation, the photopolymerized sol–gel (PSG) monolithic column developed by Quirino et al. [70,194] was also used to perform on-line preconcentration with solvent gradient and sample stacking in

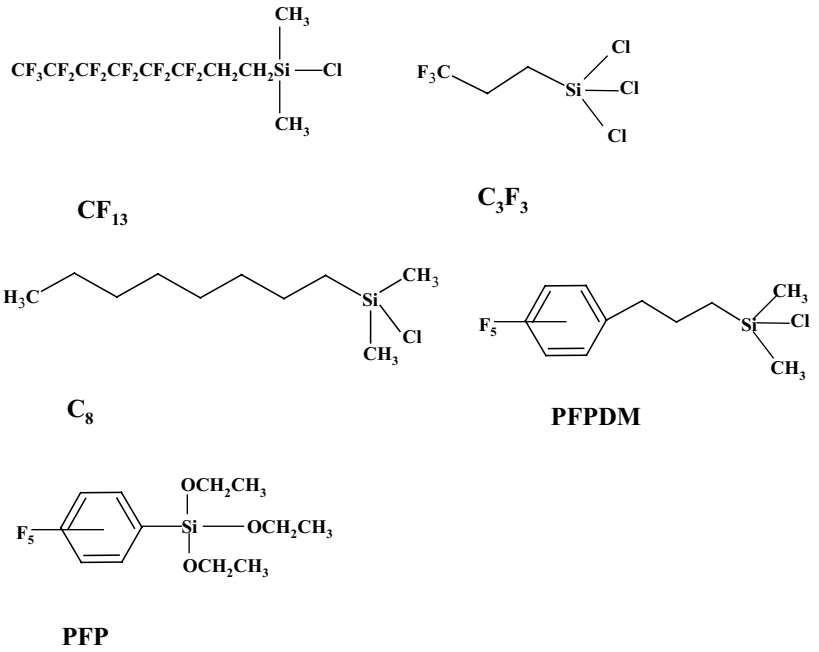


Fig. 19. Structure of the silane coupling reagents. Adapted from [104].

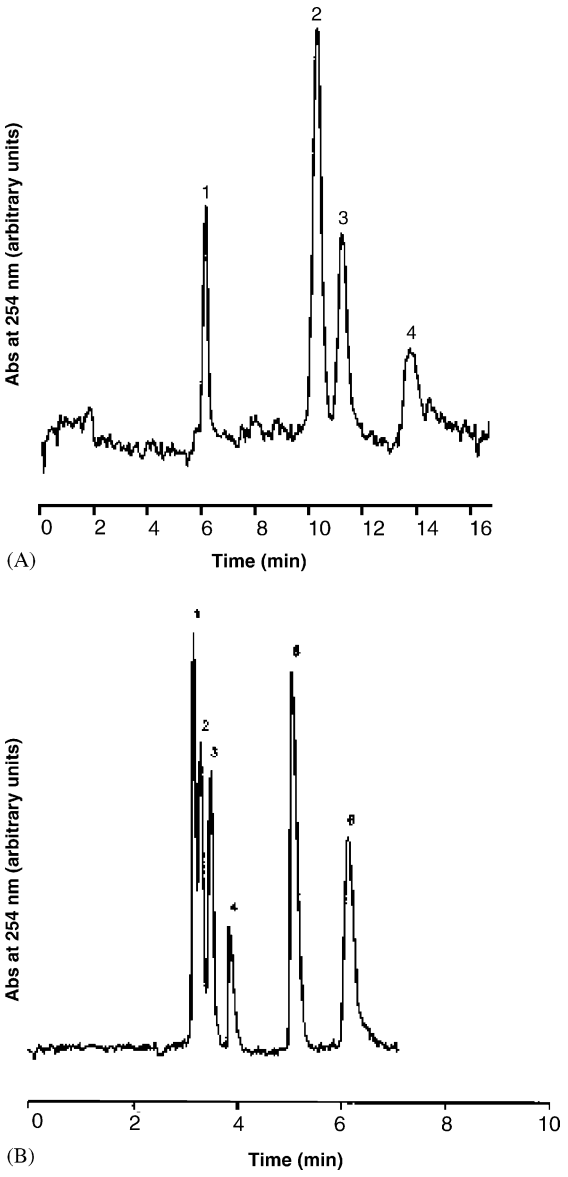


Fig. 20. Electrochromatogram of the separation of (A) nucleosides on a photomoplymerized sol–gel monolithic column. Conditions: PSG NH_2 monolith, column length: 10 cm; mobile phase 50 mM phosphate (pH 8)–water–acetonitrile (0.5:3.5:6, v/v/v); electromigration injection, plug length is 0.1 mm; running field strength is −577 V/cm; Peak identifications: (1) inosine; (2) uridine; (3) guanosine; and (4) cytidine. Adapted from [104]; (B) cationic peptides on a photopolymerized sol–gel monolithic column. Conditions: PSGPFP monolithic column, length 15 cm; Mobile phase, 50 mM ammonium acetate (pH 4.3)–water–acetonitrile (1:4:5, v/v/v); pressure injection, 30 s at 0.5 psi, running field strength 385 V/cm; peak identifications: (1) angiotensin I; (2) bradykinin; (3) angiotensin II; (4) Gly–Gly–Gly; (5) Val–Tyr–Val; and (6) methionine enkephalin. Adapted from [104].

CEC. The porous PSG monolith with a high mass-transfer rate enabled the preconcentration of dilute analytes. The extent of the preconcentration is quite significant. A 1000-fold increase in peak height was obtained for the preconcentration of peptides [70].

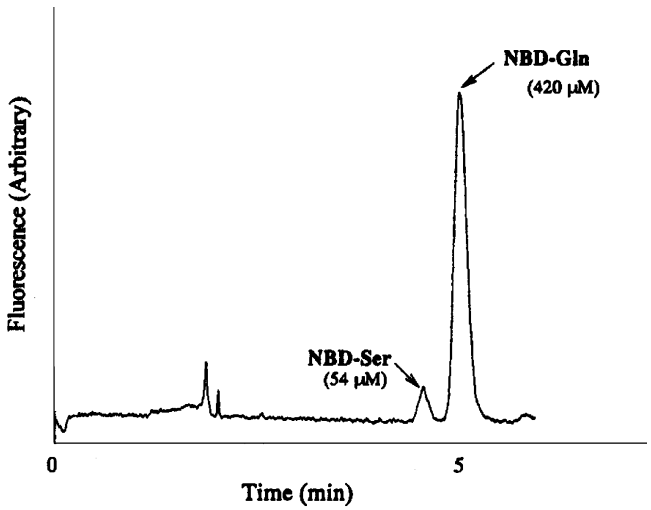


Fig. 21. Electrochromatogram of a rat CSF sample. Conditions: sample, 22 mM NBD-amino acids; mobile phase, 50 mM phosphate buffer (pH 2.5)–water–acetonitrile (1:1:8, v/v/v); capillary, end-capped column; applied voltage, 210 kV; injection, 25 kV (5 s); detection, excitation source is an argon ion laser. Adapted from [133].

Chiral separation is an important area where CEC with its inherent high separation efficiency can play a significant role in resolving chiral compounds into individual enantiomers. Separation of enantiomers is especially important for pharmaceutical industry. Since the molecular chirality greatly affects its physiological activity, chiral separation has received increasing attention in analytical sciences. However, enantiomers do not differ in their electrophoretic mobility in free solution. Therefore, they are unresolvable in an ideal free solution CE. In order to separate these isomers, chiral selectors, either in the form of buffer additives, chiral coatings, or bonded stationary phase ligands are used. Cyclodextrins (CDs) and their derivatives are the most commonly used chiral selectors in CE because of their special chemical structures [214] and selectivity for a wide range of chiral compounds.

In order to perform chiral separation in CEC, Tanaka et al. [193] developed a method to physically adsorb chiral stationary phase (CSP) physically on the sol–gel monolithic silica column. The adsorption process is mainly due to two interactions. They are the hydrophobic interaction between the avidin molecules and the solvent water, and the electrostatic attraction between the negatively charged silica surface and the positively charged avidin molecules. It was found that the electrostatic interaction played a predominant role in the adsorption process. Compared to open-tubular CEC, the resulting columns prepared by this method demonstrated more powerful separation capability due to its improved phase ratio in CEC and capillary liquid chromatography (CLC). Table 5 summarizes the theoretical plate number, resolution and separation time for twelve chiral compounds tested. High separation efficiency and good reproducibility are observed on these columns. Chen et al.

Table 5
Chiral compounds resolved

Compound	cLC			CEC		
	Efficiency (1000 plates/m)	R_s	Separation time (min)	Efficiency (1000 plates/m)	R_s	Separation time (min)
Dns–Ser	36/31	1.55	25	107/100	3.26	10
Dns–Trp	99/53	2.42	27	88/59	2.18	17
4-Fluoromandelic acid	94/76	1.70	19	128/68	1.71	5
Menadione sodium bisulfite	88/85	1.56	21	148/103	1.60	7
Abscisic acid	81/65	2.19	23	169/128	2.01	10
3-Phenylbutyric acid	122/36	1.72	21	119/49	2.12	7
Fluribiprofen	45/18	1.94	30	55/30	2.42	12
Warfarin	100/95	2.69	30	192/196	4.00	15
Chrysanthemic acid	108/101/79/55	3.35/4.20/2.79	28	203/242/136/129	4.83/11.59/3.96	18
(1*R*,2*R*)- and (1*S*,2*S*)-*N*-methyl-pseudoephedrine	89/11	2.95	25	25/16	1.94	5
Trp	47/38	1.98	16	–	–	–
PTH–Ser	41/32	1.86	15	–	–	–

Adapted from [193].

[58] reported the preparation of sol–gel monolithic columns chemically modified by β- or γ-CD. The separation of dansyl amino acid enantiomers was successfully achieved by using the obtained γ-CD modified monolithic column, and the β-CD modified monolithic column has been successfully applied for the enantioseparation of racemates of benzoin and several dansyl amino acids as well as the separation of the positional isomers of *o*-, *m*-, and *p*-cresols. Fig. 22 shows the chemical structure of γ-CD-modified monolithic sol–gel column. The same research group [122,123] reported the successful separation of dansyl amino acid enantiomers using sol–gel monolithic columns modified by L-phenylalaninamide and L-prolinamide in ligand-exchange capillary electrochromatography (LE-CEC) or by using Cu(II) complexes with L-amino acid amide as chiral selector or chiral stationary phase in CE, CEC and micro-LC. Recently, they described a method for the preparation of monolithic sol–gel columns modified with L-hydroxyproline as a ligand exchange chiral stationary phase [195]. The prepared monolithic chiral stationary phase has been shown to be effective in the enantioseparation of dansyl amino acids, free amino acids, hydroxy acids and dipeptides by both CEC and micro-LC.

Kang et al. [121] developed a method for the preparation of sol–gel chiral monolithic column. The chiral stationary phase they used was Chirasil-γ-Dex, which was statically coated on the surface of silica matrix. The immobilization of Chirasil-γ-Dex was performed by thermal treatment. The chromatographic performance of the obtained columns was evaluated by separating selected enantiomers. Fig. 23 shows electrochromatograms illustrating the chromatographic per-

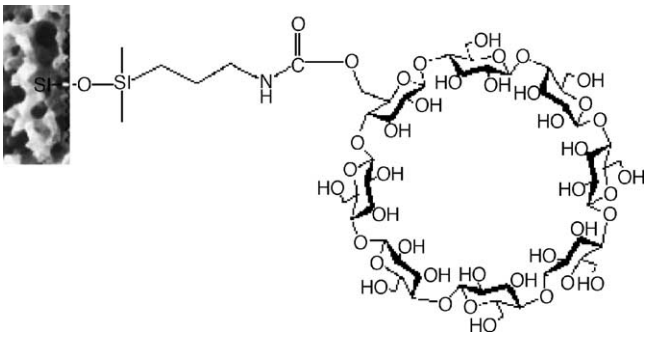


Fig. 22. Chemical structure of γ-CD-modified monolithic sol–gel silica column. Adapted from [58].

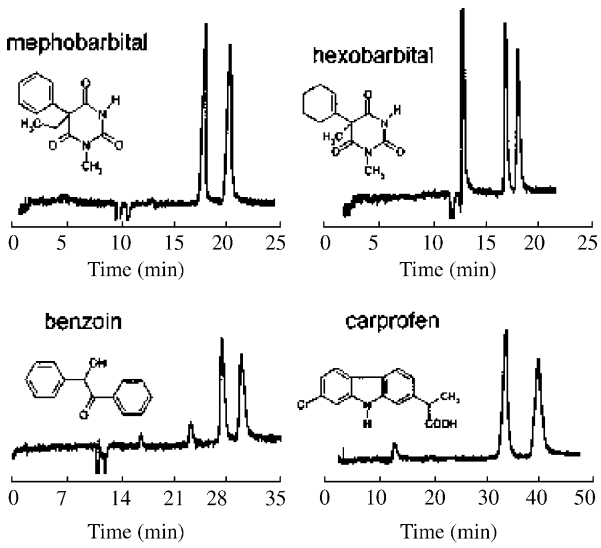


Fig. 23. Separation of enantiomers on the Chirasil-Dex monolithic column. Conditions: fused silica capillary column, 25 cm (effective length) × 50 μm i.d.; UV detection was performed at 210 nm. For mephobarbital, hexobarbital and benzoin: mobile phase, MES–Tris buffer (pH 6)–methanol (90:10, v/v); applied field strength, 0.4 kV/cm; samples injection at 3 kV for 4 s; carprofen: mobile phase, MES–Tris buffer (pH 6)–methanol (60:40, v/v); applied field strength, −0.4 kV/cm; sample injection at −3 kV for 4 s. Adapted from [121].

Table 6
Summary of sol–gel stationary phases used in CEC

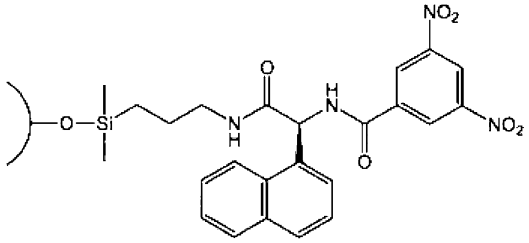
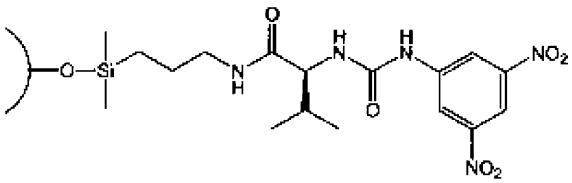
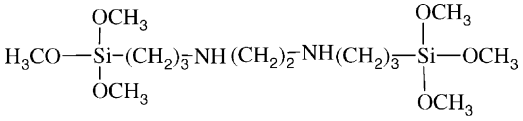
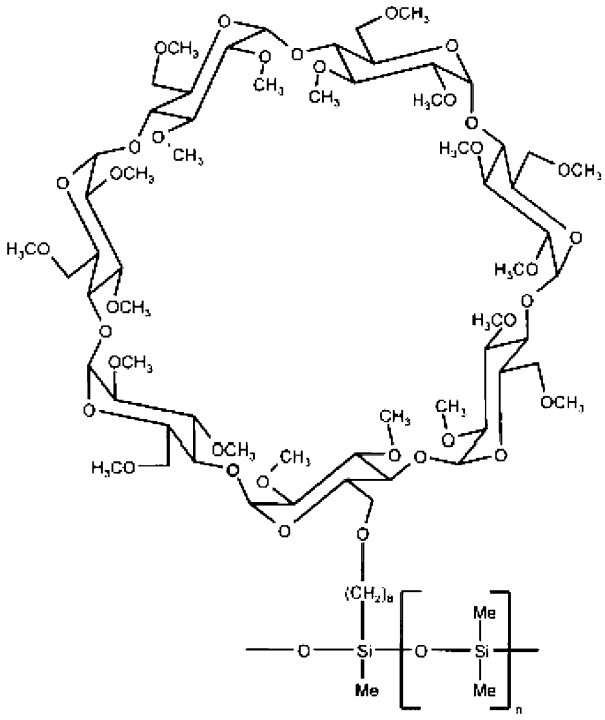
Name of the stationary phase	Structure	Application
(*S*)-*N*-3,5-Dinitrobenzoyl-1-naphthylglycine		Chiral selector for amino acids in packed column CEC [203]
(*S*)-*N*-3,5-Dinitrophenylaminocarbonyl-valine		Chiral selector for amino acids in packed column CEC [203]
Octadecyl and propylsulfonic acid functional groups in mixed-mode	As shown in Fig. 5A	Separation of neutral aromatic compounds, aromatic hydrocarbon homologues [162] PAHs, corticosteroids, alkaloids [163] in packed column CEC
C_{18}	–	Separation of retinyl esters in packed column CEC [165]
C_{30}	–	Separation of retinyl esters in packed column CEC [167]
Bis[3-(trimethoxysilyl)propyl]-ethylenediamine*		Separation of monosaccharides, alkylbenzoate homologues and 1-phenyl-3-methyl-5-pyrazolone (PMP) derivatives of aldopentose isomers in packed column CEC [168]
Permethylated beta-cyclodextrin (Chirasil-Dex)		Enantiomer separation of barbituric acids, benzoin, carprofen, ibuprofen in packed column CEC [225] Enantiomer separation of mephobarbital, hexobarbital, benzoin and carprogen in monolithic column CEC [121]

Table 6 (*Continued*)

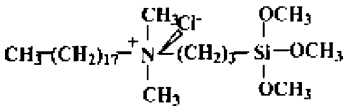
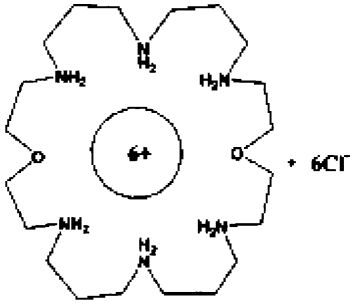
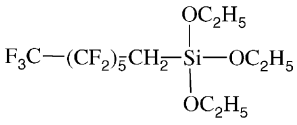
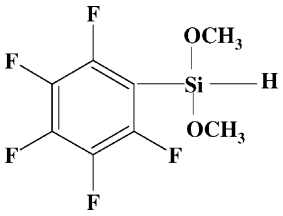
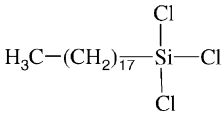
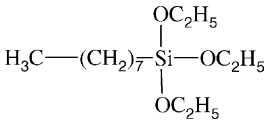
Name of the stationary phase	Structure	Application
N-Octadecyldimethyl[3-(trimethoxysilyl) propyl]ammonium chloride [J.D. Hayes 987] Also named as 3-(trimethoxysilyl)propyl] octadecyldimethyl ammonium chloride (TODAC) [D. Allen 1249]		Separation of PAHs, aromatic aldehydes and ketones, benzene derivatives in open-tubular column CEC [101] and monolithic column CEC [100] Separation of alkyl benzenes, anilines, phenylthiohydantoin amino acids (PTH-AA) in monolith column CEC [126]
1,4,7,10-tetraazacyclotridecane-11,13-dione (dioxo[13]aneN_4)	As shown in Fig. 13A	Separation of isomeric nitrophenols and benzenedios, isomeric aminophenols, diaminobenzenes, dihydroxybenzenes, and biogenic monoamine neurotransmitters in OTCEC [146]
2,6-Dibutyl-β-cyclodextrin (DB-β-CD)	As shown in Fig. 13B	Separation of isomeric nitrophenols and benzenedios, isomeric aminophenols, diaminobenzenes, dihydroxybenzenes, and biogenic monoamine neurotransmitters in OTCEC [181]
Macrocyclic compound, [28] ane-N_6O_2·6HCl		Separation of monophosphorylated nucleotides in OTCEC [226]
Tridecafluoro-1,1,2,2-tetrahydrooctyl-triethoxysilane (F_{13}-TEOS)		Separation of fluorinated organic compounds and halogenated organic compounds in OTCEC [180]
(Pentafluorophenyl)dimethylsilane*		Separation of amino acids in OTCEC [131]
Octadecyltrichlorosilane		Separation of aryl alkyl ketones, *N*-alkylanilines, diuretics, and some basic pharmaceutical drugs in OTCEC [178]
C_8-TEOS*		Separation of PAHs in OTCEC [89,90]

Table 6 (*Continued*)

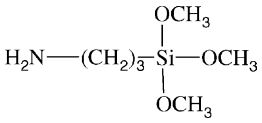
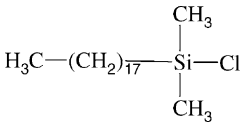
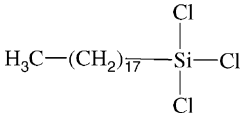
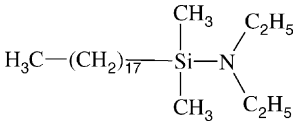
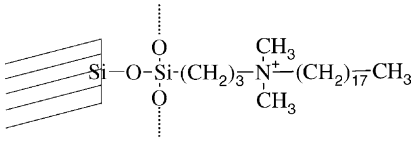
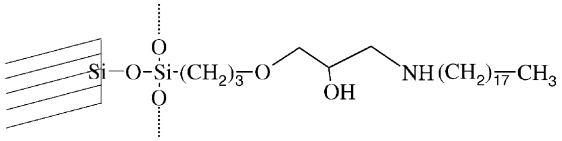
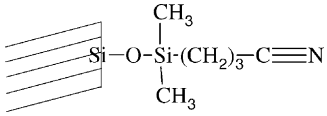
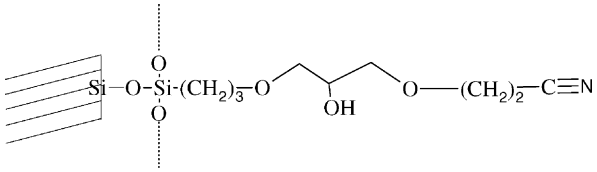
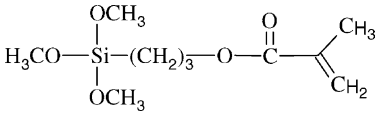
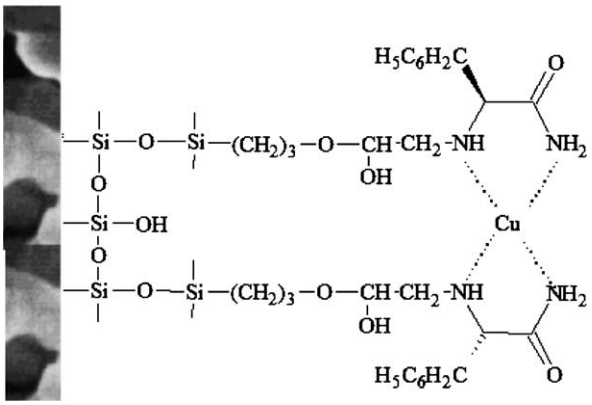
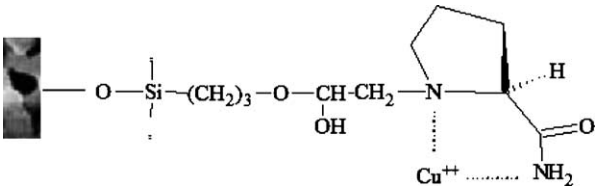
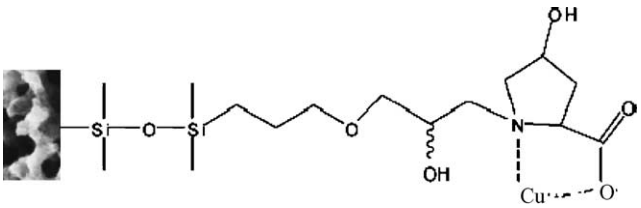
Name of the stationary phase	Structure	Application
(3-aminopropyl)trimethoxysilane (APS)*		Separation of peptides in OTCEC [179]
Dimethyloctadecylchlorosilane*		Separation of the mixture of acetophenone and valerophenone in monolithic column CEC [188]
Octadecyltrichlorosilane*		Separation of alkyl benzenes in monolithic column CEC [191]
Octadecyldimethyl-*N*,*N*-diethylaminosilane*		Separation of alky benzenes, PAHs in monolithic column CEC [128]
Reaction product between chloropropyltrimethoxysilane (CPTS) and *N*,*N*-dimethyloctadecylamine (DMODA)		Separation of alkyl benzenes in monolithic column CEC [126]
Reaction product between (γ-glycidoxypropyl) trimethoxysilane (γ-GPTS) and octadecylamine.		Separation of alkyl benzenes, anilines, phenylthiohydantoin amino acids (PTH-AA) and some standard proteins in monolithic column CEC [126]
3-Cyanopropyldimethylchlorosilane (3-CPDCS)		Separation of a mixture of some model compounds (toluene, DMF, formamide and thiourea) in monolithic column CEC [192]
Reaction product between (γ-glycidoxypropyl)trimethoxysilane (γ- GPTS) and 3-hydroxypropionitrile (3-HPN)		Separation of some model compounds, and phenols, nucleic acids, nucleosides, nitrophenyl derivatives of mono- and oligosaccharides in monolithic column CEC [192]
Methacryloxypropyltrimethoxysilane (MPTMS)*		Separation of PAHs, alkyl benzenes, and alkyl phenyl ketones [105], separation of 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD) derivatized amino acids [133] in monolithic column CEC On-line preconcentration of PAHs and alkyl phenyl ketones (APKs) in monolithic column CEC [70,194]
Bonded-phases of pentafluorophenylpropyldimethyl, pentafluorophenyl, 3,3,3-trifluoropropyl, *n*-octadimethyl, perfluorohexyl, and aminopropyl	As shown in Fig. 19	Separation of nucleosides, positively charged peptides in monolithic column CEC [104]

Table 6 (*Continued*)

Name of the stationary phase	Structure	Application
Avidin	Not available	Separation of racemic 4-fluoromandelic acid, acidic chiral and basic compounds in monolithic column CEC [193]
γ-Cyclodextrin	As shown in Fig. 22	Separation of dansyl amino acids enantiomers and positional isomers of *o*-, *m*-, and *p*-cresols in monolithic column CEC [58]
L-Phenylalaninamide corporated with Cu^{2+}		Enantioseparation of dansyl amino acids in monolithic column CEC [123]
L-Prolinamide corporated with Cu^{2+}		Enantioseparation of dansyl amino acids and hydroxy acids in monolithic column CEC [122]
L-Hydroxyproline corporated with Cu^{2+}		Enantioseparation of dansyl amino acids, free amino acids, hydroxy acids, and dipeptides in monolithic column CEC [195]

Structures accompanied with (*) were added by the authors of this review paper.

formance of the prepared columns in enantioseparation of mephobarbital, hexobarbital, benzoin and carprofen.

An interesting approach to exploring enantioselectivity of sol–gel based monolithic columns was carried out by Toyo'oka and co-workers [124,145,196]. They have developed a protein-encapsulation technique for the preparation of chiral monolithic capillary columns for CEC using the sol–gel method. Bovine serum albumin (BSA) was encapsulated in capillary column using sol–gel technique. The prepared sol–gel matrix was a tetramethoxysilane-based hydrogel for which the enantioselectivity was evaluated. The effect of various factors, such as pH and concentration of the running buffer or the nature of the organic modifier on chromatographic performances, as well as binding characteristics of BSA for D,L-tryptophan (Trp) were examined. Table 6 summarizes recent developments in silica-based sol–gel stationary phases for capillary electrochromatography, and provides the identity of the sol–gel precursors and/or chromatographically active ligands together with references to the original publications.

Pharmaceutical and biochemical analytes are of great importance in analytical chemistry. Currently, HPLC is the commonly used technique for the separation and quantitation of these analytes. A number of studies have shown the advantages of CEC in these applications [43,51–55]. Sol–gel stationary phases, as shown in Table 6, have also been used to analyze peptides [104,195], proteins [126] and basic pharmaceuticals [178]. These publications have already demonstrated the potential of sol–gel stationary phases in the CEC analyses important for pharmaceutical and biomedical industries. Further applications of CEC with sol–gel stationary phases can be expected in these areas in the near future.

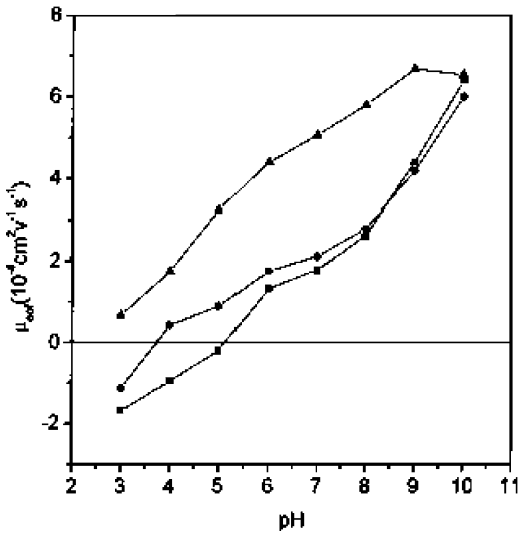


Fig. 24. Comparison of EOF profiles in CE obtained for (▲) bare fused-silica capillary; (■) magnesia–zirconia coated capillary and (●) SO_4^{2-} modified magnesia–zirconia coated capillary. Conditions—capillaries: 59 cm (effective length 51.6 cm) × 25 μm i.d.; applied voltage: 25 kV; electrolyte: 20 mmol/l Tris–HCl. Adapted from [221].

7. Nonsilica-based sol–gel stationary phases for CEC

As discussed in earlier sections, silica-based sol–gel materials are most commonly used type of sol–gel stationary phase in CEC. Some alkoxide of transition metals such as titanium, vanadium, zirconium, and Group IIIB metals such as boron and aluminum are also used as precursors in sol–gel process. Alumina-, zirconia-, and titania-based sol–gel stationary phases have been used in high-performance liquid chromatography [215–217] and capillary electrophoresis [218,219]. In the study of sol–gel columns for CE, zirconia has been used to modify the inner surface of fused silica capillaries [220–224]. As an amphoteric metal oxide, zirconia may be protonated or deprotonated depending on the pH of the solution. Thus, by coating the capillary inner surface with sol–gel zirconia material, its net surface charge can be effectively controlled making it either positively or negatively charged [220,222]. In addition, Xie et al. [221] developed the method to prepare magnesia-zirconia modified open-tubular sol–gel column for CEC. The obtained column exhibited switchable electro-osmotic flow (EOF) whose magnitude and direction can be manipulated by changing the pH of running electrolyte. Fig. 24 shows the effect of magnesia–zirconia coating and SO_4^{2-} modified magnesia–zirconia coating on the EOF. The optimum conditions were determined for the separations of six basic compounds by the SO_4^{2-} modified magnesia–zirconia coated capillary with reversed EOF. For the magnesia–zirconia coated capillary modified by alkylphosphonate, the alkyl moiety on the inner wall provided electrochromatographic separation of PAHs.

8. Conclusion

Sol–gel chemistry provides an effective methodology for the fabrication of organic–inorganic hybrid materials under mild thermal conditions, and is especially suitable for CEC column and stationary phase technology. This review summarizes a variety of sol–gel stationary phases that have been developed for CEC in the recent past. Silica-based sol–gel stationary phases in different column formats comprises the primary direction in the sol–gel stationary phase development today, although transition metal oxide-based sol–gel stationary phases are gradually getting introduced and have the potential to play an important role in the near future.

The presented review clearly demonstrates rapid developments taking place in the area of sol–gel stationary phases and column technology for capillary electrochromatography. Sol–gel chemistry presents a versatile tool for creating stationary phases with desired chromatographic and surface characteristics. It provides an effective pathway to creating hybrid organic–inorganic stationary phases that combine advantageous properties of both organic and inorganic material systems, which ultimately translates into creation of stationary phases with enhanced and tunable chromatographic selectivity. The sol–gel approach can be used under extraordinarily mild thermal conditions (frequently at room temperature) to create CEC stationary phases in the form of nanoparticles, surface coatings, and monolithic beds. At the present state, monolithic format constitutes the leading direction in the sol–gel stationary phase and column technology in CEC, although sol–gel stationary phases in the form of surface coatings in open tubular columns might find wider applications in the future because of inherent advantages in terms of simplicity in preparation and convenience in hassle-free operation. Chiral separation using sol–gel stationary phases is a rapidly growing area in CEC. Thus far, sol–gel stationary phases have been primarily used for the separation small molecules, although properly designed sol–gel stationary phases should have great potential in the separation biological macromolecules as well. A new wave of research and development in this area is very likely to take place in the very near future. The application of sol–gel stationary phases has recently been extended to electrochromatography in microchannels, and further growth in the applications of sol–gel stationary phases can be expected in this area. In short, sol–gel stationary phases represent a rapidly growing area in CEC, and may play a defining role in shaping up the future of stationary phase technology in capillary electrochromatography.

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