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Review

# Recent advances in preparation and application of hybrid organic-silica monolithic capillary columns

Hybrid organic-silica monolithic columns, regarded as a second generation of silica-based monoliths, have received much interest due to their unique properties over the pure silica-based monoliths. This review mainly focuses on development in the fields of preparation of hybrid monolithic columns in a capillary and their application for CEC and capillary liquid chromatography separation, as well as for sample pretreatment of solid-phase microextraction and immobilized enzyme reactor since July 2010. The preparation approaches are comprehensively summarized with three routes: (i) general sol–gel process using trialkoxysilanes and tetraalkoxysilanes as coprecursors; (ii) "one-pot" process of alkoxysilanes and organic monomers concomitantly proceeding sol–gel chemistry and free radical polymerization; and (iii) other polymerization approaches of organic monomers containing silanes. The modification of hybrid monoliths containing reactive groups to acquire the desired surface functionality is also described.

**Keywords:**
Monolithic columns / Organic-silica hybrid materials / Ring-opening polymerization / Sol–gel
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## 1 Introduction

During the last two decades, several advancements have been made in the chromatographic columns for separation sciences. Due to its key role in the separation process, the column is rightfully considered as the heart of the separation system, particularly, covering GC, HPLC, capillary liquid chromatography (CLC), and CEC and so on. Although the family of columns has continuously enlarged, researchers continue to pursue new columns to broaden their applications. Monolithic columns possess a unique structure and exhibit some exceptional characteristics, which are regarded as new generation of stationary phases. Their much higher external porosity compared to conventional particle-packed columns minimize the pore diffusion and mass transfer resistance, resulting in higher permeability and low-pressure drop with higher separation efficiency [1–4]. As a result, monolithic columns become an excellent tool in the analytical laboratory, not only for separation fields covering RP, ion-exchange, hydrophilic interaction, size exclusion, and affinity chromatography etc., but also for sample preparation including SPE or solid-phase microextraction (SPME), and as basis for immobilized enzyme reactors (IMERs) [5–11].

To date, two most common monolithic materials used for columns are the organic polymers such as polymethacrylates, polyacrylamide, and polystyrenes, and the silica-based inorganic polymers. The former monoliths can be generally prepared by in situ polymerization of organic monomers and crosslinkers in the presence of porogenic solvents, and the latter ones may be fabricated via a sol–gel process following a chemical modification of the matrix with different silylation reagents. They therefore exhibit different advantages and disadvantages. A number of review articles have been published in the last decade describing progresses on their preparations, properties, and applications [12–18]. It can be easily found that the problems of both the swelling or shrinkage of organic

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**Abbreviations:** **AA**, amino acid; **AIBN**, a,a′-azobisisobutyronitrile; **APTES**, aminopropyltriethoxysilane; **C8-TES**, *n*-octyltriethoxysilane; **CLC**, capillary liquid chromatography; **GPTMS**, glycidoxypropyltrimethoxysilane; **IMER**, immobilized enzyme reactor; **MAA**, methacrylic acid; **MIP**, molecularly imprinted polymer; **MPTMS**, 3-mercaptopropyltrimethoxysilane; **MTMS**, methyltriethoxysilane; **NHSG**, non-hydrolytic sol–gel; **POSS**, polyhedral oligomeric silsesquioxane; **PSG**, photopolymerized sol–gel; **PTES**, phenyltriethoxysilane; **SPME**, solid-phase microextraction; **TEOS**, tetraethoxysilane; **TMOS**, tetramethoxysilane; **VTMS**, vinyltrimethoxysilane; **$\gamma$-MAPS**, $\gamma$-methacryloxypropyltrimethoxysilane

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monoliths in some solvents and the tedious fabrication procedures of the silica-based monoliths always encumber the researchers and limit the applications. An alternative to address these issues is organic-silica hybrid monolithic column, which is receiving more and more attention as it possesses the advantages of easy fabrication, more pH stability, and less shrinkage.

Although organic-silica hybrid materials have been implemented as thin films in open-tubular capillary columns for GC [19], LC [20], and CEC [21] and as particulates in traditional columns for HPLC [22], their use in the monolithic format had not attracted considerable attention until Malik and coworkers [23] first prepared a C18-incorporated organic-silica monolithic column for RP CEC separation. Similar to common inorganic-silica monoliths, organic-silica hybrid monoliths are generally fabricated via a sol–gel process consisting of hydrolysis and condensation reactions of tetraalkoxysilanes and alkyltrialkoxysilanes under particular conditions, in which organic moiety is not involved in hydrolysis/condensation reactions to form the silica monolith but directly introduced into the monolith center without a post-modification procedure of common inorganic-silica monoliths. The detailed fabrication process may be found in an excellent review paper presented by Colon and Li [24]. After that, a variety of hybrid monoliths were further developed and applied for both separation and sample pretreatment fields. We reviewed the progress on preparation and application of hybrid monolithic capillary columns before July 2010 [25]. To continually track this topic, this review mainly summarizes and discusses the literatures since July of 2010.

## 2 Preparation of organic-silica hybrid monolithic columns

It is well known that numerous organic–inorganic hybrid materials with the formats of membranes, amorphous solids, and nanostructured particles, also defined as nanocomposites with organic and inorganic components, have been developed for many applications due to their extraordinary properties, which were generally synthesized with versatile sol–gel chemistry. The nature of the interface between organic and inorganic moieties may be exploited to classify these hybrid materials into two classes [26, 27]. Hybrids of class I are based on weak interaction such as van der Waals forces, hydrogen bondings, electrostatic interactions between organic and inorganic parts, and obtained by impregnation, doping, or physical entrapment of organic, bioorganic, or organometallic species into sol–gel matrices. Hybrids of class II correspond to all systems where organic components are strongly attached to the siloxane network via covalent bonds, and are typically prepared via cocondensation of alkoxysilane and organosilane reagents [28]. As one format of hybrids, porous monolithic hybrids, in particular, organic-silica-based monoliths have been widely developed for separation research field since last decade. To date, the hybrid organic–inorganic monoliths are synthesized and processed with several chemical routes. As shown in Fig. 1, the processes are mainly based on: (1) the encapsulation of organic components into silica monolith via sol–gel process (Route A), (2) the attachment of the desired organic functionality to sol–gel matrices by covalent bond formation (Route B), (3) direct synthesis into the final hybrid monoliths by using a functional organosilanes (Routes C, D, and E). Several good reviews have presented the works related to the former two preparation processes [4, 29–31]. Herein, we will focus on the latter process that involves the direct synthesis organic–inorganic hybrid monoliths where the organic functionality is covalently attached to porous matrices.

### 2.1 Sol–gel process of trialkoxysilanes and tetraalkoxysilanes

The conventional approach to fabricate silica-based monolithic columns is via a sol–gel process consisting of hydrolysis and condensation reactions of tetraalkoxysilanes such as tetramethoxysilane (TMOS) or tetraethoxysilane (TEOS). Hydrolysis is initially performed to form hydroxyl derivatives that consequently undergo condensation liberating alcohol and/or water, and Si–O–Si linkages are produced, while polycondensation concomitantly occurs with multiple linkages to form cyclic oligomers, eventually resulting in the formation of 3D silicate network [24]. Similar to silica monoliths, the organic–inorganic hybrid monoliths is generally prepared by using a mixture of silane precursors via sol–gel process under acidic, basic, or two-step catalysis (Route C, Fig. 1). In the mixture of two precursors, one has four functional alkoxides, $Si(OR)_4$ (e.g. TMOS or TEOS), while another contains at most three functional alkoxides $R'Si(OR)_3$ (e.g. methyltrimethoxisilane, MTMS). The hydrolysis and condensation reactions proceed not only between same precursor molecules but also between two different precursor species (as shown in Fig. 2). The alkyl group, represented by R', does not participate in the hydrolysis and condensation reactions, but is directly introduced into the resulting organic-silica hybrid monolith. It is expected that part of alkyl groups is buried in the bulk of hybrid matrices, and the other part is located at the surface and available to participate in chromatographic interactions.

Various commercially available sol–gel precursors were directly used to fabricate the organic–inorganic hybrid monoliths. As shown in Fig. 3, trialkoxysilanes with hydrophobic organic moieties, such as *N*-octadecyldimethyl [3-(trimethoxy silyl)propyl]ammonium chloride [23], *n*-octyltriethoxysilane (C8-TES) [32–35], phenyltriethoxysilane (PTES) [36–38], propyltrimethoxysilane (C3-TMS) [39], MTMS [40–44], and other trialkoxysilanes with active organic functionalities, such as aminopropyl triethoxysilane (APTES) [45–49], *N*-(β-aminoethyl)-γ- aminopropyl-triethoxysilane [50], *N*-(β-aminoethyl)-γ-aminopropylmethyldimethoxysilane [51], 3-chloropropyltrimethoxysilane [52], 3-mercaptopropyltrime thoxysilane (MPTMS) [53], *N*-trimethoxysilylpropyl- *N*,*N*,*N*-trimethylammonium chloride [54], allyl-trimethoxysilane

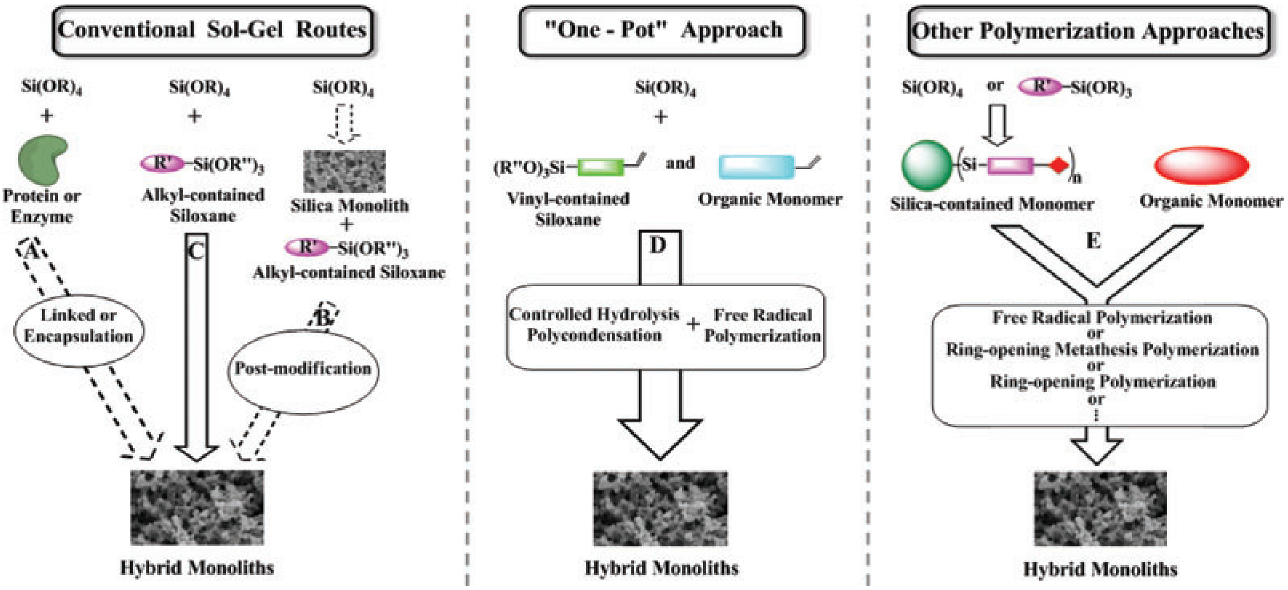

**Figure 1.** Scheme of the main routes for the synthesis of porous organic–inorganic monolithic hybrids.

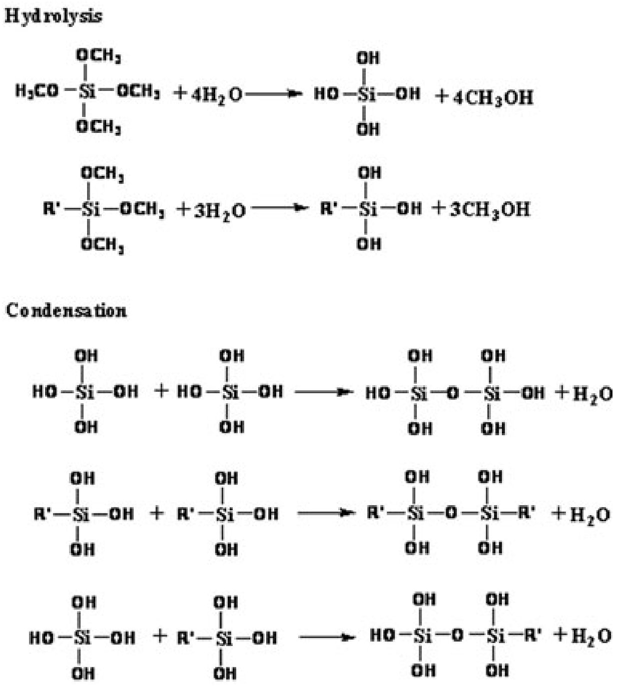

**Figure 2.** Simplified hydrolysis and condensation reactions of TMOS with alkyltrimethoxisilane during sol–gel processing.

[55, 56], and vinyltrimethoxysilane (VTMS) [57] have been selected as coprecursors with tetraalkoxysilanes, TMOS, or TEOS. Additionally, bridged silanes such as 1,4-bis(triethoxysily1)benzene [58] have also been used for preparation of hybrid monoliths. The morphology of these hybrid monoliths was mainly affected by the ratio of tetraalkoxysilane to alkyltrialkoxysilane and the processing parameters such as temperature, porogenic agent, acidic, basic, or two-step catalytic system, as well as other additives. Although longer time may be taken to individually optimize every sol–gel system, the satisfied hybrid organic-silica monoliths with desired structures and characteristics, particularly suitable for chromatographic separation would be obtained. To date, several kinds of hybrid monoliths with different organic moieties, for example, C18, C8, C3, phenyl, vinyl, allyl, aminopropyl, etc. have been successfully fabricated and applied for CEC and CLC separation or SPME and so on.

Hybrid methyl-silica monolith was initially developed by feeding MTMS into the TMOS sol in order to reduce the serious wall defects during the sol–gel process and following drying or modification process of silica monolith. The incorporation of methyl groups into the silica matrices hinders the condensation of hydroxyl groups and therefore reduces the shrinkage. The structure of the methyl-silica hybrid monoliths is more flexible, and may endure the stress generated by the shrinkage [59]. Tallarek et al. [60] presented a confocal laser scanning microscopy method to investigate the structural (especially radial) heterogeneity of different capillary methyl-silica hybrid monoliths with different pore and skeleton sizes. They also concluded that careful preparation conditions can increase the overall structural homogeneity of a silica monolith, whereas adding MTMS may increase the reproducibility of a hybrid monolith. It is hopeful that the approach of confocal laser scanning microscopy-visualized and analyzed wall effects promises a substantial improvement in column performance.

The introduction of the organic moiety during the fabrication of hybrid monoliths theoretically aimed at elimination of the postmodification procedure used to bind organic groups at the surface of monolith to impart a desired surface functionality. Laschober and Rosenberg [61] synthesized a hybrid monolith by using MTMS as a sole precursor via sol–gel process. The resulting stationary phase was

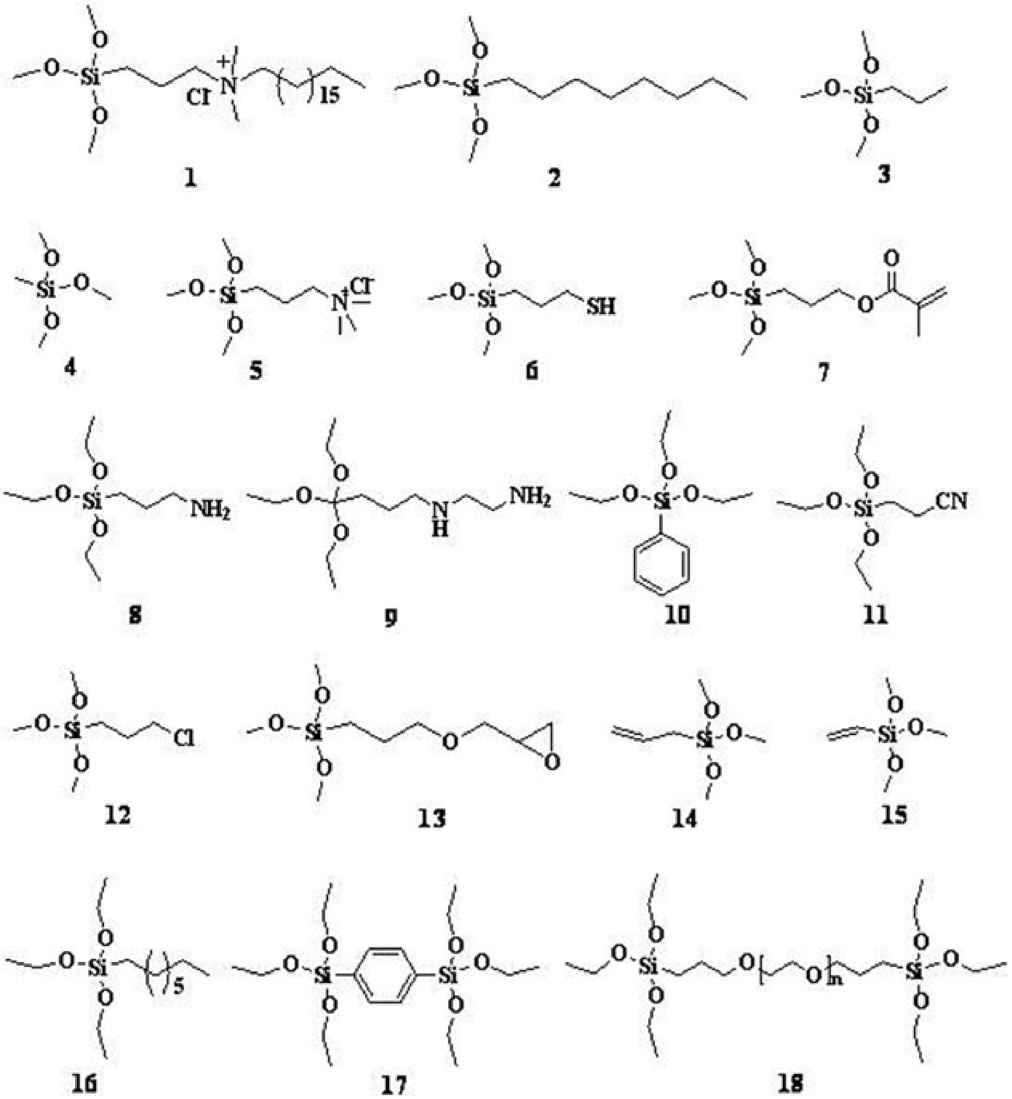

**Figure 3.** List of various silane precursors for organic-silica hybrid monolithic columns. 1, *N*-octadecyldimethyl[3-(trimethoxysilyl)propyl]ammonium chloride (C18-TMS), 2, *n*-octyltrimethoxysilane (C8-TMS), 3, propyltrimethoxysilane (C3-TMS), 4, MTMS, 5, *N*-trimethoxysilylpropyl-*N*,*N*,*N*-trimethylammonium chloride (TMSPTMA), 6, 3-mercaptopropyltrimethoxysilane (MPTMS), 7, 3-(trimethoxysilyl) propyl methacrylate (γ-MAPS), 8, aminopropyltriethoxysilane (APTES), 9, *N*-(β-aminoethyl)-γ-aminopropyl-triethoxysilane (AEAPTES), 10, phenyltriethoxysilane (PTES), 11, 2-cyanoethyltriethoxysilane (CN-TES), 12, 3-chloropropyltrimethoxysilane (CPTMS), 13, glycidoxypropyltrimethoxysilane (GPTMS), 14, allyltrimethoxysilane (Allyl-TMS), 15, vinyltrimethoxysilane (VTMS), 16, *n*-octyltriethoxysilane (C8-TES), 17, 1,4-bis(triethoxysily1)benzene (BTEB), 18, diglycerylsilane (DGS).

assessed on the basis of methylene selectivity, exhibiting a hydrophobicity comparable to commercial C8 column. As a result, it is necessary to improve hybrid monolithic columns with surface modification to achieve high retention capacities and high resolution without loss of efficiency. Once fabricating the hybrid monoliths, their surface may be functionalized through conventional silane chemistry to anchor the stationary phase (e.g. C18) by means of surface Si–O–Si–C linkage [41]. One drawback of this approach is that the poor hydrolytic stability of the bonded stationary phase leads to a limited usability of mobile phases with pH range of 2–8. Fortunately, this problem can be solved by having an organo-silica hybrid that contains a derivatizable moiety attached at the surface through a more hydrolytically stable Si–C bond. In 2005, allyl-functionalized hybrid silica monolith was directly prepared by using allyl-trimethoxysilane and TMOS, in which allyl groups are attached through a Si–C bond [55], and easily further modified with *n*-octyl-dimethylsilane and benzyl-dimethylsilane by means of hydrosilylation [56]. This promising approach provides a platform for the fabrication of hybrid monoliths with versatile stationary phases in a simple and efficient manner. Several novel hybrid monoliths were fabricated in this way.

Recently, Zeng et al. first prepared a hybrid monolith containing vinyl groups by in situ cocondensation of VTMS and TMOS via sol–gel process, and then a self-synthesized calix

[4], open-chain crown ether [57], and a novel terminal-vinyl liquid crystal crown ether (2-[4-(3-undeceny-1-yloxy)-phenyl]-2-[4′- (4′-carboxybenzo-15-crown-5)-phenyl] propane) [62], as well as an ionic liquid containing allyl group termed 1-allyl-3-methylimidazolium chloride [63], were chemically immobilized by free radical polymerization using a,a'-azobisisobutyronitrile (AIBN) as an initiator, respectively. The resulting two hybrid monoliths were successfully applied for separation of polycyclic aromatic hydrocarbons and other analytes by CEC.

Yao and coworkers [64] recently exploited click chemistry to modify the vinyl-functionalized hybrid monolithic column, which is a modular synthetic approach toward the assembly of new molecular entities. Since click chemistry was pioneered by Sharpless in 2001 [65], this practical approach receives more and more attention in almost all areas of modern chemistry from drug discovery to materials science [66]. In Yao's work, vinyl-functionalized silica hybrid monolith was synthesized via the co-condensation of VTMS and TMOS, and then mercaptoethylamine, noctadecanethiol, and 3-mercapto-1-propanesulfonate were easily reacted with the vinyl group on the hybrid matrices via thiol-ene click reaction, providing a long carbon chain (C18) and increasing the hydrophobicity. This work indicates that the hybrid silica monolith with desired vinyl groups might be of great prospect for many further surface modifications.

Ionic liquids containing relatively large organic cations and inorganic anions recently received great attention in separation and analysis field because of their low melting point, good solubility to organic compounds, low volatility, stability and the highly charged nature, and widely applied as stationary phases in GC, HPLC, CEC [67, 68]. A polymeric ionic liquid modified hybrid monolith was fabricated for CEC [69]. A mercaptopropy-functionalized silica hybrid monolith was first prepared by the in situ co-condensation of TMOS and MPTMS. The monomer 1-viny-3-octylimidazolium bromide was then polymerized on mercapto-contained hybrid monolith through surface radical chain-transfer polymerization. The resulting hybrid monolithic column could be well applied for CEC separation of aromatic hydrocarbons, alkylbenzenes, and phenols based on the multiple interactions including hydrophobic effect and π–π interactions between analytes and functionalized groups (the ring of benzene and imidazole). Due to the great chemical reactivity of the alkylchloro groups, the chloropropyl moieties on the surface of the hybrid silica monolith matrix have been conveniently modified by a tertiary amine of *N,N*-dimethyl-*N*-dodecylamine (DMDA) via the nucleophilic substitution reaction onto the chloro-contained hybrid monolithic matrix, which was synthesized via the co-condensation of 3-chloropropyltrimethoxysilane and TMOS [52]. Recently, Liu et al. [70] immobilized an ionic liquid, *N*-methylimidazole, to the chloropropyl-functionalized silica (CP-silica) hybrid monolith via the chloropropyl group. The ring of imidazole on the column provides a reversed EOF in acidic pH for CEC. It is expected that the chloropropyl-functionalized hybrid monolith is also a promising hybrid monolith for the surface modification with other nucleophiles such as NaCN or $NH_3$ to obtain –CN or $-NH_2$ functionalities on the surface for many applications.

## 2.2 "One-pot" process of alkoxysilanes and organic monomers

In 2006, PMMA-silica hybrid monolith was prepared by simultaneously using TEOS, methylmethacrylate, and 3-(trimethoxysilyl) propyl methacrylate as precursors in the presence of water and ethanol [71]. Homogeneous monolith was obtained after the drying and gelation–solidification processes, which totally took about 7 days. Due to no evidence of macro phase separation, this kind of transparent hybrid monolith could not be used as hybrid monolithic column. It gave indeed a strong hint to introduce the organic moiety into the silica-based hybrid matrices. Three years later, we presented the approach so called ''one-pot'' approach (as shown in Fig. 4) for the preparation of organic-silica hybrid capillary monolithic columns by simultaneously using organic monomers and alkoxysilanes, TMOS, and VTMS, as precursors [72]. First, the hydrolysis of alkoxysilanes of TMOS and VTMS was hydrolyzed in the mixture of acetic acid (0.01 M), PEG (10 000 MW), and urea for 1 h at 0°C to form a homogeneous solution. After adding organic monomer and initiator, AIBN, the mixture was then introduced into the pretreated capillary to form the hybrid monolith at 40 and 60°C for 12 h, respectively, for condensation and polymerization. It was found that the resulting hybrid monolith was homogeneous and permeable, resulting from phase separation and porous structure in the presence of porogenic agents (PEG and urea). Particularly, the resulting hybrid monolith exhibited good chromatographic efficiency, and could be successfully applied for CEC and CLC separation. In this process, hydrolysis and condensation/polycondensation happened at a relatively low temperature to form a vinyl-functionalized silica hybrid monolith, and then free radical polymerization occurred at a relatively high temperature to anchor the organic polymer at the surface of hybrid monolith. As a result, the "one-pot" process can not only introduce the desirable functional groups into the silica monolith by combining the merits of the meso- and macroporous structure of silica monolith and the great availability of functional chemicals, but also address the limitation and difficulty of using or synthesizing the alkyltrialkoxysilanes to prepare the hybrid monoliths but using various organic monomer or other functionalities.

Besides the monomers of allyldimethyldodecylammonium bromide and acrylamide being used to fabricate hybrid monoliths with "one-pot" process [72], the monomer of [3-(methacryloylamino) propyl]trimethylammonium chloride was also used to prepare strong anion exchange hybrid column via this ''one-pot'' approach. The resulting hybrid column was coupled with MALDI-TOF MS for the enrichment and detection of phosphopeptides from protein digest with excellent sensitivity. This strong anion exchange hybrid monolith-based platform for phosphorylation analysis integrated the merits of the monolithic column-based

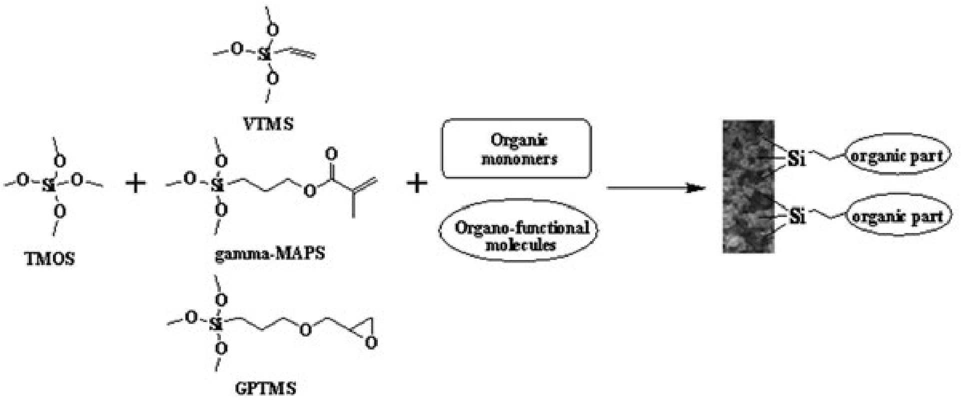

**Figure 4.** Schematic preparation of the organic-silica hybrid monolith via "one-pot" process by incorporation of organic monomers or functional biomolecules with alkoxysilanes.

treatment including microliter treatment capability and limited sample loss, as well as the excellent compatibility of MALDI-MS [73]. More recent, Lin et al. [74] adopted the same "one-pot" manner to synthesis of organic–inorganic hybrid affinity monoliths by using TMOS and γ-MAPS as coprecursors and 4-vinylphenylboronic acid as a functionalized organic monomer. The resulting hybrid affinity monoliths was evaluated for in-tube SPME of glycoproteins from a real sample, exhibiting excellent specificity toward *cis*-diol-containing biomolecules and glycoproteins. It is expected that the hybrid affinity monolithic column will become a promising tool for glycoproteomic analysis.

In the "one-pot" approach described, two-step thermal-treatment was required to form a hybrid monolith skeleton with desired pores and permeability at low temperature, and to further ensure that organic monomers are incorporated into the silica matrices at a relatively high temperature [72]. These tedious preparation procedures would likely lead to a lowering of the reproducibility of hybrid monolithic columns. Hence a simple single-step thermal-treatment "one-pot" approach for the preparation of hybrid monoliths was developed by using γ-MAPS and TMOS as precursors, as well as using two commercially available organic monomers, [2-(methacryloyloxy)ethyl]-dimethyl-(3-sulfopropyl)-ammonium hydroxide and 2-methacryloyloxyethyl phosphorylcholine (MPC), respectively [75]. After carefully optimizing the TMOS/γ-MAPS molar ratio, content of monomer, composition of porogenic solvent, and reaction temperature, the [2-(methacryloyloxy)ethyl]-dimethyl-(3-sulfopropyl)-ammonium hydroxide-silica and 2-methacryloyloxyethyl phosphorylcholine-silica hybrid monolithic columns exhibited good permeability and mechanical stability, particularly, possessed hydrophilicity at higher organic solvent contents (>50% ACN) in the separation of various neutral, basic, and acidic analytes, as well as small peptides by CLC. Uniform hybrid monolith with large through-pores looks like a pure silica (as shown in Fig. 5), and a column efficiency varying from 50 000 to 110 000 N/m was achieved for these compounds at the optimal flow rate. Compared to the previous two-step thermal-treatment "one-pot" approach, the single-step approach is simpler and more convenient, demonstrating that this improved "one-pot" approach can be used as a general method for preparing different types of monolithic columns.

The organic monomers selected in the above-mentioned "one-pot" approach for preparing the hybrid monolithic capillary columns were of hydrophilicity as all reactions were accomplished in aqueous solution containing PEG and urea. Consequently, the incompatibility between hydrophobic organic monomer and aqueous prepolymerization mixture limits to form a homogeneous solution in the "one-pot" process. To address this problem, we [76, 77] further developed a novel polymerization system composed of water, organic solvent (methanol and DMF), and CTAB, which acts as supramolecular template to form desired pores in the sol–gel monolith. We first synthesized a chiral monomer, mono(6$^A$-N-allylamino-6$^A$-deoxy)-perphenylcarbamoylated-β-CD (Ph-β-CD), and then the hybrid Ph-β-CD-silica monolith was successfully fabricated by a "one-pot" approach via the polycondensation of VTMS and TMOS and in situ copolymerization of chiral monomer [76]. More than ten racemates could be well enantioseparated on the resulting hybrid monolith by CLC. Similarly, another hydrophobic monomer of benzyl methacrylate was also selected to prepare the phenyl-silica hybrid monolithic column in the same prepolymerization system through "one-pot" process. The satisfied permeability of the resulting hybrid benzyl-silica monolithic column was calculated as $3.23 \times 10^{-13}$ m$^2$, and the minimum plate height was determined as 8.38 μm, which corresponds to 119 000 theoretical plates per meter in CLC [77]. Since this novel prepolymerization system mainly consists of organic solvents (methanol and DMF), the limitation of using water-soluble organic monomers in the previously reported "one-pot" method is circumvented. Therefore, various hydrophobic monomers of methacrylates and styrenes as well as other β-CD derivatives may be selected to prepare organo-silica hybrid monoliths with hydrophobic selectivity in the same way.

To shorten the time of sol–gel process, a novel organic–inorganic hybrid monolithic column for HPLC was synthesized via free radical copolymerization with sonocation assistance by using VTMS and a vinyl ester resin synthesized with methacrylic acid (MAA) and bisphenol A diglycidyl ether as precursors [78]. First, VTMS, PEG (10 000 MW), and acetic

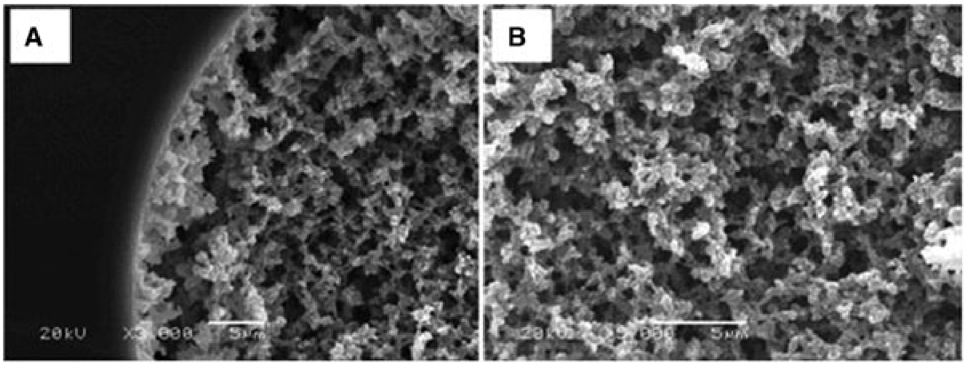

**Figure 5.** SEM images of an [2-(methacryloyloxy)ethyl]-dimethyl-(3-sulfopropyl)-ammonium hydroxide-silica hybrid monolith prepared with the improved "one-pot" approach. Magnification: (A) 3000× and (B) 5000×. Reprinted from [76] with permission.

acid (0.05 M) were mixed by sonication to form a homogeneous solution at 40°C for 20 min. After adding dodecanol, vinyl ester resin, and AIBN into the homogeneous solution with 10 min sonication, the polymerization was performed at 60°C for 12 h to form the hybrid monolith in a stainless-steel column (50 mm × 4.6 mm id). The process totally involves two major reactions: the polycondensation of VTMS, and the copolymerization of vinyl ester resin and the polycondensated product of VTMS at temperatures of 40 and 60°C, respectively. They demonstrated that the total time of preparation decreased due to the fast sol–gel process with the assistance of sonication. This strategy is expected to apply for the hybrid monolith in the confine of capillary.

The "one-pot" approach was recently advanced to use glycidoxypropyltrimethoxysilane (GPTMS) instead of VTMS or $\gamma$-MAPS as precursor for the preparation of amino acid (AA)–silica hybrid monolithic column [79]. The basic AA (L-arginine, L-lysine, and L-histidine) was covalently bonded into the hybrid matrices via the epoxy ring-opening reaction between the amine group and the glycidyl moiety in GPTMS, while the basic AA was also served as an "internal catalyst" for silica condensation in the "one-pot" process (as shown in Fig. 4). The incorporation of the AA into the hybrid monolith makes the monolith a good zwitterionic stationary phase, which could be applied for the separation of a series of polar compounds in CEC. This "one-pot" strategy offers another effective way for the easy preparation of various hybrid monolithic columns by immobilization of other functional biomolecules via the epoxy ring-opening reaction.

## 2.3 Other polymerization approaches of monomers containing silanes

It is well known that thermal- or photo-initiated free radical polymerization has been widely applied for preparation of organic polymer-based monolithic columns, while hydrolysis and polycondensation reactions in sol–gel process have been most frequently exploited to fabricate the silica-based inorganic monoliths as well as hybrid organo-silica monoliths. Bonn et al. [80] prepared a novel hydrophobic organo-silane-based monolithic capillary columns with thermally initiating free radical polymerization. They first synthesized a new crosslinker, namely bis(*p*-vinylbenzyl)-dimethylsilane (Fig. 6), by Grignard reaction of *p*-vinylbenzylchloride and dichlorodimethylsilane. Then, the crosslinker was copolymerized with *p*-methylstyrene to prepare a silica-containing organic monolith in the presence of 2-propanol and toluene for 24 h at 65°C. The resulting hybrid monoliths were proved to be mechanically stable with swelling propensity factors of 0.78–1.10. The porous properties could be easily adjusted by changing monomer content, microporogen content, and nature, and hence separation of biopolymers such as proteins, peptides as well as oligonucleotides was achieved within few minutes. This in situ process of incorporating silica into polymeric monolith opened an avenue for the easy preparation of the organic-silica hybrid monolithic column by using organic monomers containing silanes via free radical polymerization.

Due to the large surface area, nanoparticles have been exploited to modify the monolithic media to enhance the interactions between the analytes and the stationary phases, which were successfully applied for GC, CEC or CLC separation, SPE, and other fields [81]. Polyhedral oligomeric silsesquixanes (POSS) with sizes of from 1 to 3 nm in diameter can be thought of as the smallest possible organic-silica hybrid particles, as building blocks of interest in many applications of POSS-containing materials such as medical polymers, high-temperature composites, dendrimers, and liquid crystals [82]. We [83] first adopted a POSS reagent of POSS-methacryl substituted (POSS-MA) (Fig. 6) as the crosslinker to prepare a hybrid monolithic column via free radical copolymerization with a self-synthesized monomer of *N*-(2-(methacryloyloxy)ethyl)-dimethyloctadecylammonium bromide using toluene/dodecanol as porogenic solvents and AIBN as initiator at 55°C for 12 h. The preparation process was as simple as common organic monoliths instead of using methacryl-containing POSS as the nanosized hybrid blocks (crosslinker), and the morphology and permeability could be easily adjusted by changing the composition of polymerization mixture. The resulting POSS-containing hybrid monoliths exhibited good mechanical and pH stability, as well as high column efficiency in both CEC and CLC separation. This pioneer work gives an interesting clue for the preparation of hybrid monolithic column by using other multifunctional hybrid materials or reagents as the basic nanoblocks for monoliths through either grafting or

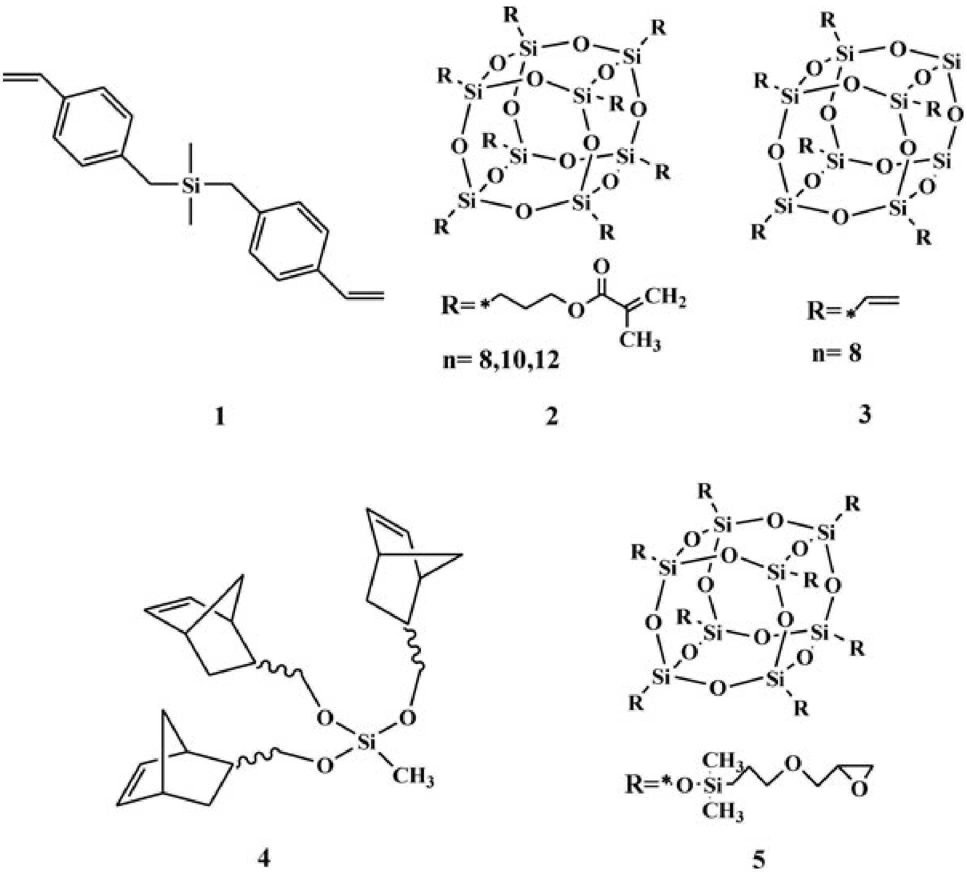

**Figure 6.** Molecular structure of monomers containing silanes. 1, bis(*p*-vinylbenzyl)-dimethylsilane (BVBDMS); 2, POSS-methacryl substituted (POSS-MA); 3, polyhedral vinylsilsesquioxane; 4, tris(5-norborn-2-enemethoxy) methylsilane ($(NBE\text{-}CH_2O)_3SiCH_3$); 5, octaglycidyldimethylsilyl POSS (POSS-epoxy).

copolymerization. More recently, three organic monomers, butyl methacrylate (BuMA), lauryl methacrylate (LMA), and MAA, were copolymerized with this methacryl-containing POSS in the presence of 1-propanol and 1,4-butanediol to prepare the poly(POSS-*co*-BuMA), poly(POSS-*co*-LMA), and poly(POSS-*co*-MAA) monoliths, respectively [84]. The resulting hybrid monoliths possessed good permeability and mechanical stability, and different selectivity as well.

Nischang and coworkers [85] also used a POSS reagent containing eight vinyl groups, polyhedral vinylsilsesquioxane (Fig. 6), to prepare hierarchically structured porous hybrid monolith in THF and PEG (200 MW) porogenic solvents via radically initiated polymerization at 60°C for 24 h. The mesopores and macropores could be facilely tuned by changing the ratio of THF and PEG. It was greatly surprised that a nanoporous structure with surface areas of approximately 800 $m^2/g$ and a pore volume of 0.53 $cm^3/g$, originating from micro ($\leq$2 nm) and mesopores ($<$10 nm), was revealed by nitrogen adsorption/desorption analysis (as shown in Fig. 7). The high surface area maybe originates from the assembly of the nanometer-sized, bulky rigid cages. The results also suggested that various substituents could be introduced via thiol-ene click reaction due to the existence of a number of residual vinyl groups in the surface of hybrid monoliths. This strategy of single-step preparation of a functionalizable high-surface-area material with a hierarchical pore space offers a facile and highly flexible route to tailor the porous materials with the desired surface property for liquid chromatographic, extractive, and catalytic applications.

Since the metathesis reaction has emerged in the mid 1970, it became a useful tool in organic synthesis, which awarded the 2005 Nobel Prize in chemistry to Chauvin, Grubbs, and Schrock. Buchmeiser and coworkers first developed various organic monolithic columns with inner diameter ranging from 50 μm to 5 cm via ring-opening metathesis polymerization for HPLC and CLC [87]. They also prepared the hybrid monolithic columns via ROMP by using *cis*-cyclooctene (COE), tris(cyclooct-4-enyl-1-oxy)methylsilane as monomers, 2-propanol and toluene as porogens, and $RuCl_2(Py)_2(IMesH_2)(CHC_6H_5)$ (Py = pyridine, $IMesH_2$ = 1,3-dimesityl-4,5-dihydroimidazolin-2-ylidene) as initiator within the confines of 200 μm id fused-silica capillary [87]. Recently, 5-norborn-2-enemethyl bromide ($NBE\text{-}CH_2Br$) and tris(5-norborn-2-enemethoxy)methylsilane ($(NBE\text{-}CH_2O)_3SiCH_3$) (Fig. 6) were used as monomers to prepare ROMP-derived hybrid monoliths [88]. The different rates of polymerization for $NBE\text{-}CH_2Br$ and $(NBE\text{-}CH_2O)_3SiCH_3$ allowed to form a highly crosslinked, permeable, and mechanically stable monolithic matrix. The resulting monoliths were converted poly ($NBE\text{-}CH_2Br$) chains into weak anion exchangers via reaction with diethyl amine, demonstrating relatively high ion exchange capacity and allowing for fast and highly efficiency separation of double-stranded DNA fragments.

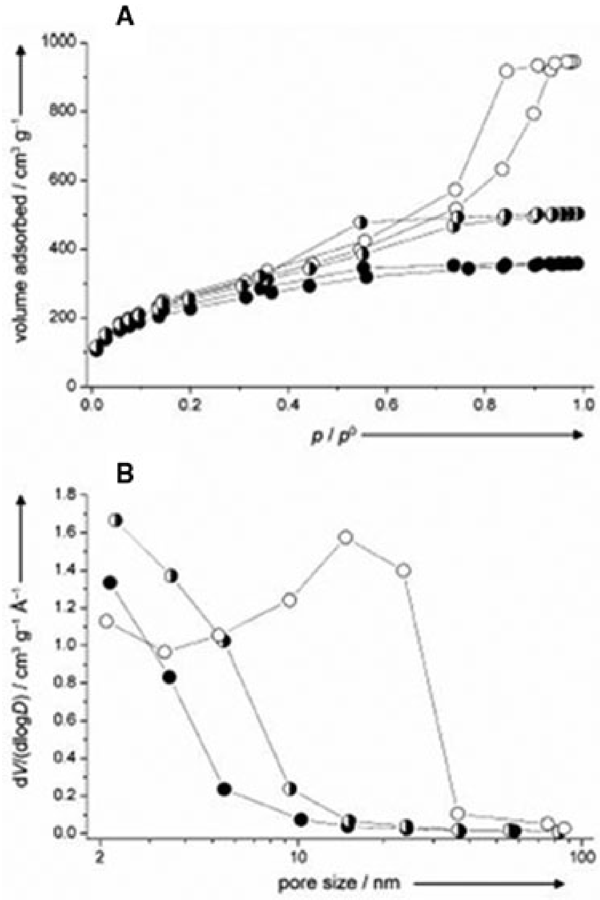

**Figure 7.** (a) Nitrogen adsorption/desorption isotherms and (b) pore size distribution curves from adsorption according to Barrett–Joyner–Halenda (BJH) for different polymers. Reprinted from [85] with permission.

Additionally, ring-opening polymerization has also been adopted to prepare the organic monoliths by using epoxy-contained monomers and diamines [89, 90]. More recently, we [91] utilized a POSS reagent of octaglycidyldimethylsilyl POSS (POSS-epoxy) (Fig. 6) as an epoxy monomer for preparation of a series of hybrid monoliths via ring-opening polymerization with diamines, such as 1,6-diaminohexane, 1,8-diaminooctane, 1,10-diaminodecane and 1,12-diaminododecane. Due to the hydrophobicity of POSS-epoxy and the existence of the numerous macro through-pores, the resulting hybrid monoliths were perfectly suitable for chromatographic separation in RP mode. Particularly, ultra-high column efficiencies for alkylbenzenes etc. (all over 100 000 N/m, and the highest one reached about 140 000 N/m) were achieved on these monolithic columns. This novel approach shows high versatility for preparation of series of porous, functionalizable hybrid monoliths with well-controlled 3D skeletal structure and high surface tailorability, which not only can be applied for chromatographic separation with high efficiency, but also are promising to match other potential applications.

## 3 Application of hybrid monolithic columns

### 3.1 Microscale separation

Monoliths have the hierarchical mesoporous/macroporous structure of enhanced permeability, resulting in fast mass transfer kinetics and lower backpressure during separation. This property makes the monoliths an ideal choice to traditional packed columns for fast and efficient analysis of complex mixtures, such as drugs, metabolites, and peptides, particularly suitable for fast separation in 2nd dimension of 2D separation system [11,92,93]. As a branch of monolithic materials, hybrid monoliths not only possess the same properties of organic and silica-based monoliths such as good permeability, fast mass transfer, and ease of modification, but also have excellent features of high pH stability of organic monoliths and mechanical stability of pure silica monoliths. These qualities make the hybrid monoliths an alternative in micro-column formats for CLC [34, 41, 42].

CEC has been regarded as one of the most powerful separation techniques due to the plug profile flow of mobile phase driven by the EOF in a capillary column or a microchannel of microfluidic chip, which combines the high efficiency of CE and the high selectivity of LC. Since a uniform porous C18-containing hybrid monolithic column for RP CEC was presented in 2000 by Malik and coworkers [23], considerable investigations have been pursued in an effort to use hybrid silica-based monolithic columns in CEC. Hybrid monolithic stationary phase functionalized with octyl- or phenyl- groups was commonly synthesized by a two-step acid/base-catalyzed hydrolysis/cocondensation of TEOS and C8-TES [32–35] or PTES [36–38], respectively, for CEC or other applications.

A silica-based CEC monolithic column with mixed modes of RP and weak anion exchange has been successfully prepared by using TEOS, C8-TES, and APTES with a self-catalyzed sol–gel technique [94]. Fast and efficient separation of six aromatic acids was obtained using acidic mobile phase with column efficiency up to 160 000 plates/m on the resulting C8-functionalized hybrid monolith. In 2010, a C8-functionalized hybrid monolithic column with sulfonic acid groups was prepared by changing the composition of TEOS, C8-TES, and MPTMS in the mixture of precursors via sol–gel chemistry [95], which was similar to that of hybrid monolith using TEOS, PTES, and MPTMS as precursors [37]. Then, the obtained hybrid monolith was oxidized using hydrogen peroxide (30%, w/w) to yield sulfonic acid groups. The sulfonic acid group, served as strong cation-exchanger, and generated stable EOF in a wide range of pH. As shown in Fig. 8, five anilines can be well separated at pH range from 4.5 to 9.0. The resulting hybrid column exhibited strong cation exchange /RP mixed-mode separation mechanism in CEC, where positively charged anilines had strong cation exchange and hydrophobic interaction with the stationary phase at the low pH, and the separation of anilines mainly depended on hydrophobic interaction when the buffer pH increased to 8.0.

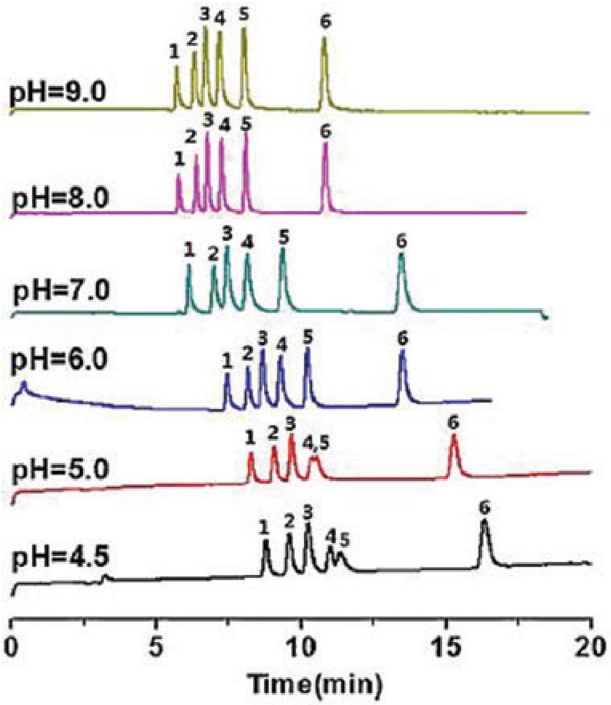

**Figure 8.** Electrochromatograms for separation of anilines at different pH values. Experimental conditions: monolithic capillary column, 75 μm id × 45 cm (effective 30 cm); mobile phase: 25 mM PBS buffer, containing 60% (v/v) ACN; injection: 15 kV × 3 s; UV detection at 254 nm; applied voltage: 18 kV. Order of peaks: 1, thiourea; 2, o-nitroaniline; 3, aniline; 4, o-toluidine; 5, *N*-methylaniline; 6, *N*,*N*-dimethylaniline. Reprinted from [95] with permission.

Molecular imprinting is a powerful technique for constructing a synthetic polymer receptor with tailor-made binding sites, that is, molecularly imprinted polymers (MIPs), which are highly cross-linked polymer containing spatial and functionality memory for template molecules, and provide the specific selectivity when used as molecular selective stationary phases in chromatography [96, 97]. Monolithic MIPs can be prepared in a capillary or stainless steel column either by *in situ* copolymerization of functional monomers and crosslinkers in the presence of template molecules [98] or by post-grafting of MIPs on the silica [99] or organic-silica hybrid [100, 101] monolithic matrices. A novel approach of room temperature ionic liquid (RTIL)-mediated non-hydrolytic sol–gel (NHSG) was explored for the fabrication of new molecularly imprinted hybrid monoliths for CEC enantioseparation by Yan's group [102] using MAA and γ-MAPS as precursors. The RTIL-mediated NHSG protocol involved free-radical copolymerization and NHSG process, which offers the potential for the reduction or elimination of residual silanol groups in the hybrid and in turn the nonselective absorbance of the analytes. Hence, NHSG hybrids can subtly increase the selectivity of the hybrid monolithic MIPs [103]. Similarly, a MIP hybrid monolith was also prepared by using acrylamide and γ-MAPS as precursors, methanol and toluene as porogenic solvents, AIBN as initiator, as well as four isomers of ractopamine as multitemplate molecule [104]. The imprinted hybrid monolith had good selectivity for four isomers of ractopamine, and four isomers of ractopamine could be separated completely in CEC under the optimal conditions.

## 3.2 Sample pretreatment

The chromatographic analysis of analytes in environmental, food or biological samples always encounters some problems, such as low concentrations of analytes, a complicated matrix and a limit of available sample volume for analysis. To overcome these problems, a process of sample preparation/pretreatment such as SPE or SPME is required to remove the endogenous interferences, and to concentrate the analytes prior to chromatographic determination. Due to the unique properties of various chemistry, fast mass transfer and ease of preparation, the monolithic material is particularly suitable for handling the samples with satisfied efficiency. Capillary monolithic columns can be easily integrated into the chromatographic system of GC, LC and CE, offering possible miniaturization and automation. Numerous applications have been presented for online or inline preconcentration coupled with capillary monoliths, and many new devices have been designed and developed for offline detection, which were listed in excellent reviews [9, 105].

Capillary hybrid monoliths have also been used for SPME with off-line mode, exhibiting some advantages over the organic monoliths and pure silica monoliths. Hu and coworkers [106] have prepared a bimodal porous hybrid monolith using AEAPTMS and TMOS as precursors by sol–gel technology, and the resulting monolith was successfully used as capillary microextraction (CME) column for aluminum (Al) fractionation following detection with electrothermal vaporization ICP MS (ETV–ICP–MS). Under the optimized conditions, the limit of detection was 1.6 ng/L for Al with an enrichment factor of 436-fold and a sampling frequency of 9 $h^{-1}$, indicating an excellent pH tolerance, solvent stability and long lifetime even for more than 250 times without decreasing adsorption efficiency. They [107] recently exploited the APTES-based hybrid monolith synthesized with APTES and TEOS via sol–gel process for the preconcentration of trace elements such as Mn, Co, Ni, Cu, Cd, and Pb in human hair and urine samples. The developed capillary microextraction-ICP-MS had several advantages of simple, sensitive, low cost, efficient for trace/ultratrace element quantification in complex samples, and favorable for those samples with limited amount. Due to the existence of amine groups in this APTES-based hybrid monolith, the modification was subsequently acquired by glutaraldehyde, and then activated with (aminomethyl) phosphonic acid, followed by $Ti^{4+}$ chelation to prepare a hybrid monolithic $Ti^{4+}$-IMAC column [48]. Such a hybrid IMAC column was successfully utilized to selectively capture 224 unique phosphopeptides in digest of mitochondrion-enriched component extracted from rat liver, corresponding to 148 phosphoprotein groups recognized with high confidence. The results demonstrate great potential of the hybrid monolithic $Ti^{4+}$-IMAC column in large-scale phosphoproteome analysis.

On-line in-tube SPME coupled to HPLC has received widely acceptance and applications due to fast operation, easy to automate, solvent-free and requirement of small sample volume. Feng and co-workers [35, 108, 109] developed several kinds of hybrid monolithic columns for in-tube SPME online hyphenated with capillary HPLC using UV or MS detection. First, a C8-incorporated hybrid monolith was synthesized using TEOS and C8-TES as two precursors by a two-step acid/base-catalyzed hydrolysis/cocondensation. The resulting hybrid monolith was applied for in-tube SPME preconcentration coupled to HPLC analysis. The limits of detection (S/N = 3) for 6 polycyclic aromatic hydrocarbons were found to be between 2.4 and 8.1 ng/mL with a UV absorbance detector, which are 299–456 times lower than those obtained without preconcentration [35]. Second, a hybrid monolith was synthesized with the simultaneous incorporation of TEOS, C8-TEOS, and MPTMS, which facilitated strong cation exchange sites generated by oxidization using hydrogen peroxide (30%, w/w), and applied for the determination of sulfonamide residues in milk samples [108]. The monolith was contained 4.8% of sulfur and 31.6% of carbon with elemental analysis, which is much higher than traditionally bonded silica materials. The results also indicated that the hybrid monolith retained excellent extraction performance for sulfonamides after being treated with 0.1 M HCl (pH 1.0) for 12 h and 5 mM phosphate solution (pH 10.5) for 12 h. Third, a novel hybrid organic–inorganic silica monolith with cyanoethyl functional groups was prepared by using 2-cyanoethyltriethoxysilane (CN-TES) and TEOS as precursors, and then a rapid, sensitive, and automated in-tube SPME/LC-MS method was developed for the analysis of ten antidepressants in urine and plasma [109]. The LODs for these antidepressants were calculated to be 0.06–2.84 ng/mL in urine and 0.07–2.95 ng/mL in plasma, while the LOQs were 0.19–9.45 ng/mL in urine and 0.23–9.83 ng/mL in plasma. These results proved that the hybrid monoliths advocate an environmentally friendly, inexpensive, and rapid sample pretreatment technique, and the automated online in-tube SPME gets a higher throughput, improves precision of analysis, and protects the analysts from exposure to toxic solvents.

Preconcentration techniques including sample stacking, ITP, field amplified injection, sweeping, and pH-mediated stacking have been employed in CE to enhance the detection sensitivity for environmental and biological analysis. The sample stacking was widely used to concentrate the analytes at the front of a capillary column with inline mode, which can be formed by constructing a small monolith bed at the front of a CE capillary or by coupling a short monolith capillary column to the inlet of a CE capillary [9]. Zhou et al. [50] presented the use of a new hybrid monolithic column prepared in situ by polymerization of *N*-(β-aminoethyl)-γ-aminopropyl-triethoxysilane and TEOS via sol–gel process for extraction of DNA following CE separation. The main extraction mechanism was based on the Coulombic force between DNA and the amino groups of monolithic column. The inline DNA extraction with CE was also performed by using another hybrid monolith, which was synthesized with TEOS and *N*-(β-aminoethyl)-γ-aminopropylmethyldimethoxysilane as precursors via sol–gel process [51]. Hu et al. [38] also fabricated a phenyl-functionalized hybrid silica monolithic column using PTES and TEOS as precursors according to previous reports [36], and the hybrid monolith was used for the extraction of bisphenol A from cola samples. The pretreated samples were further concentrated and separated using large-volume stacking and sweeping MEKC.

### 3.3 Immobilized enzymatic reactor

The enzymatic microreactor has attracted great interest in past decades and applied in a variety of areas from inhibitor screening to proteolysis in proteomics research. The monolithic materials have been proved to be ideal matrices for preparation of IMERs due to their unique structural characteristics [110]. To date, a lot of porous organic monoliths and silica monoliths were successfully used as supports for immobilization of proteins and preparation of both stationary phases for bioaffinity chromatography and enzymatic reactors, exhibiting much higher activity compared to the reactions in solution [6]. Herein, we mainly focus on the preparation approaches of IMERs in hybrid organic-silica monolithic matrices. Several strategies were developed and could be divided into two diverse categories: (i) entrapment of proteins in hybrid organic-silica matrices via biofriendly sol–gel processing and (ii) covalently bonding the enzymes to the hybrid monoliths containing reactive groups.

A previous widely used method is entrapment of proteins in silica matrices using TMOS or/and MTMS as precursors via sol–gel method to form the monolithic composites, which has emerged as a promising platform for encapsulation of biomolecules such as enzymes, antibodies, and cells [111]. In order to enhance their stability and activity, biofriendly sol–gel processing was developed to fabricate highly biocompatible sol–gel-derived materials [112]. Kato et al. [113] added a very small amount of chitosan TMOS sol for the preparation of the BSA-encapsulated column. They found the column durability was improved by the addition of chitosan, indicating the chitosan/TMOS column was promising as a protein-encapusulation column. Hodgson et al. [114] developed protein-entrapped biocompatible hybrid silica materials prepared using a mixture of the protein-compatible silica precursor diglycerylsilane, poly(ethylene oxide) (MW 10 000), APTES, and a buffered solution of the protein of interest. The results demonstrated that diglycerylsilane and sugar-modified silanes such as maltonamidylsilane [115] and *N*-(3-triethoxysilylpropyl)gluconamide [116] could maintain the activity of a wide variety of proteins during the sol–gel process.

Obviously, the above-mentioned method of protein encapsulation for fabrication of hybrid IMERs has some disadvantages of usually requiring the addition of other additives, that is, PEG and dextrin to stabilize the structure and time consuming (ca. 3–15 days). In addition, large molecules such as proteins may not be able to diffuse through the nanopores of a network. Several new approaches were encouragingly developed to improve the IMER efficiency that involves the preparation of macroporous hybrid monoliths followed by surface modification and enzyme immobilization, just like those on general organic or pure silica monolithic matrices. Kato et al. [117, 118] prepared two miniaturized IMERs by coating enzymes-containing gel on two kinds of porous hybrid monoliths. First, the hybrid monolith was fabricated with TMOS and MTMS via sol–gel process, while trypsin-encapsulated sol was also prepared by the sol–gel approach, and then coated and gelled on the hybrid monolith to form a thin trypsin-encapsulated sol–gel film after 2–3 days [117]. Second, a photopolymerized sol–gel (PSG) monolith was fabricated by using γ-MAPS as precursors. The pepsin-encapsulated sol–gel film was similarly coated on the surface of PSG hybrid monolith without loss of enzyme activity [118]. Zare et al. [119] developed a trypsin-immobilized microreactor in a reactive hydrophilic macroporous PSG-PEG monolith, which was prepared in situ in a fused-silica capillary by using γ-MAPS and PEG dimethacrylate as precursors via a combination of sol–gel chemistry and photopolymerization, followed by functionalization of the hybrid monolith surface with trimethoxysilylbutyraldehyde containing an aldehyde group. The resulting trypsin–PSG–PEG microreactor exhibited long-term stability and high proteolytic activity for the on-column digestion of large peptides, which was enhanced more than 2000 times that in free solution [119]. This approach was similarly transferred into a microchannel for producing an on-chip enzyme immobilized hybrid monolith microreactor that integrates a microfluidic electrochemical cell for rapid characterization of enzymatic kinetics. The monolith was generated using TMOS and MTMS via sol–gel chemistry, followed by polyethylenimine functionalization and enzyme immobilization via electrostatic attraction between electronegative enzymes and electropositive polyethylenimine polymers [120].

Due to the activation groups such as amino-, epoxy-, and allyl-groups existing in the surface of several hybrid monoliths, they enable a variety of routes to the immobilization of an enzyme. First, the 3-aminopropyl-contained hybrid capillary monoliths developed by Zhang' group [46] was synthesized by using TEOS and APTES as precursors, and further activated using a common bifunctional reagent, glutaraldehyde. The trypsin was finally covalently immobilized on the aldehyde-activated hybrid monolith, following a reduction reaction of unstable aldimine functionality to a secondary amine with sodium cyanoborohydride to enhance the IMER stability. This approach eliminated the activation procedure of aminolysis that was generally required for enzyme immobilization starting from an epoxide-modified support. The resulting hybrid monolith of IMER was further integrated with micro-RP LC coupled with MS/MS (μRPLC-MS/MS) for online digestion in proteome research, demonstrating that such an online platform opened a route for high-throughput proteome analysis and improved identification of low-abundance proteins [47, 49]. Recently, they [121] developed a metal-ion chelate IMER supported on hybrid silica monolith for rapid digestion of proteins. The preparation procedures were simply illustrated in Fig. 9. The monolithic support was in situ prepared in a capillary via the polycondensation between TEOS hydrolytic sol and iminodiacetic acid conjugated GPTMS. After activated by $Cu^{2+}$, trypsin was immobilized onto the monolithic support via metal chelation. The successful application of such an IMER for efficient digestion of rat liver extract demonstrated that the IMER supported on hybrid monolith exhibited high enzymatic activity, good regeneration capacity, and improved protein identification [121]. An expoxy-functionalized hybrid monolith was also directly employed to prepare an IMER, which was synthesized by using GPTMS and TEOS as precursors. Trypsin was then immobilized via the reaction between vicinal diol groups, which were obtained from hydrolysis of epoxy groups, and the amino groups of trypsin. The present IMER has potential for high throughput digestion [122].

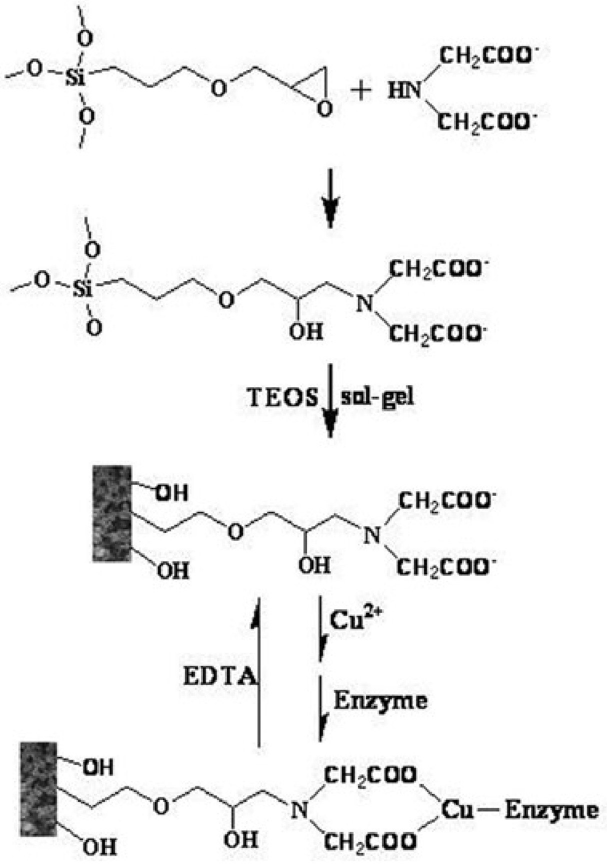

**Figure 9.** Synthetic scheme of organic–inorganic hybrid silica monolith for enzyme immobilization via metal ion chelation.

Chen et al. [123] presented a novel approach to prepare monolithic enzyme microreactor via thiol-ene click chemistry. The hybrid monolithic capillary column with methacrylate functionality was fabricated by sol–gel process using TMOS and γ-MAPS as precursors. The disulfide bonds of trypsin were reduced by tris(2-carboxyethyl)phosphine hydrochloride to form free thiol groups. Then the trypsin containing free thiol groups was immobilized on the hybrid monolith via thiol-ene click chemistry to form a trypsin microreactor. It was found that the microreactor with hydrophilic spacer could deliver the highest activity and yield a rapid reaction rate, indicating that thiol-ene "click" chemistry for the construction of IMER is a promising way for the highly efficient immobilization of proteins under mild conditions, especially enzymes with free thiol radicals [123].

## 4 Concluding remarks

As the development of a number of pure silica-based monoliths, hybrid organic-silica monolithic columns were initially developed to overcome some problems in the fabrication of silica monolith such as tedious preparation procedures, unsatisfied reproducibility due to the shrinkage in the gelling process. They were in situ synthesized by using commercially available tetraalkoxysilanes and alkyltrialkoxysilanes via sol–gel chemistry, offering the improved characteristics over silica-based monoliths including good mechanical stability and without in need of postmodifications. The organic moieties were directly incorporated into the silica support through a Si–C bond, which is more hydrolytically stable than a Si–O–Si–C linkage obtained by using different silylation reagents for surface derivatization of pure silica-based monoliths. As a result, hybrid monoliths are becoming an attractive and popular alternative to pure silica-based monoliths.

Although some developments on hybrid monoliths are just refinements of the methods developed previously, some notable new approaches are still emerging and growing. Compared to the direct synthesis approach of hybrid monoliths, which are limited in the use of commercial alkyltrialkoxysilanes, a postmodification or postfunctionalization certainly represents a complementary and flexible technique for the preparation of various monolithic stationary phases by directly exploiting the versatile reactive groups of hybrid monoliths, for examples, amino, epoxy, vinyl. The postmodification of hybrid monolithic columns allows thoroughly independent of controlling their morphology and chemistry, therefore, the reoptimization of the preparation conditions of hybrid monoliths can be avoided due to any change of desired chromatographic functionality. "One-pot" process represents a novel simple preparation approach of hybrid monolithic stationary phases, in which various organic functional monomers can be directly used in the preparation of hybrid monoliths with desired functionalities. Although the change of organic monomers may affect the porous property and phase separation, requiring optimization of synthesis conditions each time, the limitation of organo-functionalized silanes can be circumvented with "one-pot" approach. Many growing applications in RP, ion exchange, and hydrophilic interaction chromatography, as well as affinity chromatography have performed on hybrid monoliths for chiral separations and proteome analysis. Other polymerization such as free radical polymerization, ring-opening polymerization, and ring-opening metathesis polymerization, controlled free radical polymerization, polyaddition, and polycondensation, widely used for the preparation of organic monolithic materials, may be adopted to fabricate the hybrid monoliths using monomers containing silanes. This provides various facile ways to increase the mechanical and thermal stability of organic polymer-based monolithic column without sacrifice the merits such as good pH stability and easy preparation.

Even though the hybrid monolithic stationary phases in the traditional sizes (4.6 mm id) are not commercially available, there is no doubt that the development of hybrid monolithic stationary phases has opened a new avenue in chromatographic separation science, and more widely applications of hybrid monoliths in both separation and sample pretreatment will be expected. We can also optimistically expect novel discoveries related to miniaturization of separation devices based on hybrid monoliths in microfluidic chips.

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