Review

Recent progress of polar stationary phases in CEC and capillary liquid chromatography

The stationary phases including the nonpolar and polar stationary phases in microscale separations have been greatly developed. This review describes the recent progress of the polar stationary phases (PSPs) with the focus on their preparation and the interesting applications in the field of CEC and capillary liquid chromatography (CLC) covering the literatures from the middle of 2006 to the middle of 2008. The PSPs described herein were summarized into three types as open-tubular, particle-packed and monolithic stationary phases for either CEC or CLC, and the separation mechanisms are of hydrophilic interaction, chiral selection, ion-exchange and/or electrostatic interaction, etc. After overviewing the literatures published in the last 2 years, the research efforts on PSPs for CEC and CLC have been remaining in the fabrication of open-tubular and monolithic capillary columns; whereas, the research endeavor of PSPs on particulate-packed format seemed to decline most likely due to the commercial availability of various packing particles for CEC and CLC.

Keywords:
Capillary liquid chromatography / CEC / Polar stationary phase

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1 Introduction

Microscale separations have been gaining the rising attentions due to the distinct merits of the microscale columns applied in the field of chromatography, such as the low solvent consumption, high column efficiency and good sensitivity by coupling with ESI-mass spectrometers. The major output of the prepared stationary phases was the application in CEC and capillary liquid chromatography (CLC), covering the most research areas in the field of microscale separations.

Technically, CEC combines the advantages of HPLC and CE with the versatility of stationary phases and the great separation performances. The columns in CEC can be categorized into three types: the open-tubular, the particulate-packed and the monolithic columns. The corresponding separation modes in CEC, thus, can be simply called as the open-tubular CEC (OT-CEC), the packed CEC (P-CEC) and the monolithic CEC, respectively. The particulate-packed columns were fabricated by packing the traditional HPLC-particulates in capillaries with the supporting frits at both ends of the packed materials. The particulate-packed capillary column was ever the most favorable column style for CEC due to the mature arts and crafts in providing the versatility of stationary phases and the great choices of surface functionality and particle structure. As the development of CEC, the inherent limitations of the particulate-packed capillary columns, such as tedious packing procedure, frequent bubble formation, difficult fabrication of supporting frits and the lack of column reproducibility, etc., have come into the view of practices [1, 2]. The OT capillary columns have frequently been applied in CEC without the above-mentioned shortcomings. Unfortunately,
the blemish in the low phase ratio for conventional OT capillary columns was obvious in CEC separations. Certainly, the endeavors in increasing the phase ratios of OT capillary columns have been proposed, including surface etching, the coating of polymeric and silica layers, the binding of the nanoparticles on the inner walls of capillaries [3–7]. Rather than the OT and the particulate-packed capillary columns, the monolithic capillary columns have gained the increased attentions. The main benefits of using the monolithic column are the elimination of the problems associated with packed column, such as the difficulty in packing, the bubble formation in running due to the supporting frits of bed. Additionally, the much higher phase ratio of monolithic column than that of the OT capillary column also accelerated its wide applications in CEC.

CLC uses the capillary columns instead of the regular columns with the bigger inner diameters for HPLC. In CLC, due to the decrease of column inner diameter, the separation efficiency has been extremely improved as compared to that of conventional HPLC. Moreover, due to the resultant great sensitivity by coupling capillary columns to ESI-mass spectrometers, the CLC-MS systems have been widely applied in the fields of proteomics, genomics and metabolomics [8–10]. Generally, the inner diameter of a capillary used in CLC was in the range of 50–500 μm, and particle size for capillary packing was between 1.5 and 5 μm. Obviously, the difficulty in packing the small particles in capillaries is still the problem for CLC. Instead of the tedious packing process, the monolithic capillary columns were synthesized by in situ preparation of polymer- or silica-based monoliths with various specific functionalities arising from the versatile in situ chemical reactions and/or post-modifications. Importantly, the in situ prepared monoliths are chemically attached onto the inner wall of capillaries, and no retaining frits are required to support the monolithic matrices.

The stationary phases in CEC and/or CLC have been greatly developed for the demands of microscale separations. The major applications of these diverse stationary phases are of the reserved-phase separation using the nonpolar stationary phases, which have been well discussed and reviewed by various purposes [11–15]. Rather than the nonpolar stationary phases the polar stationary phases (PSPs) as the complementary stationary phases are also playing the irreplaceable role in microscale separations. In recent years, many kinds of PSPs have been developed for CEC and/or CLC based on the separation mechanisms of ion-exchange, chiral selection, hydrophilic interaction, etc. For giving the comprehensive view of PSPs, an early review on PSPs in CEC covering the literatures from 2004 till the middle of 2006 has been published [16]. To continually track the development of PSPs, this review is going to cover the literatures from the middle of 2006 to the middle of 2008, and trying to broaden the applications from CEC to CLC because of the distinct advantages and quick development of CLC in recent years.

## 2 PSPs in CEC

### 2.1 PSPs for OT-CEC

#### 2.1.1 Physically adsorbed PSPs

#### 2.1.1.1 Polymer coating

The coating of stationary phases on the inner walls of capillaries via the physical adsorption represents the convenient approach for the preparation of OT capillary columns. As for the separation of proteins, Liu et al. [17] coated a cationic Gemini surfactant of alkanediyl-2,2'-bis(dimethylalkylammonium bromide) (m–s–m, chemical structure shown in Fig. 1) onto the inner surface of capillary via the self-assembly coating. After the coating of the positively charged Gemini surfactant, the irreversible adsorption of proteins on silanol groups (Si–OH) of capillary was extremely suppressed, and the separation of proteins with high separation efficiency, fine reproducibility and recovery was then carried out. They found that the long alkyl chains (m ≥ 14) would provide a good coating stability, and the stability would increase as the alkyl chain length (m) of the surfactant increases. Using poly(methacryloxyethyltrimethylammonium chloride) and its poly(ethylene glycol) (PEG)-grafted analogue as the coating materials, Wiedmer et al. [18] demonstrated the separation of four basic proteins with column efficiency of 780 000–920 000 plates/m for OT-CEC due to the attachment of positively charged polyelectrolytes on capillary for avoiding the irreversible adsorption of proteins on silanol groups. Luces et al. [19] demonstrated the physical coating of polyelectrolyte multilayers on capillary with positively and negatively charged polymers, as shown in Fig. 2.

By taking the advantage of the hydrophilicity of PVA, Carter and Ajit Sharma [20] achieved the separation of polyamidoamine dendrimers based on the electrophoretic migration and the hydrogen bonding interaction on the PVA-coated capillary. Comparing to the bare fused silica, the PVA-coated capillary provided a stable surface with an optimum hydrophobic–hydrophilic balance for dendrimers separation without the need of any cumbersome washing procedures, buffer additives or data normalization. Via the electrostatic and hydrogen bond interaction, Mora and Garcia [21] reported a simple procedure of coating fused-silica capillaries with poly(diallyldimethylammonium

![Figure 1. Chemical structure of Gemini surfactant of alkanediyl-2,2'-bis(dimethylalkylammonium bromide (m–s–m).](image-url)
chloride) (poly-DADMAC) and montmorillonite for the effective separation of environmental phenolic compounds with a significant improved resolution. Due to the use of the quaternary ammonium ions, the EOFs of the capillaries were remained constant in alkaline solutions (pH \( \geq 7 \)), which allowed the optimization of the separation conditions for phenolic compounds.

2.1.1.2 Nanoparticle coating

Due to the high surface area of nanoparticles, the coating of nanoparticles on the inner wall of capillary would improve surface area of OT capillary column and thus increase the column capacity for separation. For ion-exchange separation, Zhang et al. [22] prepared a dual-layer (strong anion-exchange/strong cation-exchange – SAX/SCX) latex-coated ion-exchange column for the preconcentration of cations by first coating a base layer of cationic quaternary ammonium anion-exchange Dionex AS5A latex particles (60 nm diameter) on the negatively charged inner wall of capillary and then coating a second layer of anionic sulphonated cation-exchange Dionex CS3 latex particles (300 nm diameter) upon the first layer via the electrostatic attractions. The dual-layer columns exhibited a moderate and pH-independent EOF (ca. \( 26 \times 10^{-3} \text{m}^2 \text{V}^{-1} \text{s}^{-1} \)) with ion-exchange capacity of 57 \( \mu \text{equiv.} / \text{g} \). By coupling the 8 cm dual-layer latex-coated OT capillary and a 72 cm untreated bare fused-silica capillary, the on-line preconcentration and separation of monovalent organic bases, alkali metal ions and alkaline earth metal ions was carried out with low detection limits of analytes in OT-CEC.

For investigating the oxidation of LDL, an OT-CEC method by using the LDL-coated OT capillary columns has been developed [23, 24]. Recently, for examining the oxidation of human very-low-density lipoprotein (VLDL), an OT-CEC approach using VLDL-coated capillary was applied [25]. Interestingly, the VLDL-coated OT capillary demonstrated the better resolution and higher stability for the separation of steroids (at pH 7.4) as compared to the LDL-coated capillary. And the coated layer was further confirmed as the monolayer on the inner wall of capillary by atomic force microscopy using the radiolabelled VLDL. Similarly, the high-density lipoproteins (HDLs), the highly heterogeneous particles of lipoproteins with the smallest diameter (7–12 nm) and densest structure in all plasma lipoproteins, have also been used to coat the OT capillary columns [26], and which were then applied in the investigation of particle structure and transformations of HDL.

2.1.2 Chemically bonded PSPs

2.1.2.1 Bonded polymer

Chemically bonded polymers could serve as the favorable and stable stationary phases for OT-CEC as compared to the physically coated ones. Due to the abundant silanol groups on the inner wall of capillary, the modification of OT capillary could be realized via direct silinization, sol–gel process or polymerization with varieties of functional polymers. Lin et al. [27] demonstrated the immobilization of macrocyclic polyamines of 4,8,12,18,22,26-hexaaza-1,15-dioxacyclooctaeicosane ([28]ane-N6O2) (chemical structure shown in Fig. 3) onto the inner wall of \( \gamma \)-glycidoxypropyltrimethoxysilane (GPTMS) silylized fused capillary via a
ring-opening reaction. The obtained OT capillary column was used for the selenium speciation by OT-CEC with detection of inductively coupled plasma MS, and the urinary quantitative analysis of selenium speciation was thus carried out with high sensitivity and great selectivity. Recently, a 32-membered octaazamacroyclic molecule ([32]ane-N8, similar with [28]ane-N6O2) was also chemically bonded on the inner wall of capillary, and served as an anion receptor for the separation of some carbohydrates in gradient elution mode by taking advantages of the combination of the anion coordination, anion-exchange and shape discrimination [28]. Using a thermally initiated radical polymerization for crosslink, a multilayer poly(butadiene-maleic acid) was immobilized onto the inner wall of the fused-silica capillary. And the fast and efficient separations of common inorganic cations were thus achieved in less than 6 min with a 60 cm poly(butadiene-maleic acid)-bonded capillary column by coupling a contactless conductivity detector [29].

The 5,11,17,23-tetra-tert-butyl-25,27-diethoxy-26,28-dihydroxy-calix[4]arene (Calix[4]) as the functional selector for the separation of isomeric compounds was introduced by Tian et al. [30] via the immobilization on the wall of a fused-silica capillary by the sol–gel technology. Namely, Calix[4] was initially reacted with 3-GPTMS (KH-560) to form a sol–gel precursor (Calix[4]–KH-560), and then mixed with tetraethoxysilane (TEOS) and coated on the wall of capillary for OT-CEC as shown in Fig. 4. Due to the stereo-specificity of Calix[4], the Calix[4]-modified sol–gel capillary column showed the great performance in the separation of structurally isomeric compounds of toluidines, nitrophenols, picolines and five neurotransmitters (Fig. 5). The separation of these isomeric compounds on this stationary phase was mainly attributed to the hydrophobic interaction, π–π interaction, hydrogen interaction and host–guest inclusion.

Additionally, based on the chemical bonding approach, a tentacle-type metal-chelating polymer chains could be fabricated by introducing the iminodiacetate acid (IDA) on a poly(glycidyl methacrylate) (poly(GMA))-modified capillary via the procedures of the synthesis of monomer, silanization of capillary inner wall, in situ polymerization with GMA,
The MCM-41 type mesoporous silica nanoparticle [36, 37] has been widely studied in chemistry of materials because of its structural simplicity and ease in preparation as well as its prominent features: such as well-defined pore shapes (hexagonal/cylindrical); narrow distribution of pore sizes; negligible pore networking or pore blocking effects. Ordered MCM-41 mesoporous nanoparticles can provide much better adsorption capacity [38–41] than conventional adsorbents due to its large pore volume and very high surface area. In addition, the MCM-41 nanoparticles can provide abundant surface silanol groups (about 40–60%) [42], which can strongly adsorb other functional ligands or materials (such as cellulose Tris(3,5-dimethylphenyl-carbamate) – CDMP) via the hydrogen bonds or chemical bonding for further necessary modification. To enhance the phase ratio of OT capillary column, Dong et al. [43] introduced the MCM-41 nanoparticles onto the inner wall of a fused-silica capillary. Based on the MCM-41-nanoparticles-modified capillary, the CDMP as the chiral selector was statically coated upon the nanoparticle layer. Comparing with the bare fused-silica capillary column coated with CDMP, the CDMP-coated MCM-41 OT capillary column offered much higher enantioselectivity, which confirmed the improvement of the increase of the phase ratio in OT-CEC.

### 2.2 PSPs in P-CEC

In the past decade, CEC has been proved as a potential technique for enantioseparation due to its advantages in low consumption of sample, solvent and stationary phases. A number of CSPs such as CDs [44–46], celluloses derivatives [47–50], proteins [51], macrocyclic antibiotics
[52–55], etc., used for HPLC have been applied on CEC enantioseparations. Lammerhofer et al. [56] reported that the hydrophilic matrix was preferable for chiral separation due to the reduction of non-enantioselective interaction. Based on the hydrophilic silica matrix, the coated cellulose derivatives were applied for the separations of enantiomers [47, 57–61]. Karlsson et al. [62] had immobilized the macrocyclic antibiotics of vancomycin on the silica gel for enantioseparation in CEC in 2000, which showed powerful chiral recognition ability for β-blockers and thalidomide, especially with a nonaqueous polar organic mobile phase. Recently, Aturki et al. [63] applied the vancomycin immobilized CSP for the separation of novel antidepressant mirtazapine and its metabolites of 8-hydroxymirtazapine and N-desmethylmirtazapine. The simultaneous enantioseparation of these three pairs of enantiomers was achieved on this CSP within 30 min.

Andre et al. [64] bound the L-RNA aptamer to biotin, which was then grafted on the streptavidin-modified porous silica particles for the enantioseparation of a series of herbicide molecules in CEC, as shown in Fig. 6. When adding Mg2+ divalent cations in the mobile phase, the separation efficiency as well as the peak shape could be improved by stabilizing the secondary structure of aptamer and improving the mass transfer kinetics in the ligand-aptamer binding process. This L-RNA CSP was demonstrated to be effective in the enantioseparation with good stability and, as well, in the determination of the enantio-merization barrier and Eyring activation parameters of flamprop pesticide.

Lin et al. [65] immobilized the perphenylcarbamoylated β-CD on silica particles as the CSP for enantioseparation in pressure-assisted CEC. Seven chiral analytes were successfully resolved under the optimized conditions, and five of them could be baseline-separated within 12 min. For this CSP, the phosphate buffer seemed to be the more effective BGE versus triethylammonium acetate for enantioseparation. Avidin-immobilized magnetic particles (MPs) were also developed for enantioseparation in P-CEC [66]. The avidin-immobilized MPs were first prepared by physically immobilizing avidin on the carboxylated MPs, and then packed into a capillary by applying a magnetic field to form a fritless packed column. The obtained column showed good enantioselectivity toward ketoprofen with good repeatability under the optimized condition.

2.3 PSPs in monolithic CEC

2.3.1 Silica-based monolith

Silica-based monoliths were used as the valuable PSPs in CEC due to the presence of the silanol groups of the silica skeleton. Using the 3-aminopropyltrimethoxysilane-modified silica monolith, Ye et al. [67] performed a mixed-mode of hydrophilic and weak anion-exchange (WAX) mechanisms in CEC. The amino groups on the silica monolith not only provide the anodic EOF under acidic conditions but also served as the weak anion-exchanger. Polar compounds including phenols, nucleic acid bases and nucleosides were separated using a mobile phase with high ACN content based on the separation mechanisms of hydrophilic interaction, WAX and electrophoresis. Similarly, a diethylene-triaminopropyl-modified silica monolith was also applied in CEC as the PSP for the separation of neutral and basic solutes [68] with an anodic EOF generated under the acidic condition. The separation of neutral solutes including toluene, DMF, thiourea was achieved based on the hydrophilic interaction using the mobile phase containing 95% ACN, while the separation of basic tetracycline antibiotics was realized not only because of the hydrophilic interaction but also the electrophoretic migration. By modifying silica monolith with 3-glycidoxypropyltriethoxysilane and followed by a subsequent covalent immobilization of phenylalanine via a ring-opening reaction, Hu et al. [69] prepared a zwitterionic stationary phase that the direction and the magnitude of EOF could be easily manipulated by adjusting the pH value of mobile phase. The separations of acidic and basic compounds were respectively performed on this zwitterionic stationary phase with the separation mechanisms including weak hydrophobic and ion-exchange interactions. Interestingly, Hutchinson et al. [70] prepared the anion-exchange latex-coated silica monolith for IE-CEC. Compared to their previous prepared latex-coated OT

![Figure 6. Enantioseparation of (A) flamprop (fla), (B) 2-phenoxypropionic acid (2ppa), (C) diclofop (df), (D) haloxyfop (ha), (E) fluazifop (fl). Mobile phase: ACN/2 mM acetate buffer at pH 6.0, 10 mM NaCl, 5 mM MgCl2 0.15/0.85 v/v, temperature 30°C. (Reprinted from [64] with permission.)](image-url)
capillary column [71, 72], the latex-coated silica monolithic column showed the significant increase in retention of inorganic anions due to the high phase ratio and ion capacity of monolithic matrix.

By introducing the chiral selector onto the silica monoliths, the silica-based monolithic CSPs could be fabricated via the post-modification. Preinerstorfer et al. [73] immobilized the SCX-type chiral selector of (S)-N-[4-[allyloxy]-3,5-dichlorobenzoyl]-2-amino-3,3-dimethylbutane phosphonic acid onto the 3-mercaptopropyltrimethoxysilane-modified silica monolith for the separation of chiral basic drugs in nonaqueous CEC. Comparing with a particulate-packed capillary column with the same chiral selector, the monolithic silica capillary column showed better performance in system robustness and column longevity in CEC. Also, by immobilizing cinchona alkaloid-derived anion-exchange-type chiral selector, namely O-9-(tert-butylcarbamoyl)quinidine, onto the silica monolith [74], the simultaneous separation of four stereoisomers of N-benzylxycarbonyl phosphinic pseudodieptide methyl ester benzylxycarbonyl-homophenylalanine was achieved with nonaqueous buffers. By immobilizing the macrocyclic antibiotic vancomycin onto the silica monolith, Dong et al. [75] realized the enantioseparation of five β-blockers and thalidomide with column efficiency of 48 000–210 000 plates/m in either nonaqueous organic or aqueous mobile phases.

### 2.3.2 Polyacrylamide-based monolith

Polyacrylamide-based monoliths demonstrate the great hydrophilicity, and thus can be used as the PSPs for CEC separations. Guryca et al. [76] prepared a polyacrylamide-based monolithic column for the separation of oligosaccharides via in situ copolymerization of acrylamide (AAm) and N,N'-methylenebisacrylamide (MBA) in the presence of aqueous porogens of DMF, H2O and PEG. Rather than the monomer of AAm, a more hydrophilic monomer of N-[Tris(hydroxymethyl)methyl]acrylamide was applied in the preparation of monolithic column. Not as expected, the obtained N-[Tris(hydroxymethyl)methyl]acrylamide monolithic column did not perform better than the poly(AAm-co-MBA) monolith. Generally, the polyacrylamide-based monoliths prepared in the presence of aqueous porogenic solvents would provide the soft monolithic skeleton. To obtain the rigid polyacrylamide-based hydrophilic monolithic column, Dong et al. [77] used the fully organic porogenic solvent consisting of DMSO and dodecanol via in situ copolymerization of AAm, MBA and 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS). The incorporated sulfonic groups from AMPS on the monolithic column not only served as the generator of EOF, but also played as the SCX sites for the recognition of enantiomers. Twelve pairs of enantiomers including acidic, neutral and basic analytes were mostly baseline separated under acidic or basic aqueous mobile phases with the assistance of the stable EOF over a wide pH range arising from the quaternary ammonium groups of the monolithic column.

### 2.3.3 Polymethacrylate-based monoliths

The polymethacrylate-based monoliths generally showed the strong hydrophobicity because of the hydrophobic skeleton derivatized from the polymerization of methacrylates. For obtaining the hydrophilic or ion-exchange properties based on the polymethacrylate-based monoliths, the introduction of monomers with ion-exchange or hydrophilic moieties to the monoliths are generally required. Lin et al. [82] fabricated the polymethacrylate-based monoliths with dual properties of hydrophilic/cation-exchange and RP/cation-exchange via in situ copolymerization in capillaries. Where, the poly(GMA-co-3-sulfopropyl methacrylate-co-ethylene dimethacrylate (EDMA)) monolith was first prepared by polymerizing of GMA, 3-sulfopropyl methacrylate and EDMA. After that, the epoxy groups of GMA on the
obtained column was then hydrolyzed by hydrochloric acid to generate the diol groups and enhanced the hydrophilicity of polymethacrylate monolithic matrix. This diol monolithic column was then applied in the separation of five alkaloids by CEC using high ACN content mobile phase. The separation mechanism in this case was confirmed as a mixed-mode of hydrophilic and cation-exchange interaction due to the co-existence of diol and sulfonic groups. Based on this mixed-mode, the phenols were thus successfully separated with the high efficiency [83]. However, when decreasing the content of the organic solvent (ACN) to 50% in mobile phase, the separation of compounds including anilines, nucleic acid bases and basic narcotic pharmaceuticals were then achieved on the RP/cation-exchange mode. Shortly, the mixed separation mechanism of hydrophilic interaction/cation-exchange was observed with high ACN content (>80%) in mobile phase, whereas the mixed-mode of RP and cation-exchange was then carried out with low ACN content (<70%) in mobile phase.

By attaching the chiral selectors on the monolith matrices via post-modifications, the enantioseparations could be achieved. Messina et al. [84] prepared a CSP by introducing the ergot alkaloid derivative of (±)-1-(4-amino- butyl)-(5R,8S,10R)-terguride onto poly(GMA-co-EDMA) monolith via the ring-opening reaction between the epoxy and primary amino groups. On this monolithic stationary phase, the anodic EOF was generated due to the positively charged amino groups under low pH value. And the successful enantioseparation of seven 2-aryloxypropionic acids in CEC was subsequently realized with a nonaqueous (ACN:MeOH = 9:1 v/v) mobile phase consisted of 5 mM TEA/acetic acid (TEA/acetic acid = 0.013–0.024 v/v).

### 2.3.4 Organic–inorganic hybrid monolith

As the derivative of silica monolith, the organic–inorganic hybrid monoliths were synthesized by sol–gel process based on the hydrolysis and co-condensation of siloxane and organo-siloxane precursors [85]. Ding et al. [86] prepared a hybrid silica monolith by the sol–gel technology using precursors of TEOS, aminopropyltriethoxysilane and octyltrimethoxysilane. The incorporated amino groups on this hybrid silica monolith served as the WAX groups, while aromatic acids were separated based on the combined mechanism of
electrophoretic migration, the weak ion-exchange and RP interactions. And for basic compounds, the peak tailing was dramatically reduced as the amino groups on the surface shielding the strong adsorption of positively charged analytes onto the silica monolithic matrix. Lin et al. [87] prepared an anion-exchange hybrid silica monolith by sol–gel method using N-trimethoxysilylpropyl-\(N,N,N\)-trimethylammonium chloride as the functional organic precursors. They found that the adding time of PEG (the phase separation catalyst in sol–gel process) could dramatically affect the pore structure and the performance of the resulted hybrid silica monolith. By controlling the addition of PEG at 2 h after the prehydrolysis of TMOS, the maximum selectivity of inorganic anions on the obtained monolith and column efficiency could be achieved. The separation of anionic analytes was attributed to the anion-exchange and electrophoretic migration. Recently, Zheng et al. [88] adopted methacryloxypropyltrimethoxysilane (MPTMS) as the precursor in the preparation of organic–inorganic hybrid silica monolith for the separations of 16 PAHs and 11 alkyl phenyl ketones in CEC. It was observed that the \(\pi-\pi\) interaction between the aromatic analytes and the methacryloxy residues did contributed to the CEC separation along with the primary hydrophobic interaction.

Tian et al. [89] prepared a Calix[4] open-chain crown ether-modified hybrid silica monolith for CEC separation by introducing the Calix[4] open-chain crown ether (\(p\)-tert-butyl-calix[4]arene–1,3-bis(allyloxyethy) ether) onto a vinyl-functionalized silica monolith. Comparing to the vinyl-functionalized hybrid silica monolithic column, the Calix[4]-modified silica monolith showed better performance in the separation of nucleotides, \(\beta\)-blockers, neurotransmitters and PAHs due to the host–guest and intermolecular hydrogen bonding interactions. Wang et al. [90] introduced a room temperature ionic liquid-mediated nonhydrolytic sol–gel protocol using MPTMS as the precursor, carboxylic acids as the functional monomers and zolmitriptan as the template for preparing the molecularly imprinted hybrid silica monoliths. The incorporated room temperature ionic liquid could reduce the gel shrinkage and act as the pore template, and hence improved the recognition of analyte on the prepared hybrid silica monolith. The excellent chiral separations of \((R)/(S)\)-zolmitriptan were thus carried out in CEC with the molar ratios of methacrylamide to MPTMS at 1:4 and 1:2.

### 2.3.5 Particle-fixed monolith

By fixing the particulates within the confines of capillaries, the particle-fixed fritless monolithic columns could be prepared for CEC separations. Schmid et al. [91] embedded silica-based CSP particles (3 \(\mu\)m, modified with teicoplanin aglycone or ristocetin A) in the copolymerized matrices of methacrylamide, piperazine diacrylamide, ammonium sulfate and charge-providing agent of VSA or DADMAC. The introduced VSA and DADMAC produced the cathodic and anodic EOF, respectively, and a number of racemic amino acids and dipeptides were resolved. However, the column efficiencies (ca. 10 000 plates/m) after the entrapping process were lower than those of the particulate-packed column using the same particles. Additionally, by fixing the \(1,4\)-hydroxyproline-modified particles in a capillary, the enantioseparations of amino acid enantiomers were realized in CEC and CEC based on the ligand-exchange mechanism [92]. Via the ring-opening metathesis polymerization, Gatschelhofer et al. [93] encapsulated the teicoplanin aglycone-modified silica particles within the capillary using the monomers of norborn-2-ene, 1,4,4a,5,8,8a-hexahydro-1,4,5,8,exo-endo-dimethanonaphthalen. Twelve pairs of glycyli dipeptides were successfully resolved with most of them at baseline separation on the prepared column.

### 3 PSPs in CEC

Besides the application of PSPs in CEC, the PSPs applied in CEC have been demonstrated in the analysis of polar compounds including metabolites, peptides, proteins and carbohydrates. A lot of efforts have been contributed to the development of PSPs in CEC with improved stability, resolution, sensitivity and the flexibility as hyphenated with mass spectrometers. In this part, the PSPs in CEC were reviewed in three column types of OT, packed and monolithic columns, respectively, with the emphasis on the monolithic capillary columns due to its continuously quick development in recent years.

#### 3.1 PSPs in OT- and P-CLC

For OT capillary column, recently, Kuban et al. [94] obtained a multilayered stationary phase with quaternary amino groups for anion-exchange CEC. The multilayered PSP on the inner wall of a capillary was prepared by a successive coating approach via the condensation polymerization of methylamine and 1,4-butanediol diglycidyl ether. Up to 25 successive porous polymeric layers could be obtained, and the increase of the porous polymer layer could correspondingly increase column capacity and chromatographic performance in the OT–CLC mode. The baseline separation of a suite of inorganic anions (\(F^{-}\), \(Cl^{-}\), \(NO_{3}^{-}\), \(Br^{-}\), \(NO_{2}^{-}\)) could be achieved by using the anion-exchange PSP with 25 porous polymeric layers.

In P-CLC, the PSPs used recently were most commercially available ion-exchange materials, and could be used for the separation of polar analytes based on the different mechanisms [10, 95, 96]. The progress of PSPs in P-CLC will not be expanded further in this review.

#### 3.2 Monolithic PSPs in CEC

#### 3.2.1 Hydrophilic monolith

Hydrophilic interaction liquid chromatography (HILIC) is a kind of version of the normal phase liquid chromatography,
In HILIC, the (hydrophilic) aqueous layer is formed near the surface of the stationary phase against the (hydrophobic) organic mobile phase, which thus generates environment for the separation of analytes with the mechanisms including the two-phase partitioning, the hydrogen bonds interactions and as well as the electrostatic interactions under the highly organic solvent conditions. The PSP is widely applied in HILIC, because the stronger PSPs would provide the greater hydrophilic interaction.

A hydrophilic monolithic stationary phase was prepared by Jiang et al. [97] via the copolymerization of N,N-dimethyl-N-methacryloyloxyethyl-N-(3-sulfopropyl)ammonium betaine and EDMA within the confine of a 100 μm id capillary. The obtained monolith demonstrated the good selectivity for neutral, basic and acidic polar analytes in HILIC with a high organic content (ACN% > 60%) in mobile phase. The good mechanical strength with no swelling or shrinking was also observed. By changing the hydrophilic monomer, another methacrylate-based hydrophilic monolith was also obtained via the in situ copolymerization of N-(hydroxymethyl) methacrylamide and EDMA with the porogens of 1-propanol and butane-1,4-diol [98], which could be used for the separation of oligonucleotides with a mobile phase containing high content of ACN.

By the polycondensation of Tris(2,3-epoxypropyl) isocyanurate and 4-[(4-aminocyclohexyl)methyl]cyclohexylamine as shown in Fig. 8, a polymer-based monolithic PSP was prepared by Hosoya et al. [99], which possessed an excellent homogenous structure as seen in Fig. 9. In the mobile phase containing 60% of ACN, the prepared monolithic column demonstrated the hydrophobic property, and could be used for the separation of alkylbenzenes in RP mode with a column efficiency of 40 000 plates/m. However, using the higher ACN content (100%) in mobile phase, the hydrophilic interaction and high column efficiency up to 60 000 plates/m was observed because of the existence of the polar hydroxyl and amine functional groups on the polymer backbone.

Upon the silica-based monolith, Ikegami et al. [100] prepared a weak cation-exchange (WCX) monolithic column by first modifying the silica monolithic matrix with N-{3-triethoxysilylpropyl} methacrylamide and then being copolymerized with acrylic acid to introduce the carboxylic groups as the WCX moieties. Pyridylamino-sugars and peptides including a tryptic digest of BSA were separated on the poly(acrylic acid)-coated monolith in HILIC, while proteins and nucleosides were analyzed in WCX mode. Due to the greater hydrophilicity of poly(acrylic acid), pyridylamino-sugars showed the greater retention on this poly(acrylic acid)-coated monolith than that on a more hydrophilic polyacrylamide-coated monolith for comparison [101].

### 3.2.2 Ion-exchange monolith

Suzuki et al. [102] introduced cetyltrimethylammonium bromide onto the silica monolith to form an anion-exchange stationary phase for capillary ion chromatography. Because of the favorable permeability of silica monolith, rapid separation of five anions was achieved on this column with low inlet pressure. In addition, the stability of the prepared column was improved by the addition of small amount of cetyltrimethylammonium chloride in the eluent. Using the amphoteric surfactant, O’Riordain et al. [103] introduced N-dodecyl-N,N-(dimethylammonio)undecanoate onto the silica monolith for the separation and determination of inorganic anions as well. Wieder et al. [104] presented an SAX poly(GMA-co-DVB) capillary column for the separation of nucleotide and oligonucleotides. The introduction of SAX groups onto the surface of monolith including two steps: the ring opening of the epoxide groups by diethylamine, and the following alkylation of obtained tertiary amine with diethyl sulfate to produce the quaternary ammonium functionality. The increase of pH value of mobile phase led to a stronger retention of oligonucleotides, which revealed the stronger electrostatic interaction between SAX stationary phase and more deprotonated oligonucleotides at higher pH.

For the preparation of SCX monolith, Gu et al. [105] prepared a column by in situ photopolymerization with AMPS and poly(ethylene glycol)diacrylate (PEGDA) as the...
monomers. Because of the high content of AMPS (40%) in monolith, the obtained column showed the high ion-exchange capacity. As shown in Fig. 10, very nice separations of peptides or protein digest were obtained with high resolution, peak capacity and efficiency with the separation mechanisms of electrostatic and RP interactions. The excellent performance was mainly attributed to the application of much hydrophilic and biocompatible crosslinker, PEGDA. Afterwards, another SCX monolith was prepared by using VSA and PEGDA for polymerization [106], which demonstrated much lower hydrophobicity when used propylparaben as the test analyte. Further nice separation of peptides, standard proteins and HDLs were observed under cation-exchange mode. Recently, Wang et al. [8] prepared a polyacrylamide-based SCX monoliths by in situ polymerization of ethylene glycol methacrylate phosphate and MBA in a trinary porogenic solvent of DMSO, dodecanol and DMF, which was used as the trap column in nanoflow liquid chromatography. Such phosphate monolithic column presented higher dynamic binding capacity, faster kinetic adsorption of peptides, and more than ten times higher permeability than the particulate packed SCX column. By coupling the phosphate monolithic column with an RP column in 2D-LC/MS analysis, the efficient separation of tryptic digest of yeast proteins were obtained with the identification of 1522 distinct proteins. Moreover, by integrating 10 cm SCX monolith and 65 cm RP monolith in one single 100 μm id capillary in two steps: (1) the preparation of 10 cm phosphate monolith; (2) the preparation of poly(lauryl methacrylate-co-EDMA) monolith within the pre-prepared capillary at the first step, a biphase integrated monolithic column was prepared by Wang et al. [107] with SEM images shown in Fig. 11. This SCX/RP integrated monolithic column was used for the online multidimensional separation of yeast protein digestes in LC-MS, and good separation performance was obtained with the identification of 780 proteins by five-step gradient elutions.

Besides the above-mentioned monolithic PSPs, Bakry et al. [108] developed a particle-fixed monolith for separation of nucleotides in μ-HPLC by introducing the anion-exchange silica particles into capillary and encapsulated by poly(styrene-divinylbenzene) via in situ polymerization. Such fritless particle-fixed monolith showed favorable stability and mechanical strength.

3.2.3 Chiral monolith

Several CSPs based on the silica monoliths have been developed in the past 2 years. Chankvetadze et al. [109] modified a silica monolith with amylase Tris(3,5-dimethylphenylcarbamate) to form a CSP for enantioseparation in CLC. The CSP was evaluated by ten selected racemates, and the superior enantioselectivity was obtained. Fast separations of 1,2,2,2-tetraphenylethanol and 2,2'-dihydroxy-6,6'-dimethylbiphenyl were also achieved within 1 min due to the favorable hydrodynamic properties of the silica monolith. By covalent immobilization of cellulose derivatives onto the silica monolith [110], the silica monolithic column also showed high stability and powerful enantioselectivity.

4 Conclusions

The preparation and application of PSPs will remain its attraction in the field of microscale separations including CEC and CLC. As the quick development of biology-related
areas, the great challenge to the analytical separation scientists is the analysis of the highly complex biological mixtures including a number of polar biological compounds. The progress on the PSP as the complement of the commonly used nonpolar stationary phases will extremely enhance the power in separating complex mixtures. The PSPs based on OT capillary column are still promising as the simplicity and convenience in separations. The endeavor for improving the phase ratio of OT capillary column is most likely welcome to overcome the inherent disadvantage. It is worth to investigate the application of nanostructure materials and monolithic porous layer in OT capillary for enhancing the phase ratio and column capacity. The monolithic capillary columns could offer the great performance in the microscale separations. The flexibilities in chromatographic operation and column preparation along with the versatile approaches for post-modification have made the monoliths as the most promising stationary phases.

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5 References


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Figure 11. SEM image of a biphasic monolithic capillary column (100 µm id) at magnification of 5000×. (Reprinted from [107] with permission.)
phoresis 2008, 29, 928–935.


[85] Hu, J. W., Xie, C. H., Tian, R. J., He, Z. K., Zou, H. F., Electro-

[86] Ding, G. S., Da, Z. L., Yuan, R. J., Bao, J. J., Electro-


[90] Wang, H. F., Zhu, Y. Z., Lin, J. P., Yan, X. P., Electro-

[91] Schmid, M. G., Koidl, J., Freigassner, C., Tahedl, S. et al., Electro-


