13
Capillary Electrochromatography

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13.1
Introduction

Capillary electrochromatography (CEC) is a hybrid separation technique that combines the high selectivity of high-performance liquid chromatography (HPLC) and the high efficiency of capillary electrophoresis (CE) [1–9]. In CEC, the mobile phase is driven through the chromatographic stationary phase by electro-osmotic flow (EOF) that is the motion of liquid induced by an applied potential across a capillary tube. Different from parabolic that is driven by a pressure in HPLC, CEC holds plug-like flow profile driven by EOF and, therefore, generates narrow peaks and achieves much more efficient. In addition, CEC allows for a small diameter packing beads to achieve high efficiencies due to no back pressure. Compared to CE, the use of stationary phase of CEC results in the better separation of solutes (especially for uncharged sample components). CEC has presented its unique advantages such as high separation power, high selectivity, high sensitivity, short analysis time, low consumption of samples and chemicals, and good compatibility with mass spectrometry (MS). Thus, a great attention has been paid to applications of CEC. Separation by CEC depends on several important parameters, such as applied voltage, stationary phase, and mobile phase composition. Many excellent CEC reviews have been published in the past 10 years [10–27]. The aim of this chapter is to highlight recent, significant developments within the stationary phases and CEC applications.

13.2
Stationary Phase

The stationary phase is crucial to CEC separation, because it decides the electrochromatographic migration behavior of analytes. To date, three classes of capillary columns have been widely used in CEC including fused silica packed columns, open tubular coating columns, and monolithic columns [11,12,18,19].

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13.2.1 Packed Column CEC

The particulate packing material is a popular type of CEC stationary phase, because HPLC packing material provides a wide range of choices for CEC. The capillaries are typically packed with 3 μm, 5 μm, or 1.8 μm functional silica particles, and two retaining frit are used to fix beads. The detection window is immediately adjacent to one of the frits, thereby allowing on-column detection. A variety of packed columns have been prepared and applied to CEC, and a large number of works have been published in the past 30 years [11]. Compared to HPLC, CEC displays super high column efficiency using the same packed column due to faster intraparticle mass transfer. Good capillaries packed in CEC should generate at least 150 000 plates per meter.

Bead pore size and morphology have been considered to have an important effect on separation efficiency in packed column CEC. Fanali *et al.* recently evaluated and compared the performance of the full porous and core–shell (nonporous)-based capillary columns in nanoliquid chromatography (nano-LC) and CEC separation. Their results demonstrated that intraparticle pore flow is the major contributor to improved peak efficiency in CEC compared to nano-LC and core–shell materials may be quite useful under CEC conditions [28]. This group also compared the separation of cytochrome *c* tryptic digest using packed C_{18} silica-based column in nano-LC and CEC. Their results demonstrated that the use of CEC revealed a higher separation efficiency and shorter analysis time [29].

Packed column has been widely used in pressurized CEC (pCEC) and multiple separation system [30]. Recently, Yan's group developed a 2D separation platform using micro-LC and pCEC for the analysis of complex samples [31]. Samples were fractionated by the first-dimension micro-LC using packed strong cation-exchange (SCX) column and then injected into the second-dimension pCEC with packed C_{18} column for further separation, as shown in Figure 13.1. The 2D system permits three separation modes involving CE, SCX, and reversed phase chromatography and, therefore, provides high selectivity, high resolution, and high peak capacity.

In traditional packed column, the two frits, which are fabricated by sintering a small section of silica beads, are necessary to entrap the stationary phase into the capillary. However, this approach results in severe problems such as bubble formation and unstable EOF, band broadening, fragility of frit, poor reproducibility, and adsorption of analytes onto the frits. As an alternative to traditional frits, an organic polymer inlet frit [32] or outlet frit [33] can address the above issues. In addition, soft ferrite-based micromagnetic particles have been reported as novel frits for CEC [34]. Furthermore, the approach was combined with a T-junction connector for CEC postcolumn derivatization [35]. Recently, a solvothermal reduction method for the synthesis of uniform magnetite spheres was developed for the preparation of magnetically immobilized frits for packed CEC [36]. Meanwhile several physical fritting methods have been successfully applied to

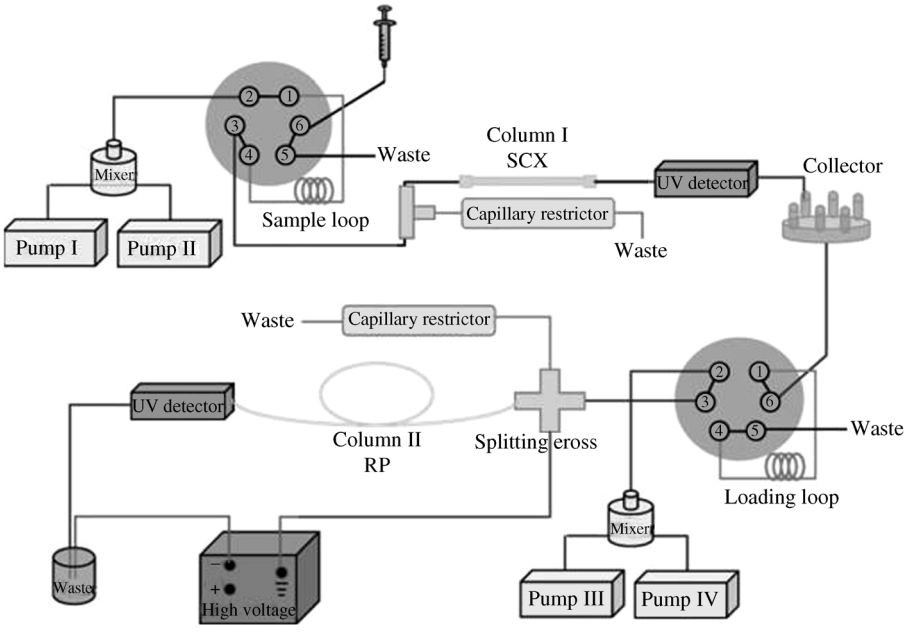

Figure 13.1 Schematic of 2D SCX-μLC/RP-pCEC. (Reproduced with permission from Ref. [31]. Copyright John Wiley and Sons.)

CEC [37,38]. Compared to traditional frits, these novel frits and approaches have showed their potential and good properties for CEC.

13.2.2 Open Tubular Column CEC

The open tubular (OT) capillary column, which does not require the fabrication of frits formation and particles' packing, contains the stationary phase as a coating on the inner wall of the tubes by chemically linking or physically or dynamically adsorbing. Compared to other forms of stationary phase or pseudo-stationary phases in CEC, the coating of OT capillary column has some advantages, such as higher stability, higher separation efficiency, and ease of preparation. Therefore, both of macromolecules and small molecules have been successfully separated in OT-CEC [12]. In recent years, several new materials have been characterized as the stationary phase for OT-CEC.

Nanoparticles (NPs) have attracted tremendous interest in separation science because of their unique physical and chemical properties derived from the "quantum size effect" [22,39]. Recently, Hamer, Yone, and Rezzano reported a new method of immobilization of gold nanoparticles (AuNPs) on a fused silica to improve the efficiency of separation and the selectivity of selected solutes [40]. This study indicates that the immobilization of AuNPs on the capillary wall

could significantly alter the electrophoretic mobility and improve the separation of tryptic peptides. Miksik *et al.* developed the bare GNP-based stationary phase for OT-CEC separation. Their study indicated that OT-CEC stationary phase might be a potential tool for identification of nonenzymatic and enzymatic post-translational modification of proteins [41].

Graphene and functional graphene (e.g., GO and GOOH) have drawn considerable attention in separation science research due to their unique physicochemical properties such as ultrahigh specific surface area and strong Π-Π electrostatic stacking property, and facile modification. Qu, Gu, and Hu recently reported the application of G and GO as coating material for OT-CEC [42]. The GO-coated capillary column was fabricated, as shown in Figure 13.2. Generally, the inner interface of the capillary column was first washed and dried, and then modified with 3-aminopropyldiethoxymethyl silane (3-AMDS). The capillary was subsequently washed with GO solution to produce a layer of GO. Interestingly, a pH-dependent EOF was observed in the G-coated column, while a constant EOF was shown in the GO oxide coated column. Their study showed that separation

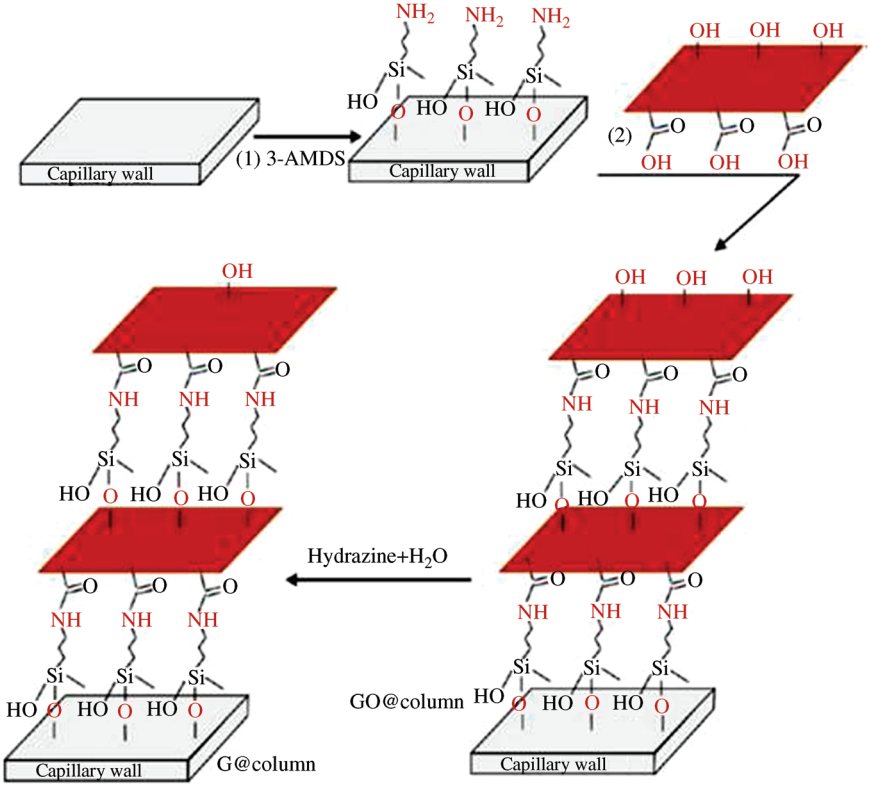

Figure 13.2 Schematic representation of the fabrication processes of GO and G-coated capillary columns. (Reproduced with permission from Ref. [42]. Copyright American Chemical Society.)

of neutral analytes was obtained in a typical reversed phase mode in GO oxide-coated column. However, the separation got poor in G-coated column due to the strong Π-Π stacking and hydrophobic interactions between graphene and poly-aromatic hydrocarbons. Liu *et al.* also used GO and reduced graphene oxide (GOOH) sheet as stationary phases for OT-CEC separation [43]. In this work, GO or GOOH was immobilized on the inner walls of a bare capillary column by electrostatic interaction between negatively charged GO or GOOH and positively charged poly(diallyldimethylammonium chloride) (PDDAC) layer modified on the inner surface of the capillary. They performed OT-CEC for the separation of various analytes, including acid nitrophenol, isomers, basic nitroaniline isomers, and neutral polycyclic aromatic hydrocarbons (PAHs). Their results indicated that OT-CEC separations depend solely on the interaction between the solutes and the stationary phase for neutral PAHs and weak basic nitroaniline isomers, while the separation replies on both electrophoresis and stationary–solute interactions for negatively charged acid nitrophenol isomers.

Metal-organic frameworks (MOFs) are attractive as novel separation medium due to their distinguished properties such as large surface area, accessible tunnels, and diverse structures. Yan's group first used MOFs as the stationary phase for chromatographic separation [44,45]. This group further developed CAU-1@PMMA-coated capillary, which was generated by incorporating MOF into polymethyl methacrylate (PMMA) [46]. Due to the increases of surface area and EOF, the CAU-1@PMMA-coated capillary column presented higher column efficiency, larger column capacity, and shorter separation time for baseline separation of aromatic carboxylic acids than the PMMA-coated capillary column. Xu *et al.* recently prepared an MIL-100(Fe) coated OT capillary column for CEC though *in situ*, layer-by-layer self-assembly approach [47]. As a new coating material, MIL-100(Fe) was evaluated and proposed as a separating medium to carry out the chromatographic process with CEC. The separation of some neutral, acidic, and basic analytes was obtained due to the size selectivity of lattice aperture and hydrophobicity of the organic ligands.

Chen *et al.* developed a novel electrochromatographic technique to immobilize living biofilm-forming bacteria on fused silica capillaries [48]. They selected *Staphylococcus aureus* as model bacterium. *S. aureus* formed a biofilm on the inner wall of a fused silica capillary coated with poly(L-lysine). They optimized the conditions for biofilm formation including bacterial concentration, growing time, and the stability of the ensemble. Interactions between *S. aureus* biofilms and different antibiomicrobial agents were further studied for CEC. The effect of five antibiotics (penicillin G, oxacillin, fusidic acid, rifampicin, and vancomycin) on biofilms was examined in terms of retention factors and reduced mobilities of the antibiotics. The antibiotic susceptibility profile for *S. aureus* is similar as the result of minimal inhibitory concentrations registered on the 96-microtiter well plates for both planktonic and biofilm cells. The technique allows a highly efficient and easy characterization of interactions between *S. aureus* biofilms and potentially active antimicrobial compounds under different conditions.

In OT-CEC, the ionic functional group can be conjugated or adsorbed to the capillary wall to provide a means of controlling the ionic characteristics and the EOF as a function of pH. Recently, Moore *et al.* synthesized a zwitterionic molecular micelle, poly-epsilon-sodium-undecanoyl lysinate (poly-epsilon-SUK), which contains both carboxylic acid and amine groups, as a coating in OT-CEC for protein separation [49]. The EOF can be adjusted or reversed depending on the pH of the background electrolytes, because the zwitterionic poly-epsilon-SUK can be either protonated or deprotonated. The separation of four basic and six acidic proteins can be achieved under the optimized condition. Poly-ε-SUK coating presents a unique property for the simultaneous separation of acidic and basic proteins.

Xu and Sun developed a phenylalanine (Phe) functionalized tentacle-type polymer-coated capillary column for protein separation by OT-CEC [50]. Both cathodic and anodic EOF could be obtained by varying the pH values of the mobile phase, due to the amphoteric functional groups of the Phe bonded to the tentacle-type polymer stationary phase. Their result indicates that the migration behavior of the four proteins depends on the interplay of chromatographic retention and electrophoretic migration.

Shen *et al.* reported a new amphipathic block copolymer, poly(*tert*-butyl acrylate)$_{(127)}$-block-poly(glycidyl methacrylate)$_{(86)}$ as the coating in OT-CEC [51]. The modified capillary inclined to hydrolyze generate EOF under basic conditions by ring-opening reaction. In addition, the coating could form micelle-like aggregates by self-assembly. Compared to bare capillary, this coating could act as a surfactant and lead to improve the separation of steroids.

Zhou *et al.* developed a hydrophilic polysaccharide, carboxymethylchitosan (CMC)-modified OT-CEC for the separation of basic proteins [52]. CMC was covalently bonded to the capillary inner wall and exhibited a remarkable tolerance and chemical stability against 0.1 mM HCl, 0.1 mM NaOH, or some organic solvents. Their work demonstrated that the presence of the molecular micelle in the PEM coating decreased the adsorption of proteins onto the capillary wall and enhanced the protein–PEM interaction, and resulted in better protein separation than the use of a simple cationic polymer alone.

Polyelectrolyte multilayer (PEM) coating is a kind of extremely stable coating that is constructed by the use of several electrostatic interactions involving ion-exchange. Luces *et al.* used four cationic polymers, namely, poly-L-lysine, poly-L-ornithine, poly-L-lysine-serine, and poly-L-glutamic acid-lysine), and three anionic molecular micelles, namely, sodium poly(*N*-undecanoyl-L-leucyl-alaninate) (poly-L-SULA), sodium poly(*N*-undecanoyl-L-leucyl-valinate) (poly-L-SULV), and sodium poly(undecylenic sulfate) (poly-SUS) as PEM coating for enhanced protein separation in OT-CEC [53]. The separation of four proteins was investigated by changing cationic polymer concentration, number of bilayers, temperature, applied voltage, and pH of the BGE. The use of molecular micelles to form PEM coatings resulted in better separation than single cationic coatings. Their work demonstrated that the presence of the molecular micelle in the PEM coating decreased the adsorption of proteins onto the capillary wall and enhanced the

protein–PEM interaction, and resulted in better protein separation than when a simple cationic polymer is used alone.

13.2.3 Monolithic Column CEC

Monolithic materials have been extensively evolved as the legitimate member of the large family of separation media because of their available preparation, fast mass transfer, and enhanced efficiency. Monolithic columns not only overcome the difficulties associated with standard packed column technology but also eliminate the need for end frits to retain the stationary phase in CEC [10,13,16,19,21,54,55]. In general, the monolithic columns can be categorized into three types: organic polymer-based monolithic column [16], silica-based monolithic column, and organic–inorganic hybrid monolithic columns [10,56].

Now, organic polymer-based monolithic columns have been widely used in CEC as separation media due to their characterization such as easy fabrication and functionalization. Pyell's group recently described the synthesis of a new amphiphilic monolithic stationary phase by *in situ* free radical copolymerization of cyclodextrin-solubilized *N*-adamantyl acrylamide, piperazinediacrylamide, methacrylamide, and vinylsulfonic acid in aqueous medium [57]. The synthesized monolithic stationary phases are amphiphilic and can be employed in the reversed and normal-phase mode depending on the composition of the mobile phase. The influence of the total monomer concentration on the chromatographic properties, on the electro-osmotic mobility, and on the specific permeability was further investigated. They found that the formed morphology, the pore size distribution, and the fractional volume of mesopores and macropores depend on the concentration of the lyotropic salt ammonium sulfate in the polymerization mixture [58]. Further, they investigated the influence of the synthesis parameters on the chromatographic efficiency for a homolog series of alkylphenones in the reversed phase mode at a constant composition of the mobile phase via CEC with varied electric field strength [59]. When the concentration of the lyotropic salt ammonium sulfate or the initiator ammonium persulfate was changed in the polymerization mixture, there was a strong influence on the chromatographic efficiency, while only a minor impact was observed with varied molar fraction of the charged monomer VSA.

Silica-based monolithic columns exhibit excellent mechanical stability and high separation efficiency. A well-prepared silica monolithic skeleton via sol–gel process with subsequent surface modification is quite sophisticated and time-consuming compared to those of the polymer-based ones [10]. Jiang's group reported the preparation of a new mixed mode monolithic column for CEC via a sol–gel process and following postmodification with 4,4′-dipyridine [60]. A stable and reversed EOF was generated at acidic pH due to the pyridinium groups on the surface of the stationary phase. The monolithic stationary phase exhibited reversed phase chromatographic behavior toward neutral solutes.

Meanwhile, inorganic anions and organic acids can be separated by the mixed mode mechanism comprising electrophoresis, hydrophobic, and anion-exchange interactions. This group also developed a polymeric ionic liquid (PIL)-modified monolithic column for CEC by the *in situ* cocondensation of tetramethoxysilane and 3-mercaptopropyltrimethoxysilane via a sol–gel process [61]. The PIL-modified (PlmC(8)-silica) monolithic column generated a strong reversed and relatively stable EOF in a wide range of pH. Yao's group developed a cage-like silica nanoparticle-functionalized silica hybrid monolith via "one-pot" process for CEC [62]. In this process, the polycondensation of hydrolyzed alkoxysilanes and *in situ* reaction of mercaptopropyltrimethoxysilane (MPTS) with sodium 3-mercapto-1-propanesulfonate-modified octavinyloctasilasesquioxane (MPS-OVS) simultaneously occurred in a pretreated capillary. The monolithic column not only showed typical reversed phase and cation-exchange chromatographic retention mechanisms but also gave good separation of anilines, alkylbenzenes, and phenols, indicating its excellent chromatographic performance. The best theoretical efficiency similar to 470 000 plates per meter was obtained for 2-aminophenol in CEC. Wang's group recently prepared an amino acid (AA)-silica hybrid monolithic column using "one-pot" strategy [63]. The basic AA was covalently incorporated into the silica hybrid skeleton via the epoxy ring-opening reaction, and meanwhile, the basic AA was also found to catalyze the polycondensation of tetramethoxysilane and GPTMS. The monolith afforded a zwitterionic stationary phase for CEC, the direction and magnitude of EOF can be controlled by the pH of the mobile phase used. Besides an electrophoretic mechanism, the monoliths behave in a typical hydrophilic interaction. This strategy paves the way for easy preparation of various functional silica monolithic columns, aiming at different separation purposes.

The organic–inorganic hybrid monolithic columns overcome inherent drawbacks of organic polymer-based monoliths and silica-based monoliths and, therefore, show better properties such as good mechanism strength, high permeability, wide pH range tolerance, and high column efficiency. To date, the fabrication of organic–inorganic hybrid monolithic column has been widely investigated [64–67]. Zou's group recently developed a novel inorganic–organic hybrid monolithic column using polyhedral oligomeric silsesquioxane (POSS) reagent as inorganic–organic hybrid cross-linker and *N*-(2-(methacryloyloxy) ethyl)-dimethyloctadecylammonium bromide(MDOAB) as functional monomer [68]. The POSS reagent was thought to enhance the mechanical and thermal stability of the monoliths and the MDOA–POSS hybrid monolith possessed excellent properties as separation media. Their results showed this monolithic column offers an opportunity for the separation of tryptic peptides. The group also prepared hydrophobic organic–inorganic hybrid monolithic columns via copolymerization of butyl methacrylate (BuMA), laurylmethacrylate (LMA), and methacrylic acid (MAA) with POSS reagent [69]. The poly(POSS-*co*-LMA-*co*-MAA) monolith exhibited better selectivity for the separation of polar compounds. They further used the POSS reagent containing a methacrylate group as functional monomer and dimethacrylate (BPADMA) and ethylene dimethacrylate

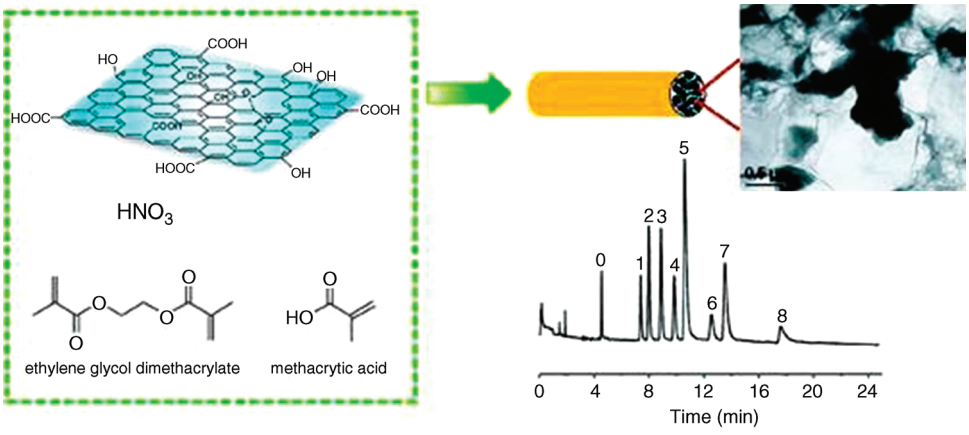

Figure 13.3 The graphene oxide nanosheets incorporated into a monolithic column for CEC separation. (Reproduced with permission from Ref. [72]. Copyright American Chemical Society.)

(EDMA) as cross-linkers to fabricate hybrid monoliths [70]. The obtained hybrid monoliths exhibited good separation for alkylbenzenes, phenols, anilines, and PAHs in CEC or cLC. In addition, this group developed a novel hybrid monolith by the ring-opening polymerization of POSS-epoxy and 1,4-butanediamine (BDA) [71]. The resulting column possessed a well-defined 3D skeleton, high mechanical stability, and good CEC separation for neutral and polar compounds.

New materials incorporating monolithic columns have received interest for their improved properties and potential applications. Yan's group reported the fabrication of GO nanosheets incorporating monolithic column via one-step room-temperature polymerization for CEC (Figure 13.3) [72]. GO sheets first were dissolved and dispersed in cyclohexanol, and then were mixed with MAA and EDMA in cyclohexanol. After fornicating, the monophasic solution was introduced into the fused silica capillary to react for 24 h. Their results showed that GO modified EOF by ionizing oxygen-containing functional groups and greatly increased interactions between the neutral analytes and the stationary phase by providing hydrophobicity and Π-Π electrostatic stacking. The study indicates that the GO-incorporated monolithic columns are promising for CEC separation. Zhang's group incorporated the core–shell silica nanoparticles of Fe_3O_4@SiO_2/NH_2, worm-like and hexagonal SBA-15 silica, into polymethacrylate monolithic columns as stationary phase for CEC [73]. Their study showed that embedding nanoparticles into monolithic column enhances the CEC selectivity and results in a relative 290 000 plates/m high column efficiency. The silver nanoparticles (AgNPs) recently were embedded into lauryl methacrylate monoliths by the *in situ* and *ex situ* approaches for CEC [74]. They evaluated the chromatographic properties of the polymer matrix for two methods and further opined that the two monoliths both exhibited satisfactory reproducibilities in their electrocromatographic behavior.

13.2.4 Molecularly Imprinted Polymers

Molecularly imprinted polymers (MIPs) are synthetic materials that have imprinted cavities with specific recognition for the desired target molecule [14,75]. MIPs are typically prepared with a reaction mixture composed of a target template, a functional monomer, a cross-linking monomer, and a polymerization initiator in a solvent. During polymerization, a complex is formed between the template and the functional monomer, and the complex is surrounded by the cross-linking monomer, yielding a three-dimensional polymer network. After the completion of reaction, the template molecules are trapped and finally eliminated to leave template-imprinted cavities by washing. Due to the advantages such as selectivity, robustness, and economy in terms of cost, MIPs have become attractive materials from the recognition of small molecular [25,76] to the captioning of protein [77] and protein post-translational modifications [78]. Here, we introduce several new works in MIP CEC.

Recently, Nilsson's group developed a new approach based on miniemulsion polymerization for synthesis of MIP nanoparticles with "monoclonal" binding behavior where (*S*)-propranolol is used as a template [79]. The performance of the MIP nanoparticles is characterized for the CEC analysis of racemic propranolol. In contrast to previous report, a unique property is that there is no apparent tailing for the enantiomer peaks, and baseline separation with 25 000–60 000 plate number is achieved. Authors attributed the effects to the reduction of the MIP site heterogeneity by means of peripheral location of the core cross-linked NP and to MIP-binding sites with the same ordered radial orientation.

Yan's group recently developed a room-temperature ionic liquid (RTIL)-mediated nonhydrolytic sol–gel (NHSG) protocol for the fabrication of MIP silica-based hybrid monoliths [80]. Three carboxylic acids were examined as both functional monomers and catalysts for the NHSG condensation of methacryloxypropyltrimethoxysilane (MPTMS) to form silica-based framework. The results showed that RTIL was incorporated both to reduce gel shrinkage and to act as the pore template. The column further showed excellent chiral recognition for a basic template zolmitriptan. The incorporation of RTIL is proved to increase porosity and hence improve the selectivity of the prepared hybrid monoliths.

Liu's group recently developed an MIP coating grafted to a trimethylolpropane trimethacrylate (TRIM) core material for CEC. The core monolith was prepared with a solution of 20% (w/w) TRIM in a mixture of porogen and a polymerization precursor, which can generate a stable EOF due to the formation of ionizable groups after the polymerization hydrolization. Strong recognition ability for *S*-amlodipine and resolution of enatiomer separation were obtained on the resulting grafted imprinted monolith in CEC mode. These results suggest that the method of grafted polymerization is a good alternative to prepare CEC-based monolithic MIPs [81]. This group also investigated the preparation of D-zopiclone MIP nanoparticles using precipitation polymerization. Compared to the previously reported MIP microparticles, the MIP nanoparticles showed good peak

symmetry and an ability of high-speed separation in CEC mode [82]. Additionally, they prepared MIP nanoparticles in the presence of liquid crystalline monomer as physical cross-linker to replace the most of the chemical cross-linker. The resulting D-zopiclone imprinted nanoparticles can retain affinity and specificity for the template even when prepared with a level of cross-linker as low as 5% [83].

Jang *et al.* developed a monolithic layer of MIP for the simultaneous separation of phospholipid (PL) molecular structures by OT-CEC. Their results demonstrated that introducing an MIP-based monolith along with charged species at the OT column made it possible to separate PL molecules. Additionally, a simple nanospray interface utilizing a sheath flow was developed for the interface of OT-CEC with ESI-MS–MS and the resulting OT-CEC–ESI-MS–MS was able to separate PL standards. The developed method was applied to human urinary lipid extracts and resulted in the separation and structural identification of 18 molecules by data-dependent collision-induced dissociation [84].

Cheong's group recently developed long open tubular *S*-ketoprofen MIP capillary columns (1–3 m) for both chrial and nonchiral CEC separation. Chiral separation of racemic ketoprofen and nonchiral separation of other profen drugs have been successfully achieved with the number of theoretical plates over a million. This study has shown the outstanding prospect of the strategy to form a porous OT layer in a moderately long (1 m) capillary column in molecule-imprinted polymer-CEC studies [85]. They further developed four OTMIP capillary columns using atenolol, sulpiride, methyl benzylamine (MBA), and (1-naphthyl)-ethylamine (NEA) as templates by the pre-established generalized preparation protocol. Four different MIP thin layers showed quite different morphologies. This study demonstrates a generalized MIP preparation protocol that is valid for both acidic and basic templates.

13.3 Application

Due to its unique advantages such as high separation power, high selectivity, short analysis time, and low consumption of samples, CEC has been successfully applied in a wide verity of real samples including polycyclic aromatic hydrocarbons (PAHs), pharmaceutical analytes, amino acids, enantiomers, carbohydrates, nucleic acids, peptides, proteins, and antibodies [11,15–17,20,23]. In the past 5 years, the number of papers published on CEC application has been greatly increased. Here, we summarize two important applications of CEC: separation of chiral compounds and analysis of peptides and proteins.

13.3.1 Chiral Separation by CEC

In this chapter, we summarize the chiral CEC separation according to their column types and stationary phase, as shown in Table 13.1. In addition to MIP,

Table 13.1 Chiral separation by CEC.

Mode	Stationary phase	Sample	Ref.
OT-CEC	Protein avidin	Omeprazole enantiomers and its metabolites	[105]
Chip-based OT-CEC	BSA-gold nanoparticle	Ephedrine enantiomers and norephedrine isomers	[106]
Chip-based OT-CEC	BSA-conjugated polydopamine graphene oxide (PDA/GO) nanocomposites	Enantiomers of tryptophan, threonine, and dipeptide	[86]
OT-CEC	Polystyrene (PS) nanoparticles coated by BSA	D- and L-tryptophan	[107]
Chip-based OT-CEC	Polydopamine platform conjugated by BSA	D- and L-tryptophan	[108]
OT-CEC	β-cyclodextrin-modified gold nanoparticles	Enantiomers of chlorpheniramine, zopiclone, and tropicamide	[88]
OT-CEC	MIP-ketoprofen	Ketoprofen enantiomers.	[109]
OT-CEC	(3-glycidyloxypropyl)trimethoxysilane-chitosan	Ibuprofen enantiomers	[110]
OT-CEC	Chitosan in sol–gel phases	Phenylglycine enantiomers	[111]
OT-CEC	Nanochitosan cross-linked with polyacrylamide	Tryptophan enantiomers	[112]
OT-CEC	3-Chloro-2-hydroxypropyl methacrylate-L-histidine-based ligand exchange	Six pairs of amino acid enantiomers	[113]
OT-CEC	Vancomycin	Aromatic racemates	[114]
OT-CEC	Homochiral helical MOF	Racemic flavanone and praziquantel	[103]
Packed CEC	Phospholipid-BSA	D- and L-tryptophan	[115]
Packed CEC	Copper ligand exchange on a *trans*-(1S,2S)-1,2-bis-(dodecylamido) cyclohexane (1) gel	Five pairs of dansylated amino acids	[116]
Packed CEC	Vancomycin	Simultaneous separation of two β-blockers with multiple stereogenic centers	[117]
Packed CEC	Porous and core–shell silica polysaccharide cellulose tris(3,5-dichlorophenylcarbamate)	Seven pairs of drug enantiomers (etozoline, praziquatel, and temazepam)	[28]
Packed CEC	Polysaccharide-based silica–gel with cellulose tris(3-chloro-4-methylphenylcarbamate	Six neutral racemic analytes (etozoline, lorazepam, and oxazepam)	[99]

Packed CEC	Polysaccharide-based cellulose tris (4-chloro-3-methylphenylcarbamate)	Amlodipine enantiomers	[98]
Packed CEC	(*S*)-*N*-(3,5-dinitrobenzoyl)leucine-*N*-phenyl-*N*-propylamine	π-acidic, π-basic, and α-amino acid amides	[118]
Packed CEC	Sulfated and sulfonated polysaccharide	Sixty-six racemic analytes	[100]
Packed CEC	Amylose tris(5-chloro-2-methylphenylcarbamate)	Racemic flavanone and temazepam lopirazepam and etozoline	[119]
Packed CEC	Four chlorine-containing polysaccharide-based stationary phases	Forty-eight pairs of drug enantiomers	[120]
Packed CEC	18-crown-6-tetracarboxylic acid-bonded silica	α-Amino acids	[121]
Monolithic CEC	Room-temperature ionic liquid-mediated nonhydrolytic sol–gel silica-based hybrid MIP monolith	Zolmitriptan enantiomers	[80]
Monolithic pCEC	MIP- tyrosine	Tyrosine and its amino acid derivatives	[122]
Monolithic CEC	(MIP) coating grafted onto a trimethylolpropane trimethacrylate	Racemic amlodipine, zopiclone, and ofloxacine	[81]
Monolithic CEC	MIP-D-zopiclone imprinted nanoparticles	Racemic zopiclone	[82]
Chip monolithic CEC	MIP-L-tyrosine and L-tryptophan	Amino acid enantiomers	[123]
Monolithic CEC	BSA-conjugated gold nanoparticles	Ten pairs of amino acid enantiomers	[87]
Monolithic CEC	β-Cyclodextrin-modified gold nanoparticles	Enantiomers of chlorpheniramine, zopiclone, and tropicamide	[124]
Monolithic CEC	Glycidyl methacrylate-bonded β-cyclodextrin monolithin	Thirty-two pairs of enantiomers	[125]
Monolithic CEC	Glycidyl methacrylate-β-cyclodextrin silica monoliths with vinylbenzyl trimethylammonium	Forty-one pairs of enantiomers	[126]
Monolithic CEC	Polyacrylamide-based monoliths with cellulose tris(3,5-dimethylphenyl-carbamate)	Fourteen pairs of enantiomers	[89]
Monolithic CEC	Cellulose tris(3,5-dimethylphenylcarbamate); silica monolithic column	Six pairs of enantiomers	[90]
Monolithic CEC	Cellulose tris(3,5-dimethylphenylcarbamate)-modified zirconia monolithic columns	Ten racemic compounds	[92]
Monolithic CEC	Cellulose 3,5-dimethylphenylcarbamate-coated zirconia monolithin	Six racemic basic compounds	[93]

(*continued*)

Table 13.1 (*Continued*)

Mode	Stationary phase	Sample	Ref.
Monolithic CEC	Monolithic silica stationary phases immobilized with cellulose tris(3,5-dimethylphenylcarbamate)	Fourteen chiral substances	[127]
Monolithic CEC	Monolithic silica capillary columns coating with cellulose tris(3,5-dimethylphenylcarbamate)	Fifteen chiral compounds	[128]
Monolithic CEC	Sol–gel coated with cellulose tris (3,5-dimethylphenyl-carbamate)	Eight racemic drugs	[91]
Monolithic CEC	Polysaccharide-based Sepapak-2 (cellulose tris(3-chloro-4-methyl-phenylcarbamate)) and Sepapak-4 (cellulose tris(4-chloro-3-methylphenylcarbamate)	Sixteen pairs of racemic pesticides	[97]
Monolithic CEC	Polybutylmethacrylate-based chiral monolithic column	Four racemic amino acids	[129]
Monolithic CEC	Chiral amino-acid-based polymeric monolith	Ephedrine and pseudo-ephedrine enantiomers	[101]
Monolithic CEC	Lindamycin phosphate-modified zirconia monolith	Six pairs of racemic compounds	[130]
Monolithic CEC	Maleopimaric acid anhydride-bonded silica monolith	Eight pairs of amino acid enantiomers	[102]

a number of chiral selectors have been widely used in chiral CEC in the recent 5 years [23].

Proteins coated onto OT capillary columns have been used routinely for the separation of enantiomers by CEC. Recently, Liang *et al.* reported a novel chip-based enantioselective OT-CEC using bovine serum albumin (BSA) conjugated polydopamine graphene oxide (PDA/GO) nanocomposites as stationary phase [86]. Figure 13.4 illustrates the fabrication of chiral OT-CEC chip. The microfluidic chip was first filled with a solution containing dopamine and GO, and then PDA/GO nanocomposites were formed and deposited on the inner wall of microchannel as permanent coating. Finally, BSA was stably immobilized in the PDMS microchannel as a protein stationary phase. The efficient separation of chiral amino acids and chiral dipeptide can be obtained using the constructed OT-CEC columns. Lu *et al.* reported that preparation of silica monolith modified with BSA–gold nanoparticles conjugates for chiral CEC [87].

So far, cyclodextrins (CDs) is the most popular chiral selector in CEC. Recently, Li *et al.* used thiolated β-CD-modified gold nanoparticles (CD-GNPs) as stationary phase to develop chiral OT-CEC. The OT capillary column was prepared by electrostatic assembly of PDDAC, followed by self-adsorption of negatively charged CD-GNPs [88].

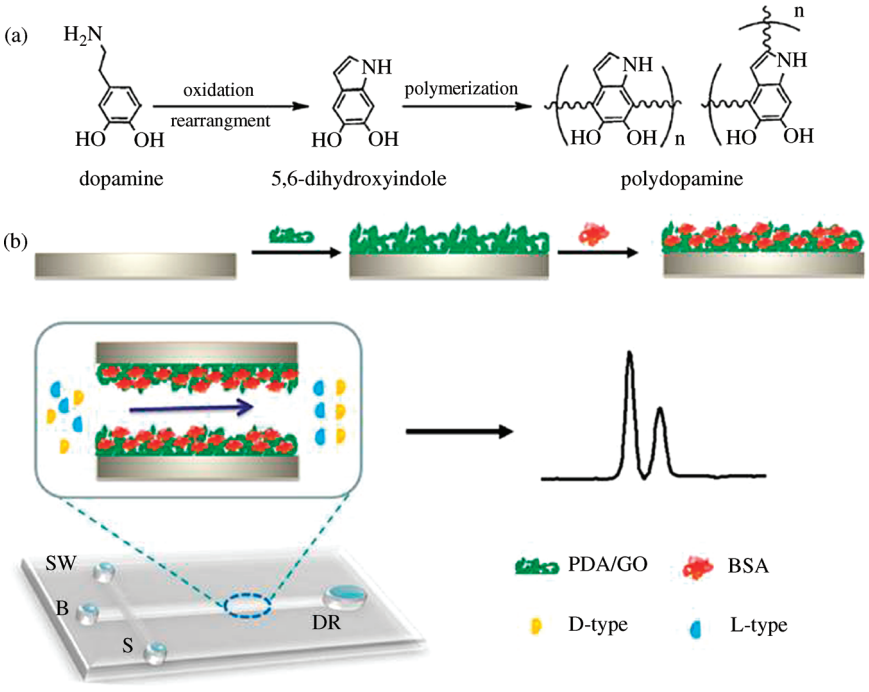

Figure 13.4 (a) Scheme of simultaneous polymerization of dopamine. (b) Scheme of the mechanism of BSA immobilized on the PDMS microchannel through the PDA/GO layer and the chip-based OT-CEC system for chiral analysis using PDA/GO/BSA stationary phase integrated with in-channel electrochemical detection. The sample reservoir (S) was filled with the mixture of enantiomer solution. (Reproduced with permission from Ref. [86]. Copyright Elsevier.)

A variety of cellulose derivatives bonded to OT, packed, and monolithic columns have been reported for chiral separation. Zou's group has reported organic, silica, and hybird monolithic capillary columns coated with cellulose tris(3,5-dimethylphenyl-carbamate) for enantioseparations in CEC [89–91]. Park group developed a series of zirconia monolith modified with cellulose derivatives for chiral CEC separation [92–96]. Perez-Fernandez *et al.* developed two novel polysaccharide-based chiral stationary phases for the chiral separation of a group of 16 pesticides [97]. The Fanali group compared the chiral separation in nano-LC and CEC using cellulose derivatives [98,99]. Zheng *et al.* developed negatively charged polysaccharide stationary phases, namely, 6-SO_4-CDMPC and CDMPC-SO_3 for normal-phase chiral CEC. Compared to conventional cellulose tris(3,5-dimethylphenyl carbamate) or CDMPC CSPs, the novel stationary phase not only shortened the analysis time but also maintained an excellent resolving power. Using the optimized conditions, a chiral pool containing 66 analytes was screened to evaluate the enantioselectivity under normal phase, polar organic phase, and reversed phase modes [100].

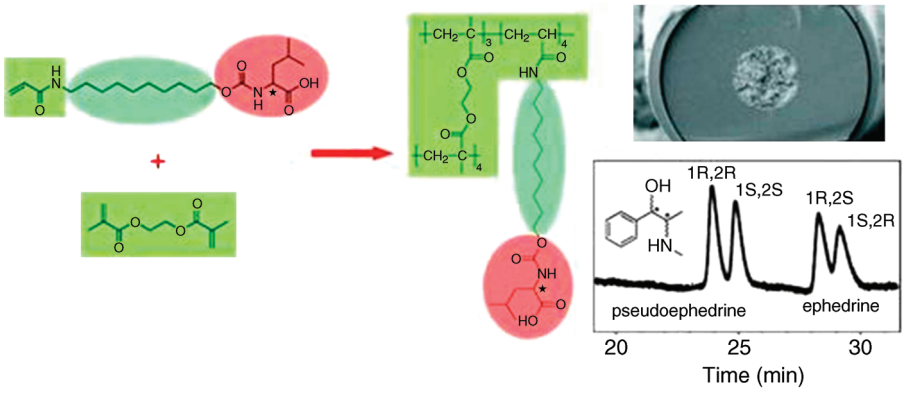

Figure 13.5 A new synthetic family of polymeric monoliths opening up possibilities for chiral separations in CEC. (Reproduced with permission from Ref. [101]. Copyright American Chemical Society.)

Although several classes of chiral selectors have been successfully used in chiral CEC, the design and discovery of new chiral materials are still highly expected. Recently, He *et al.* reported the fabrication of three novel amino-acid-based monolithic columns derived from an acryloylamide tail, a carbamate linker, and an amino acid of different chain length by a "one-pot" synthesis, as shown in Figure 13.5 [101]. The stationary phase displayed good capability in chiral CEC, and further their results suggests that the chain length of amino acid surfactant is important for improved chiral recognition. The method promises to pave the way for the discovery of an amino-acid-based polymeric monolith for chiral separations in CEC. Ye *et al.* developed maleopimaric acid anhydride-bonded silica monolith for enantioseparation of phenylthiocarbamyl amino acids by CEC [102]. Fei *et al.* used a homochiral helical MOF as chiral stationary phase for separations of isomers in OT-CEC [103].

13.3.2 Separation of Proteins and Peptides by CEC

After genomics and transcriptomics, proteomics has attracted much interest due to it representing the actual functional molecules in the cell. The proteome is very complex because each protein can be chemically modified in different ways after synthesis. Mass spectrometry-based proteomics technique have developed into a powerful tool for the large-scale study of proteins. Identifying unique patterns of protein expression, or biomarkers, associated with specific diseases is one of the most promising areas of clinical proteomics. In a typical shot-gun strategy, the proteins are first digested into peptides by trypsin and then the tryptic peptides are subjected to chromatographic separation and identification by MS. Therefore, the separation media is crucial to the quality of protein identification. CEC affords several advantages such as two separation modes of CE and

HPLC, high separation power, low consumption of samples, and good compatibility with MS [27]; therefore, it has a promising potential for the application in proteomics [17]. We summarize some new reports on the separation of peptides and proteins by CEC in the past 5 years, as shown in Table 13.2.

Table 13.2 Separation of proteins and peptides by CEC.

Mode	Stationary phase	Mobile phase	Sample	Ref.
OT-CEC	Graphene oxide sheets	5 mM phosphate buffer	Chicken egg white	[42]
OTCEC	Gold nanoparticles	20 mM potassium phosphate, pH 6.5	HAS tryptic digest	[40]
OT-CEC	Bare gold nanoparticles	20 mM sodium phosphate	Tryptic peptides of native BSA	[41]
OT-CEC	Cationic polymers and anionic molecular micelles	20 mM phosphate, pH 4	Basic proteins	[53]
OT-CEC	Etched C_5- and C_{18}-modified columns	0.3 mM phosphate and 0.19 mM tris	Human IgG and HAS	[131]
OT-CEC	Hydrophilic polysaccharide and carboxymethylchitosan	20 mM phosphate buffer (pH 2.5 or pH 3.0)	Four proteins	[52]
OT-CEC	Poly-epsilon-sodium-undecanoyl lysinate coating	Sodium phosphate; PH 3 and 11.5	Acidic and basic proteins	[49]
OT-CEC	A phenylalanine (Phe) functionalized tentacle-type polymer	10 mM phosphate/citric buffer, pH 2.5	Basic proteins	[50]
Pseudo-stationary phase	Nanoparticle-based hydrophobic interaction capillary electrochromatography	Lipid-based liquid crystalline nanoparticles suspended in 175–250 mM tricine, pH 7.5	GFP and (H) GFP	[132]
Packed column	C_{18} stationary phase	H_2O and ACN	Cytochrome *c* tryptic digest	[133]
Packed column	cIEF/pCEC 2D system	20 mM phosphate containing 0.1% HPC; catholyte, 20 mM NaOH; H2O–acetonitrile–TFA	BSA tryptic digest	[104]
Packed pCEC	Strong SCX/reversed phase	TFA–0.5 M NH4Cl – ACN; H2O–ACN–TFA	Digest of BSA	[31]
Monolithic CEC	Polymethacrylate	ACN/5 mM borate buffer	Microcystins	[134]
Monolithic CEC	Tentacle-type polymer stationary phase covalently modified with branched polyethyleneimine	30 mmol/l phosphate buffer, ACN content	Enkephalin-related peptides	[135]

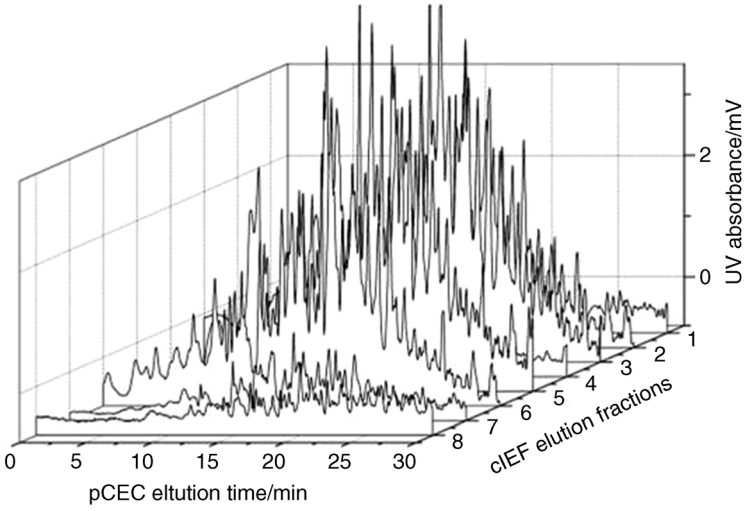

Figure 13.6 Analysis of BSA tryptic digest with online cIEF-pCEC 2D system. (Reproduced with permission from Ref. [104]. Copyright John Wiley and Sons.)

Combing new materials or addictives, OT-CEC has advantages on protein separation. Qu *et al.* have developed OT column coated with graphene oxide for glycoisoforms of ovalbumin in CEC [42]. Luces *et al.* used novel polyelectrolyte multilayer coatings for enhanced protein separation in OT-CEC [53]. Moore *et al.* synthesized novel lysine-based zwitterionic molecular micelle for simultaneous separation of acidic and basic proteins using OT-CEC [49]. Xu and Sun investigated protein separation employing a capillary coated with phenylalanine functionalized tentacle-type polymer under both cathodic and anodic EOF [50].

To date, reversed phase packed column is still the main media for the separation of complex tryptic peptides. Yan's group recently developed a comprehensive 2D separation system coupling capillary IEF (cIEF) with pCEC for protein and peptide mapping [104]. In the 2D system, sample fractions, which were focused and separated in the first dimension cIEF based on their differences in pIs, were electrically mobilized and further successively resolved by their differences in size, hydrophobicity, and electrophoretic mobility in the second-dimension pCEC. A theoretical peak capacity of approximately 24 000 has been achieved for BSA digest (Figure 13.6). The result indicates that CEC-based separation media is a potential tool for proteomics research.

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