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Review

Monolithic enantiomer-selective stationary phases for capillary electrochromatography

Monolithic materials have become a well-established format for stationary phases in the field of capillary electrochromatography. Four types of monoliths, namely particle-fixed, silica-based, polymer-based, and molecularly imprinted monoliths, have been utilized as enantiomer-selective stationary phases in CEC. This review summarizes recent developments in the area of monolithic enantiomer-selective stationary phases for CEC. The preparative procedure and the characterization of these columns are highlighted. In addition, the disadvantages and limitations of different monolithic enantiomer-selective stationary phases in CEC are briefly discussed.

Keywords: Capillary electrochromatography / Enantiomer-selective stationary phases / Enantioseparation / Monoliths

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1 Introduction

Enantioseparations represent an attractive topic of research in separation science. As it has become well recognized that enantiomers can show different biological activities when exposed to a biological environment, analysis of enantiomers is thus especially important in pharmaceuticals, agrochemicals, food additives, *etc.* Chromatography, including gas chromatography, high performance liquid chromatography, supercritical fluid chromatography (SFC), capillary electrophoresis, and capillary electrochromatography, has made great contributions in the field of enantioseparations [1–4]. One of the key points in the development of these techniques is the design and preparation of enantiomer-selective stationary phases which afford effective chiral-recognition [5].

CEC has attracted increasing interest as it combines the high selectivity of HPLC with the high efficiency of CE. In addition, as a miniaturized separation technique, it has a number of advantages such as the low consumption of stationary phases and solvents, need of smaller samples, environmental safety, and easy coupling to mass spectrometry, *etc*. During the past decade, CEC has proven its potential in enantioseparations [6–9]. Traditionally, enantiomer-selective stationary phases or chiral selectors used in HPLC have been transferred to CEC, resulting in the current use of three column technologies: packed, open-tubular, and monolithic columns for CEC. For packed columns, particulate stationary phases are packed into capillary columns and retained by frits. These columns have a high phase ratio and high enantioselectivity, but also suffer from disadvantages caused by the frits, such as non-specific interactions, bubble formation during CEC runs, and accidental breakage of the capillary columns. In addition, considerable skill is needed to prepare highly permeable and stable frits. For open-tubular columns, the chiral selectors are coated or immobilized on the inner surface of the capillaries, thus there are no packing materials in the capillaries and no frits are needed. But these columns suffer from low phase ratio, ease of overloading, and low sensitivity in UV detection. The problems encountered in these two column techniques led to the development of a new concept of packed CEC columns, *i.e.*, monolithic columns. In this class of columns, the packing materials are covalently linked together to form continuous and uniform beds (or

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Abbreviations: **AA,** acrylamide; **AGE,** allyl glycidyl ether; **AIBN,** azobisisobutyronitrile; **AMPS,** 2-acrylamide-2-methyl-1-propanesulfonic acid; **APS,** ammonium peroxodisulfate; **BMA,** butyl methacrylate; **CDMPC,** cellulose tris(3,5-dimethylphenylcarbamate); **CSP,** chiral stationary phase; **DADMAC,** diallyldimethylammonium chloride; **DADT,** *N,N′*-diallyltartardiamide; **Dns,** *N*-5-dimethylamino-1-naphthalene sulfonyl; **EDMA,** ethylene glycol dimethacrylate; **GMA,** glycidyl methacrylate; **HMAA,** *N*-(hydroxymethyl) acrylamide; **L-PheAN,** L-phenylalanine anilide; **MAA,** methacrylamide; **MIP,** molecularly imprinted polymer; **OVM,** ovomucoid; **PDA,** piperazine diacrylamide; **SFC,** supercritical fluid chromatography; **TEMED,** *N,N,N′,N′*-tetramethylethylenediamine; **TRIM,** trimethylolpropane trimethacrylate; **VSA,** vinylsulfonic acid.

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rods), which are attached to the inner wall of the capillary by covalent bonding or electrostatic forces; thus no frits are required to support the beds. This type of column has comparable phase ratio and enantioselectivity to conventional packed columns, but decreases the risk of bubble formation or breakage of the capillary. In addition, the monoliths can easily be prepared in capillaries through *in situ* polymerization reaction or the sol-gel process, and the chiral selectors can be introduced by simultaneous copolymerization of functional monomers or by post-modification strategies. No special skill is required in all these procedures, making inter-laboratory studies easy and comparable.

The monolithic enantiomer-selective stationary phases utilized in CEC can be classified into the following four categories: Particle-fixed monoliths [10–16]; silica-based monoliths [17–26]; polymer-based monoliths [27–46]; and molecularly imprinted monoliths [47–55]. Particle-fixed monoliths are prepared by sintering the silica-based packing materials or fixing the conventional packing materials into a network of silica or polymer. This kind of monolith can be regarded as the improved version of conventional packed columns, as they utilize some special techniques to stabilize the packing materials other than fabricating frits. Silica-based monoliths are prepared by a sol-gel process based on the polycondensation of alkoxysilanes, and such columns are resistant to different kinds of organic solvents, representing a promising column technique in CEC. Polymer-based monoliths are prepared by *in situ* polymerization of organic monomers with a cross-linker, thus rigid rods or homogeneous gels are formed in the capillaries. The above three kinds of monoliths have allowed almost unlimited choice of both matrix and surface chemistries. Thus, theoretically, all the chiral selectors utilized in HPLC can be transferred to CEC with monolithic columns. However, only some of them have been transferred, as enantioseparation by monolithic column CEC is still in its infancy. The reported chiral selectors utilized in CEC with monolithic columns include ligand exchangers [17–19, 33–35], Pirkle-type selectors [16, 39–46], cyclodextrin (CD) and its derivatives [10, 12, 20, 21, 27–31], chiral crown ethers [32], antibiotics [15, 36, 37], polysaccharide derivatives [23], and proteins [22, 24–26, 38]. They can be attached to the support by encapsulation, physical adsorption, or covalent bonding. Molecularly imprinted polymers (MIPs) are prepared by polymerization of functional monomers and cross-linkers in the presence of chiral templates (imprinting molecules). After removal of the templates, imprints which show enantioselectivity to the original template molecules (or their structurally related analogues), are formed. These kinds of stationary phases are achiral, and no chiral selectors are attached to the matrix. Thus, they show completely different separation principles, and represent a series of complementary alternatives to the so-called chiral stationary phases (CSPs).

Several excellent review papers [6, 7, 56–59] which discuss enantioseparations in CEC have appeared in recent years, but their emphasis is often placed on method development or the enantiomers resolved, and they often cover all types of capillary columns. A critical review paper dealing with the topic of "fritless packed columns" in enantioseparations by CEC was published in 2002 [60]. However, some new developments in this area have appeared since then. This review will discuss the state-of-the-art of the monolithic enantiomer-selective stationary phases in CEC, and highlight the preparation and characterization of this kind of column.

2 Particle-fixed monoliths

Three categories of particle-fixed monoliths can be found in the published papers: (1) particle-sintered monolithic columns, where the silica-based particles are packed first and then covalently linked by thermal treatment; (2) particle-entrapped monolithic columns, where the particles are packed first and then "glued" together by introduction of entrapping solutions; (3) particle-loaded monolithic columns, where the particulate packing materials are first suspended in the entrapping solutions and then embedded into the network formed by polymerization or polycondensation of the entrapping solution.

Wistuba *et al.* [10] prepared monolithic enantiomer-selective stationary phase by a particle sintering method. Bare porous silicas were first packed into the capillary by a conventional slurry method, and then the packed bed was sintered by heating at 380°C for 10 h to form a monolith. Chirasil-Dex (Fig. 1) was coated on the prepared monolith, and then immobilized by heating the capillary column at 235°C for three days. Enantioseparation of 11 pairs of neutral, acidic, and basic enantiomers under reversed-phase conditions was achieved on the prepared columns by CEC, and column efficiencies for most of the enantiomers were greater than 30 000 plates/m. The monolithic columns were stable and robust at high voltage (30 kV) and high pressure (400 bar). In this method, particle-based CSPs could not be sintered directly as the chiral selectors might be destroyed at the high temperature used in the sintering process, thus post-modification of the column was necessary. The whole procedure is time consuming and might lead to some reproducibility problems, as the monolith must be produced individually within a single capillary. This may be the reason why only one paper has been published on the preparation of monolithic enantiomer-selective stationary phases by a sintering method.

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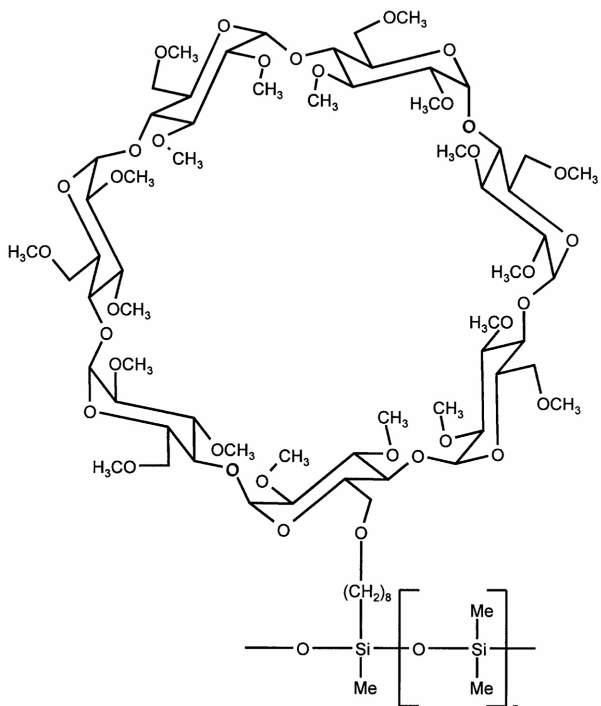

Figure 1. Structure of Chirasil-Dex (depending on the reaction conditions, the linkage to the polysiloxane may occur from the mono-2- or mono-6- position of cyclodextrin, respectively). Reproduced from [10], with permission.

CSPs could not be directly used in a particle-sintered method. To take full advantage of the commercially available CSPs, "mild" methods which can preserve the enantioselectivity of the fixed CSPs have been developed. Chirica and Remcho [11] proposed a particle-entrapped approach by filling the packed capillary with potassium silicate solution followed by gradually heating from 40 to 160°C over several days. After removing the temporary frit, the column was cured by flushing with dilute ammonium hydroxide and dried in an oven. This approach was first applied to fix silica-based reversed-phase packing materials and then to molecularly imprinted polymeric packings. In the latter case, L-dansylphenylalanine-imprinted polymer stationary phase was entrapped and the obtained capillary was used to separate D,L-dansylphenylalanine. Compared to the corresponding HPLC analysis, the CEC separation using the particle-entrapped capillary was faster and more efficient. In addition, the silanol groups on the silica network contributed to EOF, although non-specific interaction was also introduced into the separation at the same time. Using a similar principle, Wistuba *et al.* [12] prepared particle-entrapped monolithic capillaries based on Chira-Dex-silica by an *in situ* sol-gel technique. The packed bed of Chira-Dex-silica was flushed with a sol-gel solution consisting of tetraethoxysilane (TEOS), 0.1 M HCl, and ethanol, and then dried and heated at 120°C, allowing the formation of a silicate network which glued the particles together to form a monolith. The obtained monolithic capillary was used to resolve 14 pairs of enantiomers, and high column efficiencies (up to 102 000 plates/m) and resolutions were achieved (Fig. 2). Compared to its packed capillary counterpart, the monolithic capillary was found to have lower selectivity, which may be caused by masking of the chiral selector in the silica network. The monolithic capillary was stable towards high voltage (30 kV) and pressure (300 bar).

To reduce the effect of non-specific interactions generated by a silicate-based entrapping matrix, Chirica and Remcho [13] have proposed the preparation of a particle-entrapped monolith using an organic-based entrapping solution. A mixture of methacrylate-based monomers, cross-linkers, porogenic solvents, and initiator was pumped through the capillary packed with reversed-phase packing materials. After a polymerization step, an organic network formed and held the particles in place. Another advantage of this entrapping method is the ease of tuning the ionic groups on the matrix support, which makes the EOF more controllable. However, this approach has not been applied to the entrapment of enantiomer-selective stationary phases.

In the preparation of particle-sintered or entrapped monoliths, prior packing of the capillaries is required and the whole procedure is complicated and time-consuming. An alternative method of preparing this type of monolith is the particle-loaded technique, which combines packing and entrapment of the particles in a single step. In this approach, the packing materials are added to the entrapping solution to form a suspension, and then the suspension is injected into a capillary. After a polymerization or so-gel process, the particles are embedded in the formed matrix. The whole procedure is simple and fast, although the lower particle density compared to the particle-entrapment approach may lead to some decrease in separation selectivity.

Lin *et al.* [14] prepared molecularly imprinted polymers using L-phenylalanine or L-phenylalanine anilide (L-PheAN) as imprinting molecules. After crushing and sieving, the particles (diameter less than 5 μm) were suspended in the entrapping solution consisting of acrylamide (AA) and bisacrylamide. The suspension was injected into the capillary and thermostated at 40°C for 4 h to form the monolithic enantiomer-selective stationary phase. The obtained column was used to resolve phenylalanine and several other aromatic amino acids. Under the running conditions adopted, there was an insufficient density of ionizable groups on the stationary phase, thus the enantiomers migrated primarily according to the electrophoresis mechanism. Schmid *et al.* [15] embedded silica-based CSPs (3-μm particles modified

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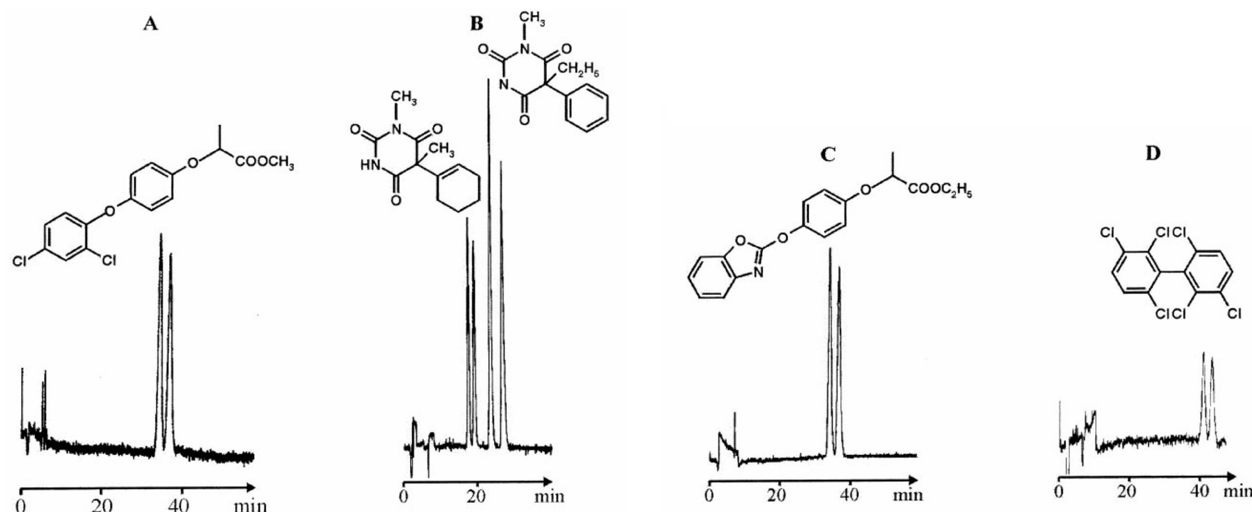

Figure 2. Electrochromatograms for resolution of enantiomers on particle entrapped monolith. Particles: Chira-Dex silica. Conditions: 20 cm (overall length 42 cm) × 100 mm id capillary; 20 mM 2-(*N*-morpholino)ethanesulfonic acid buffer, pH 6.0. (A) 25 kV; 40 bar; buffer/methanol (2:3, v/v). (B) 20 kV; 10 bar; buffer/methanol (7:3, v/v). (C) 20 kV; 10 bar; buffer/methanol (2:3, v/v). (D) 20 kV; 60 bar; buffer/methanol (3:7, v/v). Reproduced from [12], with permission.

with teicoplanin aglycone or ristocetin A) in capillaries by a polymerization process. The entrapping solution was a mixture of methacrylamide (MAA), piperazine diacrylamide (PDA), ammonium sulfate, and charge-providing agent vinylsulfonic acid (VSA) or diallyldimethylammonium chloride (DADMAC). The polymerization process was catalyzed by addition of ammonium peroxodisulfate (APS) and *N,N,N′,N′*-tetramethylethylenediamine (TEMED). The inner wall of the capillary has been pretreated with γ-methacryloxypropyltrimethoxysilane, which took part in the polymerization and attached the monolith to the capillary wall, thus prevented the particles and matrix from exiting the capillary during separation. The introduction of a charge-providing agent in the monomer made the EOF more controllable. The prepared columns were used to resolve enantiomers of amino acids, dipeptides, and α-hydroxy acids in CEC. However, their efficiencies (*ca.* 10 000 plates/m) were lower than those of their packed counterparts, which may be due to masking of the chiral selectors or the introduction of non-specific interactions by the matrix. According to the authors, this technique could be applied to embed any commercially available silica-based CSP.

Kato *et al.* [16] suspended silica-based CSPs (5-μm particles modified with (S)-*N*-3,5-dinitrobenzoyl-1-naphthylglycine or (S)-*N*-3,5-dinitrophenylaminocarbonyl-valine) and a small amount of bare silica (to improve and stabilize the EOF) in a sol-gel solution (mixture of TEOS, ethanol, and hydrochloric acid), then injected the suspension into the capillary and heated at 120°C for 1 h to embed the particles in a sol-gel process. The prepared columns were used to resolve 16 pairs of 4-fluoro-7-2,1,3-benzoxadiazole-derivatized amino acids, and most of them were baseline separated with high column efficiencies (plate heights 14–60 μm). No differences in elution pattern of the analytes were observed over three months using a single column.

3 Silica-based monoliths

Silica-based monoliths have the advantages of good mechanical strength and high flow-through. In addition, proven methods for preparing HPLC packing materials can be directly used, including those for preparing CSPs.

Chen *et al.* [17] first reported the application of silica-based monoliths for enantioseparation of dansyl (Dns) amino acids by ligand exchange. The silica monolith was prepared according to a conventional sol-gel technique. The chiral selector (L-phenylalaninamide) was immobilized on the surface of the silica monolith by a bifunctional reagent, namely (3-glycidoxypropyl)trimethoxysilane. After conditioning with Cu(II) aqueous solution, the column could be used to resolve 12 pairs of Dns-amino acids (R_s >1.50). A high column efficiency of 90 000 plates/m can be obtained for Dns-Leu under optimized chromatographic conditions. Chromatographic data obtained for the columns prepared with different batches were similar. In addition, the columns remained stable in use for at least hundreds of injections. Using the same approach, the same authors also prepared silica-based monolithic CSPs for ligand-exchange CEC with L-prolinamide [18] or L-hydroxyproline [19] as chiral selectors. Under optimized chromatographic conditions, the former showed enantioselectivities towards eight pairs

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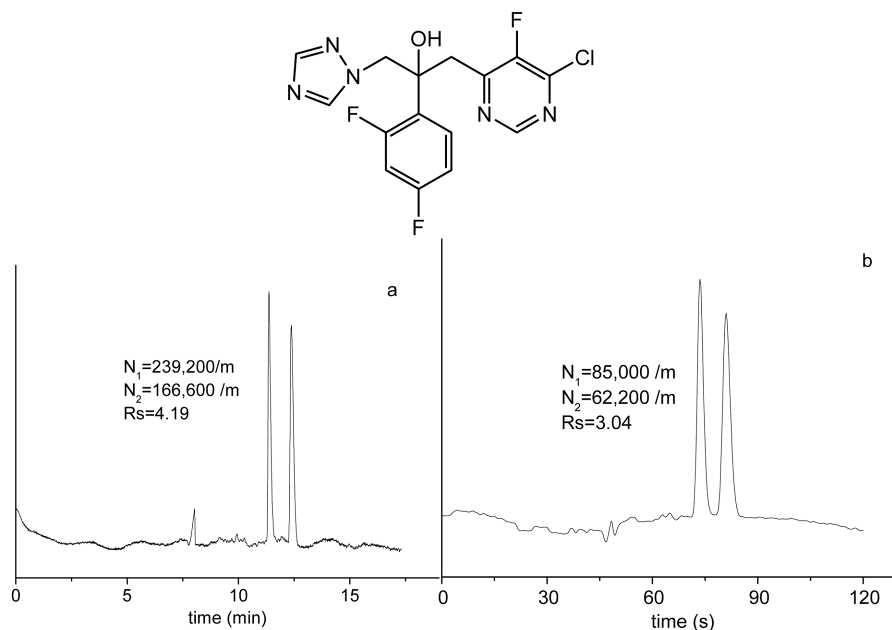

Figure 3. Electrochromatograms for resolution of enantiomers on monolithic silica capillary with coated CDMPC as chiral selector. Mobile phase: acetonitrile/phosphate buffer (2 mM, pH 6.80) (60:40, v/v). Capillary: 30.2 cm (total length) × 50 mm id monolithic silica column coated with 60 mg/mL of CDMPC in acetone. (a) Effective length, 20 cm; voltage, 10 kV. (b) Effective length, 10.2 cm; voltage, 30 kV. Reproduced from [23], with permission.

of Dns-amino acids and seven pairs of hydroxy acids, and the latter towards eight pairs of Dns-amino acids, three pairs of hydroxy acids, and two pairs of dipeptides.

Kang *et al.* [20] prepared silica-based monolithic columns according to the method published by Ishizuka *et al.* [61] with little modification. A hydrothermal treatment was employed to prevent the matrix from cracking. Chirasil-Dex, which had been used as chiral selector by Wistuba *et al.* [10], was coated and then immobilized on the silica matrix. Several neutral and negatively charged enantiomers (benzoin, mephobarbital, hexobarbital, and carprofen) were resolved under aqueous mobile phases. A high efficiency of 92 000 plates/m was obtained for the first eluted enantiomer of hexobarbital. The columns remained stable for more than a hundred runs in a period of two months. The run-to-run (RSD for retention $<1\%$) or day-to-day (RSD for retention $<8\%$) reproducibility was satisfactory, but the column-to-column reproducibility was poor. Chen *et al.* [21] described the preparation of β- or γ-CD-modified silica monoliths for enantioseparations. The CDs were immobilized by a bifunctional reagent, namely (3-isocyanatopropyl)triethoxysilane. A β-CD-modified silica monolith could resolve benzoin and two pairs of Dns-amino acids, while a γ-CD-modified one could resolve nine pairs of Dns-amino acids.

Liu *et al.* [22] described the preparation of silica-based monolithic columns with physically adsorbed avidin as chiral selector. Avidin was adsorbed on the surface of the matrix primarily by electrostatic interaction. The prepared column possessed a higher phase ratio than its previously reported open-tubular counterpart [62]. The EOF generated on the column was found to be very weak, which limited the resolution of neutral enantiomers, but allowed the resolution of acidic and basic enantiomers which possess high electrophoretic mobility. 10 pairs of enantiomers were resolved by CEC with aqueous mobile phases. The column lasted only two weeks due to the loss of avidin, which, however, could be readsorbed in a very simple procedure.

Recently, we [23] described an approach for preparing silica-based monolithic columns with coated cellulose tris(3,5-dimethylphenylcarbamate) (CDMPC) as chiral selector. CDMPC was coated onto the silica matrix by a solvent evaporation method [63], and the optimized concentration of CDMPC in acetone was found to be 60 mg/mL. The prepared column afforded sufficient EOF although coated with neutral CDMPC. Fifteen pairs of neutral and basic enantiomers were resolved with aqueous mobile phases, while two of them were resolved under non-aqueous conditions. Column efficiencies for most of the enantiomers were higher than 100 000 plates/m, and the highest was up to 240 000 plates/m (Fig. 3a). Very fast enantioseparations could be realized by short-end separation with high voltage (Fig. 3b). Chromatographic data obtained on columns prepared with different batches were similar, and the columns

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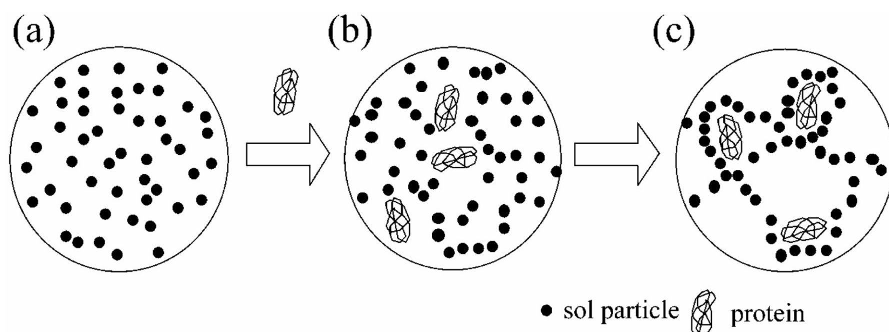

Figure 4. Scheme of protein encapsulation in the silicate matrix during sol-gel polymerization. (a) Formation of sol particles during hydrolysis and condensation. (b) Addition of protein into the sol. (c) The growing silicate network traps protein molecules. Reproduced from [24], with permission.

remained stable for at least 40 injections. This approach could be extended to prepare silica-based monolithic columns coated with other polysaccharide derivatives.

Kato *et al.* [24] reported a protein-encapsulation technique for preparation of monolithic columns for CEC. Fully or partially hydrolyzed silane, $SiOH_{4-n}(OMe)_n$, was mixed with protein solutions of bovine serum albumin (BSA) or ovomucoid (OVM). The mixture was injected into the capillary, allowing the fabrication of silica networks, and the protein was encapsulated during the growth of the networks (Fig. 4). No further thermal treatment was performed, and the silica matrix formed in the capillary was thus a hydrogel, differing greatly from other silica-based monoliths. The column preparation conditions were systematically optimized. Enantiomers of tryptophan and benzoin were resolved on a BSA-encapsulated column, while those of benzoin, eperisone, and chlorpheniramine were resolved on an OVM-encapsulated one. A high column efficiency of 72 000 plates/m could be obtained for the first eluted enantiomer of benzoin. The lifetime of the capillaries was a problem due to the loss or denaturing of the proteins. In the authors' subsequent paper [25], BSA-encapsulated columns were characterized by their attenuated total reflectance-FT-IR (ATR-FT-IR). The clusters which form the gels were found with the diameter of 1 μm, and BSA maintained its structure after being encapsulated. The same group [26] also developed silica sol-gel/organic hybrid materials for encapsulation of BSA in capillaries. Small amount of biopolymers (gelatin or chitosan) were incorporated in the gels, which changed the microenvironment around BSA and further benefited the enantioselectivity and stability of the capillary columns.

4 Polymer-based monoliths

According to their morphologies in the capillaries, polymer-based monoliths can be classified into two categories, namely "homogeneous gels" and "rigid rods". In the former case, the polymers have ideal chromatographic properties since they are non-particulate, and eddy diffusion is negligible. Such capillary columns can not be flushed by a hydrodynamic flow because of the leakage of the gels under pressure, making post-modification of the column difficult. Thus the chiral selectors are often introduced through copolymerization with the monomer and the cross-linker. In the latter case, large through-pores are formed in the polymers, permitting hydrodynamic flow through the capillaries. The chiral selectors can be introduced either through *in situ* copolymerization with the monomers or through post-modification of the column.

Koide and Ueno [27] prepared charged polyacrylamide gels incorporating carboxymethyl-β-CD polymer and/or poly-β-CD as chiral selectors in the capillaries to separate enantiomers by CEC. A mixture of chiral selectors, Tween 20, AA (monomer), *N,N′*-methylenebisacrylamide (BIS) (cross-linker), charge-providing agent, APS, and TEMED in aqueous buffer was injected into a capillary and allowed to stand at room temperature for at least 5 h to form monolithic CSPs. The prepared capillaries with homogeneous gels were equilibrated with EOF. Two pairs of cationic and one pair of neutral enantiomers were resolved on negatively charged gels by incorporating poly-β-CD and carboxymethyl-β-CD polymer as chiral selectors, while 12 pairs of anionic enantiomers were resolved on positively charged gels by incorporating poly-β-CD as chiral selector. A high column efficiency of 240 000 plates/m was obtained for the second eluted enantiomer of Dns-aspartic acid. The prepared columns can be used for two weeks without deterioration through the elution of chiral selectors. The same authors also prepared negatively [28] or positively [29] charged gels with covalently attached β-CD as chiral selector. Allyl carbamoylated β-CD (AC-β-CD) was synthesized by the reaction of β-CD and allyl isocyanate in dry pyridine followed by

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collection and purification steps. Then the obtained AC-β-CD was copolymerized with AA, BIS, 2-acrylamido-2-methylpropanesulfonic acid (AMPS), or *N*-(2-acrylamidoethyl) triethylammonium iodide in the presence of APS and TEMED to form homogeneous gels. On the column with positively charged gels, 16 acidic and two neutral compounds were resolved with column efficiencies up to 150 000 plates/m. On the column with negatively charged gels, 15 cationic and two neutral enantiomers were resolved also with high column efficiencies. In addition, the addition of achiral crown ether (18-crown-6) to the mobile phase benefited the enantioseparation of primary amino compounds [28]. The concentration of AC-β-CD in the polymerization solution affected the retention and resolution of enantiomers. Such columns were stable for at least three or four months.

Almost at the same time, Végvári *et al.* [30] used a similar concept to prepare charged gels with β-CD as chiral selectors. 2-Hydroxy-3-allyloxypropyl-β-CD (allyl-β-CD) was synthesized by a simple one-step procedure and then used as a monomer component in the polymerization solution. Eight pairs of neutral and ionic enantiomers were resolved on the prepared capillary column, and in some cases very high column efficiencies (*ca.* 500 000 plates/m) were obtained. The prepared capillaries could be used for one month without bubble formation or loss of resolution. Very recently, this method was transferred to a microchip with allyl-γ-CD as chiral monomer by Zeng *et al.* [31]. Homogeneous gels were formed in the microchannels with a width of 43 μm, a height of 17 μm, and an effective length of 36 mm on the chip, and two fluorescein isothiocyanate (FITC)-labeled-Dns-amino acids were simultaneously resolved within 100 s (Fig. 5).

As in their previous approaches, Koide and Ueno [32] also prepared negatively charged gels with covalently attached (+)-tetraallyl 18-crown-6 carboxylate or (+)-18-crown-6 tetracarboxylic acid 2-allyl ester as chiral selectors. 12 pairs of primary amino enantiomers were resolved with high efficiencies of up to 135 000 plates/m. A capillary prepared with the former selector was used to test the optical purity of L-alanine-2-naphthylamide, and 0.1% of D-form could be detected.

Schmid *et al.* [33] prepared a continuous bed by *in situ* copolymerization of MAA (monomer), PDA (cross-linker), VSA (charge-providing agent), and *N*-(2-hydroxy-3-allyloxypropyl)-L-4-hydroxyproline (chiral selector) for ligand-exchange CEC. Nine pairs of underivatized amino acid enantiomers were resolved using acidic mobile phases. Because of the low backpressure of the prepared capillaries, pressure-assisted CEC was realizable, which could speed up the separations. In their subsequent work [34], a similar continuous bed was used to resolve 11 pairs of hydroxy acid enantiomers. VSA was omitted in the synthesis of the continuous bed as it might show a negative effect on the migration of the analytes. The analytes migrated primarily according to an electrophoretic mechanism. However, the retention times were relatively long. To further speed up the separation of hydroxy acids, Lecnik *et al.* [35] used DADMAC as charge-providing agent, resulting in a positively charged continuous bed. The EOF generated was superimposed upon the electrophoretic migration of the analytes, thus considerably reducing the retention time of the analytes.

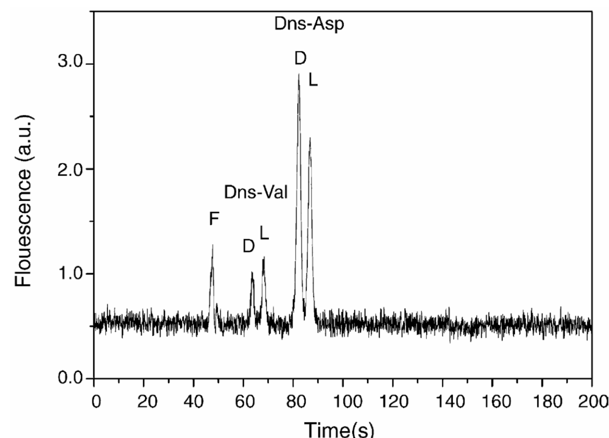

Figure 5. Electrochromatogram for simultaneous resolution of FITC labeled Dns-Val and Dns-Asp on γ-CD bonded polyacrylamide gel inside the channel on polydimethylsiloxane microchip device. Size of microchannel: width, 43 μm; height, 17 μm; effective length, 36 mm. Mobile phase: 0.1 M Tris/0.25 M boric acid in 7 M urea (pH 9.0). Voltage: 3.6 kV. Reproduced from [31], with permission.

Kornyšova *et al.* [36] prepared continuous beds by *in situ* copolymerization of *N*-(hydroxymethyl)acrylamide (HMAA), PDA, allyl glycidyl ether (AGE), and VSA. The epoxy groups on the continuous bed were converted into aldehyde groups by treatment with sodium hydroxide and then with sodium periodate solutions. The chiral selector, vancomycin, was then immobilized on the continuous bed by reductive amination. The prepared capillary column afforded high EOF with both non-aqueous and aqueous mobile phases, but only showed chiral discrimination ability in the latter case. Four pairs of enantiomers were resolved, and thalidomide was baseline resolved with a column efficiency of 120 000 plates/m for the first peak. A simplified approach was reported in their subsequent work [37]. AGE used in [36] was replaced by *N,N′*-diallyltartardiamide (DADT); thus the hydroxy groups on the polymerized DADT moieties could be directly oxidized to aldehyde groups by periodate. In addition, the cleavage of DADT during the oxidation process decreased the cross-linking density and increased the average pore size and porosity of the skeleton. Enantioseparations of several chiral drugs were conducted.

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This approach can be used for the attachment of other chiral selectors, if they have aldehyde group-reactive groups in the molecules.

Machtejevas and Maruška [38] prepared continuous beds with immobilized human serum albumin (HSA) as chiral selector. During the allyl activation of the protein and the subsequent polymerization process, acetylsalicylic acid or L-tryptophan was used as an additive to interact with the chiral active sites of the protein, thus preventing them from being blocked. It was observed that L-tryptophan was a more effective additive than acetylsalicylic acid. Enantioseparation of D,L-kynurenine by CEC was achieved on the prepared monolithic column with an efficiency of about 110 000 plates/m for the first eluted enantiomer.

Peters *et al.* [39] reported the preparation of chiral "moulded" rigid monoliths in capillaries by a single-step approach. A mixture of 2-hydroxyethyl methacrylate, (*N*-L-valine-3,5-dimethylanilide) carbamate (chiral monomer), ethylene glycol dimethacrylate (EDMA), butyl methacrylate (BMA) or glycidyl methacrylate (GMA) (comonomer), 2-acrylamide-2-methyl-1-propanesulfonic acid (AMPS), propan-1-ol and butane-1,4-diol (porogenic solvents), and azobisisobutyronitrile (AIBN) was injected into the capillaries for copolymerization *in situ* at 60°C for 12 h. Due to the rigidity of the resulting monolith, its anchoring to the capillary wall was not required. The capillary containing the most hydrophilic diol functionalities, which were obtained by the hydrolysis of the epoxide moieties of GMA, showed the lowest non-specific effect on the enantioseparations. Enantioseparation of *N*-(3,5-dinitrobenzoyl)leucine diallylamide was achieved with column efficiencies of 61 000 plates/m for the first peak and 49 500 plates/m for the second one.

Similarly, Lämmerhofer *et al.* [40 – 42] prepared monolithic capillaries by *in situ* copolymerization of *O*-[2-(methacryloyloxy)ethylcarbamoyl]-10,11-dihydroquinidine (chiral monomer 1 in Fig. 6), GMA or 2-hydroxyethyl methacrylate (HEMA), and EDMA in the presence of porogenic solvents. The polymerization was initiated either thermally or by UV light, with the former approach affording better chromatographic properties. The monolith adhered to the inner wall of the capillary by electrostatic forces between the basic quinuclidine functionalities of the monolith and the acidic silanol groups of the capillary wall. The quinidine functionality exposed on the surface of the polymer acted as both charge-providing agent to generate EOF and chiral selector to resolve the enantiomers. Chemical composition and porous structure of the polymers affected the chromatographic properties, and they could be easily controlled by varying the percentage of the monomers and porogenic solvents in the reaction mixture. In addition to the characters of

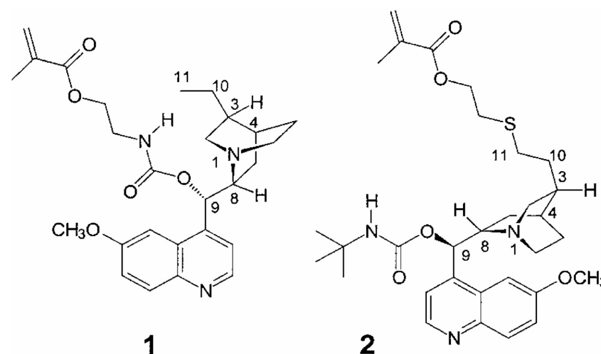

Figure 6. Structure of the chiral monomers used for preparation of monoliths. 1, *O*-[2-(methacryloyloxy)ethylcarbamoyl]-10,11-dihydroquinidine. 2, *O*-9-(*tert*-butylcarbamoyl)-11-[2-(methacryloyloxy)ethylthio]-10,11-dihydroquinine. Reproduced from [43], with permission.

the monoliths, other variables such as the type and composition of the eluent, ionic strength, and column temperature affected the enantioseparations [42]. Enantioseparation of a broad spectrum of derivatized amino acids was achieved. For the separation of *N*-2,4-dinitrophenyl-Val, a very high column efficiency of up to *ca.* 250 000 plates/m could be obtained. Shortening the effective length of the monolith in the capillary column could decrease the run times without sacrificing the separation ability. Subsequently, the same authors [43] also reported monolithic capillaries prepared by an approach similar to that reported in [41], except that the chiral monomer was substituted with *O*-9-(*tert*-butylcarbamoyl)-11-[2-(methacryloyloxy)ethylthio]-10,11-dihydroquinine (chiral monomer 2 in Fig. 6). 9-Fluorenylmethoxycarbonyl-, Dns-, 7-dimethylaminosulfonyl-1,3,2-benzoxadiazol-4-yl-, and carbazole-9-carbonyl-derivatized amino acids were resolved on the prepared capillaries with column efficiencies higher than 100 000 plates/m. Capillaries prepared with chiral monomer 2 showed enhanced enantioselectivities and reduced separation times compared to chiral monomer 1. In addition, as the chiral monomers (1 and 2) behaved like pseudoenantiomers, reversed elution orders were observed on the corresponding capillary columns, which offered the freedom to choose the enantiomer migration order when necessary.

Chiral selectors could be introduced not only by copolymerization with the monomers, but also by post-modification of the columns. Preinerstorfer *et al.* [44] prepared a monolith of poly(GMA-co-EDMA), then converted the epoxy groups on the surface of the matrix into 3-mercapto-2-hydroxy-propyl residues by nucleophilic substitution with sodium hydrogen sulfide. The reaction conditions were optimized to make the conversion of epoxy groups quantitative and reproducible. The resulting thiol functionalities enabled the attachment of different kinds of ligands by appropriate reaction. *O*-9-*tert*-Butylcar-

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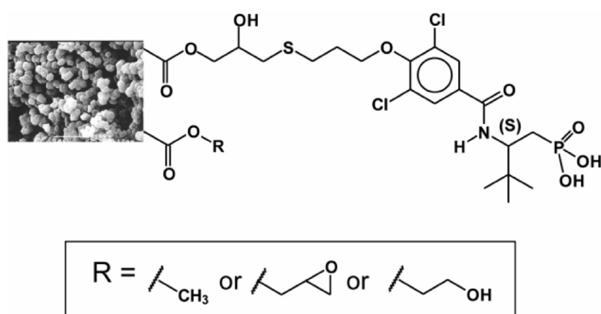

Figure 7. Structure of the chiral monoliths based on the selector (*S*)-*N*-(4-allyloxy-3,5-dichlorobenzoyl)-2-amino-3,3-dimethylbutanephosphonic acid and pendant side chain residues stemming from the distinct comonomers employed. Reproduced from [45], with permission.

bamoylquinine was attached to the monolith through a radical addition reaction, creating a brush type chiral stationary phase for enantioseparation of *N*-3,5-dinitrobenzoyl-leucine in anion exchange mode. Using a similar concept, the same group also attached another chiral selector, namely (*S*)-*N*-(4-allyloxy-3,5-dichlorobenzoyl)-2-amino-3,3-dimethylbutanephosphonic acid, to three kinds of monoliths with different hydrophilicities (Fig. 7). Enantioseparations of mefloquine and mefloquine *tert*-butylcarbamate were performed under strong cation exchange conditions. Capillaries prepared with the more hydrophilic matrix of poly(GMA-co-HEMA-co-EDMA) showed higher enantioselectivity due to the reduced non-specific interactions.

Recently, Messina *et al.* [46] prepared a monolith of poly(-GMA-co-methyl methacrylate-co-EDMA), and the chiral selector, namely (+)-1-(4-aminobutyl)-(5*R*,8*S*,10*R*)-terguride, was attached by reaction with the epoxy moieties on the surface of the monoliths (Fig. 8). Enantioseparation of seven pairs of 2-acryloxypropionic acid enantiomers on the prepared capillaries was accomplished with a non-aqueous mobile phase. The columns remained stable for hundreds of analyses.

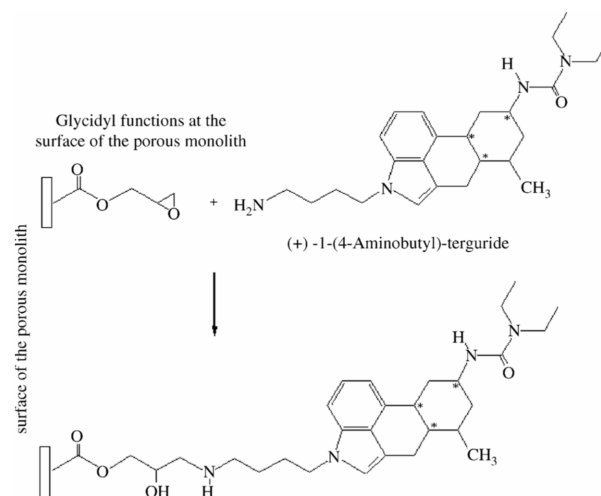

Figure 8. Reaction scheme for the functionalization of poly (GMA-co-methyl methacrylate-co-EDMA) monolith with (+)-1-(4-aminobutyl)-(5*R*,8*S*,10*R*)-terguride. Reproduced from [46], with permission.

5 Molecularly imprinted monoliths

Molecularly imprinted polymers (MIP) have been used as enantiomer-selective stationary phases in CEC since the introduction of so-called superporous monoliths by Schweitz *et al.* [47]. The imprinting molecules first formed complexes with the functional monomers in a polymerization solution containing cross-linkers and initiators; after a polymerization reaction the imprinting molecules were removed, thus resulting in a matrix with cavities whose shape, size, and chemical functionality are complementary to those of the imprinting molecules. Enantiomer-selective stationary phases of this type always show predetermined enantioselectivities, and the imprinted enantiomer is always strongly retained.

In their first report, Schweitz *et al.* [47] dissolved imprinting molecules of *R*-propranolol or *S*-metoprolol, MAA (functional monomer), trimethylolpropane trimethacrylate (TRIM) (cross-linker); and AIBN in dried toluene. This mixture was polymerized to prepare MIPs in capillaries at −20°C with a 350-nm UV source as irradiator (Fig. 9). The porous structure was controlled by careful timing of the polymerization reaction. Lower temperature benefited the formation of better-defined imprinting sites in the resultant polymer. The remaining monomer, radical initiator, and imprinting molecule could be easily flushed out by hydrodynamic pumping. The whole procedure was quick and simple, and could be finished in 3 h. Propranolol or metoprolol could be resolved on the MIP columns by CEC. Fast enantioseparation of propranolol could be realized in less than 2 min at high voltage (30 kV) in combination with a pressure of 7 bar. Capillaries prepared by this approach could be continuously used for several weeks with good reproducibility. The influence of the composition of the polymerization mixture including the type and amount of functional and cross-linking monomers, the molar ratio of the imprinting molecule to the monomers, and the type of the porogenic solvents on the enantioselectivity of the resultant MIP was systemically investigated with *S*-ropivacaine as imprinting molecule [48]. It was observed that the use of 1–25% isooctane as porogenic solvent led to the formation of super-porous monoliths. Among the adopted cross-linking monomers, TRIM afforded the best resolutions. In a further study [49] with *S*-propranolol as

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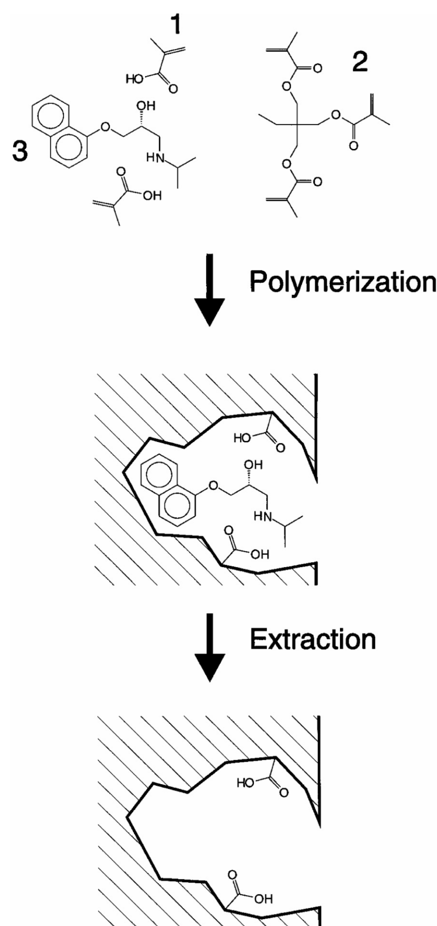

Figure 9. Schematic representation of imprint formation. Methacrylic acid (1) was used as functional monomer, trimethylolpropane trimethacrylate (2) as cross-linking monomer, and *R*-propranolol (3) as imprinting molecule. Reproduced from [47], with permission.

imprinting molecule, it was found that changes in the type and composition of functional monomers resulted in differences in polymer morphology, and thus in electrochromatographic properties. Partly replacing MAA by other functional monomers such as methyl, butyl, or epoxypropyl methacrylate might improve the efficiency and resolution. In addition, the feasibility of using multiple imprinting molecules in a single capillary column was also investigated. *R*-Propranolol [50, 51] imprinted capillary has been utilized to resolve several β-adrenergic antagonists, which are structural analogues of propranolol. Efficiencies of about 35 000 to 70 000 plates/m were obtained for the first eluted enantiomers (Fig. 10). In another report [52], a short end of capillary column with *S*-propranolol as imprinting molecule was prepared using a similar approach. Thus, very fast enantioseparation of racemic propranolol (less than 1 min) was achieved at high voltage.

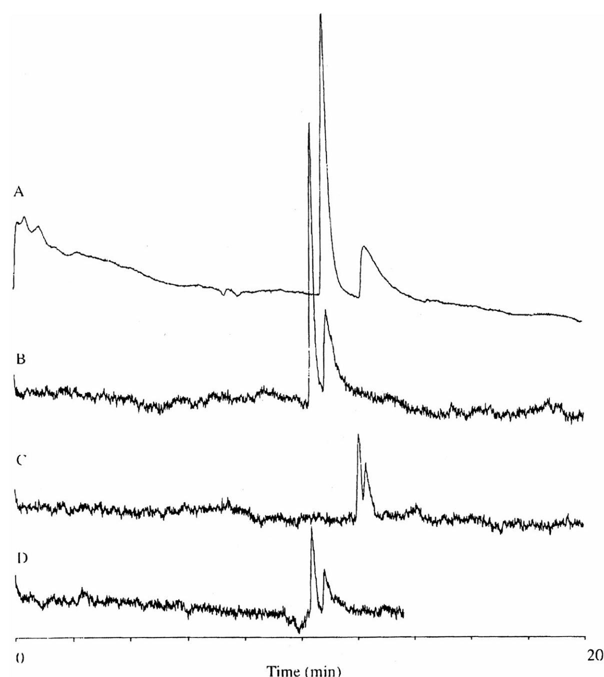

Figure 10. Electrochromatograms for resolution of propranolol (A), pindolol (B), prenalterol (C), and atenolol (D) on a MIP-based monolithic column (90 cm × 75 mm id) by CEC (imprinting molecule, *R*-propranolol). Reproduced from [51], with permission.

Lin *et al.* [53] proposed a thermally induced polymerization approach for preparation of MIP monoliths in capillaries. They employed a different protocol to introduce vinyl moieties onto the inner wall of the capillaries, *i.e.*, by treating the capillaries with thionyl chloride following Grignard reaction. L-PheAN (imprinting molecule), MAA and/or 2-vinylpyridine (functional monomers), EDMA (cross-linker), AIBN, and ammonium acetate (conducting agent for electrophoretic exchange of solvents) were dissolved into chloroform, and allowed to polymerize in capillaries at 60°C. Replacing part of the MAA with 2-vinylpyridine as functional monomer benefited the enantioseparations. It seems that the obtained polymers in the capillaries could not be flushed by hydrodynamic pumping, as the change from the polymerization solvents to electrolyte was performed electrophoretically.

Liu *et al.* prepared *R*-1,1′-bi-2,2′-naphthol [54] and *S*-naproxen [55] imprinted monoliths in capillaries, respectively, by an *in situ* polymerization with thermal initiation. The polymerization solution consisted of the imprinting molecules, MAA, EDMA, AIBN, and toluene/isooctane. The influence of several parameters on the electrochromatographic properties of the monoliths was investigated [54]. It was observed that a higher molar ratio of the imprinting molecule to the functional monomer and a higher content of porogenic solvents benefited the enantioseparations. Under optimized running

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conditions, baseline enantioseparation of racemic binaphthol and naproxen, respectively, could be achieved.

6 Conclusions

Monolithic enantiomer-selective stationary phases have emerged as potential alternatives to packed columns in enantioseparations by CEC. Capillary columns with monolithic enantiomer-selective stationary phases need no frits to retain the packing materials, and disadvantages such as bubble formation and accidental breakage of the capillaries are avoided. It is apparent that each of the technologies described above for preparation of monolithic enantiomer-selective stationary phases has its own advantages and disadvantages; however, none of them has evidently proved to be superior to the others. Particle-fixed monoliths have the advantage that a broad spectrum of commercially available CSPs can be utilized; however, a drop in enantioselectivity and efficiency is often observed. Silica-based monoliths with high mechanical stability appear promising in CEC. They have the advantages that modification methods for preparing particulate packing materials can be directly used. But most chiral selectors can only be attached by post-modifications of the columns, making the procedures complicated and possibly causing reproducibility problems. In addition, the inadequate stability of the silica-based matrix under high pH conditions is another limitation. Polymer-based monoliths can be fabricated in the capillaries in a single step with *in situ* polymerization, or followed by post-modifications of the columns, but the tuning of pore diameter and morphology of the matrix is a challenging subject. MIP-based monoliths provide predetermined selectivity for the imprinted enantiomers, which represent interesting alternatives to other chiral selectors. Due to the limitations of the present imprinting strategy, only limited numbers of enantiomers have been imprinted, thus new imprinting strategies are expected in the future to extend the spectrum of enantiomers and to improve the column efficiencies. In conclusion, much progress has been achieved in the preparation of monolithic enantiomer-selective stationary phases for CEC during the past decade. However, for the purpose of practical use, these techniques are expected to develop further to overcome the current disadvantages, to cover more chiral selectors with different separation principles, and to improve the inter-laboratory or column-to-column reproducibility.

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7 References

[1] Maier, N. M., Franco, P., Lindner, W., *J. Chromatogr. A* 2001, *906*, 3–33.

[2] Ward, T. J., *Anal. Chem.* 2002, *74*, 2863–2872.

[3] Ward, T. J., Hamburg, D. M., *Anal. Chem.* 2004, *76*, 4635–4644.

[4] Zhang, Y., Wu, D. R., Wang-Iverson, D. B., Tymiak, A. A., *Drug Discovery Today* 2005, *10*, 571–577.

[5] Okamoto, Y., Yashima, E., *Angew. Chem. Int. Ed.* 1998, *37*, 1020–1043.

[6] Fanali, S., Catarcini, P., Blaschke, G., Chankvetadze, B., *Electrophoresis* 2001, *22*, 3131–3151.

[7] Wistuba, D., Schurig, V., *J. Chromatogr. A* 2000, *875*, 255–276.

[8] Kang, J. W., Wistuba, D., Schurig, V., *Electrophoresis* 2002, *23*, 4005–4021.

[9] Lämmerhofer, M., *J. Chromatogr. A* 2005, *1068*, 31–57.

[10] Wistuba, D., Schurig, V., *Electrophoresis* 2000, *21*, 3152–3159.

[11] Chirica, G., Remcho, V. T., *Electrophoresis* 1999, *20*, 50–56.

[12] Wistuba, D., Banspach, L., Schurig, V., *Electrophoresis* 2005, *26*, 2019–2026.

[13] Chirica, G. S., Remcho, V. T., *Anal. Chem.* 2000, *72*, 3605–3610.

[14] Lin, J. M., Nakagama, T., Uchiyama, K., Hobo, T., *Chromatographia* 1996, *43*, 585–591.

[15] Schmid, M. G., Koidl, J., Freigassner, C., Tahedl, S., Wojcik, L., Beesley, T., Armstrong, D. W., Gübitz, G., *Electrophoresis* 2004, *25*, 3195–3203.

[16] Kato, M., Dulay, M. T., Bennett, B., Chen, J. R., Zare, R. N., *Electrophoresis* 2000, *21*, 3145–3151.

[17] Chen, Z. L., Hobo, T., *Anal. Chem.* 2001, *73*, 3348–3357.

[18] Chen, Z. L., Hobo, T., *Electrophoresis* 2001, *22*, 3339–3346.

[19] Chen, Z., Nishiyama, T., Uchiyama, K., Hobo, T., *Anal. Chim. Acta* 2004, *501*, 17–23.

[20] Kang, J. W., Wistuba, D., Schurig, V., *Electrophoresis* 2002, *23*, 1116–1120.

[21] Chen, Z. L., Ozawa, H., Uchiyama, K., Hobo, T., *Electrophoresis* 2003, *24*, 2550–2558.

[22] Liu, Z., Otsuka, K., Terabe, S., Motokawa, M., Tanaka, N., *Electrophoresis* 2002, *23*, 2973–2981.

[23] Qin, F., Xie, C., Feng, S., Ou, J., Kong, L., Ye, M., Zou, H., *Electrophoresis* **2006**, *27*, 1050–1059.

[24] Kato, M., Sakai-Kato, K., Matsumoto, N., Toyo'oka, T., *Anal. Chem.* 2002, *74*, 1915–1921.

[25] Sakai-Kato, K., Kato, M., Nakakuki, H., Toyo'oka, T., *J. Pharm. Biomed. Anal.* 2003, *31*, 299–309.

[26] Kato, M., Saruwatari, H., Sakai-Kato, K., Toyo'oka, T., *J. Chromatogr. A* 2004, *1044*, 267–270.

[27] Koide, T., Ueno, K., *Anal. Sci.* 2000, *16*, 1065–1070.

[28] Koide, T., Ueno, K., *J. Chromatogr. A* 2000, *893*, 177–187.

[29] Koide, T., Ueno, K., *HRC J. High Resolut. Chromatogr.* 2000, *23*, 59–66.

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[30] Végvári, Á., Földesi, A., Hetényi, C., Kocnegarova, O., Schmid, M. G., Kudirkaite, V., Hjertén, S., *Electrophoresis* 2000, *21*, 3116 – 3125.

[31] Zeng, H. L., Li, H. F., Lin, J. M., *Anal. Chim. Acta* 2005, *551*, 1 – 8.

[32] Koide, T., Ueno, K., *J. Chromatogr. A* 2001, *909*, 305 – 315.

[33] Schmid, M. G., Grobuschek, N., Tuscher, C., Gübitz, G., Végvári, Á., Machtejevas, E., Maruška, A., Hjertén, S., *Electrophoresis* 2000, *21*, 3141 – 3144.

[34] Schmid, M. G., Grobuschek, N., Lecnik, O., Gübitz, G., Végvári, Á., Hjertén, S., *Electrophoresis* 2001, *22*, 2616 – 2619.

[35] Lecnik, O., Gübitz, G., Schmid, M. G., *Electrophoresis* 2003, *24*, 2983 – 2985.

[36] Kornyšova, O., Owens, P. K., Maruška, A., *Electrophoresis* 2001, *22*, 3335 – 3338.

[37] Kornyšova, O., Jarmalaviciene, R., Maruška, A., *Electrophoresis* 2004, *25*, 2825 – 2829.

[38] Machtejevas, E., Maruška, A., *J. Sep. Sci.* 2002, *25*, 1303 – 1309.

[39] Peters, E. C., Lewandowski, K., Petro, M., Svec, F., Fréchet, J. M. J., *Anal. Commun.* 1998, *35*, 83 – 86.

[40] Lämmerhofer, M., Svec, F., Frechét, J. M. J., Lindner, W., *J. Microcolumn Sep.* 2000, *12*, 597 – 602.

[41] Lämmerhofer, M., Peters, E. C., Yu, C., Svec, F., Frechét, J. M. J., Lindner, W., *Anal. Chem.* 2000, *72*, 4614 – 4622.

[42] Lämmerhofer, M., Svec, F., Frechét, J. M. J., *Anal. Chem.* 2000, *72*, 4623 – 4628.

[43] Lämmerhofer, M., Tobler, E., Zarbl, E., Lindner, W., Svec, F., Fréchet, J. M. J., *Electrophoresis* 2003, *24*, 2986 – 2999.

[44] Preinerstorfer, B., Bicker, W., Lindner, W., Lämmerhofer, M., *J. Chromatogr. A* 2004, *1044*, 187 – 199.

[45] Preinerstorfer, B., Lindner, W., Lämmerhofer, M., *Electrophoresis* 2005, *26*, 2005 – 2018.

[46] Messina, A., Flieger, M., Bachechi, F., Sinibaldi, M., *J. Chromatogr. A* (in press).

[47] Schweitz, L., Andersson, L. I., Nilsson, S., *Anal. Chem.* 1997, *69*, 1179 – 1183.

[48] Schweitz, L., Andersson, L. I., Nilsson, S., *J. Chromatogr. A* 1997, *792*, 401 – 409.

[49] Schweitz, L., Andersson, L. I., Nilsson, S., *Analyst* 2002, *127*, 22 – 28.

[50] Nilsson, S., Schweitz, L., Petersson, M., *Electrophoresis* 1997, *18*, 884 – 890.

[51] Schweitz, L., Andersson, L. I., Nilsson, S., *Chromatographia* 1999, *49*, S93 – S94.

[52] Schweitz, L., Andersson, L. I., Nilsson, S., *Anal. Chim. Acta* 2001, *435*, 43 – 47.

[53] Lin, J. M., Nakagama, T., Uchiyama, K., Hobo, T., *J. Pharm. Biomed. Anal.* 1997, *15*, 1351 – 1358.

[54] Liu, Z. S., Xu, Y. L., Wang, H. F., Yan, C., Gao, R. Y., *Anal. Sci.* 2004, *20*, 673 – 678.

[55] Xu, Y. L., Liu, Z. S., Wang, H. F., Yan, C., Gao, R. Y., *Electrophoresis* 2005, *26*, 804 – 811.

[56] Schweitz, L., Andersson, L. I., Nilsson, S., *J. Chromatogr. A* 1998, *817*, 5 – 13.

[57] Lämmerhofer, M., Svec, F., Frechét, J. M. J., Lindner, W., *Trends Anal. Chem.* 2000, *19*, 676 – 698.

[58] Gübitz, G., Schmid, M. G., *Electrophoresis* 2004, *25*, 3981 – 3996.

[59] Mangelings, D., Maftouh, M., Vander Heyden, Y., *J. Sep. Sci.* 2005, *28*, 691 – 709.

[60] Fujimoto, C., *Anal. Sci.* 2002, *18*, 19 – 25.

[61] Ishizuka, N., Minakuchi, H., Nakanishi, K., Soga, N., Nagayama, H., Hosoya, K., Tanaka, N., *Anal. Chem.* 2000, *72*, 1275 – 1280.

[62] Liu, Z., Otsuka, K., Terabe, S., *J. Sep. Sci.* 2001, *24*, 17 – 26.

[63] Chankvetadze, B., Yamamoto, C., Tanaka, N., Nakanishi, K., Okamoto, Y., *J. Sep. Sci.* 2004, *27*, 905 – 911.

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