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Review

# Chiral silica-based monoliths in chromatography and capillary electrochromatography

Dorothee Wistuba

*Institute of Organic Chemistry, University of Tübingen, 72076 Tübingen, Germany*

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ABSTRACT

Chiral-modified silica-based monoliths have become well-established stationary phases for both high performance liquid chromatography (HPLC) and capillary electrochromatography (CEC). The silica-based monoliths were fabricated either *in situ* in the capillaries for nano-HPLC and CEC or in a mould for "conventional" HPLC. The present review summarizes the chiral modification of silica monoliths and the recent development in the field of enantioselective separations by nano-HPLC and CEC.



**Contents**



## 1. Introduction

Columns with particulate silica beds are well known and are extensively used for separation in HPLC and CEC. For increasing the performance of traditional HPLC columns, sorbents of progressively more narrow grain size composition and finer particles were used. The result was a significant increase in the pressure that should be necessary to ensure the optimal flow of the mobile phase. A novel concept in column technology has received considerable attention in recent years. Monoliths consisting of one single piece of a porous solid were employed as highly efficient stationary phase in chromatography and electro-driven methods [1–3]. Monolithic columns represent a good alternative to particle-packed columns for both HPLC and CEC separations because of their enhanced mass transfer and lower pressure drop. Little is known about the use of monolithic columns in GC [4]. Despite the fact that the monolith is characterized by high porosity, it significantly resists the carrier gas flow. The permeability was about three times lower than that of open tubular capillary. As expected a higher loadability but lower HETP values could be found.

*E-mail address:* dorothee.wistuba@orgchem.uni-tuebingen.de.

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**Table 1**
Chiral silica-based monolithic columns for "conventional" HPLC.

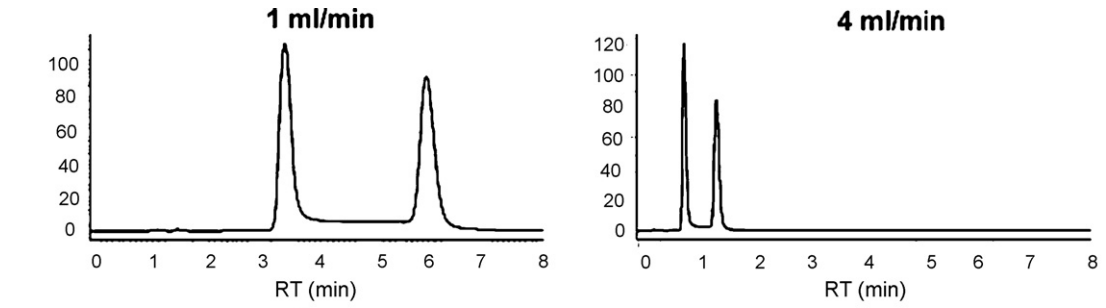
| Silica backbone | Chiral selector | Analyte | Mobile phase | Reference |
|---|---|---|---|---|
| Chromolith Si | β-CD | Chromakalim, prominal, norgestrel, methadone, MTH-phe, PTH-phe, metoprolol, oxazepam | Hydro-organic, polar organic | [8] |
| Chromolith, RP-18e | Cellulose tris(3,5-dimethylphenyl carbamate) | 2,2,2-trifluoro-1-(9-anthryl)ethanol, piprozolin, 2,2′-dihydroxy-6,6′-dimethylbiphenyl | Apolar organic | [9] |
| Chromolith Si | Cellulose tris(3,5-dimethylphenylcarbamate) | 2,2,2-trifluoro-1-(9-anthryl)ethanol, benzoin, 2,2′-dihydroxy-6,6′-dimethylbiphenyl, flavanone 2-phenylcyclohexanone, 1,2,2,2-tetraphenyl-ethanol, Tröger's base, Co(III) acac | Apolar organic | [10] |
| Chromolith-$NH_2$ | Penicillin G acylase | 2-Aryloxyalkanoic acids | Hydro-organic | [11] |
| Chromolith Si | 2,3-Methylated 3-mono-acylated 6-*O*-*tert*-butyldimethylsilylated β-CD 2,3-methyl 6-O-tert butyldimethyl-silylated β-CD | 14 and 7 compounds, respectively | Hydro-organic | [12] |
| Chromolith Si | *tert*-Butylcarbamoylquinine | *N*-derivatized amino acids, suprofen | Hydro-organic | [13] |
| Onyx monolith | Proline-derived chiral selector | *N*-(3,5-dinitrobenzoyl)amino acids | Apolar organic | [14] |
| Chromolith Si | $\alpha_1$-acid glycoprotein | Warfarin, propranolol | Hydro-organic | [15] |
| Chromolith Si | HSA | trp, warfarin, ibuprofen | Aqueous, hydro-organic | [16] |

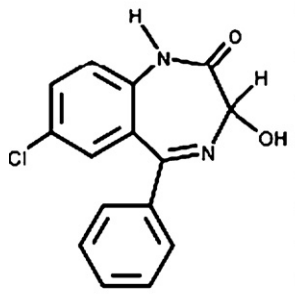
The monolithic stationary phases can be classified into two types: (i) silica-based monoliths prepared by sol–gel technology, by entrapping particles in inorganic gels or sintering silica beds and (ii) rigid organic polymer-based monoliths prepared by polymerization of suitable organic monomers in the presence of a porogen. In the present review only silica-based monolithic columns were considered. Silica monoliths were prepared by sol–gel technology either in a mould to produce a rod column (e.g., 4–7 mm I.D.) or *in situ* in a fused silica capillary (e.g., 50–530 μm I.D.) [1]. Once formed, the silica monoliths could be modified by covalently attaching chiral selectors to the silanol groups of the silica surface. To the author's best knowledge no enantiomeric separation with monolithic stationary phases in GC was described before.

## 2. Chiral-modified silica monolithic columns in "conventional" HPLC (see Table 1)

Chiral monoliths can be used in both in "conventional" HPLC (columns with internal diameters in a range of 2–4.8 mm) and nano-HPLC (capillaries with internal diameters up to 500 μm) systems. Nakanishi and Soga [5] developed a new sol–gel process which allows the preparation of monolithic materials with a bimodal pore structure (throughpores and mesopores) suitable for chromatography. The production is based on acid-catalyzed hydrolysis and polycondensation of alkoxysilanes. Tanaka and coworkers [6], and Lubda et al. [7] used this method for preparing monolithic silica columns of conventional format with high efficiencies and low backpressures for HPLC. The first commercially available monolithic silica column (Chromolith) was introduced by MerckKGaA in 2000. Classical HPLC columns with monolithic separation beds of this size are prepared in a column mould in which the monolith can later be replaced. In a further step the monolithic silica has to be coated or clad with a suitable material such as PEEK (poly(ether ether ketone) to which the column end fittings can be attached for use in HPLC-Systems [1]. *In situ* polymerization, the usually used method for preparing monolithic silica capillaries, is not applicable for conventional HPLC columns due to shrinkage. However, conventional HPLC apparatus which are available in nearly every lab, can be equipped with a classical monolithic column.

In the year 2003, the first enantiomeric separations with chiral-modified silica monoliths were described by Lubda et al. [8] and by Chankvetadze et al. [9]. As silica backbones both groups used commercially available monoliths (Chromolith) and modified the silica surface with chiral selectors. Lubda et al. [8] compared the chromatographic behaviour of a β-cyclodextrin modified silica monolith with a commercially available β-cyclodextrin bonded particulate material (ChiraDex). While the enantioselectivities of both columns were comparable, the retention factors and thus the analysis time were in most cases lower on the silica monolith than on the packed column. Even if the amount of β-cyclodextrin bound to the silica monolith was only half of the amount of β-cyclodextrin bound to particles (ChiraDex) good separation factors were achieved for several chiral drugs such as chromakalin, prominal, oxazepam and methadone (see Fig. 1). Due to the flat van Deemter curve, fast enantiomeric separation could be observed. To modify the plain silica monolith two different synthesis routes were described: (i) batch modification of the unclad silica monolith and (ii) *in situ* modification of silica monolith in the clad column in the flow-through mode. Unsatisfactory heterogeneity of the surface modification were found for the batch modification, thus in situ modification (ii) was preferred. Chankvetadze et al. [9] described the enantiomeric separation of 2,2,2-trifluoro-1-(9-anthryl)ethanol, 2,2′-dihydroxy-6,6′-dimethylbiphenyl and piprozolin on a silica monolith with octadecyl-functionalities coated with cellulose tris(3,5-dimethylphenylcarbamate). The baseline separation of the enantiomers of 2,2,2-trifluoro-1-(9-anthryl)ethanol was accomplished in less than 30 s (see Fig. 2). A disadvantage of the coated-type polysaccharide monoliths is the solubility or swelling of the material. To overcome this, cellulose tris(3,5-dimethylphenylcarbamate) was covalently attached *in situ* onto native silica monoliths clad in a 50 mm × 4.6 mm PEEK HPLC column [10]. The chiral modification of the silica monolith occurs in three steps: (i) reaction with γ-glycidoxypropyltrimethoxysilane, (ii) modification with the polysaccharide derivative and (iii) treatment with 3,5-dimethylphenylisocyanate in order to convert the hydroxyl groups of cellulose into carbamate moieties. However no significant improvement of enantiomer resolving ability was observed for the covalently modified monolith. Massolini et al. [11] immobilized




| Flow rate | | | | |
|---|---|---|---|---|
| Factor | 1 ml/min | 2 ml/min | 3 ml/min | 4 ml/min |
| $t_{R1}$ min | 3.46 | 1.70 | 1.11 | 0.82 |
| $t_{R2}$ min | 5.95 | 2.91 | 1.89 | 1.39 |
| $k_1$ | 1.06 | 1.05 | 1.02 | 1.00 |
| $k_2$ | 2.54 | 2.51 | 2.44 | 2.39 |
| α | 2.40 | 2.39 | 2.39 | 2.39 |
| $Asym_1$ | 1.68 | 1.52 | 1.43 | 1.46 |
| $Asym_2$ | 1.16 | 1.18 | 1.17 | 1.22 |
| Pressure [bar] | 50 | 77.5 | 103 | 128 |
| $R_S$ (USP) | 6.29 | 5.55 | 5.17 | 4.67 |
| $N_1$ [$m^{-1}$] | 15790 | 13470 | 12680 | 10810 |
| $N_2$ [$m^{-1}$] | 28860 | 21410 | 18310 | 14630 |

**Fig. 1.** Effect of increasing flow rate on the separation of oxazepam by HPLC in methanol/0.02 M $H_3PO_4$, pH 2.07, 20/80 (v/v) at 20 °C using a β-cyclodextrin monolith [8].

penicillin G acylase (PGA) on a monolithic silica support (Chromolith-$NH_2$) to create an HPLC-enzyme reactor for studying the enantioselectivities of ester hydrolysis catalyzed by PGA. Bayer et al. [12] transferred the excellent chiral GC selectors 2,3-methylated 3-monoacetylated 6-*O*-*tert*-butyldimethylsilylated β-cyclodextrin (only one of seven methyl groups in 3-position was substituted by an acetyl group) and 2,3-methyl 6-*O*-*tert*-butyldimethylsilylated β-cyclodextrin to HPLC. The derivatized cyclodextrins were covalently attached onto aminopropyl functionalized monolithic silica HPLC columns. Due to the broader enantioselectivity 2,3-methyl 6-*O*-*tert*-butyldimethylsilylated β-cyclodextrin (enantiomers of 14 of 33 compounds were separated) predominates the usability of 2,3-methylated 3-monoacetylated 6-*O*-*tert*-butyldimethylsilylated β-cyclodextrin (enantiomers of 7 of 33 compounds were separated) as a chiral stationary phase in HPLC (see Fig. 3). Particular brush-type chiral stationary phases are well known for enantiomeric separation in HPLC. The excellent enantiomer separation properties have inspired several scientists to prepare the monolithic counterpart. In 2004, Lubda and Lindner [13] presented a monolithic silica column with covalently bonded *tert*-butylcarbamoylquinine chiral anion-exchanger selector (via a thio ether bridge) as a stationary phase for enantiomeric separation of *N*-derivatized amino acids and suprofen. It was found that the enantioselectivity of the chiral monolith is basically comparable with the similar modified particular counterpart, even though the total amount of *tert*-butylcarbamoylquinine is less than half of that value reached with the particular material. The influence of hydro-organic and polar organic mobile phase was investigated. The coupling of six 10 cm monolithic silica columns serially is possible due to the low backpressure and allows the baseline separation of suprofen enantiomers, upon increase of flow rate from 1 to 4 ml/min, even in less than 10 min (see Fig. 4). Recently, Slater et al. [14] also reported on the preparation of a brush-type chiral stationary phase using click chemistry. First, the silica surface was modified with 3-(azidopropyl)trimethoxysilane, then a proline-derived chiral selector (*N*-(*N*-(5-hexynoyl)prolinoyl)-5-aminoindan) was immobilized in the presence of a copper

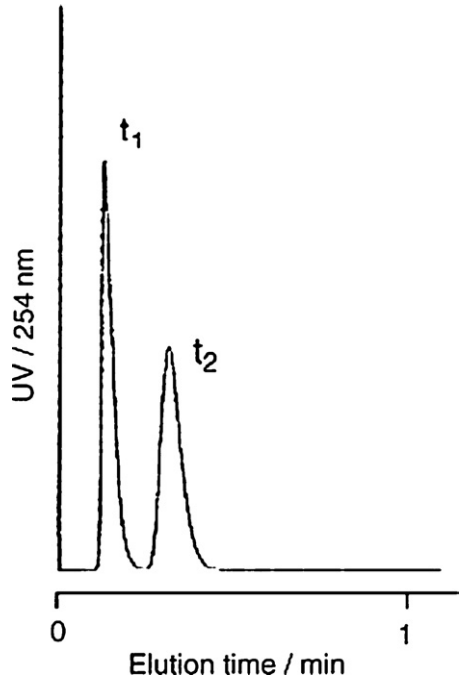



**Fig. 2.** Enantioseparation of 2,2,2-trifluoro-1-(9-anthryl)ethanol using cellulose tris(3,5-dimethylphenylcarbamate) *in situ* coated 12% (w/w) on Chromolith SpeedRod as CSP. Column: 4.6 mm × 50 mm; mobile phase: hexane/2-propanol 90/10 (v/v); flow rate: 20 ml/min [9].

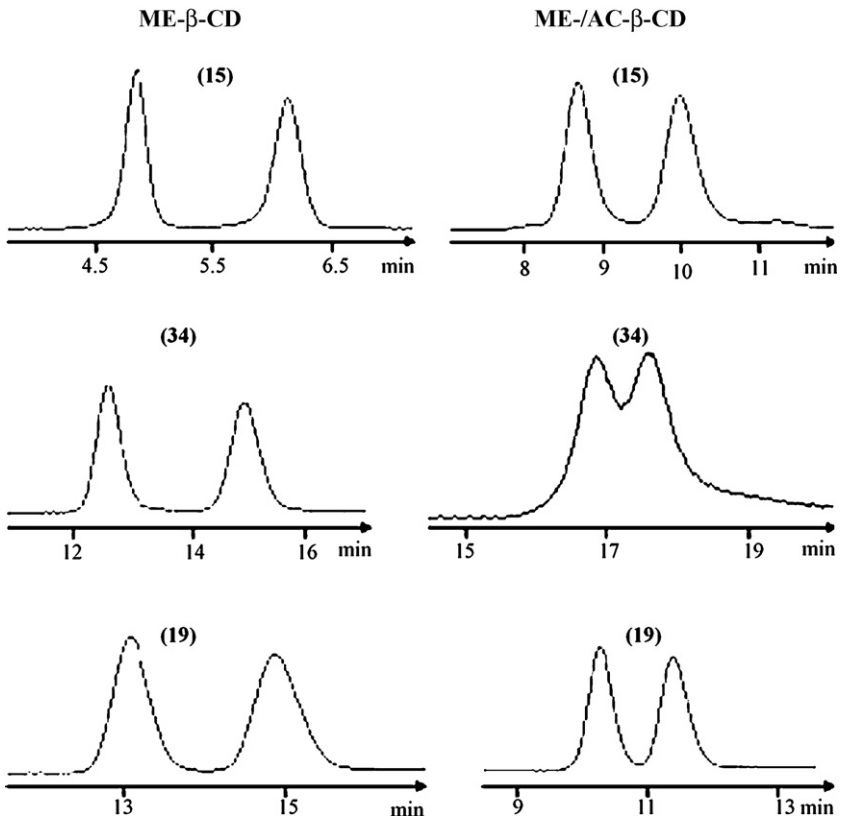



**Fig. 3.** Comparison of the enantiomeric separation α-ionone (15), 2,2,2-trifluoro-1-(9-anthryl)ethanol (34) and flavanone (19) on monolithic columns modified with 2,3-methylated 6-*O*-*tert*-butyldimethylsilylated β-cyclodextrin (ME-AC-β-CD) and with 2,3-methylated 6-*O*-*tert*-butyldimethylsilylated β-cyclodextrin (ME-β-CD) by HPLC in acetonitrile/0.1 M dihydrogen phosphate, pH 4 [12].

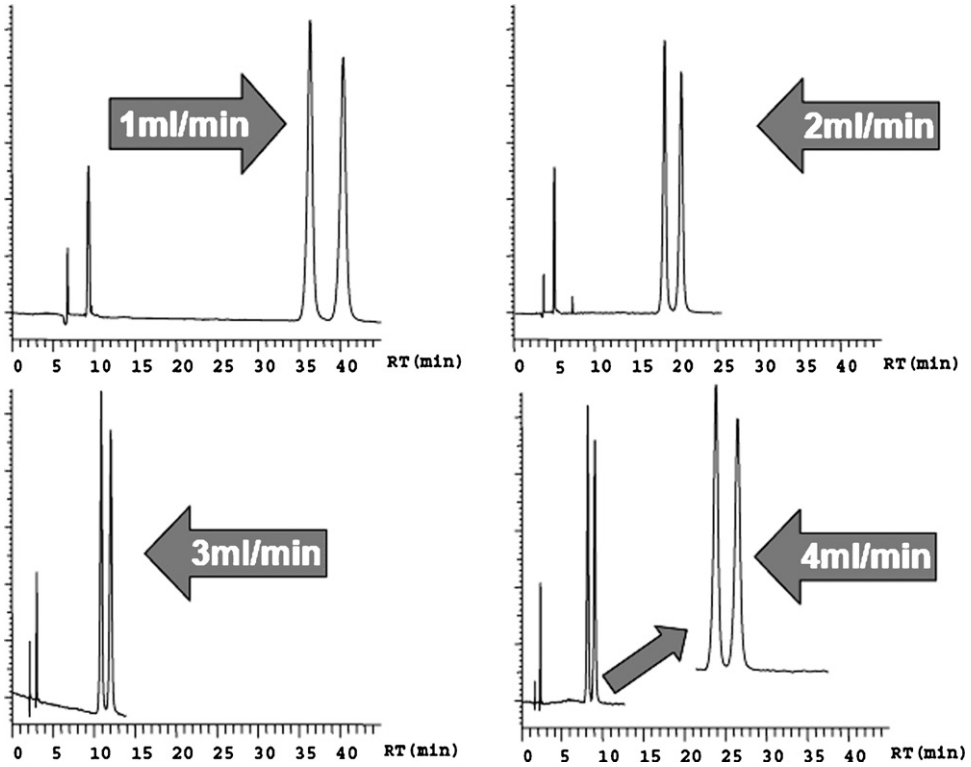



**Fig. 4.** Effect of increasing flow rate on the separation of suprofen in methanol/glacial acid 99.5/0.5 (v/v) using a 60 cm long *tert*-butylcarbamoylquinine-modified monolithic silica column [13].

(I) catalyst. Compared with the analogue, the chiral separation factors of four *N*-(3,5-dinitrobenzoyl)amino acid dialkyl amide are much lower and peak tailing occurred. The cause of this poor behaviour is the large amount of unreacted silanol groups which leads to extensive nonspecific interactions. To improve the performance, the chiral-modified silica monolith was end-capped with trimethylsilyl groups by *in-column* treatment with 1,1,1,3,3,3-hexamethyldisilazane. This resulted in an enhancement of the chiral separation factor of all four investigated model compounds. Mallik et al. [15] reported on affinity silica monoliths (Chromolith) containing immobilized $\alpha_1$-acid glycoprotein (AGP) chiral selector for the enantiomeric separation of warfarin and propranolol. The chromatographic results were compared with data obtained when used AGP immobilized silica particle or monoliths based on a glycidyl methacrylate-*co*-ethylene dimethacrylate. The surface coverage of AGP in the silica monolith was about 18% higher than that obtained with silica particles (i.e., 5.8 versus 4.9 nmol AGP/m$^2$) and about 61% higher than observed for the organic polymer-based monolith. The silica monolith is superior in terms of resolution factors $R_S$, which are dissatisfying for the other two stationary phases. In 2008, the same group published an immobilization protocol for the covalent linkage of HSA (human serum albumin) to a silica monolith [16]. In this method, a diol silica monolith was first oxidized by using periodic acid to give aldehyde groups on the interior surface of the monolith. In a second step, the amine groups reacted with the aldehyde groups on the monolith to form a Schiff's base. The more stable secondary amine linkage was formed in a reduction reaction with cyanoborohydride. The enantioseparation of tryptophan, warfarin and ibuprofen was described. The chromatographic results were compared with data obtained for the same protein when coupled to silica particles or to a rigid organic monolith. The surface coverage but not the absolute amount of HSA of the three kinds of HSA CSPs (chiral stationary phases) based on: (i) the silica monolith, (ii) the particulate silica and (iii) the rigid organic monolith were nearly the same. But the HSA silica monolith gave higher or leastwise comparable resolutions and efficiencies.

## 3. Chiral-modified silica monolithic capillaries in nano-HPLC and capillary electrochromatography (CEC) (see Table 2)

Silica capillaries with a monolithic separation bed covalently attached to the tube wall have been prepared *in situ* for use in nano-HPLC and CEC, whereas, capillaries with small internal diameters are favourable for covalent bonding between the tube and the monolithic silica skeleton. This kind of silica-based monoliths are prepared by the sol–gel technology, whereas, the chiral selectors are immobilized by (i) covalent bonding, (ii) by physical adsorption or (iii) by encapsulation. Monoliths can also be prepared by particle-fixation technique such as embedding the chiral-modified silica particles into a sol–gel matrix, into an organic polymer, by gluing it via a sol–gel process or by sintering unmodified silica particles at high temperature followed by chiral derivatization [17–19].

### 3.1. Monoliths prepared by sol–gel technique

The sol–gel method based on the *in situ* polycondensation of alkoxysilanes, also with the capillary inner wall, resulted in a monolithic stationary phase with low flow resistance (high flow throughput) and good mechanical strength. The chiral selector is either covalently bonded via a spacer to the silica backbone or adsorbed at the silica surface. For enantiomeric separation, any of the well-known CSPs such as ligand-exchange-type CSP [20–22], cyclodextrins and modified cyclodextrins [23,24,37], proteins and glycoproteins [25–28], cellulose and amylose derivatives [29–32,39], anion-exchange-type CSPs [33–36] and macrocyclic

**Table 2**
Chiral silica-based monoliths for nano-HPLC and CEC prepared by sol–gel technique (see 3.1).

| Silica backbone[a] | Chiral selector | Analyte | Method | Mobile phase | Reference |
|---|---|---|---|---|---|
| TMOS/PEG/acetic acid | L-Pro-amide, L-Phe-amide L-Ala-amide | Dansyl-aas, hydroxy acids aas, dipeptides | nano-HPLC, CEC | Hydro-organic | [20–22] |
| TMOS/PEG/acetic acid | β- and γ-CD | Dansyl-aas, benzoin, | CEC | Hydro-organic | [23] |
| TMOS/PEG/acetic acid | Permethyl-β-CD (Chirasil-β-Dex) | Mephobarbital, hexobarbital, benzoin, carprofen | CEC | Hydro-organic | [24] |
| TMOS/PEG/acetic acid | Avidin | aas-derivatives, organic acids, menadione, warfarin, *N*-methyl-pseudoephedrine | nano-HPLC, CEC | Hydro-organic | [25] |
| MTMS/TMOS/HCl o. TMOS/HCl gelatine, chitosan | BSA, OVM | trp, benzoin, eperisone, | CEC | Aqueous, hydro-organic | [28–28] |
| TMOS/MTMS/PEG/urea/acetic acid | Amylose- o. cellulose-tris(3,5-dimethylphenylcarbamate) | 10/11 Compounds | nano-HPLC | Organic | [29,30] |
| TMOS/MTMS/PEG/urea/acetic acid | 3,5-disubstituted phenyl-carbamate derivative of cellulose and amylase | 10 compounds | nano-HPLC | Organic | [31] |
| TMOS/PEG/acetic acid | tris(3,5-dimethylphenylcarbamate) | 15 Compounds | CEC | Hydro-organic non-aqueous | [32] |
| Chromolith Si CapRod (Merck) | *S*-*N*-(4-Allyloxy-3,5-dichlorobenzoyl-1-amino-3,3-dimethylbutane-phosphonic acid | 17 Basic compounds | CEC | Non-aqueous | [33] |
| Chromolith Si CapRod (Merck) | *O*-9-(*tert*-Butylcar-bamoyl)-quinidine | Phosphinic acid pseudodipeptides | CEC | Non-aqueous | [34] |
| Chromolith Si CapRod (Merck) | *trans*-(1*S*,2*S*)-2-(*N*-4-allyl-oxy-3,5-dichlorobenzoyl)-aminocyclohexanesulfonic acid | 8 Basic drugs | CEC, nano-HPLC | Non-aqueous, aqueous | [35,36] |
| TMOS/PEG/acetic acid | β-CD | Dansyl-leu, baclofen | CEC | Hydro-organic | [37] |
| TMOS/PEG/acetic acid | Vancomycin | 7 Basic drugs, benzoin | CEC | Aqueous non-aqueous polar | [38] |
| TMOS/PEG/acetic acid | Cellulose-tris(3,5-dimethylphenylcarbamate) | 6 Neutral and basic compounds | CEC | Hydro-organic | [39] |
| MPTMS/carboxylic acids/RTIL | Template: zolimitriptan | Zolimitriptan | CEC | Hydro-organic | [41] |

[a] TMOS: Tetramethoxysilane; MTMS: methyltrimethoxysilane; MPTMS: 3-methacryloxypropyltrimethylsilane; RTIL: room temperature ionic liquid.

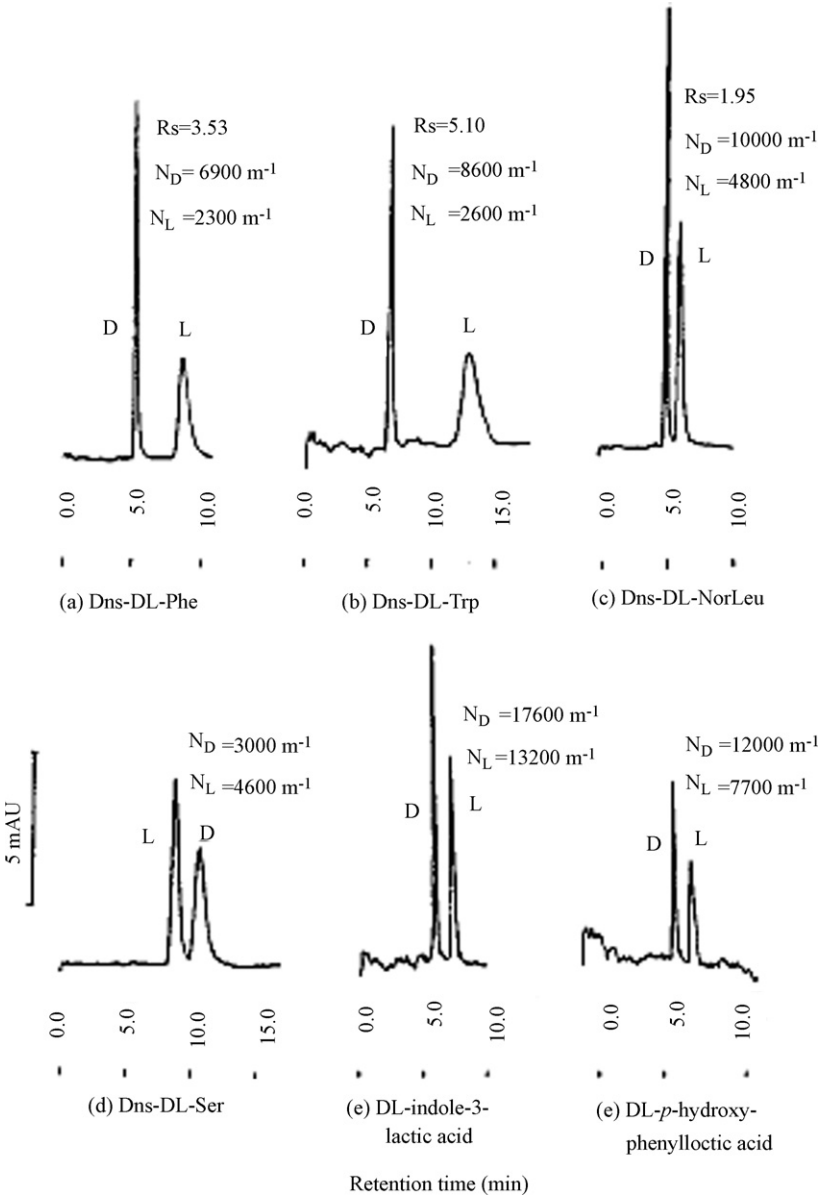



**Fig. 5.** Enantiomeric separation of several dansyl-amino acids by CEC on a chiral monolithic column. Conditions: capillary: 34 cm × 100 μm I.D.; mobile phase: acetonitrile/0.5 mM $Cu(Ac)_2$–50 mM $NH_4Ac$ (7:3), pH 6.5; −13.6 kV, 37 μA [21].

antibiotics [38] have been used (see Table 2). These chiral-modified monolithic capillaries can be used both in nano-HPLC and in CEC.

### 3.1.1. Ligand-exchange-type CSPs

The first chiral-modified silica monolith for nano-HPLC [20,21] and CEC [21,22] based on the ligand-exchange principle was described by Chen et al. These chiral-modified monolithic capillaries were successfully used for the enantiomeric separation of hydroxyl acids and dansyl-amino acids (see Fig. 5). The continuous silica skeleton, prepared inside a capillary (100 μm I.D.) was covalently modified with chiral selectors, such as L-phenylalaninamide, L-alaninamide and L-prolinamide via the bifunctional (3-glycidoxypropyl)trimethoxysilane spacer. The chiral discrimination occurs by the exchange of one ligand in the Cu(II) complex of the CSP with an analyte ligand, forming ternary-mixed copper complexes with different stabilities. Based on the thermodynamic data, the chiral recognition mechanism is mainly under enthalphic control [20]. Resolution factors $R_s$ in the range of 0.47–1.84 for nano-HPLC [20] and $R_s$ values up to 3.53 [21] for CEC could be observed. In CEC, the EOF was found to be dependent on applied electric field strength, the pH, and the composition of the mobile phase and was directed from the cathode to the anode. Scanning electron micrograph showed that monolithic capillaries have the morphology of continuous silica skeleton and large through-pore.

### 3.1.2. Oligo- and polysaccharide-based CSPs

Cyclodextrins and cellulose or amylose derivatives are suitable chiral selectors in CEC and HPLC. While cellulose and amylose derivatives are either covalently linked to the silica monolith or coated to silica, cyclodextrins were always covalently immobilized.

*3.1.2.1. Cellulose and amylose derivatives.* Polysaccharide derivatives such as tris(3,5-disubstituted phenylcarbamate) of cellulose or amylose first coated on the silica backbone and later covalently immobilized were suitable as CSPs in CEC and nano-HPLC. Chankvetadze et al. [29] prepared a cellulose tris(3,5-dimethylphenylcarbamate) (CDMPC) coated silica monolith and used it for enantioseparation in nano-HPLC. The coating method

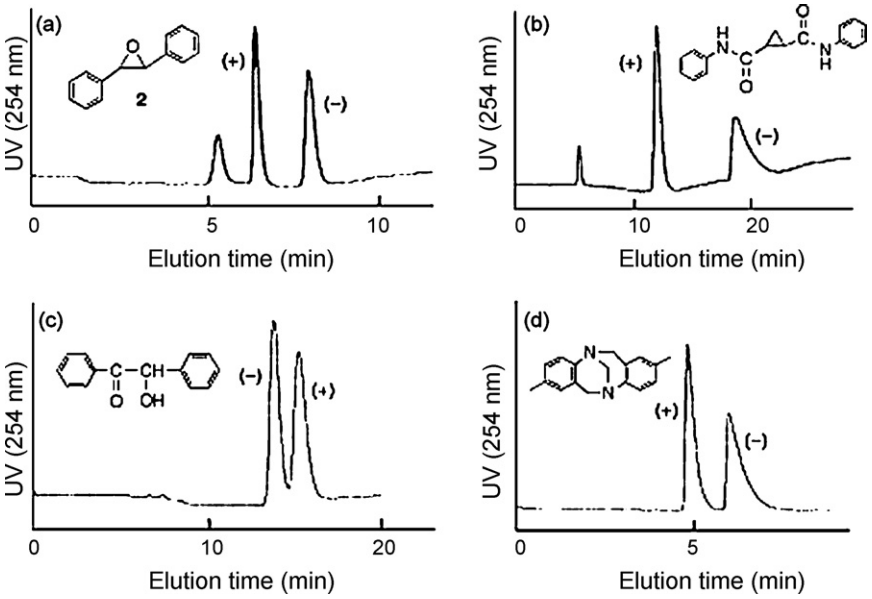



**Fig. 6.** Enantioseparation on a monolithic fused silica capillary column modified with a 50 mg/ml ADMPC solution in chloroform by nano-HPLC. Capillary: 20 cm × 100 μm I.D. Applied pressure: 0.5 MPa. Mobile Phase: *n*-hexane/2-propanol 9/1 (v/v). Detection: 254 nm [30].

appears to be fairly simple and fast. The enantiomers of several chiral test compounds were separated with high efficiencies either with less polar *n*-hexane/2-propanol mobile phase or in the reversed phase mode with water/acetonitrile. The baseline separation of the enantiomers of 2,2,2-trifluoro-1-(9-anthryl)ethanol was achieved in less than 30 s. The same group used amylose tris(3,5-dimethylphenylcarbamate) (ADMPC) instead of the cellulose analogue coated on the same silica backbone [30] for enantiomeric separation in nano-HPLC (see Fig. 6). The effect of the coating on peak performance and chromatographic parameters such as retention, resolution and separation factor was investigated. By enhancing the loading of ADMPC on the surface of monolithic silica, retention, separation factor and resolution increased significantly. But an unfavourable effect of the increased loading was a decreased efficiency. Similar effects have been observed with conventional 4.4 mm I.D. packed columns. The monolithic silica capillary was compared with a common, 4.6 mm I.D. HPLC column packed with particulate silica. It was demonstrated that owing to a lower density of ADMPC on the silica surface the retention was significantly lower using the monolithic capillary (with one exception). As a consequence a lower separation factor and a shorter analysis time were also observed for the most test analytes. In 2006, Chankvetadze et al. [31] immobilized several 3,5-disubstituted phenylcarbamate dervatives of cellulose and amylose in a two-step process onto a native silica monolith. First, 3,5-disubstituted phenylcarbamate derivatives of cellulose and amylose bearing methacryloyloxy groups were polymer-coated onto the monolith surface, second, immobilization via copolymerization with 2,3-dimethylbutadiene containing AIBN (*N*,*N*-azobisisobutyronitrile) took place. The effects of the nature of polysaccharide and the substituents, as well as the multiple covalent immobilization of the chiral selectors on the performance, were studied. Zou and co-workers [32,39] transferred both kinds of CDMPC-modified monolithic capillaries, the covalently bonded and the coated one, to CEC. Fifteen neutral and basic compounds were enantioseparated with resolution $R_s$ up to 8.67 under aqueous or non-aqueous mobile phase using the CDMPC-coated monolithic capillary. Baseline separation of the enantiomers of benzoin occurs in less than 90 s. The covalent bonding of CDMPC enabled the use of THF in mobile phase which is not possible with the physically coated CDMPC-modified monolithic capillary. However, lower resolution was obtained. The capillaries were stable under the aqueous and non-aqueous mobile phases and the column-to-column reproducibility was satisfactory.

*3.1.2.2. Cyclodextrins and cyclodextrin derivatives.* For the first time, Kang et al. [24] described a cyclodextrin-functionalized monolith for the enantiomeric separation of mephobarbital, hexobarbital, benzoin, and carprofen by CEC (see Fig. 7). The chiral monolithic stationary phase was produced in a two-step process. First, a porous monolith was prepared inside the capillary by the sol–gel technique. After gelation a hydrothermal treatment at 100 °C was performed to prevent the sol–gel matrix from cracking during the drying process. Second, Chirasil-Dex (a polydimethylsiloxane-bound permethylated β-CD) was coated and thermally immobilized to give a non-extractable CSP. A reduction in the EOF was observed caused by shielding of a part of silanol groups on the silica surface. A high column efficiency of about 92,000 plates per metre was obtained for the first eluted enantiomer of the model analyte hexobarbital. It was shown that the resolution factor $R_s$ decreased with an increase in the buffer concentration and the amount of the organic modifier methanol. Columns were stable with a good run-to-run reproducibility. Unfortunately, the column-to-column reproducibility was quite poor. Chen et al. [23] described a chiral silica monolith with β- or γ-CDs covalently linked to the silanol groups of the silica matrix via a (3-isocyanatopropyl)triethoxysilane spacer for CEC. The EOF generated by the remaining unmodified silanol groups appeared to be very weak. The enantiomeric separation of dansyl-amino acids on the γ-CD-modified monolith and that of benzoin, dansyl-aspartic acid, and dansyl-glutamic acid on the β-CD-modified monolith was demonstrated. The separation efficiency of 49,000 theoretical plates per metre for dansyl-threonine was obtained in a BGE (background electrolyte) consisting of 50 mM MES (70%) and methanol (30%). The chiral monolithic column showed a good stability over a period of more than a hundred runs. A new method for preparing a β-CD-modified monolith in a single-step sol–gel process was described by Hsieh et al. [37]. β-CD silicon alkoxides were synthesized via a SN2 reaction of 6-*O*-monotosyl-β-CD with aminopropyltriethoxysilane. Copolymerization of the CD derivatives with TMOS (tetramethoxysilane) in an acid-catalyzed sol–gel reaction, resulted in a monolithic CSP, which enables the

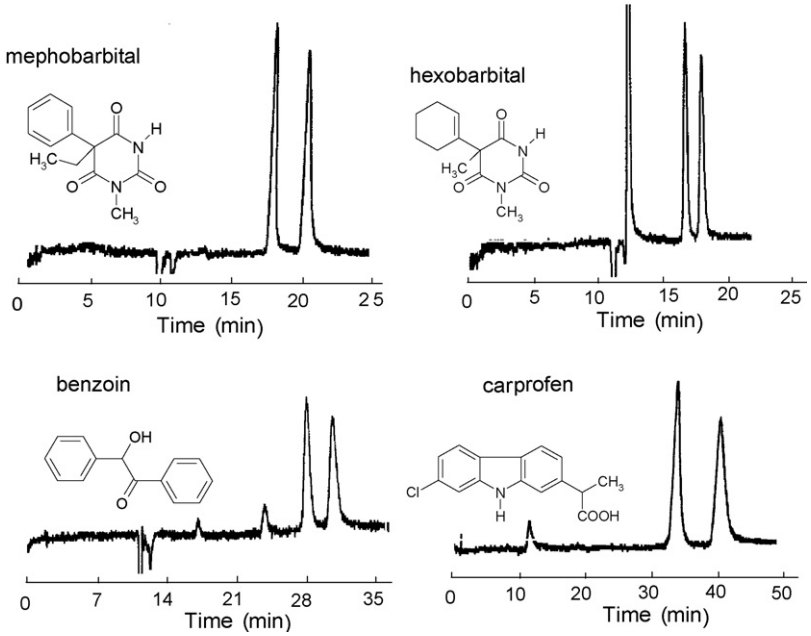



**Fig. 7.** Enantiomeric separation on a Chirasil-Dex modified silica monolithic column by CEC. Column: 25 cm × 50 μm I.D. Conditions for mephobarbital, hexobarbital and benzoin: MES–TRIS buffer (pH 6)/methanol (90/10, v/v); applied field strength: 0.4 kV/cm. Conditions for carprofen: MES–TRIS buffer (pH 6)/methanol (60/40, v/v); applied field strength: −0.4 kV/cm. UV detection: 210 nm [24].

enantioseparation of dansyl-leucine and baclofen. The fabrication of the β-CD-modified monolith could also be carried out in a one-pot reaction by integrating the two steps mentioned above. The monolithic capillaries have a good lifetime with an excellent run-to-run reproducibility of the migration time and a good day-to-day stability. But unfortunately the column-to-column reproducibility is relatively poor (RSD < 12%).

### 3.1.3. Protein- and glycoprotein-based CSPs

Liu et al. [25] reported on the fabrication of silica-based monolithic capillaries with physically adsorbed avidin as chiral selector. Enantiomeric separations of amino acid derivatives, several organic acids, menadione sodium bisulfite, warfarin and *N*-methylpseudoephedrine were achieved in both nano-HPLC and CEC mode (see Fig. 8). Theoretical plate numbers of 122,000 per metre for nano-HPLC and 242,000 per metre for CEC were observed. In the CEC mode only a very weak EOF was generated which limited the analysis of neutral enantiomers. However, the separation of acidic and basic enantiomers was suitable with resolution factors $R_S$ up to 11.59. As expected for the most investigated analytes, the resolution was higher in the CEC than in the HPLC mode. The reproducibility in relation to the retention time, retention factor and

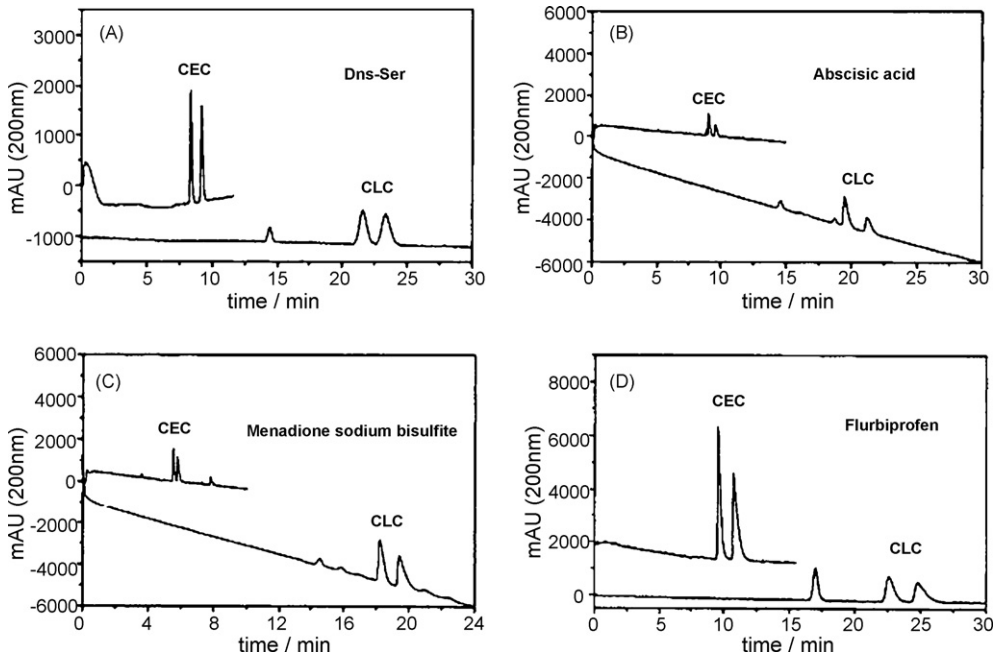



**Fig. 8.** Enantiomeric separation of acidic chiral compounds by nano-HPLC (CLC) and CEC. Conditions: column: 20 cm × 50 μm I.D. monolithic silica capillary with physically adsorbed avidin stationary phase; mobile phase: 10 mM phosphate buffer (pH 5.95) containing different concentrations (v/v) of methanol (A, B, C 15%; D 30%); pressure for nano-HPLC: 138 kPa; voltage for CEC: −15 kV [25].

separation factor was good (RSD < 1.3%) in both the electro-driven and pressure-driven systems. The RSD values for the theoretical plate number and resolution were acceptable (RSD < 6.6%). Unfortunately the columns have a very short lifetime (about two weeks) that resulted from the loss of the physically adsorbed avidin. Kato et al. [26] developed a novel sol–gel method for the preparation of protein-encapsulated monolithic columns for CEC. BSA (bovine serum albumin) or OVM (ovomucoid) were encapsulated into TMOS-based silica matrix in a single step within a capillary. Because no further thermal treatment was performed, a monolith composed of a TMOS-based hydrogel was formed without shrinking. Enantiomeric separation of tryptophan and benzoin was achieved on a BSA-encapsulated monolith and the enantiomers of eperisone, chlorpheniramine and benzoin were resolved on an OVM-encapsulated monolith. Under optimized conditions, theoretical plate number for the first eluted enantiomer of benzoin reached 72,000 plates per metre. While the run-to-run repeatability was quite satisfactory, the lifetime of the monolithic capillary was a problem due to the loss or denaturing of the proteins. In a further paper of the same group [27], BSA-encapsulated monoliths were characterized by their attenuated total reflectance-FT-IR (ATR-FT-IR). It was found that the EOF has close relationship with the contents of residual silanol groups in the hydrogel. The influence of the preparatory conditions for BSA-encapsulated columns on the enantioseparation of tryptophan was investigated. By using the natural polymers gelatine or chitosan as copolymers during the sol–gel process, a more stable BSA-encapsulated silica monolith with somewhat higher enantioselectivity was generated [28].

### 3.1.4. Ion-exchange-type CSPs

In a series of papers, Preinerstorfer et al. [33–36] reported on silica monoliths modified with anion- or cation-exchange-type chiral selector. On-column modification of a silica monolith occurred by activation with 3-mercaptopropyl trimethoxysilane and subsequent covalent bonding of vinyl group containing selectors through a radical addition reaction. By immobilization of (*S*)-*N*-(4-allyloxy-3,5-dichlorobenzoyl)-2-amino-3,3-dimethylbutane phosphonic acid [33], a strong chiral cation-exchange column for the enantiomeric separation of several chiral bases by non-aqueous CEC was obtained. Resolution factors $R_s$ up to 4.8 could be observed. For the ion-exchange process effective counter-ions like 2-aminobutanol or TEMED were needed to perform reasonably fast separation with high efficiency (30,000–90,000 theoretical plates per metre). Weak counter-ions such as Huenig base (*N*,*N*-diisopropylethylamine) showed much better enantioselectivities but long elution times. The above described monolithic column was compared to columns prepared from analogously functionalized 3.5 μm silica particles as well as to methacrylate-based organic monoliths. The particle-packed column possesses a higher enantioselectivity compared to the silica-based CSP which was mainly attributed to somewhat lower selector coverage. However, compared to the inorganic supports, the separations on the organic polymethacrylate monoliths were faster (about three times) at the expense of a slightly lower performance. An anion-exchange-type chiral monolith was obtained by coupling *O*-9-(*tert*-butylcarbamoyl)quinidine to a silica-based monolith according to above mentioned immobilization protocol [34]. With a non-aqueous CEC method the simultaneous separation of the four stereoisomers of the *N*-benzyloxycarbonyl phosphinic pseudodipeptide methyl ester Z-hPhe$\Psi$($PO_2HCH_2$)Phe-$OCH_3$ as well as the corresponding *N*-2,4-DNP-derivative with free *C*-terminal carboxylic group was suitable. It was demonstrated, that the enantioseparation in the CEC mode on monolithic CSPs is superior to the HPLC mode on particulate CSPs. By immobilization of *trans*-(1*S*,2*S*)-2-(*N*-4-allyloxy-3,5-dichlorobenzoyl)amino cyclohexanesulfonic acid a novel strong cation-exchange monolith for CEC and nano-HPLC was prepared [35]. Enantiomers of various basic pharmaceuticals (see Fig. 9) were resolved with resolution factors $R_s$ up to 9.99. The performance was investigated in both aqueous and non-aqueous mobile phase. The highest selectivity factors and resolutions were observed under aqueous conditions in both CEC and nano-HPLC. Theoretical plate heights of 3–5 μm could be obtained in CEC. The concentration of the chiral selector moieties at the monolithic stationary phase influenced the enantioselectivity and the resolution. First, an increase enhanced the enantioselectivity and the resolution, but over a certain level the parameters were diminished due to inaccessibility of the binding sites when ligands are too close to each other. The monolithic phase showed high run-to-run repeatability with RSDs far below 1%. The contributions of electrokinetic and chromatographic processes on the enantioselective separations were investigated by variation of the applied electric field strength and the pressure support and also the ionic strength of the counter-ion in the mobile phase [36]. Nonlinear electrokinetic effects could be negligible. CEC migrations could be modelled by a simple combination of individual migration contributions ($k_{CLC}$, $\mu^*_{eo}$, $\mu_{ep,eff}$). Reasonable agreement between CEC mobilities calculated from the individual increments and experimentally determined ones could be found with deviations less than 5%.

### 3.1.5. Macrocyclic antibiotic-based CSPs

To the best knowledge of the author, only one silica-based monolith modified with a macrocyclic antibiotic was described [38]. Dong et al. attached vancomycin via a 3-glycidoxypropyl trimethoxysilane spacer to the monolithic silica skeleton and resolved eight neutral and basic racemates by CEC. Non-aqueous polar organic or aqueous mobile phase were used as BGE. The effects of the polar organic mobile phase composition were studied, and it was found that the ratio of organic modifiers had a significant effect on the efficiency and resolution. Resolution factors up to 2.93 and theoretical plates per metre up to 217,000 were observed. Good run-to-run repeatabilities (RSD < 2.8%) but less satisfactory RSD values (<10.5%) for the column-to-column testing were found.

### 3.1.6. Molecularly imprinted polymer-based CSPs

Organic polymer-based molecularly imprinted polymers (MIPs) with a memory for the template are extensively applied for enantiomeric separation because of their excellent pH stability and the easy availability of the monomers. But when they were exposed to different organic solvents, shrinking or swelling deformed the MIP receptors and influenced the recognition ability [40]. Silica-based MIPs prepared by conventional hydrolytic sol–gel process often require curing and ageing at high temperature. Cracking and shrinking also became a problem. An alternative approach to fabricate the silica-based MIPs without curing and shrinking is the non-hydrolytic sol–gel (NHSG) process due to no or only little water involved. Wang et al. [41] reported on the preparation of a MIP silica-based hybrid monolith for enantiomeric resolution of a basic drug, zolimitriptan, by a room temperature ionic liquid (RTIL)-mediated NHSG methodology. RTIL was incorporated to reduce shrinking and also operated as the pore template. Three different carboxylic acids were investigated for their capability to act as both the functional monomers and the catalysts for the NHSG condensation of methacryloxypropyltrimethoxysilane (MPTMS) to form a silica-based framework. Two reactions are involved, one is the free-radical polymerization of the C=C double bonds, and the other is the NHSG condensation of the siloxanes. A baseline separation of zolimitriptan within about 15 min by CEC under hydro-organic conditions was suitable.

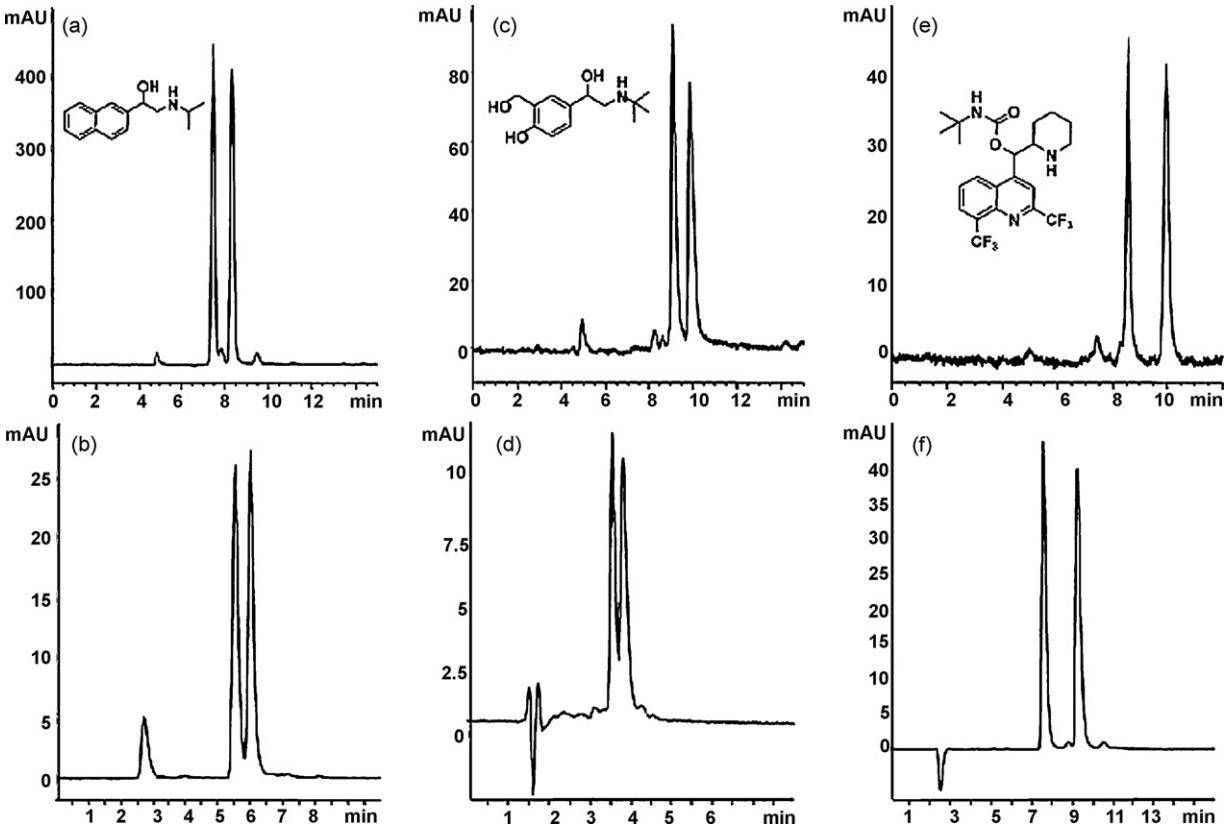



**Fig. 9.** Enantiomeric separation of (a and b) propranolol, (c and d) salbutamol, and (e and f) mefloquine-*t*-butylcarbamate by CEC (a, c and e) and nano-HPLC (b, d and f) with a SCX-modified silica monolith. Mobile phase: acetonitrile/methanol (80:20, v/v) containing 25 mM formic acid and 12.5 mM (*R*,*S*)-2-amino-1-butanol). Conditions CEC: capillary: 8.5 cm × 100 μm I.D.; applied voltage: 7 kV. Conditions nano-HPLC: capillary 33.5 cm × 100 μm I.D.; flow rate: 0.35 μl/min [35].

### 3.2. Monoliths prepared by particle-fixation technique

Two different kinds of chiral monoliths based on the particle-fixation technique have been described: (i) silica particles were fused together either by sintering at high temperature or by gluing in a sol–gel process (particle linked monolith) and (ii) modified silica particles were embedding in silica gel by a sol–gel technique (particle embedded monolith). The monoliths prepared by immobilizing of silica particles into a silica matrix by the sol–gel technique can be subdivided into two classes. Depending on the density of silica particles, monoliths with different structures were obtained. Low particle density leads to embedded silica particles in a silica network (particle embedded monolith) [43]. In the case of high particle density, silica particles were glued together and linked to the inner wall of capillaries during the sol–gel process [11,23] (particle linked monolith).

#### 3.2.1. Particle linked monolith

Wistuba and Schurig [42] prepared a robust and stable Chirasil-Dex monolith by sintering a packed bare silica bed, previously treated with sodium carbonate, at 380 °C. The silica skeleton was polymer-coated with Chirasil-Dex, a permethylated β-CD covalently linked via an octamethylene spacer to dimethylpolysiloxane, which was then thermally immobilized. Immobilization is believed to result from the presence of residual Si–H moieties, still available from the synthesis of Chirasil-Dex. The enantiomeric separation of various compounds such as barbituric acids (see Fig. 10), benzoin, methyl-α-phenylsuccinimide, MTH-proline, mecoprop methyl, fenoxaprop methyl, carprofen and ibuprofen by CEC and pressure-supported CEC was feasible. Comparison of CEC and nano-HPLC modes with a single column using a unified experimental setup showed the CEC to deliver approximately two to three times higher efficiency. In the CEC mode efficiencies up to 88,000 plates per metre could be observed. The Chirasil-Dex monolith allows application of voltage up to 30 kV and a pressure in excess of 400 bar. But the permeability and the pressure drop is similar to that of packed capillaries. After a four-month operation period of a single column, only minor shifts in elution time and loss of resolution were observed. Chirica and Remcho [44] used potassium silicate for entrapping chiral molecular imprinted polymeric (MIP) packing material for the enantiomeric separation of dansyl-phenylalanine by CEC. The enantiomeric separation of dansyl-phenylalanine occurred more rapidly and more efficiently than with the corresponding HPLC method. Enantioselective monoliths can be prepared by using chiral packing materials often used in HPLC. Kato et al. [45] described a immobilizing method for a chiral particle glued monolithic column for CEC. (*S*)-*N*-3,5-dinitrobenzoyl-1-naphthylglycine or (*S*)-*N*-3,5-dinitrophenylaminocarbonyl-valine modified silica particles were suspended in a solution consisting of TEOS (tetraethoxysilane), ethanol, and aqueous hydrochloric acid and was filled into a capillary. Heat treatment was subsequently carried out at 120 °C until the silica particles were immobilized. The enantiomeric separation of amino acids derivatized with the fluorogenic reagent 4-fluoro-7-nitro-2,1,3-benzoxadiazole were performed. A sol–gel-glued cyclodextrin-modified silica monolith was fabricated by Wistuba et al. [46] by packing first the capillary with permethyl-β-cyclodextrin-modified silica particles (Chira-Dex-silica) and subsequently fusing the packing bed by an *in situ* sol–gel process. The Chira-Dex-silica was prepared by linking permethyl-β-cyclodextrin via a mono-2-thioether spacer to bare silica particles [47]. The resulting CD monolith is stable toward voltage (30 kV) and pressure (300 bar) and possesses a high efficiency

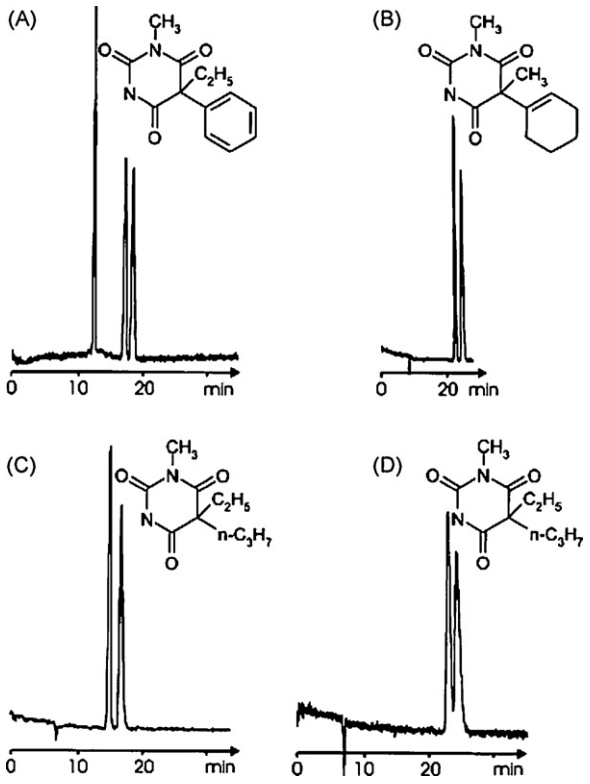



**Fig. 10.** Enantiomeric separations on barbituric acids on a sintered silica monolith modified with Chirasil-Dex by CEC. Conditions A: capillary 20 cm × 100 μm I.D., 20 kV, 12 bar, 20 mM MES, pH 6/methanol (1:1, v/v). Conditions B, C and D: capillary 20 cm × 100 μm I.D., 20 mM MES, pH 6/methanol (7:3, v/v), 20 kV, 12 bar. Detection: UV, 230 nm [42].

(up to 100,000 plates per metre). The enantioseparation of barbituric acids, thiopental, MTH-proline, methyl-α-phenylsuccinimide, chlorinated alkyl phenoxypropanoates, PCBs, carprofen and 2,2,2-trifluoro-(9-anthranyl-ethanol) was demonstrated. In the pressure-assisted CEC mode the efficiency is twice as high as in the nano-HPLC mode. The scanning electron microscope (SEM) analysis of the monolith shows, that the CD-modified silica particles are fused and not embedded in a sol–gel matrix (see Fig. 11A). A comparison with the Chirasil-Dex modified silica monolith fabricated by the sol–gel process [24] reveals a quite different structure of the silica backbone (see Fig. 11B).

### 3.2.2. *Particle embedded monolith*

This kind of monolith with a low density of silica particle immobilized in a silica matrix, as described by Dulay et al. [43], was not used for chiral analysis until now.

## 4. Comparative considerations

Columns with monolithic stationary phases display the desirable features of robustness, long-time stability, and high sample capacity. But a main problem is that the preparation of well-reproducible silica monoliths by sol–gel technology is not trivial. Minor experimental variations in the composition of the solution during the polymerization process may lead to significant differences in the morphological structure. Also cracking and shrinking represents a serious problem. The silica-based monolithic phases for CEC and nano-HPLC could be prepared *in situ* into the capillaries while the monolithic stationary phase for "conventional" HPLC have to be fabricated in a mould and subsequently clad by a polymeric material. Unfortunately, both kinds of chiral-modified monolithic columns are not commercially available. Using monolithic capillaries, the possibility of switching between the CEC and nano-LC mode operating in a single (unified) instrumental setup consists by coupling an HPLC pump to the inlet vial of the capillary electrophoresis system [Ref. [47] and literature cited therein]. When comparing these two methods employing the same column in an unified equipment, CEC shows a higher column efficiency at comparable elution times and hence better resolution factors. A benefit of monolithic columns for "conventional" HPLC, is the compatibility with the classic HPLC apparatus which is available at nearly every laboratory.

Often monolithic capillaries represent an alternative to packed capillaries for CEC separations, offering several advantages: retaining frits, the cause of many problems, are not necessary; movement of charged CSPs in an electrical field is not possible; long capillaries can be prepared without packing problems, and preparation is often easier than packing small particles into a narrow capillary. Frits are difficult to fabricate reproducibly with respect to permeability and mechanical stability. They are also fragile and often active, leading, e.g., to peak tailing for basic analytes. Irreproducible column performance and bubble formation are consequences of retaining frits. Bubble formation inside the capillary leads to a noisy baseline, an instable current, and, under unfavourable circumstances to the breakdown of the current. An advantage of packed column is the availability of a high number of chiral HPLC packing materials with different selectivities. The benefit of particle-fixed monoliths over monolithic stationary phases fabricated by a sol–gel process is the

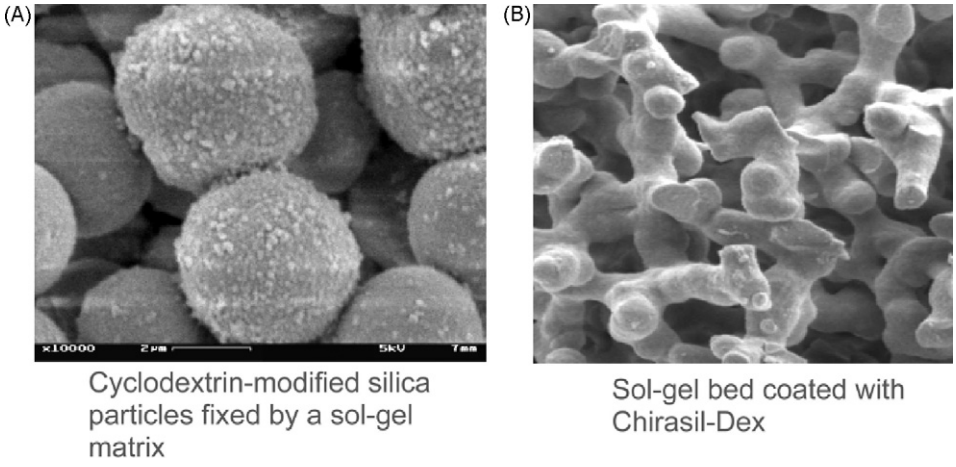



**Fig. 11.** SEM of a Chirasil-Dex-silica particle-glued monolith (A) and a sol–gel silica monolith modified with Chirasil-Dex (B) [46].

possibility to use nearly all commercially available chiral-modified silica particles used in HPLC. The most silica-based monoliths fabricated by sol–gel technology have to be post-modified by attaching the chiral selectors to the silica surface. But a drawback of the particle-loaded monolithic stationary phase is the relatively low permeability which is comparable to those of packed capillaries.

An alternative stationary phase in nano-HPLC and CEC to inorganic silica-based monoliths represents rigid organic monoliths. They were prepared *in situ* in a capillary or on a microfluidic chip by a single-step polymerization of organic polymers in the presence of a crosslinker and a porogen. To generate a stable EOF in CEC an ionizable monomer is often copolymerized. A benefit of the organic polymeric monolith is that a wide range of monomers are available for the polymerization process. However, silica-based monoliths showed porosities higher than 80%, which surpass the rigid organic monoliths and the particulate columns by 15–20%. The higher permeability compared to particle-packed columns is effected by a larger through-pore-size/skeleton size ratio [2].

Due to the discussed benefits of chiral monolithic stationary phases, a further development, leading also to a great number of commercially available chiral monolithic columns, is desirable.

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