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Review

Monoliths with chiral surface functionalization for enantioselective capillary electrochromatography

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ABSTRACT

The state-of-the-art in CEC enantiomer separations with monolithic capillary columns is comprehensively reviewed. The various types of monolithic columns comprising *in situ* organic polymer monoliths, molecularly imprinted polymer (MIP) monoliths, silica monoliths and monoliths made from particles are discussed with a focus on materials' synthesis, chemistry and properties as well as column aspects. Monolithic MIP-type porous layer open-tubular (PLOT) columns are treated herein as well. From this survey of the literature, the authors come to the conclusion that monolithic silica capillaries appear to become the preferred column type for CEC enantiomer separations of low-molecular drugs and other chiral pharmaceuticals or chemicals.

Contents

Abbreviations: AA, acrylic acid; AB, 2-aminobutanol; AAm, acrylamide; Ac, acetyl; AC-β-CD, allylcarbamoylated β-cyclodextrin; AcOH, acetic acid; AETMA, [2-(acryloyloxy)ethyl]trimethylammonium methyl sulfate; AMTEA, *N*-(2-acrylamidoethyl)triethylammonium iodide; AGE, allyl glycidyl ether; AIBN, α,α′-azobis(isobutyronitrile),2,2′-azobis(2-methylpropionitrile); AlaA, alanine amide; allyl-β-CD, 2-hydroxy-3-allyloxy-propyl-β-cyclodextrin; AMPS, 2-acrylamido-2-methyl-1-propane sulfonic acid; APS, ammonium persulfate; APVE, 3-amino-1-propanol vinylether; BA, butyl acrylate; BDDA, 1,3-butanediol diacrylate; BIS, *N*,*N*′-methylenebisacrylamide; BMA, butyl methacrylate; BSA, bovine serum albumin; Bz, benzoyl; CC, carbazole-9-yl-carbonyl; CD, cyclodextrin; CDMPC, cellulose tris(3,5-dimethylphenylcarbamate); CE, capillary electrophoresis; CEC, capillary electrochromatography; CGE, capillary gel electrophoresis; CLC, capillary liquid chromatography; CM-β-CD, carboxymethyl-β-CD; CSP, chiral stationary phase; CTAB, cetyl trimethylammonium bromide; DATD, diallyltartardiamide; DBD, 7-dimethylaminosulfonyl-1,3,2-benzoxadiazol-4-yl; DEA, diethylamine; DMAA, dimethylacrylamide; DMAEMA, 2-dimethylaminoethyl methacrylate; DMDAAC, dimethyldiallylammonium chloride; DMN, dimethyl naphthalenedicarboxylate; DNB, 3,5-dinitrobenzoyl; DNP, 2,4-dinitrophenyl; DNS, dansyl (5-dimethylaminonaphthalinsulfonyl); DNS-AAs, *N*-dansyl-amino acids; DNZ, 3,5-dinitrobenzyloxycarbonyl; DOPA, dihydroxyphenylalanine; DPDS, di-2-pyridyldisulfide; DVB, divinylbenzene; EDMA, ethylene dimethacrylate; EGDMA, ethylene glycol dimethylacrylate; EOF, electroosmotic flow; ESI-MS, electrospray ionization-mass spectrometry; ETMOS, ethyltrimethoxysilane; FA, formamide; FMOC, 9-fluorenylmethoxycarbonyl; GMA, glycidyl methacrylate; HB, Huenig base (*N*,*N*-diisopropylethylamine); HEMA, 2-hydroxyethyl methacrylate; HMAA, N-(hydroxymethyl)acrylamide; HP, Hydroxypropyl; hPhe, homophenylalanine; HSA, human serum albumin; Hyp, hydroxyproline; i.d., inner diameter; IPA, *N*-isopropylacrylamide; ISEC, inverse size exclusion chromatography; LA, lauryl acrylate; LE-CEC, ligand-exchange CEC; LIF, laser-induced fluorescence detection; LMA, lauryl methacrylate; LOD, limit of detection; MAA, methacrylic acid; MAAm, methacrylamide; γ-MAPS, 3-(methacryloyloxy)propyl trimethoxysilane; MBA, *N*,*N*′-methylene-bisacrylamide; MDA, mandelic acid; MEAMS, 2-(methacryloyloxy)ethyl trimethylammonium methyl sulfate; MEKC, micellar electrokinteic chromatography; MES, 2-(1-morpholino)ethanesulfonic acid; MIP, molecularly imprinted polymer; MMA, methyl methacrylate; MTH, methylthiohydantoin; MTMS, methyl trimethoxysilane; NAS, *N*-acryloyloxysuccinimide; NBD-F, 4-fluoro-7-nitro-2,1,3-benzoxadiazole; NBE, norbornene; NDA, naphthalene-2,3-dicarboxaldehyde; NH_4OAc, ammonium acetate; NHSG, nonhydrolytic sol–gel (process); NEt_3, triethylamine; NIPAAm, *N*-isopropylacrylamide; NMF, *N*-methylformamide; ODS, octadecylsilica; PDA, piperazine diacrylamide; PEG, poly(ethylene glycol); PEO, poly(ethylene oxide); PheA, phenylalanine amide; PLOT, porous-layer open-tubular (column); ProA, proline amide; PTH, phenylthiohydantoin; ROMP, ring-opening metathesis polymerization; RTIL, room-temperature ionic liquid; S, styrene; SCX, strong cation-exchange; SEM, scanning electron microscopy; SDS, sodium dodecyl sulfate; SSA, styrenesulfonic acid; TB, Tröger base; tBuCQD, *O*-9-(*tert*-butylcarbamoyl)quinidine; tBuCQN, *O*-9-(*tert*-butylcarbamoyl)quinine; TEA, triethylamine; TEAA, triethylammonium acetate; TEMED, *N*,*N*,*N*′,*N*′-tetramethyl ethylenediamine; TEOS, tetraethoxy silane (tetraethyl orthosilicate); TFA, trifluoroacetic acid; TFMAA, trifluoromethacrylic acid; THP, tetrahydropalmitine; TMOS, tetramethoxysilane; TRIM, trimethylolpropane trimethacrylate; VBC, vinylbenzyl chloride; 4-VPY, 4-vinylpyridine; VSA, vinylsulfonic acid; WAX, weak anion-exchange; Z, benzyloxycarbonyl (carbobenzoxy).

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1. Introduction

In the past decade, monolithic separation media [1] have become a preferred column packing material and morphology, respectively, for microscale separation techniques in capillaries [2–5]. Advantages such as ease of *in situ* preparation in capillaries or narrow channels of microfluidic devices [4] from reaction solutions of monomers or reactive precursors make them tentatively to ideal stationary phases for microscale separation formats. Functionalities for the respective chromatographic mode can be incorporated *in situ* via functional monomers or post-modification of the surface of monolithic materials. Other advantages for CEC application comprise the avoidance of retaining frits which are required to keep microparticulate packing materials in the capillary in case of packed columns. As a consequence much better column robustness and extended column longevity are the result which is of particular relevance for validated CEC assays that are employed to solve real world applications. Compared to open-tubular columns monoliths greatly benefit from a larger adsorption surface which makes them less prone to overloading effects. The general benefits of monolithic columns in comparison to packed and open-tubular columns have been frequently discussed in more detail elsewhere [6].

Different types of monolithic materials have been developed, which partly evolved from less successful pioneering studies of others some time earlier [1,7]. Amongst them, organic polymer monoliths emerged in the late 1980s in form of highly swollen crosslinked polyacrylamde gels, first suggested by Hjertén [8], and rigid crosslinked polymethacrylate copolymers, as proposed by Tennikova et al. [9]. Other type of organic polymer monoliths appeared later such as polystyrene monoliths, monoliths prepared by ring-opening metathesis polymerisation (ROMP), monoliths from polycondensation, etc., which have been described in detail by a recent review of Svec [7]. In the literature, polyacrylamide-type monolithic stationary phases can sometimes be found as continuous beds or gels [10–13]. Silica monoliths based on sol–gel technology have been developed by Nakanishi and Tanaka in the mid 1990s [14]. Both concepts have been later adopted for capillary columns and enantioselective (electro)chromatography. Besides, monoliths made from particles [15] as well as some column formats based on monolithic materials such as in monolithic PLOT columns will be mentioned as well. This review intends to briefly summarize the developments in the field of chiral monoliths with particular focus on their application for enantioselective CEC [16,17]. It follows earlier reviews on this topic, e.g. [18–23].

2. Chiral organic polymer monoliths

2.1. General remarks

Organic polymer monoliths historically evolved from polymeric gel materials such as employed in gel electrophoresis and later adopted for capillary gel electrophoresis. Early developments of stereoselective capillary electrophoresis (CE) with gel-filled columns or completely homogeneous separation media with chiral selectors like cyclodextrins [24,25] or bovine serum albumin [10,26] physically or chemically incorporated in slightly crosslinked neutral gel matrixes may be regarded as pioneering works that paved the way for enantioselective CEC with chiral monolithic separation media [18–21]. Due to lack of ionic functionalities of the gel matrix the separations were slow in these studies, because no EOF supported the transport of the ionic analytes through the gel columns and the permeability of the swollen gels was low. This was later overcome by incorporation of ionizable functionalities into the polymer and stronger crosslinking leading eventually to the typical organic polymer monoliths employed nowadays for CEC.

Organic polymer monoliths are most often prepared by free radical polymerization processes from monomers such as (metha)acrylamides, (meth)acrylates or styrene, by ring-opening metathesis polymerisation (ROMP), or by polycondensation processes [1,7]. For enantiomer separation, styrenic matrices seem to be less suitable. A single paper reported on polystyrene-based chiral monoliths in HPLC [27] and no reports on enantioselective polystyrene-based monolithic materials for CEC applications can be found in the literature. The hydrophobic aromatic matrix appears to be prone for non-specific interactions (hydrophobic interactions, π–π-interactions) which are detrimental for enantioselectivity, as explained recently [28,29]. ROMP-based organic polymers have been prepared for HPLC, but not for CEC enantioseparation [30–32]. Hence, the majority of studies were focusing on enantioselective poly(meth)acrylamide and poly(meth)acrylate monoliths (*vide infra*).

In general, such monoliths are prepared from a homogenous polymerization mixture containing besides the (functional) monomer(s) a suitable crosslinker, a polymerisation initiator and porogens. The functional monomer bears pendant chiral moieties (i.e. the active chiral recognition sites) in case of the *in situ* approach or reactive functionalities for subsequent derivatization of the synthesized monolith with a chiral selector. Changes in the properties of the porogenic solvent as well as modifications of the synthesis conditions allow the tailoring of the morphology, porosity and pore

Table 1
Enantioselective CEC with *in situ* prepared organic polymer monoliths.

Analytes	Chiral selector or monomer (template)	(Co)monomers	Solvent (porogens)	Mobile phase (applied voltage)	Maximum efficiency (theor.pl/m)	Ref.
Polymethacrylate monoliths						
DNB-Leu-diallylamide	2-Hydroxyethyl methacrylate (*N*-L-Val-3,5-dimethylanilide) (19.7%, w/w)	GMA (40%), EDMA (40%), AMPS (0.3%) (w/w)	70% (w/w) of reaction mixture; 1-propanol (75%), 1,4-butanediol (15%), water (10%)	ACN–5 mM phosphate buffer (pH 7) (80:20, v/v)	61,000	[46]
N-derivatized amino acids (DNB, DNZ, DNP, FMOC, Z, Bz, Ac), mecoprop, fenoprop	[O-2-(methacryloyloxy) ethylcarbamoyl]-10,11-dihydroquinidine (20%, w/w)	HEMA (60–70%), EDMA (10–20%) (w/w)	60% (w/w) of reaction mixture; cyclohexanol, 1-dodecanol (various ratios)	0.4 mol/L AcOH + 4 mmol/L NEt_3 in ACN–MeOH (80/20, v/v)	240,000	[36,37,39,52]
DNB-Leu, DNZ-Leu, FMOC-Leu, FMOC-Ser, CC-Ser, DNS-Ser, DBD-Ser	*O*-9-(*tert*-butylcarbamoyl)-11-[(2-methacryloyloxy)ethylthio]-10,11-dihydroquinine (10 or 20%, w/w)	HEMA (60–80%), EDMA (10–20%)	60% (w/w) of reaction mixture; cyclohexanol, 1-dodecanol (various ratios)	0.4 mol/L AcOH + 4 mmol/L NEt_3 in ACN–MeOH (80/20, v/v)	200,000	[38]
Polyacrylamide gels and monoliths						
Benzoin, terbutaline, chlorpheniramine, 1,2-diphenylethanol, propranolol	Poly-β-CD (50 mg/mL) and CM-β-CD (50 mg/mL) (physically incorporated)	8% T (AAm, BIS, AMPS), 5% C, 5.5% S (see Ëfinitions and Symbols¨)	0.1 mol/L Tris–0.15 mol/L boric acid (pH 8.1), 5% (v/v) Tween 20	0.2 mol/L Tris–0.3 mol/L boric acid (pH 9), 5% (v/v) Tween 20; 20 kV (12 μA)	127,000	[47,53]
DNS-amino acids, phenylmercapturic acid, warfarin	Poly-β-CD (80–120 mg/mL) (physically incorporated)	8.6% T (AAm, BIS, AMTEA), 4.7% C, 5.6% S	0.1 mol/L Tris–0.15 mol/L boric acid (pH 8.1)	0.2 mol/L Tris–0.3 mol/L boric acid (pH 8.1)	240,000	[53]
Propranolol, terbutaline, benzoin	AC-β-CD (50 mg/mL)	5% T (AAm, BIS, AMPS), 10% C, 5.6% S	0.1 mol/L Tris–0.15 mol/L boric acid (pH 8.1)	0.2 mol/L Tris–0.3 mol/L boric acid (pH 9)	84,000	[48]
Terbutaline, metaproterenol, isoproterenol, propranolol, pindolol, chlorpheniramine, TrpOMe, TrpOEt, α-methyltryptamine, clenbuterol; 1-(1-naphthyl)ethanol, methyl mandelate	AC-β-CD (50–100 mg/mL)	5 or 7% T (AAm, BIS, AMPS), 5 or 10% C, 5.5, 5.6 or 6% S	0.1 mol/L Tris–0.15 mol/L boric acid (pH 8.1)	0.2 mol/L Tris–0.3 mol/L boric acid (pH 7 or 9)	144,000	[54]
1-Aminoindan, 1,2,3,4-tetrahydro-1-naphthylamine, 1-(1-naphthyl)ethylamine, primaquine	AC-β-CD (50 mg/mL)	5% T (AAm, BIS, AMPS), 5% C, 5.5% S	0.1 mol/L Tris–0.15 mol/L boric acid (pH 8.1)	0.2 mol/L Tris–0.3 mol/L boric acid (pH 7) + 5–10 mmol/L 18-crown-6	150,000	[54]
DNS-amino acids, phenylmercapturic acid, warfarin, 2-phenoxypropionic acid, FMOC-Val, benzoin, 1-(1-naphthyl)ethanol	AC-β-CD (10–50 mg/mL)	5.4% T (AAm, BIS, AMTEA), 9.3% C, 5.6% S	0.1 mol/L Tris–0.15 mol/L boric acid (pH 8.1)	0.2 mol/L Tris–0.3 mol/L boric acid (pH 8.1)	150,000	[55]
Hexobarbital, mephobarbital, warfarin, tropicamide, ibuprofen, propranolol, mephenytoin, hydrobenzoin	Allyl-β-CD (37.5–150 μmol/mL)	5% T (AAm, BIS, AMPS), 3% C, 17% S	0.1 mol/L Tris–0.15 mol/L boric acid (pH 8.2)	0.1 mol/L Tris-0.15 mol/L boric acid (pH 8.2)	570,000	[12]
Ibuprofen, warfarin, mephobarbital	Allyl-β-CD (50 μmol/mL)	5% T (AAm, BIS, DMDAAC), 3% C, 17% S	0.1 mol/L Tris–0.15 mol/L boric acid (pH 8.2)	0.1 mol/L Tris–0.15 mol/L boric acid (pH 8.2)	65,000	[12]
Ibuprofen, metoprolol	Polyrotaxane-based polyacrylamide continuous bed formed from anionic and cationic β-cyclodextrin derivatives	21.4% T (MAAm, PDA, IPA), 41.7% C	$(NH_4)_2SO_4$ in 50 mM phosphate buffer (pH 7)	50% Aqueous MeOH buffered with AcOH-TEMED (0.05% each)		[56,57]
1-Aminoindan, 1-(1-naphthyl)ethylamine, 1-phenylethylamine, alanine-2-naphthylamide, α-methyltryptamine, 1,2-diphenylethylamine, tryptophanol, 2-amino-1,2-diphenylethanol, TrpOMe	Mono or tetraallyl 18-crown-6-tetracarboxylate (20–40 mM)	5% T (AAm, BIS, AMPS), 5% C, 5.5% S	0.1 mol/L Tris–0.15 mol/L borate (pH 8.1)	0.2 mol/L triethanolamine–0.3 mol/L boric acid (pH 6 or 7) with 0–50% ACN (v/v)	135,000	[58]

Table 1 (*Continued*)

Analytes	Chiral selector or monomer (template)	(Co)monomers	Solvent (porogens)	Mobile phase (applied voltage)	Maximum efficiency (theor.pl/m)	Ref.
Amino acids (Asn, DOPA, α-methyl-DOPA, α-methyl-Phe, Tyr, Phe, Thr, Trp, Ser)	*N*-(2-hydroxy-3-allyloxypropyl)-L-4-Hyp (10–20%) (25 μL)	PDA (22 mg), MAAm (18 mg), VSA (30%) (3 μL)	10 mg $(NH_4)_2SO_4$ in 175 μl 50 mM phosphate buffer (pH 7–8)	50 mM NaH_2PO_4/0.1 mM Cu(II) pH 4.6; 7 kV; 12 bar on inlet		[11]
Hydroxy acids like mandelic acid (MDA), 3-hydroxy-MDA, 4-hydroxy-MDA, 4-bromo-MDA, 4-methoxy-MDA, 3,4-dihydroxy-MDA, 3-hydroxy-4-methoxy-MDA, 4-hydroxy-3-methoxy-MDA, atrolactic acid, 3-(4-hydroxyphenyl)lactic acid, 3-phenyllactic acid	*N*-(2-hydroxy-3-allyloxypropyl)-L-4-Hyp (10–20%) (25 μL)	PDA (22 mg), MAAm (18 mg)	10 mg $(NH_4)_2SO_4$ in 175 μl 50 mM phosphate buffer (pH 8)	20 mM NaH_2PO_4/0.1 mM Cu(II) pH 4.5; −7 to −15 kV		[35]
Hydroxy monocarboxylic acids and hydroxy dicarboxylic acids	*N*-(2-hydroxy-3-allyloxypropyl)-L-4-Hyp (10–20%) (25 μL)	PDA (22 mg), MAAm (18 mg), DMDAAC	10 mg $(NH_4)_2SO_4$ 175 μl 50 mM phosphate buffer (pH 8)	50 mM CH_3COONH_4; 0.1–5 mM $Cu(CH_3COO)_2$ pH 4.4; −5 kV		[59]
Tryptophan and kynurenine	Allyl-activated HSA in methacrylamide–piperazine diacrylamide polymer matrix	PDA (11.0 mg), MAA (9.0 mg)	APS (5 μL, 5%, w/v) and TEMED (5 μL, 5%, v/v)	20 mM sodium phosphate pH 7.2	110,000	[60]

size distributions, and therefore of the flow properties of the monolith which depend mainly on the size distribution of the macropores (typically diameters in the range between 0.5 and 1.5 μm).

The chosen mold (usually a fused-silica capillary or channels of microfluidic devices [33,34]) is filled with the reaction mixture by a syringe. The copolymerization of the vinyl monomers can then be triggered by either one of different initiation processes (i.e. redox catalyst, thermal, UV light, γ-ray or e-beam radiation initiated polymerization) [7] and leads finally to a macroporous microglobular monolithic polymer matrix with a more or less unimodal pore distribution. Poly(meth)acrylamide monoliths are commonly synthesized by redox-initiation with ammonium persulfate (APS)/*N*,*N*,*N*′,*N*′-tetramethyl ethylenediamine (TEMED) [11,12,35]. Thermally initiated polymerization with α,α′-azobis(isobutyronitrile) (AIBN) as radical initiator has been the standard method for chiral polymethacrylate monoliths and only in a few studies a UV initiation protocol has been implemented [36–39]. So far, γ-ray and e-beam radiation initiated polymerization have not been utilized for preparation of enantioselective capillary columns.

The use of silica capillaries with vinylized inner wall, obtained usually by using 3-(methacryloyloxy)propyl trimethoxysilane (γ-MAPS) as vinylization reagent, allows the crosslinking of the polymer with the silica wall leading to monolithic capillary columns that have better mechanical properties and improved robustness. The microglobular polymer matrix is formed by a nucleation and growth mechanism as outlined in Ref. [40]. Briefly, polymer nuclei are initially formed which are highly swollen with monomers because the monomers are thermodynamically better solvents than the porogens. Thus polymerization occurs preferentially in the already existing polymer nuclei leading to their growth rather than formation of new polymer nuclei. At a certain stage they become insoluble in the polymerization mixture leading to phase separation and the typical macroporous microglobular structure. At the end of the polymerization the porogenic solvent fills completely the voids between the polymer skeleton and thus the pores. The onset of phase separation has a decisive role for the macropore size distribution and can be, amongst others, governed by the type of porogens and the composition of binary or ternary porogenic mixtures. The total porosity and the phase ratio, respectively, of the resultant monolithic polymer can be adjusted by the monomer-to-porogen ratio in the reaction mixture. Typically employed monomer-to-porogen ratios are 40:60 (w/w) or 30:70 (w/w) for rigid polymethacrylate type monoliths. However, the total monomer content may be as low as 5–20% for polyacrylamide gels.

Three basically distinct approaches can be adopted for the introduction and creation of active chiral recognition sites:

(i) incorporation of chiral moieties into the polymer matrix employing chiral functional monomers,
(ii) molecular imprinting with a single enantiomeric chiral template leading to chiral cavities, and
(iii) *post*-polymerization modification (post-modification) in which chiral functionalities are introduced by derivatization of the achiral (reactive) monolith matrix.

Both first and second approaches represent single-step *in situ* fabrication protocols which are straightforward from a technical point of view. In the first approach, the *in situ* incorporation of a selector, the chiral recognition sites are introduced directly by copolymerization of a functional monomer bearing chiral moieties. Papers that have been published employing this approach are summarized in Table 1. However, *in situ* incorporation of active chiral recognition sites may also be accomplished by the creation of chiral cavities through a molecular imprinting pro-

Table 2
Enantioselective CEC with monolithic capillaries prepared by MIP technologies.

Analytes	Chiral template	Monomers	Solvent (porogens)	Mobile phase (applied voltage)	Maximum efficiency (theor.pl/m)	Ref.
MIP-type polymethacrylate monoliths						
Propranolol	(R)-propranolol (0.03 M)	MAA (0.24 M), TRIM (0.24 M)	Toluene	ACN–4 mol/L NH_4OAc (pH 3) (80/20, v/v)		[51]
Metoprolol	(S)-metoprolol (0.03 M)	MAA (0.24 M), TRIM (0.24 M)	Toluene	ACN–2 mol/L NH_4OAc (pH 3) (80/20, v/v)		[51]
Ropivacaine, mepivacaine, bupivacaine	(S)-ropivacaine (0.02–0.24 M)	MAA (0.24–0.48 M), TRIM (0.24–0.48 M)	Isooctane (1–25%) in toluene	ACN–25 mM phosphate or citrate buffer (pH 2–6.5) (80/20, v/v)		[50]
β-Blockers (propranolol, pindolol, prenalterol, atenolol)	Various	various		Various		[71–73]
Amino acids (Phe, Tyr, Phg)	(S)-Phe-anilide (0.16 M)	MAA (0.35 M), 2-VPY (0.35 M), EDMA (3.3 M)	NH_4OAc (0.1 M)–$CHCl_3$	ACN–CH_3COOH–water (80:10:10, v/v/v); T = 60 °C; 400 V/cm		[65,66]
1,1′-Bi-2-2′-naphtol	(*R*)-1,1′-bi-2-2′-naphtol	MAA, EDMA	Toluene, toluene/isooctane	ACN (60–80%, v/v)–10–50 mM acetate buffer; 10 kV	40,000	[67]
Naproxen	(*S*)-naproxen	MAA, EDMA	Toluene/isooctane	ACN (60–90%) acetate (10–50 mM, pH range 2–5) T = 25–35 °C, surfactants; 15 kV		[68]
Ibuprofen	(*S*)-ibuprofen	4-VPY, EDMA, γMAPS	Toluene/isooctane	ACN (40–80%, v/v)–acetate buffer (diff concentrations) (pH 2.5–5)	128,700	[69]
Tröger's base, tetrahydropalmatine	(5*S*,11*S*)-(−)-Tröger's base, L-tetrahydropalmatine	MAA, EDMA	Toluene/dodecanol	ACN–acetate buffer (different concentrations)		[74]
Ibuprofen	(S)-ibuprofen	4-VPY	Toluene/isooctane	25 mmol L-1 NH_4Ac; 25 mmol L-1; TEA–acetate buffer; 25 mmol L-1 NH_4CO_3; and also diff %ACN in 12.5 mmol L-1 TEA–phosphate buffer pH 3.0.	30,000	[75]
Tyrosine and its amino acid derivs.	L-Tyrosin	MAA, 4-VPY and ethylene glycol dimethacrylate	Toluene/dodecanol	ACN buffer (80/20, v/v) (pH 3–6); ACN (60–90%, v/v)–acetate buffer 50 mM (pH 4.0)		[76]

cess, the second approach, in which achiral functional monomers are assembled around an enantiomeric template molecule during polymerization driven by strong non-covalent binding forces. The result is a molecularly imprinted polymer with cavities providing a three-dimensional receptorial structure that is capable for enantioselective rebinding of the template enantiomers and/or of structurally closely related molecules. A summary of MIP-type enantioselective monoliths and their CEC application can be found in Table 2. The third approach, post-modification, adds to the flexibility because it allows the introduction of chiral recognition sites directly on the surface of the monolith subsequent to its *in situ* preparation. Typically, the monolithic support formed in the first step carries reactive moieties that can be subsequently derivatized with chiral functionalities in a second step. An increasing number of reports employing this concept can now be found in the literature and an overview is given in Table 3.

For effective CEC application of chiral monolithic capillary columns a strong and stable electroosmotic flow (EOF) over a wide pH range is desirable or required. The controlled incorporation of ionizable comonomers allows the adjustment of the ζ-potential of the stationary phase surface and thus the tailoring of the EOF in terms of its direction and strength. For the mere purpose of EOF generation it is often sufficient if such ionizable monomers are introduced in a low concentration (e.g. often less than 1% related to total monomers). The addition of a low percentage of weakly acidic (like acrylic acid) or strongly acidic monomers (like vinylsulfonic acid, VSA, or 2-acrylamido-2-methyl-1-propanesulfonic acid, AMPS) leads to columns affording cathodic flow (EOF directed from anode to cathode). Anodic EOF is instead obtained through copolymerization of a positively ionizable monomer such as [2-(acryloyloxy)ethyl]trimethylammonium methyl sulfate (AETMA). In both of the cases permanently charged comonomers are usually preferred, since they maintain a strong EOF over the entire pH range. The suitable ionizable comonomer has to be selected in view of the given separation problem, in case of ionic analytes occasionally depending on their electrophoretic migration direction. AMPS copolymerized at a level as low as 0.3% in polymethacrylate monoliths afforded strong cathodic EOF, which increased with its content in the polymerization mixture until it reached a saturation level at about 1.8% AMPS [41]. Similar trends were found for polyacrylamide gel columns. EOF increased in the range of about 1–15% AMPS or VSA of the total monomers which then leveled off [42,43]. The higher rigidity of the polymethacrylate columns as compared to soft polyacrylamide gels appears to be favorable in terms of EOF velocities that can be achieved. This result is probably to be ascribed to the different polymer morphologies and in particular pore structures of these two types of materials. Independent studies with monolithic materials were made in this direction and as a result it was found that the pore size of polymethacrylate monoliths significantly affects the flow [37,41,44]. The linear EOF velocity was directly proportional to the size of macropores under otherwise identical conditions and polymer compositions, respectively. Varying the pore size by modifying the porogenic solvent type and ratio has lead to differences in tortuosity of the structure

Table 3
Enantioselective CEC with organic polymer monoliths prepared by post-modification of monolithic beds.

Analytes	Chiral selector (CS), activation and coupling of CS	(Co)monomers	Solvent (porogens)	Mobile phase (applied voltage)	Maximum efficiency (theor.pl/m)	Ref.
Polyacrylamide matrix						
Thalidomide, warfarin, coumachlor, felodipine	Vancomycin; immobilized on hydrolyzed epoxy-polymer after oxidation to aldehyde via reductive amination	HMAA (0.16) (g/mL), PDA (0.08), AGE (0.08), VSA (30%) (40 μL)	$(NH_4)_2SO_4$ in 50 mM phosphate buffer (pH 7)	ACN–triethylammonium acetate (pH 5) (15/85, v/v)	120,000	[13]
Thalidomide, bupivacaine, warfarin ketoprofen, felodipine, atenolol	Vancomycin; *N*,*N*′-DATD monomer with diol functionality, it can be readily converted to aldehyde groups via periodate treatment	HMAA/DATD/PDA/VSA	1 mL of 50 mM Sodium phosphate buffer (pH 7.0) +40 μL of 10% aqueou TEMED and 40 μL of 10% aqueous APS	Various	120,000	[84]
Warfarin, tetrahydropalmatine, Troeger's base, 1,1′-bi-2-naphthol, benzoin, pindolol, indapamide, praziquantel	Cellulose tris(3,5-dimethylphenyl-carbamate) (CDMPC); coated onto poly(acrylamide-co-*N*,*N*′-methylene-bisacrylamide) monolith	MBA (40 mg), AA (20 mg), MEAMS (40 μL), AIBN (1 mg, 1 %, w/w, with respect to the monomers)	1-Dodecanol (200 μL) and DMSO (280 μL)	40% ACN 4 mM TEA–phosphate buffer pH 6.8; 40% ACN, 4 mM phosphate buffer pH 6.8	83,000	[82]
Poly(meth)acrylate matrix						
(*R*,*S*)-DNB-Leu	*O*-9-(*tert*-butylcarbamoyl)-quinine; epoxy monolith activated with hydrogen sulfide followed by radical addition reaction (thiol click)	Polymethacrylate (GMA/EDMA)	30% (w/w) Cyclohexanol and 30% (w/w) 1-dodecanol	ACN–MeOH (80:20, v/v), 400 mM CH_3COOH, 4 mM TEA		[85]
Mefloquine, mefloquine-*tert*-butylcarbamate	(*S*)-*N*-(4-allyloxy-3,5-dichlorobenzoyl)-2-amino-3,3-dimethylbutanephosphonic acid; epoxy monolith activated with hydrogen sulfide followed by radical addition reaction (thiol click)	Polymethacrylate (GMA/EDMA or GMA/HEMA/EDMA)	Cyclohexanol and 1-dodecanol (diff %)	Aqueous: MeOH–H_2O or ACN–H_2O (80:20, v/v), nonaqueous: MeOH–ACN (80:20 or 20:80, v/v)	47,500	[86]
2-Aryloxypropionic acids	(+)-1-(4-Aminobutyl)-(5*R*,8*S*,10*R*)-terguride (ergot alkaloid)	Polymethacrylate (GMA/MMA/EDMA)	Formamide and 1-propanol;	5 mM TEA–acetic acid (molar ratio: 0.024) in acetonitrile–methanol (9:1, v/v)		[87,88]
Ibuprofen, naproxen	β-Cyclodextrin deriv. bearing 4-dimethylamino-1,8-naphthalimide	Poly(GMA-co-EDMA) monolith	35% (v/v) 1-Propanol, 20% (v/v) 1,4-butanediol and 5% (v/v) H2O	ACN–MeOH (90–10 to 50–50, v/v), CH_3COOH/TEA (70:1) 50–350 mM	86,000	[89]
Phenylalanine, tyrosine, tryptophan, alanine, lysine, histidine, arginine, mexiletine hydrochloride, oxybutynin chloride	β-CD, Asp-β-CD, NH_2-β-CD, HP-β-CD	Poly(GMA-co-EDMA) monolith	Cyclohexanol (0.405 g) and 1-dodecanol (0.045 g)	5 mM Phosphate buffer or acetate buffer (pH 3.5–8.5)		[90]
Flavanone	β-Cyclodextrin (CD) via a triazole ring utilizing the copper(I)-catalyzed 1,3-dipolar cyclo-addn. reaction; propargylamine-modification followed by click chemistry with azido-modified CD	Poly(NAS-co-EDMA)	Toluene	MeOH–5 mM borate buffer (40:60, v/v)		[91]

of the monolithic support which may cause variations in conductivity and effective field strength in the packed structure. The sum of these effects translates into changes in flow velocities between different polymer morphologies [45].

Another point deserves attention. Achiral ionic moieties of comonomers, introduced for sake of EOF generation, may represent strong non-specific adsorption sites, where binding may occur non-enantioselectively for counterionic analytes. This leads

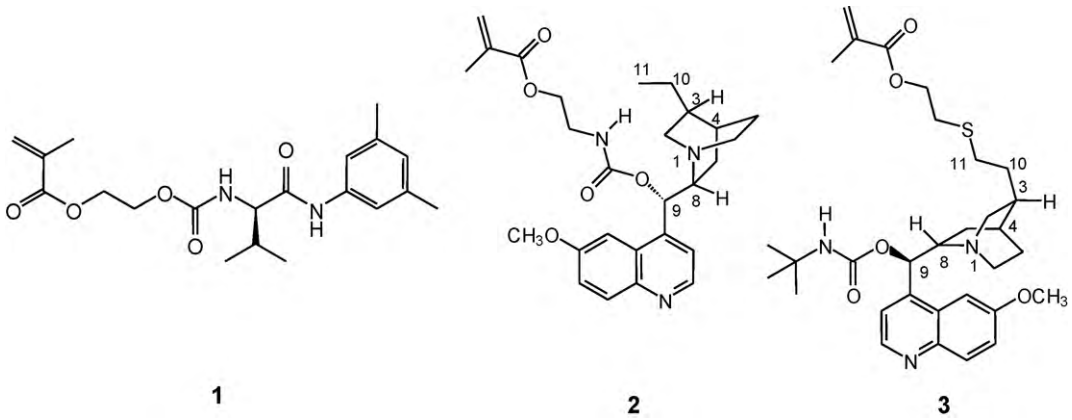

Fig. 1. Chiral monomers employed for the *in situ* preparation of chiral polymethacrylate monoliths.

to deterioration of separation factors being thus detrimental for the success of enantiomer separation. Ionizable chiral selectors may offer a significant benefit in view of this aspect, because ionizable comonomers are not needed anymore. However, it must be borne in mind that the chiral selector is usually present at higher concentrations leading to strong binding and long retention times. Sometimes it is difficult to establish conditions that properly balance the strong adsorption at the ionic sites and provide stable current without excessive Joule heating at the same time.

Overall, the challenge in the course of development of enantioselective organic polymer-type monolithic capillary columns is to get a grasp on the complex interrelations between chiral recognition, EOF control, non-specific adsorption, retention balance, rigidity and permeability without negative mutual effects upon each other in the course of tailoring each of these individual characteristics. How this has been achieved should become evident from the following examples.

Ground breaking works in this field have been published by Svec's group for the enantioselective polymethacrylate monoliths [46], Koide and Ueno for what concerns chiral polyacrylamide gels [47,48], and by Lin et al. [49] and S. Nilsson's group for monolithic MIPs [50,51].

2.2. *In situ* prepared polymethacrylate monoliths

2.2.1. *In situ* brush-type monoliths

In the pioneering work from 1998, Peters et al. prepared the first chiral polymethacrylate monoliths for enantioselective CEC by *in situ* copolymerization of a Pirkle type (donor–acceptor type) chiral monomer **1** (see Fig. 1) with ethylene dimethacrylate (EDMA), butylmethacrylate (BMA) or glycidylmethacrylate (GMA) as comonomer and AMPS as ionizable comonomer for EOF generation in presence of a porogenic solvent consisting of 1-propanol, 1,4-butanediol and water. The polymerization was carried out by thermal initiation at 50 °C with 1% (w/w; related to total monomers) AIBN as radical initiator in 100 μm inner diameter (i.d.) fused-silica capillaries that were prior modified with γ-MAPS. The pore structure could be successfully tailored with the employed porogenic mixture and yielded macropore diameters of 0.7 and 0.8 μm for the capillaries with BMA and GMA as comonomer, as determined by mercury intrusion porosimetry on dry bulk materials prepared from the same mixture in glass vials. Little less than 10% (w/w) of chiral monomer was incorporated into the polymer matrix and was sufficient to achieve enantiomer separation of the model test compound *N*-(3,5-dinitrobenzoyl)leucine diallylamide (see Fig. 2). The monolith with BMA as comonomer showed a good degree of enantioselectivity (Fig. 2a), but broad and tailing peaks precluded its practical usefulness. Upon replacement of the hydrophobic comonomer by the more hydrophilic

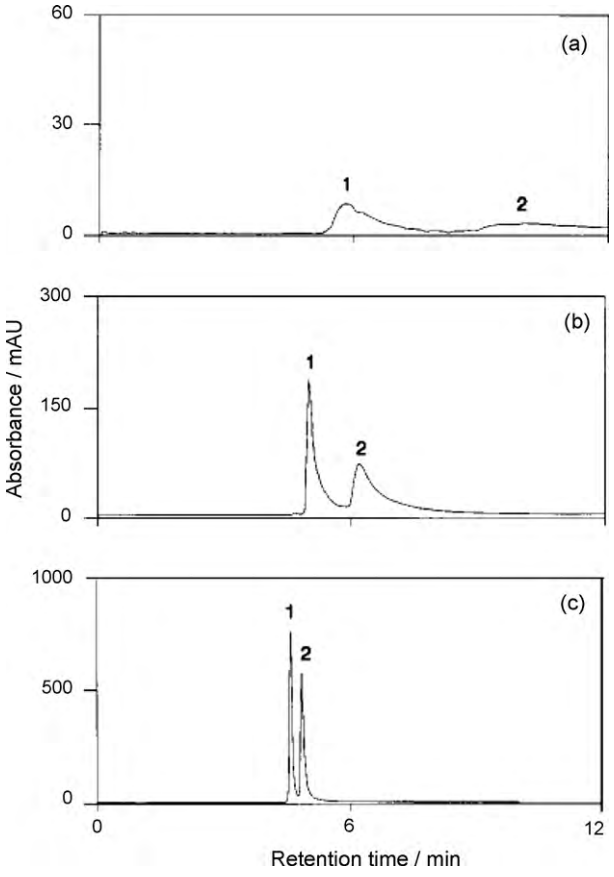

Fig. 2. Effect of surface hydrophilicity as tuned by pendant groups of the polymer matrix on the CEC enantiomer separation of *N*-(3,5-dinitrobenzoyl)leucine diallylamide: (a) Butylmethacrylate as comonomer, (b) glycidylmethacrylate as comonomer, and (c) glycidylmethacrylate as comonomer with epoxy groups hydrolyzed with 1 M aqueous sulfuric acid after polymerization. *Experimental conditions*: Reaction mixture, (a) 40% (w/w) monomers consisting of 19.7% (w/w) chiral monomer **1** (see Fig. 1), 50% (w/w) EDMA, 0.3% (w/w) AMPS and 30% (w/w) BMA were mixed with 60% porogens composed of 55% (w/w) 1-propanol, 35% (w/w) butane-1,4-diol and 10% (w/w) water; (b) 30% (w/w) monomer mixture consisting of 19.7% (w/w) chiral monomer **1** (see Fig. 1), 50% (w/w) EDMA, 0.3% (w/w) AMPS and 30% (w/w) BMA were mixed with 70% (w/w) porogenic solvent mixture composed of 75% (w/w) 1-propanol, 15% (w/w) butane-1,4-diol and 10% (w/w) water; thermally initiated polymerization at 50 °C (a and b). Pore size: 0.7 μm (a) and 0.8 μm (b) in dry state as measured by mercury intrusion porosimetry. Mobile phase, acetonitrile–5 mM phosphate buffer (pH 7) (80:20, v/v). Reprinted with permission from Ref. [46].

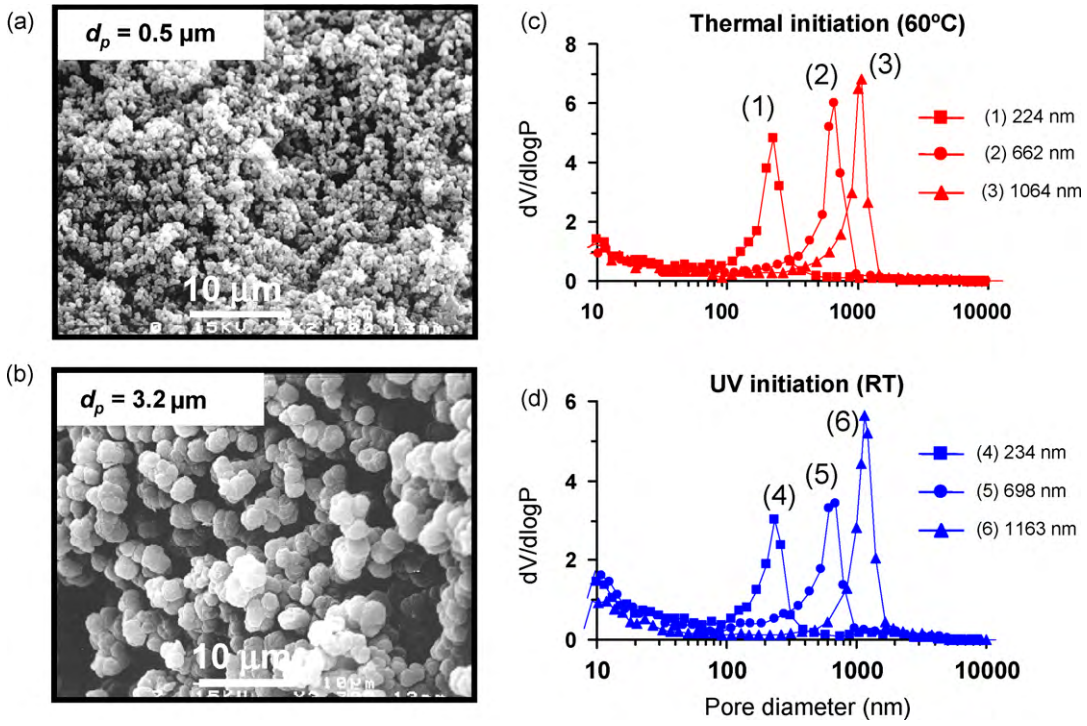

Fig. 3. Typical morphology of polymethacrylate-type poly(**2**-*co*-HEMA-*co*-EDMA) monoliths with macropore size of 0.5 μm (a) and 3.2 μm (b) as well as characteristic pore size distribution profiles of thermally initiated (c) and UV-initiated (d) monoliths with distinct modal pore diameters adjusted by the binary porogenic solvent mixture. Reprinted in modified form from ref. [36,39].

GMA, a much better chromatographic peak performance could be attained (Fig. 2b). When the epoxide moieties were hydrolyzed into hydrophilic diol residues, reasonable separations could be observed (Fig. 2c). The peaks were narrow (61,000 and 49,500 plates m^{-1}) and well resolved (R_S = 2.0). Also peak symmetry was greatly improved. It was concluded that non-specific effects deteriorate the separation in case of the more hydrophobic surface with BMA comonomer. The direct copolymerization of a hydrophilic comonomer was not envisaged at that time because it was found to be difficult in terms of creation of an appropriate pore structure. Hence, non-specific effects were reduced by use of the latent hydrophilic monomer (GMA) followed by its hydrolysis. These results emphasize the outmost importance of a fine-tuned surface chemistry for the performance of the resultant monolithic capillary column in enantioselective CEC.

2.2.2. *In situ* chiral ion-exchange type monoliths

After this proof of principle for the ability to employ a single-step *in situ* molding approach for the preparation of enantioselective polymethacrylate monoliths, the full flexibility of this method has been elucidated in subsequent investigations about dependencies of pore structure and polymer morphology on the composition of the polymerization mixture [36–39,52]. The corresponding effects on CEC characteristics and enantioselectivity were studied in detail as well [37]. While "classical" brush-type (often termed Pirkle-type) selectors such as the one described above are neutral and require the simultaneous incorporation of both a chiral and a charged monomer into the monolith matrix for CEC, a simplification of the reaction mixture can be achieved with ionizable chiral monomers. Ionizable chiral selectors thus play a dual role: they provide the active chiral distinction sites for enantioselective binding and offer charged sites for EOF generation. The viability and advantage of this strategy for CEC application has been demonstrated for cinchona-alkaloid derived chiral anion-exchange type polymer monoliths [36–39].

10,11-Dihydroquinidine and *tert*-butylcarbamoylquinine, respectively, were derivatized with methacrylic groups to obtain corresponding functional chiral monomers **2** and **3** (see Fig. 1) which typically amounted to 20% (w/w) of the total monomers (monomer-to-porogen ratio 40:60, w/w) in the reaction mixture. Characteristically, microglobular polymer morphologies typical for organic polymer monoliths that are growing by a "nucleation and growth mechanism" were formed [1,40], in which the individual microglobules were irregularly clustered and crosslinked yielding a co-continuous material with macroporous flow channels (often termed throughpores) (Fig. 3a and b). Besides thermal initiation, photopolymerization was tested as well and yielded different morphologies and pore structures from identical reaction mixtures, as expected (*vide infra*).

For suitability in chromatography and electrochromatography, a proper porous structure is essential (i) to allow the eluent to flow through the monolithic packing, (ii) to support a strong EOF (vide infra), (iii) to provide a sufficiently large surface for adsorption and separation, and last but not least (iv) to ensure an adequate chromatographic efficiency as the kinetic properties of the material are affected by the pore structure. Various factors in the synthesis have been found to be suitable instrumental tools to tailor the pore structure including temperature, reaction time, porogen composition, crosslinking degree and type of initiation as described in detail in a recent review by Svec [7]. Higher temperatures during polymerization usually translate into smaller pore diameters as a larger number of polymer nuclei are generated early in the polymerization that grow to smaller microglobules and leads to narrower pore diameters. First choice as parameter for pore tailoring is the selection of type and composition of porogenic mixtures. Typically, a binary or ternary porogenic mixture is used in which the thermodynamically poor solvent for the growing polymer chains represents the macroporogen and induces the phase separation. A common behavior is that the microglobule diameter increases with increasing macroporogen content and simultaneously the

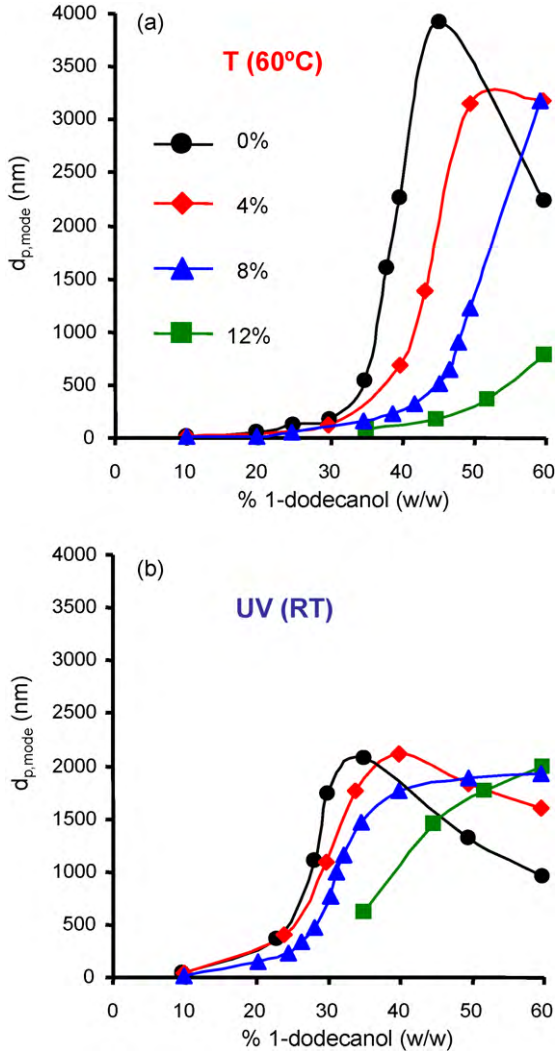

Fig. 4. Effect of percentage of chiral monomer on pore size (mode pore diameter, $d_{p,mode}$) in dependence of the macroporogen 1-dodecanol: (a) thermally initiated polymerization for 24 h at 60 °C, and (b) UV-initiated polymerization for 24 h at room temperature. Polymerization mixture: chiral monomer **2** and HEMA 24% (w/w), EDMA 16% (w/w), dodecanol + cyclohexanol 60% (w/w) (4, 8 and 12% chiral monomer **2** corresponding to 10, 20 and 30% related to the total monomer mixture). Reprinted in modified form from Ref. [36].

macropore diameter gets larger as well (*cf.* Fig. 3a and b). Often, cyclohexanol and 1-dodecanol are employed for polymethacrylate monoliths and this was also the porogen system of choice for the chiral cinchonan-derived polymethacrylate monoliths. More or less unimodal pore distributions with a large number of macropores and absence or only low number of mesopores are typically obtained by this technology (see pore distribution profiles in Fig. 3c and d). The position of the distribution, which is often characterized by the mode pore diameter ($d_{p,mode}$) which represents the maximum of the distribution curve, can be readily shifted by the ratio of cyclohexanol and 1-dodecanol (Fig. 3c and d) [36]. For this specific type of polymer, macropore sizes could be varied widely between 0.3 and 3 μm as measured by mercury intrusion porosimetry (in dry state) (Fig. 4). The slopes of the observed plots of mode pore diameter vs. macroporogen percentage are partly very steep (see ascending part of the curves in Fig. 4). This is one of the reasons why very accurate control of the composition of the polymerization mixtures is of utmost importance in order to get good reproducibilities (the same applies for temperature). Both percentage of crosslinker and of chiral monomer in the polymerization mixture have a strong effect on the resultant macropore distribution profiles and mode pore diameter ($d_{p,mode}$), respectively. The higher the crosslinker content the larger the pore volume and the smaller the pore diameters [36]. On the other hand, when the chiral monomer content in the polymerization mixture was increased at constant crosslinking degree and thermal initiation (e.g. at 60 °C), the macropore diameter became smaller. In order to end-up with the same macropore diameter, e.g. $d_{p,mode} \sim 1$ μm, the macroporogen content needs to be re-adjusted, i.e. more dodecanol was required in case of the quinidine carbamate monolith (see Fig. 4a) [36]. In agreement with above mentioned temperature effect, UV polymerization of the identical polymerization mixture at room temperature yielded typically larger pores (curves shifted to the left) (*cf.* corresponding curves in Fig. 4a and b). These morphological aspects are of key importance for the physical properties of the monoliths and their (electro)chromatographic performance (*vide infra*).

From the CEC tests performed in the course of the optimization of the polymer in above study it turned out that a chiral monomer content of 20% (w/w) related to total monomers (corresponding to 8% in the polymerization mixture) is the best compromise with regards to acceptable retention factors (increase of k with increase of chiral monomer), optimal separation factors (maximum observed at 20% monomer) and efficiencies (plate numbers increased with chiral monomer) [36]. Under typical CEC conditions, the quinuclidine moieties are protonated and generate anodic EOF (~ -1.0 to -1.5×10^{-4} cm^2 V^{-1} s^{-1}). It increased with the pore diameter. Like in the prior studies by Peters et al. it was confirmed that a more hydrophilic comonomer such as 2-hydroxyethylmethacrylate (HEMA) is favorable over other less hydrophilic monomers [36]. Separation factors for a model analyte, *N*-3,5-dinitrobenzoyl-leucine (DNB-Leu), could be increased from 1.6 with GMA as comonomer and EDMA as crosslinker to 3.4 with HEMA as comonomer. This striking effect was explained by abolishment of adverse non-specific interactions at the achiral polymer backbone that appears to become inaccessible for the solutes due to its strong solvation with polar solvent in case of HEMA.

Adjusting the degree of crosslinking was found to be the most straightforward way to tune the chromatographic performance of such anion-exchange monoliths (Fig. 5). Reducing the percentage of crosslinker in the reaction mixture, e.g. from 40 over 20 to 10% (w/w) related to the total monomer content, in batches in which the composition of porogenic solvents was modified in order to keep the macropore diameter constant at about 1.8 μm, dramatically improved the mass transfer properties (as indicated by much flatter H/u-curves, Fig. 5b). For example, the C-term could be reduced from around 40 ms (curve 1) to around 5 ms (curves 2 and 3) being more or less insignificant. Moreover by decreasing the macropore diameter from 1.8 to 1 μm in the 10%-crosslinked polymer has lead to a reduction of the A-term contribution to the zone dispersion by a factor of about 2, e.g. from around 28 (curve 1 and 2) to 14 μm (curve 3) (Fig. 5b); since the decrease of the pore diameter is associated with a concomitant decrease of the microglobule diameters of the solid matrix this effect has been expected in accordance with common chromatographic theory. On the other hand, a less crosslinked polymer may be strongly swollen with solvent so that the resultant flow channels are much narrower. This may negatively impact permeability and EOF velocities of the monolithic capillaries. In fact, greatly reduced EOF was observed for the monoliths with low crosslinking (Fig. 5a). While this effect may be less relevant for CEC application of such monolithic capillaries, their HPLC use may be discouraged due to this reason. Chiral anion-exchange type cinchona-alkaloid derived polymethacrylate monoliths obtained through an optimized synthetic procedure produced highly efficient enantiomer separations for various chiral acids with up to 250,000 plates/m (see Fig. 6) [37–39]. A point that is worth mentioning is the reversed elution order of corresponding

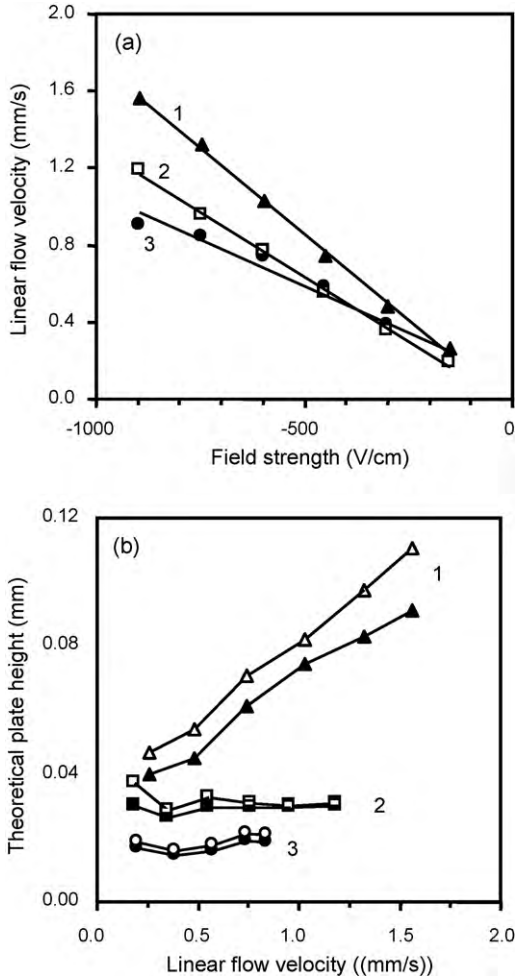

Fig. 5. Effect of crosslinking (curves 1 vs. 2) and pore size (curves 2 vs. 3) for poly(**3**-co-HEMA-co-EDMA) monoliths: (a) dependence of linear flow velocities on electric field strengths and (b) dependence of theoretical plate heights on linear flow velocities. Curve 1, 20% crosslinked with macropore diameter of 1.7 μm; curve 2, 10% crosslinked with macropore size of 1.8 μm; curve 3, 10% crosslinked with macropore size of 1.0 μm. (b) (■, ●, ▲) (*R*)-enantiomer; (□, ○, Δ) (*S*)-enantiomer. Capillary dimension, L_{total} = 33.5 cm, $L_{monolith}$ = 25 cm, $L_{effective}$ = 25 cm, 100 μm i.d., EOF marker, acetone. Sample, *N*-3,5-dinitrobenzyloxycarbonyl-leucine. Eluent, 0.4 mol/L acetic acid and 4 mmol/L triethylamine in ACN–MeOH (80:20, v/v); capillary temperature, 50 °C. Reprinted with permission from Ref. [38].

quinidine and quinine derived monoliths made from monomer **2** and **3**, respectively [38].

Although the dependencies and performances above were discussed for a single family of chiral polymethacrylate monoliths, similar relationships can be expected for other chiral monomers. Whatsoever, considering the nice performance of these chiral monoliths in enantioselective CEC it is remarkable that no further reports on *in situ* prepared chiral monolithic capillaries made from functional chiral monomers were published in the last years.

2.3. In situ prepared poly(meth)acrylamide monoliths

In situ prepared chiral poly(meth)acrylamide monoliths emerged from capillary gel electrophoresis media that were employed mainly for biopolymer separations. In the literature, such media are also often called as "continuous beds" a term that was coined by Hjérten [10]. Early attempts incorporated β-cyclodextrin (CD) and derivatives, respectively, into neutral polyacrylamide gels formed from acrylamide and bisacrylamide by either physically entrapping (polymeric) cyclodextrin into the polymer network or copolymerizing vinyl-modified CD derivatives (e.g. allylcarbamoylated-β-CD) [24,25]. These neutral cyclodextrin-modified polyacrylamide gels furnished only a low EOF thus leading to long run times, were prone to bubble formation, afforded short column life-times, and suffered from poor reproducibilities [25]. This has been overcome, at least partly, by incorporation of ionizable monomers in the polymerization mixture ending up with charged polyacrylamide gels with pendant chiral moieties as well as sufficiently strong and stable EOF, suitable for CEC.

Two basically different recipes for poly(meth)acrylamide monoliths may be distinguished amongst the studies that have been reported in the literature: (i) reaction mixture based on acrylamide (AAm) and *N*,*N*′-methylenebisacrylamide (BIS), and (ii) based on methacrylamide (MAAm) and piperazine diacrylamide (PDA) as monomers and crosslinkers, respectively.

The former protocol has been employed by Koide and Ueno [47,48,53–55,58] as well as Hjérten's group [12]. Thereby, the polymer matrix is synthesized from aqueous solutions of acrylamide and *N*,*N*′-methylenebisacrylamide at room temperature with a redox initiator (ammonium persulfate, APS/*N*,*N*,*N*′,*N*′-tetramethylethylenediamine, TEMED) typically in borate buffer. The polymerization is started with the initiator system (APS/TEMED) and the reaction mixture immediately thereafter sucked into vinylized fused-silica capillaries where polymerization is allowed to proceed usually for 1–6 h or so. Detailed information on the employed compositions of polymerization mixtures can be found in Table 1. It can be seen that the total acrylamide concentrations (% T) typically ranged between only 5–10% (for definitions of % T, % C and % S see below "Definitions and symbols"), significantly lower than in case of the above-mentioned rigid polymethacrylate monoliths. Moreover, the polymer matrix has a low crosslinking degree (% C) of only 3–10%. Due to the hydrophilicity and low crosslinking degree the hydrophilic polyacrylamide matrix is highly swollen in aqueous media which impairs the hydrodynamic flow properties through the macroporous flow channels as well as the electrokinetic permeability resulting in reduced EOF as compared to rigid analogs. A higher concentration of chargeable comonomer as compared to rigid brush type monoliths has been usually incorporated (Koide and Ueno used 5–6% S and Hjérten's group 17% S) which reduces the risk that the macropores collapse and thus helps to maintain a reasonable EOF. Both negatively and positively charged gels were prepared that afforded either cathodic or anodic electroosmotic flow dependent on the requirements dictated by the analytes (i.e. negatively charged gels with cathodic EOF for cationic solutes employing AMPS as chargeable comonomer (Fig. 7a) [47,48,53,54,58] and positively charged gels with anodic EOF for analyses of anionic solutes employing AMTEA (Fig. 7b) [53,55] or dimethyldiallylammonium chloride (DMDAAC)) [12]. In this approach of poly(meth)acrylamide monoliths, pore sizes and morphologies can be altered, for example, via the concentration of ammonium sulfate in the aqueous reaction mixture [61].

Soft polyacrylamide monoliths with physically incorporated polymeric cyclodextrins (CD) [47,53] or covalently bonded β-cyclodextrin [48,54,55] were developed by Koide and Ueno (see Fig. 7a and b), and at about the same time also by Hjerten's group (see Fig. 8a) [12]. Koide and Ueno employed randomly allylcarbamoylated-β-cyclodextrin (AC-β-CD) as functional chiral monomer and Hjertens's group an allyl-substituted β-cyclodextrin selector obtained by derivatization with allylglycidylether (see Fig. 8a). In both cases the CD derivatives possessed several vinyl groups that could simultaneously participate in the polymerization and therefore these selectors partly served as crosslinkers as well. Degrees of substitutions were not reported. The hydrophilic polyacrylamide matrix is favorable for CD-based enantiomer separations. The enantiomer separations can be carried out with

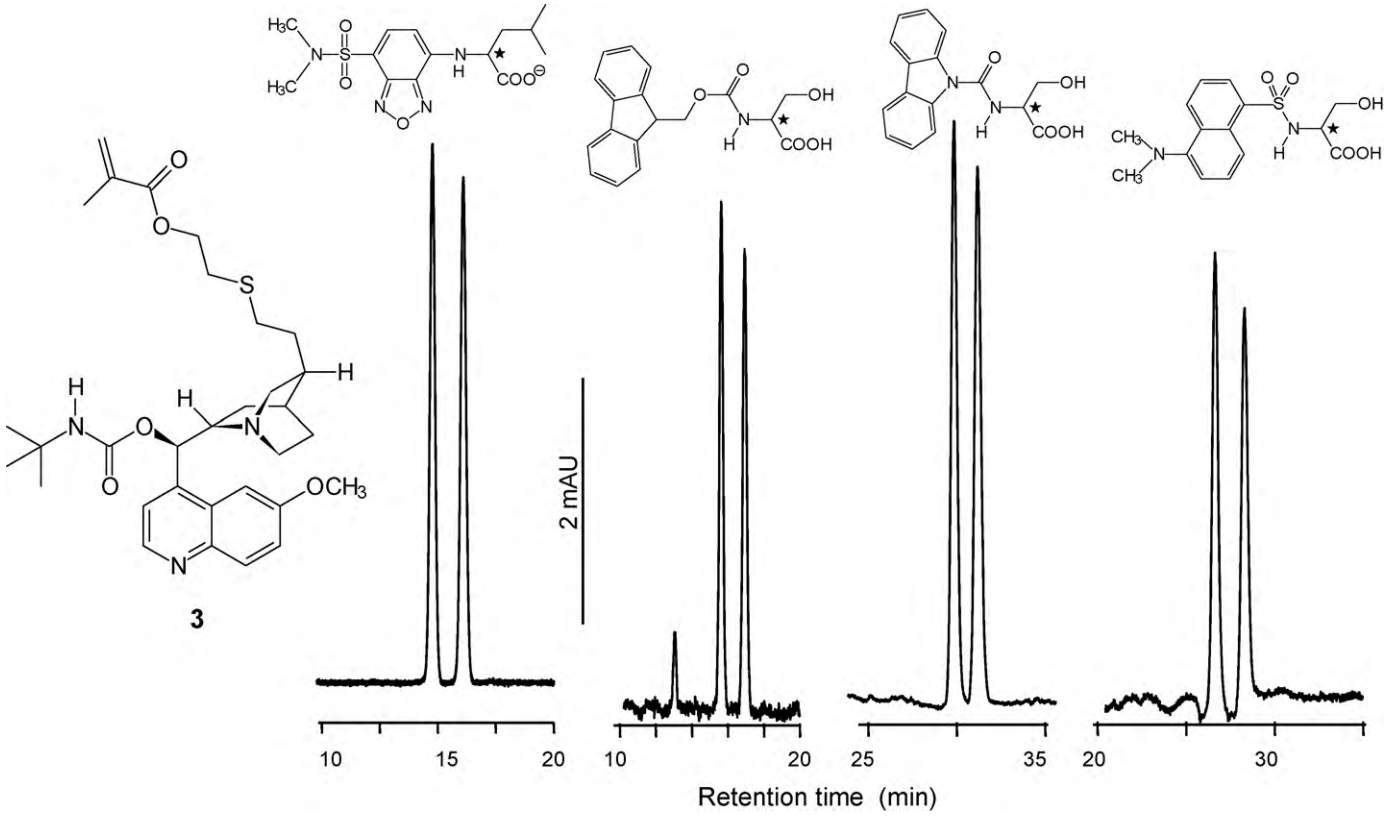

Fig. 6. CEC enantiomer separations of *N*-derivatized amino acids employing poly(**3**-*co*-HEMA-*co*-EDMA) monoliths. Monolithic column: Polymerization mixture, chiral monomer **1** 8% (w/w), HEMA 28% (w/w), EDMA 4% (w/w), cyclohexanol 15% (w/w), and 1-dodecanol 45% (w/w). UV-initiated polymerization 16 h at room temperature. Capillary dimension, L_{total} = 45 cm, $L_{monolith}$ = 20 cm, $L_{effective}$ (UV) = 36.5 cm, 0.1 mm i.d.; eluent, 0.4 mol/L acetic acid and 4 mmol/L triethylamine in ACN–MeOH (80:20); capillary temperature, 50 °C; applied voltage, −25 kV. Reprinted in modified form from Ref. [38].

completely aqueous buffered mobile phases, without need of organic modifiers that would interfere with the inclusion complexation of aromatic groups of analytes in the hydrophobic inner cavity of the cyclodextrin which is usually leading to decreased enantioselectivities. Any hydrophobic character of the polymer backbone would introduce non-specific hydrophobic interaction sites that are detrimental. If those are present, the separation needs organic modifier addition which is unfavorable as outlined above. It has been demonstrated that retention factors strongly increased with the concentration of AC-β-CD in the reaction mixture, while separation factors leveled off after an initial steep increase and resolution frequently reached maxima in the intermediate or high CD concentration range [54,55]. Végvári et al. showed that electroosmotic mobility unfortunately decreased with increase of allyl-substituted CD, probably as a consequence of its effect on pore size [12]. The spectrum of compounds that have been successfully separated into enantiomers on the CD-functionalized monoliths is quite broad and covered neutral, acidic and basic compounds carrying aromatic moieties, but also some others without aromatic group, e.g. hexobarbital (see Table 1). Due to a remarkable homogeneity of such polymeric gels, which results in reduced Eddy dispersion and greatly relaxed mass transfer resistance [10,12], efficiencies in CEC mode were partly remarkable, with plate numbers typically in the range of 30,000–150,000 m^{-1} and few cases over 200,000 m^{-1} (see Table 1). The low crosslinking degree and the low concentration of ionizable monomer (5–6% S) employed by Ueno and Koide, however, furnished in many instances slow separations. This has been partly overcome by an increase of the chargeable monomer to about 17% by Hjérten's protocol [12].

Koide and Ueno used the same approach to prepare crown ether-bonded negatively charged polyacrylamide gels (Fig. 7c) [58]. Both polyacrylamide monoliths from (+)-18-crown-6 tetraallyl and monoallyl ester functional monomers were synthesized and tested for CEC enantiomer separation. A number of chiral primary ammonium compounds (see Table 1) could be baseline separated due to stereoselective inclusion complexation into the crown ether cavity driven by triple hydrogen bonding under fully aqueous and hydroorganic conditions (aqueous buffer–acetonitrile), respectively. The two kinds of chiral polyacrylamide gels showed some complementary enantioselectivity profiles: some compounds were only separated on the tetraallyl-derived monolith, others only on the monoallyl-derived monolithic column. A typical CEC separation is shown in Fig. 9a. Alanine-2-naphthylamide enantiomers were resolved with a R_S of around 7 under analytical scale injections. In order to be able to detect minor enantiomeric impurities at low levels, a higher sample load was injected for the enantiomeric purity determination of the L-enantiomer (Fig. 9b). It can be seen that the resolution dramatically deteriorated in this case, yet 0.1% of D-form could be readily analyzed by this method. This indicates that this method may be fit for the real purpose.

It was found that polymers with *N*,*N*′-methylenebisacrylamide as crosslinker have shown suboptimal stability. Thus, in other studies BIS was replaced by piperazine diacrylamide (PDA) as crosslinker and acrylamide by methacrylamide (MAAm) as monomer [11,35,56,57,59]. The replacement of BIS by PDA has led to mechanically and chemically more stable polymers. Especially the pH stability has been improved by use of PDA instead of BIS. Vinylsulfonic acid (VSA) typically served as chargeable comonomer to create cathodic EOF [11], while DMDAAC was copolymerized for generation of anodic EOF for the separation of acids, e.g. hydroxy acids [59]. In some instances, the incorporated ionizable chiral selector provided the surface charge for EOF generation and no

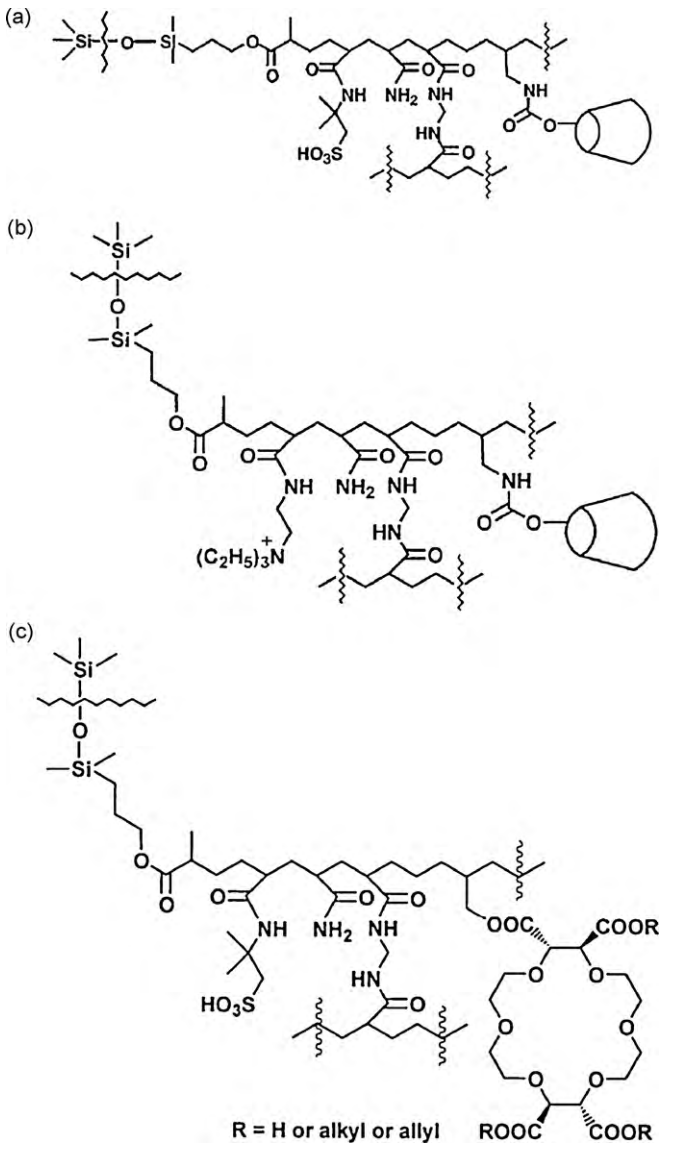

Fig. 7. Chirally functionalized polyacrylamide monoliths: β-cyclodextrin-bonded negatively charged (a) and positively charged (b) polyacrylamide gels, and crown ether-bonded gel (c). Reprinted from Ref. [19].

chargeable comonomer was included in the polymerization mixture [35,56,57].

Polyacrylamide monoliths with copolymerized *N*-(2-hydroxy-3-allyloxypropyl)-L-4-hydroxyproline as chelating selector were used in ligand-exchange mode using copper-ion containing eluents for enantioselective CEC of underivatized amino acids [11] and hydroxy carboxylic acids (Fig. 8b) [35,59]. A tentative model of the ternary analyte-metal ion-selector complex is shown in Fig. 8b. VSA was copolymerized to promote strong EOF in co-directional mode for the separation of amino acids [11]. Since sulfonic acid moieties on the surface furnish a counterdirectional separation for hydroxy acids, VSA was eliminated from the reaction mixture for these analytes and no ionizable comonomer was employed [35]. The analyte transport of the negatively charged hydroxy carboxylic acids was then accomplished by virtue of electrophoretic mobility alone. Later, VSA was substituted by DMDAAC to end up with an anodic co-electrophoretic separation mode for these hydroxy carboxylic acids [59]. Low back pressures typical for monolithic columns allowed the use of pressure-assisted CEC (pCEC) in a conventional CE instrument with an external pressurization of up to 1.2 MPa. It can be seen from Fig. 10 that much faster separations could be achieved by the pCEC mode (Fig. 10c) while resolution values were only slightly deteriorated as compared to the CEC mode (Fig. 10a) and still significantly better than in common pressure-driven nano-HPLC mode (Fig. 10b). The CEC separation using this monolith was found to be more stable than with a capillary column packed with silica-based ligand-exchange beads.

All these studies clearly demonstrate the great flexibility in terms of tailoring the surface chemistries simply by copolymerizing functional monomers with corresponding pendant selector moieties. Such an approach thus allows adapting the organic polymer monolith to virtually any separation problem.

2.4. *In situ* prepared MIP monoliths

Enantioselective binding sites in organic polymer monoliths may also be created by a molecular imprinting process with a single enantiomer template yielding molecularly imprinted polymer (MIP) monoliths with predetermined selectivity for a given solute and due to cross-reactivity also for structurally closely related analogs. If the imprinting process is performed directly in the capillary in presence of an appropriate porogen, macroporous monolithic MIP columns can be obtained in a single straightforward molding step as first successfully realized by Nilsson [50,51] and Lin [49]. This single-step column preparation approach is actually smarter than the conventional "ex-capillary" or "ex-column" preparation of MIPs, followed by grinding of the polymer, sieving to narrow particle size distribution and packing of the particulate material into column tubes for HPLC or capillaries for CEC application. Meanwhile a number of research groups adopted the *in situ* MIP preparation protocol for CEC use (see Table 2). This topic has been subject of several dedicated review articles [20,62,63] and will only be briefly discussed herein.

Like for above described *in situ* monolith preparation from functional chiral monomers, MIP monoliths can be obtained as well by either thermally initiated polymerization [49] or photopolymerizaton [50]. Photopolymerizations can be performed at low temperatures (e.g. −20 °C) and appear to afford better defined chiral cavities, because the non-covalent association complexes between functional monomers and template molecule are more stable at low temperature. Pore formation occurs by a nucleation and growth mechanism, and thus the polymer has a microglobular morphology comparable to that of above described organic polymer monoliths (see Fig. 3).

The performance of the imprinted monoliths is primarily determined by two major factors: (i) the number, uniformity and fidelity of the chiral cavities created by the imprinting process, and (ii) the porous properties of the monolithic polymer. Effective imprinted binding sites with well-defined shape and properly arranged functionalities are a result of well-defined and stable self-organized template-monomer complexes during the polymerization process. They can be obtained by proper selection of suitable functional monomers and solvent (porogens). Functional monomers, crosslinkers and porogens that have been employed for the preparation of MIP-type monolithic capillaries are summarized in Table 2 along with the template molecules and target analytes, respectively. Most successfully ionizable functional monomers such as methacrylic acid (MAA), trifluoromethacrylic acid, vinyl pyridines (VPY) have been used for imprinting of counterionic template molecules because they lead to the formation of stable template-monomer assemblies driven by strong Coulombic interactions during polymerization in apolar (preferentially aprotic) porogenic solvents like isooctane, toluene, chloroform and acetonitrile that do not interfere with the non-covalent binding. These ionizable functional monomers are moreover responsible for generation of EOF and charged comonomers are therefore not

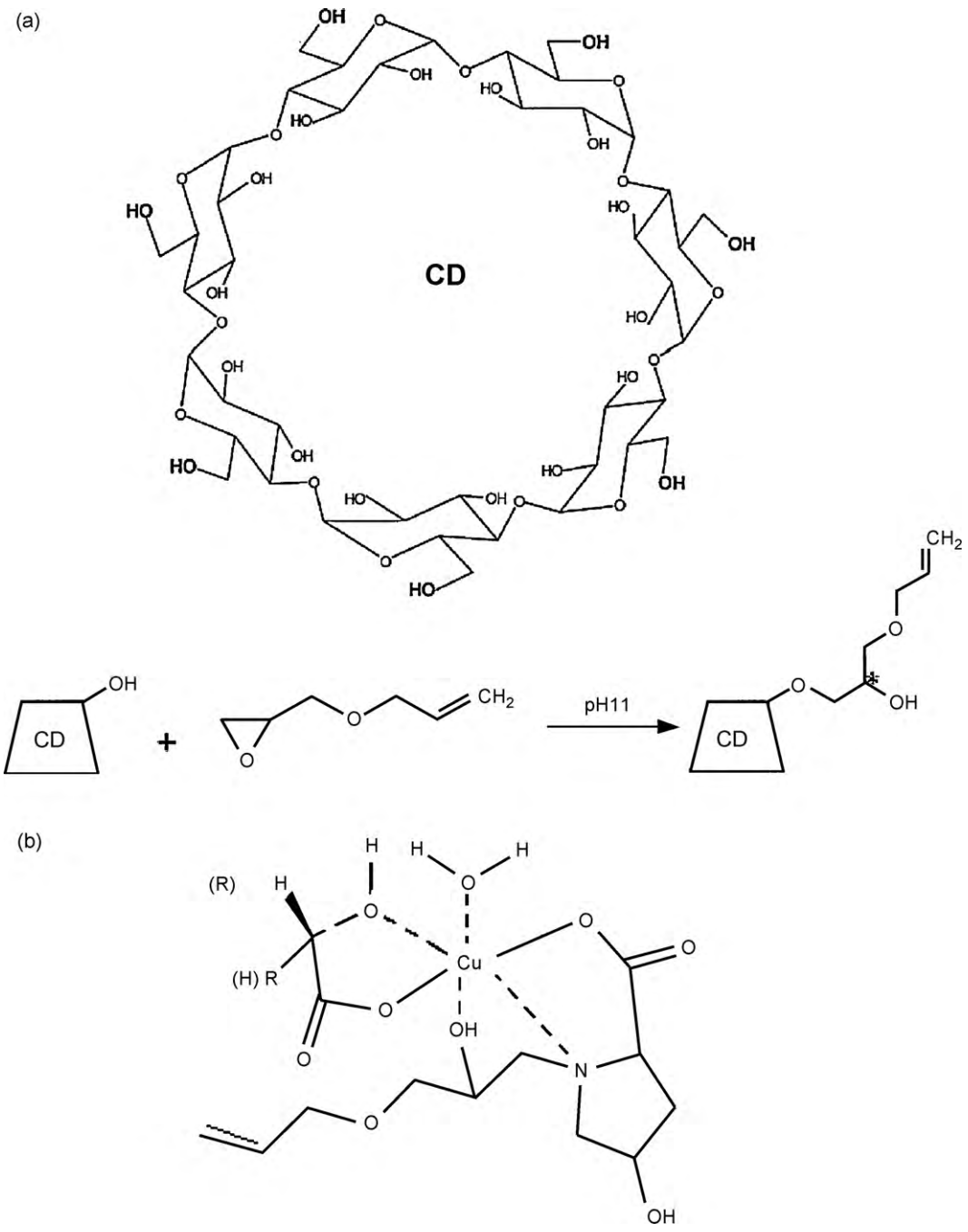

Fig. 8. Chirally functionalized polyacrylamide monoliths: (a) molecular structure of β-cyclodextrin (CD) and reaction scheme for preparation of allyl-modified β-CD functional chiral monomer. (b) Ternary metal complex between allylglycidylether-derivatized hydroxyproline (functional chiral monomer) and hydroxy carboxylic acid with Cu(II) (in corresponding diastereomeric complexes R and H groups are exchanged). Reprinted from Refs. [12] (a) and [35] (b).

needed. A high crosslinking degree ensures structural rigidity of the imprinted material and persistence of the enantioselective receptor sites. Trimethylolpropane trimethacrylate (TRIM), pentaerythritol triacrylate, and pentaerythritol tetraacrylate are preferred over ethylene dimethacrylate, as they assure a high crosslinking degree at lower molar content. A lower percentage of crosslinker, in turn, allows the use of higher contents of functional monomers which may yield higher densities of effective imprinted sites and consequently a better sample loading capacities, increased enantioselectivities and improved resolutions. Two basically different strategies were propagated for tailoring a suitable pore structure in MIP monoliths for CEC application, viz. addition of proper porogenic solvents to the monomer mixture [50] or termination of the polymerization reaction before completion [51]. The latter requires both perfect timing and precise control of reaction conditions, and is therefore no longer pursued. The former approach turned out to be advantageous as it appears to be more reproducible. With photopolymerization MIP-type monolithic capillary columns were operational within 3 h of the start of capillary preparation [51].

One of the limitations of the MIP technology is the narrow analytical applicability spectrum of such capillary columns that is restricted essentially to the template molecule. However, cross-reactivities may slightly extend the application spectrum to structurally closely related compounds. MIP-type monolithic capillaries for β-adrenergic antagonists [51,64], local anesthetics [50], amino acids and derivatives [65,66], 1,1′-bi-2,2′-naphthol

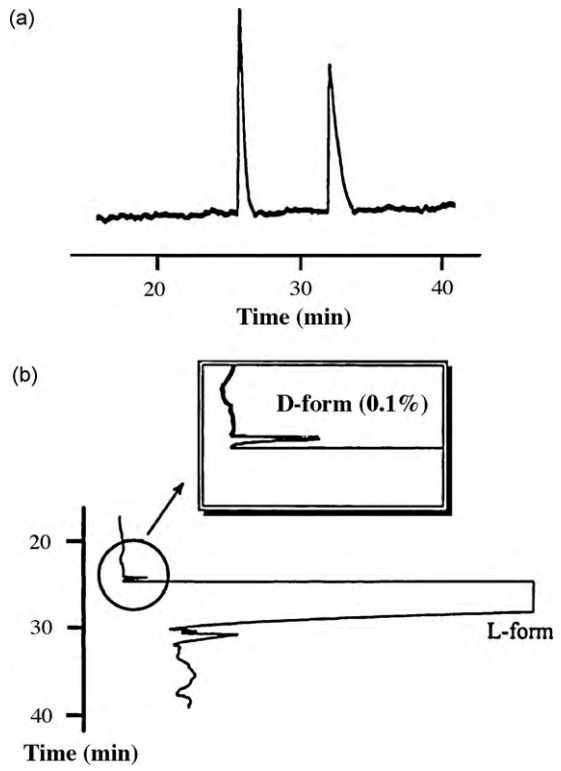

Fig. 9. CEC enantiomer separation of alanine-2-naphthylamide on (+)-18-crown-6 tetraallyl ester bonded monolithic capillary column: (a) Injection of racemate and (b) of L-enantiomer under overload conditions for enantiomeric impurity determination. Experimental conditions: Mobile phase, 0.2 mol/L triethanolamine–0.3 mol/L boric acid (pH 6) with 20% acetonitrile (v/v); applied field strength, 188 (a) and 239 V cm^{-1} (b). Reprinted from Ref. [58].

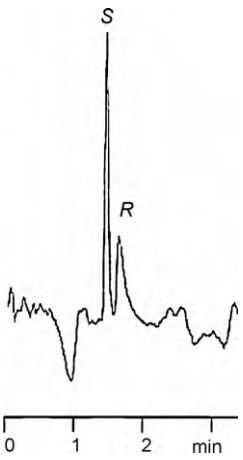

Fig. 11. CEC enantiomer separation of propranolol by (*S*)-propranolol-imprinted monolithic polymer capillary column. *Experimental conditions*: Monomers, MAA and TRIM; mobile phase, acetonitrile–4 M acetate pH 3.0 (80:20, v/v); injection, 5 kV for 3 s; applied voltage, 30 kV; UV detection, 214 nm; capillary temperature, 60 °C; applied pressure, 7 bar. Reprinted with permission from ref. [51].

[67], naproxen [68], ibuprofen [69], Tröger's base, tetrahydropalmatine [70] have been reported. In some instances, surfactants such as cationic cetyltrimethylammonium bromide (CTAB), anionic sodium dodecyl sulfate (SDS) and especially non-ionic polyoxyethylene sorbitane monolaureate (Tween 20) were added to the BGE and improved enantioresolution in CEC separations [67,68]. Some peak shape problems, however, still existed.

While the functional monomer MAA was favorable for the imprinting of basic templates, this functional monomer was less successful for acidic templates such as ibuprofen [69]. While the replacement of MAA by 4-vinylpyridine (4-VPY) as functional monomer turned out to be successful in terms of imprinting chiral recognition sites the resultant polymers prepared with 4-VPY were neutral with the employed eluents and thus no EOF could be measured on the corresponding MIPs. To alleviate this problem, Deng et al. admixed a small percentage of [3-(methacryloyloxy)propyl]trimethoxysilane (γ-MAPS) to the polymerization mixture (ca. 1.4 mol% related to the vinyl monomers). Thus, γ-MAPS was copolymerized with 4-VPY/EDMA using toluene/isooctane as porogenic solvents. Immediately after polymerization, the Si–O–C ester bonds of γ-MAPS at the MIP surface were hydrolyzed. The resultant silanols in the monolithic bed furnished then a reasonably strong EOF. Ibuprofen enantiomers could be baseline resolved by CEC with plate counts for the first eluted non-imprinted *R*-enantiomer of 128,700/m. Efficiency for the imprinted and thus second eluted *S*-enantiomer was 3400 m^{-1}.

Such unequal peak performance for imprinted (second eluted) and non-imprinted (first eluted) enantiomers are characteristic for all MIP-type materials. Although electrically driven flow significantly enhanced column efficiencies compared to HPLC separations with MIP stationary phases, the deleterious effect of polydispersity of the binding sites created by the imprinting process and mass transfer limitations on peak performance, especially for the high-affinity enantiomer, still persisted in all the presented studies (Fig. 11).

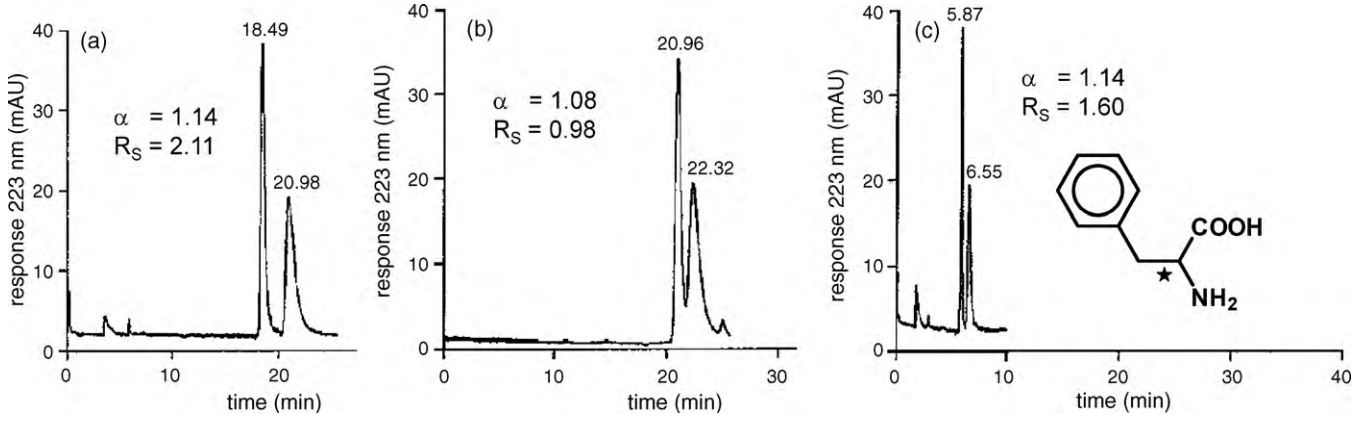

Fig. 10. Enantiomer separation of Phe by (a) CEC (30 kV), (b) nano-HPLC (12 bar on inlet side), and (c) pressure-assisted CEC (30 kV and 12 bar on inlet side). *Experimental conditions*: Capillary dimension, 26 cm × 75 μm i.d.; eluent, 50 mM NaH_2PO_4/0.1 mM Cu(II), pH 4.6; injection, 10 kV for 6 s. Reprinted with permission from Ref. [11].

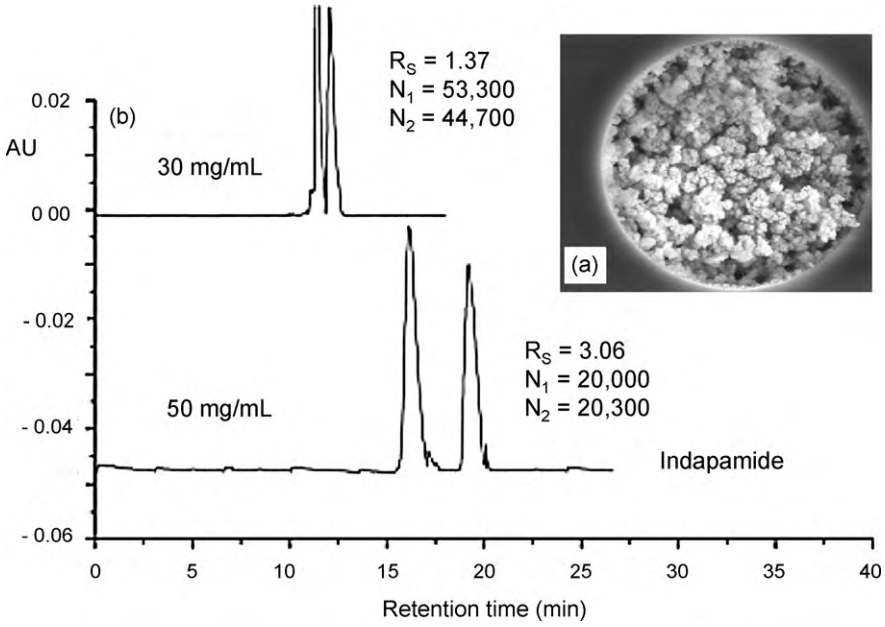

Fig. 12. Effect of selector coverage on CEC enantiomer separation performance on CDMPC-coated monolithic columns exemplified for indapamide. Experimental conditions: monolithic polyacrylamide capillary column coated with 30 or 50 mg/mL of CDMPC (in acetone); mobile phase, 2 mM phosphate buffer containing 35% acetonitrile at pH 2.7; applied voltage, −15 kV; injection, −10 kV for 1 s. Reprinted in modified form from Ref. [82].

2.5. Organic polymer monoliths obtained by post-modification with chiral selectors

All the above *in situ* synthesis approaches towards organic polymer monoliths are remarkably simple and offer great flexibility to adjust surface chemistries with regards to chromatographic and electroosmotic properties. However, it may be quite tedious to find suitable conditions which provide a permeable macroporous polymer and efficient separations simultaneously. A time consuming optimization with quest for large amounts of functional monomer or enantiomeric template which may not always be available is the consequence. Moreover, with exchange of the functional monomer or any other component in the reaction mixture a full re-optimization is necessary. Hence, nowadays more generic on-column *post-modification* strategies are becoming more and more popular which allow the immobilisation of various chromatographic ligands onto pre-optimized (reactive) monolithic beds.

Various methodologies have been pursued for surface functionalization of monoliths and the introduction or attachment of chromatographic ligands, respectively, on the monolith surface comprising photografting [77,78], living polymerization [79–81], chemical derivatization via reactive moieties of the monolith (*vide infra*), and coating of polymeric chromatographic selectors onto the monolith surface [82]. Svec and coworkers proposed the photografting of porous three-dimensional materials for functionalization of its pore surface using a benzophenone-initiated surface photopolymerization within the pores of a macroporous polymer monolith contained in fused-silica capillaries. Various functional monomers were grafted for ion-exchange, biomolecule coupling, etc. onto monolith surfaces in capillaries and microchips, yet the approach was not yet elucidated for preparation of chiral monoliths [7]. Another appealing and likewise flexible surface functionalization concept represents the living polymerization as for example pursued by Buchmeiser and coworkers. Norbornadiene-based monoliths prepared by ring-opening metathesis polymerization (ROMP) provide materials with active species on the surface where the polymerization may be reinitiated with various ROMP-active monomers. This way cyclodextrin-functionalized ROMP-derived monoliths have been prepared and successful HPLC enantiomer separations could be demonstrated, e.g. for proglumide [79,80]. The concept has not been adopted for CEC enantiomer separation, yet.

The studies that involved a post-modification strategy for preparation of organic polymer monoliths in context of enantioselective CE are summarized in Table 3. One straightforward approach constitutes monolith functionalization by coating of polymeric selectors. The viability of this route has been demonstrated by Zou and coworkers [82]. No reactive anchor groups for covalent attachment are required because the polymeric selector is insoluble under employed operation conditions and remains precipitated on the surface during CEC runs. Conditions which dissolve the selector are prohibited and must be avoided. Along this line, cellulose tris(3,5-dimethylphenylcarbamate) (CDMPC) has been coated onto polyacrylamide monoliths synthesized from acrylamide and *N*,*N*′-methylene-bisacrylamide with incorporated permanently positively charged 2-(methacryloyloxy)ethyl trimethylammonium methyl sulfate (MEAMS) for generation of anodic EOF [82]. In the first step, the polyacrylamide bed with the common microglobular polymer morphology (Fig. 12a) was synthesized. For coating of the polymeric selector, the polysaccharide derivative was dissolved at a concentration of either 20 mg/mL or 50 mg/mL in acetone and pumped into the monolith column with an HPLC pump. A slow drying step at ambient temperature over 7–12 days followed. As can be seen from Fig. 12b the monolith prepared from the 50 mg/mL selector solution showed higher retention, better enantioselectivity and resolution whilst plate numbers were lower, trends that are in agreement with those described by Chankvetadze for packed particulate columns and polysaccharide coated monolithic silica-based capillary columns as well [83].

Functionalization by coating is mainly amenable for polymeric selectors. Other selectors have been immobilized covalently by derivatization of reactive polyacrylamide-type [13,84] or polymethacrylate-type [85–88,90,92] monolithic supports. Such a strategy usually entails the preparation of an organic monolith with reactive groups that can be efficiently derivatized under mild conditions in the capillary, either dynamically, i.e. in the flow-through mode or statically, i.e. the capillary is filled with the reaction mix-

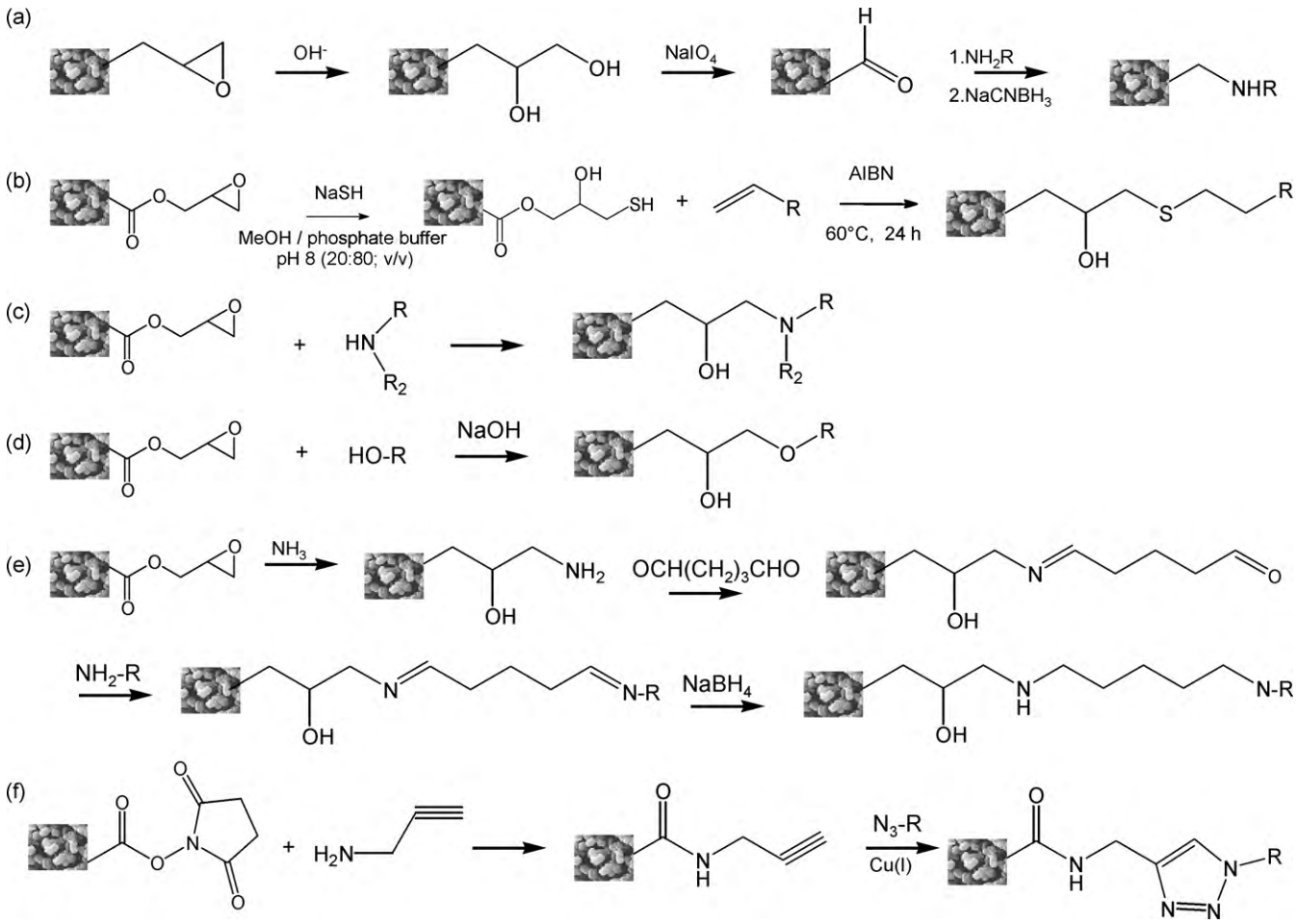

Fig. 13. Overview of employed derivatization schemes for in-capillary monolith surface functionalization (R is chiral selector moiety): (a) reductive amination, e.g. with vancomycin [13,84], (b) sulfhydryl activation of epoxy-monolith followed by thiol click with quinine carbamate [85] or aminophosphonic acid selectors [86], (c) nucleophilic substitution with amino-modified selectors, e.g. 4-aminobutyl-terguride [87] or amino-CD [90], (d) nucleophilic substitution with hydroxyl-group containing selectors, e.g. β-CD or hydroxypropyl-β-CD [90], (e) glutaraldehyde activation followed by reductive amination, e.g. with amino-modified β-CD [90], (f) alkyne activation of reactive hydroxysuccinimide monolith followed by click reaction with azido-β-CD [91].

ture, capillary ends sealed and the reaction allowed to proceed for a certain time under specific conditions.

Amongst the most popular substrates for this purpose are epoxy-functionalized monoliths. Maruska and coworkers made use of epoxy-monoliths by a popular multi-step immobilization procedure via aldehyde functionalities and reductive amination [13,84]. The polyacrylamide monolith with epoxy groups from copolymerized allyloxyglycidylether (AGE) was hydrolized to yield diol moieties which were oxidized to aldehyde groups by sodium periodate (Fig. 13a) [13]. Onto this aldehyde surface, vancomycin was, via its amino group, covalently anchored to the monolith by reductive amination with sodium cyanoborohydride. Copolymerized vinylsulfonic acid assured a reasonable EOF. Thalidomide could be separated into enantiomers in the hydroorganic RP mode (see Fig. 14) indicating that the surface concentration of selector moieties was sufficient to generate useful enantioselectivities. In a modified protocol, *N,N′*-diallyltartardiamide was incorporated into the polyacrylamide polymer instead of AGE; by sodium periodate treatment aldehyde groups could be created in a single step, unlike with AGE [84]. Moreover, by cleavage of the central C–C bond of tartramide shifts in the pore size distribution profiles were noticed in the inverse size exclusion chromatography (ISEC) profiles leading to larger pore volumes and porosity, respectively.

In several studies, poly(glycidylmethacrylate-*co*-ethylene dimethacrylate), poly(GMA-*co*-EDMA), monoliths have been employed as reactive supports [85–88,90,92]. They enable straightforward and efficient derivatization with nucleophilic reagents and selectors, respectively, by nucleophilic substitution reaction (SN_2) at the epoxy group. For example, poly(GMA-*co*-EDMA) monoliths were activated with sodium hydrogen sulfide

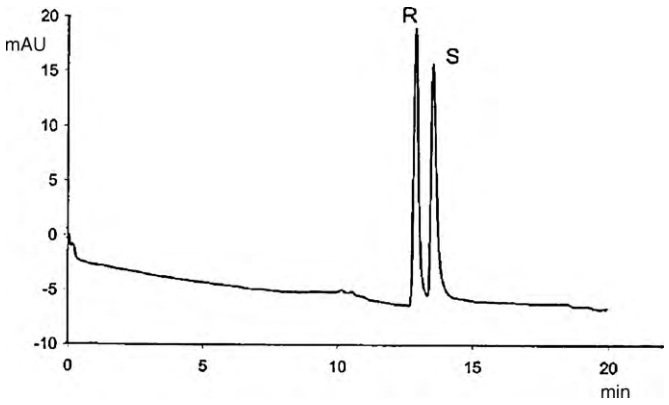

Fig. 14. CEC enantiomer separation of thalidomide in RP mode on vancomycin-modified polymethacrylamide monolithic capillary column. *Experimental conditions*: Capillary, 250 mm × 0.1 mm i.d.; mobile phase, acetonitrile–triethylammonium acetate buffer, pH 6.5 (15:85, v/v); applied voltage, 15 kV; pressure, 10 bar; capillary temperature 15 °C; UV detection, 220 nm. Reprinted with permission from Ref. [13].

to reactive sulfhydryl surface onto which chiral selectors with vinyl group could be immobilized by radical addition reaction (thiol click) (Fig. 13b) [85,86]. *O*-9-(*tert*-Butylcarbamoyl)-quinine selector [85] and (*S*)-*N*-(4-allyloxy-3,5-dichlorobenzoyl)-2-amino-3,3-dimethylbutanephosphonic acid [86] have been bonded this way yielding WAX-type and SCX-type chiral monoliths for enantiomer separation of chiral acids and chiral bases, respectively. The route via thiol monolith has the advantage that it is a chargeless linker: it does not introduce an additional charge that could provide a competitive non-enantioselective binding site under commonly employed weakly acidic to neutral eluents. Moreover, the thiol surface offers a possibility to follow the extent of functionalization [85]. The concentration of active sulfhydryls can be determined photometrically in the capillary by a thiol–disulfide exchange reaction with, e.g. di-2-pyridyldisulfide (DPDS). By differential analysis of sulfhydryls before and after attachment of the chiral selector, also information about the selector coverage can be obtained. Like in the *in situ* approach, the chemical composition of the polymeric backbone turned out to be of prime importance [86]. Incorporation of significant amounts of HEMA into the polymer chains gave rise to a substantial increase of separation factors, however, at expense of a slightly negative effect on the plate numbers.

Similar post-modification approaches of epoxy group containing polymethacrylate monoliths used nucleophilic substitution reaction with amino-modified selectors (Fig. 13c) [87,88,90,92] or with hydroxyls of selectors (Fig. 13d) [90], the former being less nucleophilic than above described thiols but more nucleophilic than the latter hydroxyls. Messina et al. utilized polymethacrylate-based monolithic stationary phases that were prepared from GMA, MMA (methyl methacrylate) and EDMA using formamide and 2-propanol as porogenic solvents [87]. The epoxy groups of such GMA-based monoliths were allowed to react with the primary amino group of the ergot alkaloid derivative (+)-1-(4-aminobutyl)-(5*R*,8*S*,10*R*)-terguride according to the scheme in Fig. 13c. With these novel weak anion-exchange CSPs seven 2-aryloxypropionic acids could be separated by nonaqueous CEC and enantioresolutions between 1.4 and 1.9 could be achieved. An optimized monolithic capillary produced efficient baseline separations with plate numbers of around 100,000 m^{-1} for the herbicide dichloroprop and was utilized to study its stereoselective degradation in soil samples [88].

Recently, a number of CD derivatives have been grafted to poly(GMA-*co*-EDMA) monoliths for CEC application [90,92]. In one study, amino-modified β-CD was decorated with 4-dimethylamino-1,8-phthalimide via an ethyl spacer (see Fig. 15a) and attached to the epoxy groups by nucleophilic substitution via the resultant secondary amino group (see Fig. 15b) [92]. This hybrid selector combines CD moieties for inclusion complexation (esp. under aqueous conditions) or hydrophilic interactions at the rim surface (esp. nonaqueous conditions), a tertiary amino group (anchor nitrogen) for electrostatic interaction and anodic EOF generation, as well as a naphthalimide moiety for π–π-interaction with aromatic groups of analytes. Unlike reported by

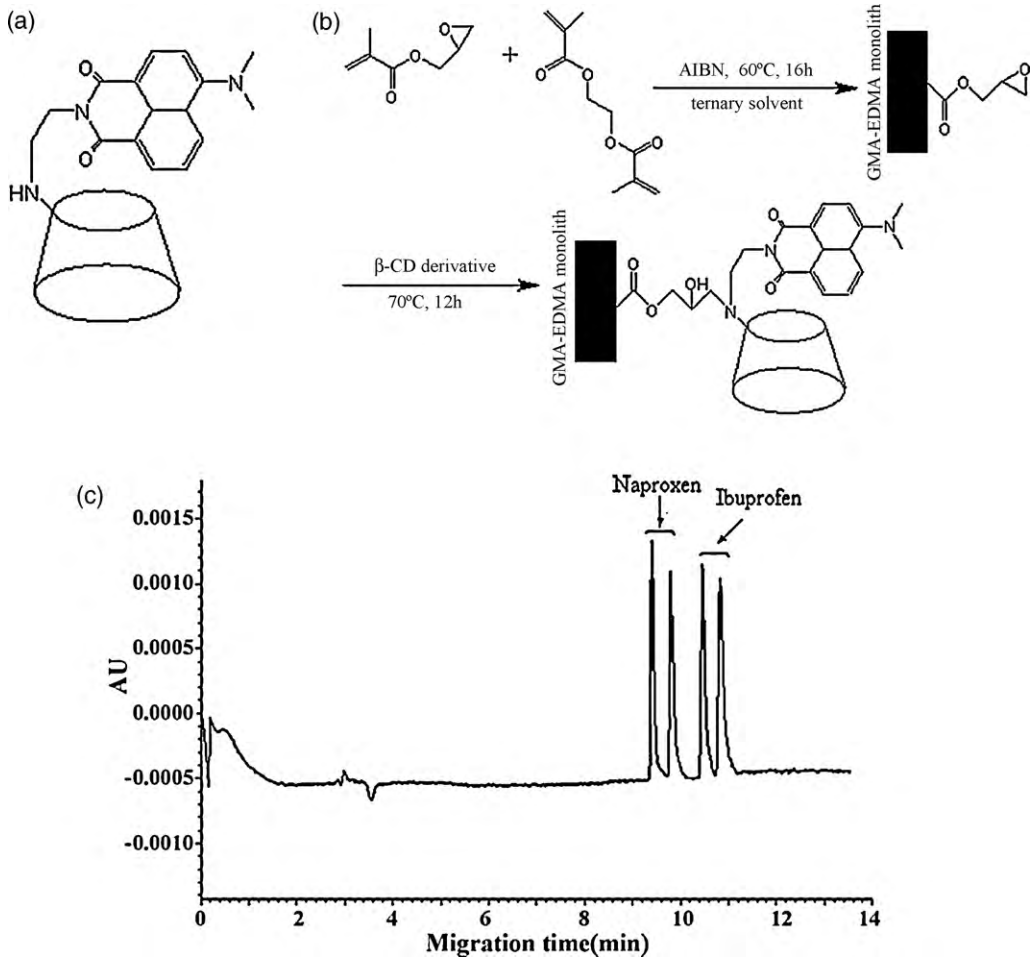

Fig. 15. Hybrid phthalimide-decorated amino-β-CD selector (a), reaction scheme for surface modification of poly(GMA-*co*-EDMA) monolith (b) and CEC enantiomer separation of ibuprofen and naproxen (c). *Experimental conditions in* (*c*): mobile phase, acetonitrile–methanol (70:30, v/v), containing 350 mM acetic acid and 5 mM triethylamine. Reprinted with permission from Ref. [92].

Peters et al. (*vide supra*), post-synthesis treatment with methanolic hydrochloric acid to hydrolyze remaining epoxide groups did not show a significant improvement in resolution. The design of the selector and surface chemistry with the tertiary amino group representing a weak anion-exchange site turned out to be favorable for the CEC enantiomer separation of acidic chiral compounds such as naproxen and ibuprofen, not least due to its generation of anodic EOF for co-directional separation process (EOF and electrophoretic mobility of solutes directed to the same electrode) (see Fig. 15c). In another study, Li et al. compared various immobilization chemistries of CD and its derivatives, respectively, on poly(GMA-*co*-EDMA) monoliths [90]. The strategies tested comprised direct immobilization of amino-β-CD and aspartic acid-modified β-CD (linked via amino group and propoxy spacer to CD) by nucleophilic substitution via amino groups (Fig. 13c) as well as of β-CD and 2-hydroxypropyl-β-CD by nucleophilic substitution via hydroxyl groups (Fig. 13d) onto poly(GMA-*co*-EDMA). Besides, amino-β-CD and aspartic acid-modified β-CD were indirectly coupled to aldehyde-modified polymethacrylate monoliths obtained by a sequence of derivatization reactions comprising aminolysis of epoxy-monoliths, reaction with glutardialdehyde followed by covalent bonding of the amino group containing selector to the aldehyde moieties to yield the corresponding imines and subsequent reduction of the imine group to a more stable amine moiety with sodium borohydride, in accordance with the derivatization scheme in Fig. 13e. The various monoliths were tested for CEC enantiomer separation of phenylalanine. A clear conclusion on which approach was preferred is hard to derive from the results.

The group of Carbonnier focused its interest on another reactive monolith type [91]. Employing UV-initiated free radical polymerization with *N*-acryloyloxysuccinimide (NAS) as functional monomer and EDMA as crosslinker in toluene as porogen, a macroporous reactive organic polymer monolith with pore diameter of about 2.25 μm could be obtained which may serve as support for various surface functionalizations. Click chemistry was then explored as subsequent post-functionalization strategy to derivatize the monolith with β-CD. Thus, poly(NAS-*co*-EDMA) monoliths were derivatized by reaction with propargylamine giving rise to alkyne-modified surface, onto which mono-(6-azido-6-deoxy)-β-CD was covalently attached by Cu(I)-mediated 1,3-dipolar Huisgen cycloaddition reaction via a 1,2,3-triazole ring (Fig. 13f). Baseline separation of flavanone could be achieved with hydroorganic eluent composed of methanol–5 mM borate buffer (40:60, v/v). A slow separation, though resulted and the broad peaks indicated that the morphology and pore structure (esp. macropore diameter) have not been fully optimized yet. As pointed out above, a macropore diameter of 2.25 μm is far from what is usually accepted as optimum which is around 1 μm or lower.

A survey of the literature on CEC enantiomer separation with organic polymer monoliths reveals that the post-modification strategy seems to become the preferred method of choice over the *in situ* approach. One striking advantage is that the post-modification strategy requires only minute amounts of chiral selector. This opens the feasibility of using expensive chromatographic selectors that are accessible in small quantities only. In sharp contrast, substantial amounts of chiral monomer are usually consumed in the course of development and optimization of an *in situ* polymerization protocol. Yet, a critical factor is the degree of surface modification and selector coverage, respectively, in the capillary. A static derivatization protocol is usually preferred over a dynamic modification. In any case, under unstirred conditions the surface coverage seems to be lower than for particles that can be synthesized in stirred reaction vessels. Reliable data on this aspect are, however, seldom reported, because it is not a trivial task to determine the surface concentrations of immobilized chromatographic ligands in a capillary column accurately. Indirect measurements for example have been performed by thiol determination before and after selector bonding with a DPDS test and allowed to derive some information [85]. Another critical factor may be the column-to-column reproducibility, especially if the derivatization scheme comprises a multi-step procedure. Data about this issue were reported by Ding et al. for a single-step coating procedure [93] and Preinerstorfer et al. for a multi-step derivatization (thiolization of epoxy groups and subsequent thiol click reaction) [85]. In both studies, column-to-column repeatability was found to be satisfactory.

3. Silica monoliths

3.1. Sol–gel process and hierarchically ordered porous silica monoliths

Inorganic silica monoliths are prepared by a sol–gel process [94,95]. In the majority of studies that have been published on monolithic silica capillaries for separation science researchers follow a protocol that has been published by Nakanishi and Soga in the early 1990s [95,96]. This process starts from an acidic solution of silica alkoxide precursor such as tetramethoxysilane (TMOS) with appropriate additives and involves acidic hydrolysis and subsequent polycondensation forming finally an inorganic polymer. Surfactants can be used to control phase separation [95]. As pointed out by Nakanishi, both onset of phase separation and sol–gel transition are governed by the kinetics of chemical bond formation. The process of phase separation starts with infinitesimal fluctuations in the solution yielding silica- and solvent-rich regions. When phase separation is induced in the unstable region of a phase diagram, a process called spinodal decomposition occurs and a spongelike, co-continuous structure is formed [97]. The final morphology is obtained by coarsening and fragmentation, yielding finally a gel-network by a dynamical freezing process via crosslinking reactions (sol–gel transition). The "frozen" structure depends on the onset of phase separation relative to the "freezing point" by sol–gel transition. Earlier phase separation in relation to sol–gel transition leads to a more coarse structure.

The morphology and macropore structure, respectively, can be controlled by the starting polymerization conditions, i.e. via the ratios between silica/solvent/additive (usually polyethylene oxide, PEO) (see Fig. 16a). The solvent volume mainly determines the porosity. With increase of the solvent and decrease of precursor, porosity and pore volume may be increased. On contrary, the domain size is controlled by additives such as poly(ethylene oxide) (PEO; polyethyleneglycol, PEG). They can form hydrogen bonds with silanols and thus govern onset of phase separation. The higher the PEO content, the smaller the macropore diameter (Fig. 16a). Overall, it implies that pore volume and macropore size can be independently adjusted. Typical porosities range between 40–80% in a mold with shrinkage and may reach more than 90% in a fused-silica capillary without shrinkage where co-condensation with the capillary wall may occur. Macropore diameters may range between 0.5 and 10 μm depending on the ternary reaction system conditions [95].

Another pecularity of the silica monolith approach is the feasibility to tailor mesopores by post-gelation aging of wet silica gels under basic and/or hydrothermal conditions to obtain hierarchical pore structures. After macropore formation by phase separation and sol–gel transition, the pore liquid can be exchanged with an aqueous ammonia solution, or alternatively, urea can be added in the starting sol from which aqueous ammonia can be generated *in situ* upon heating and decomposition of urea. Dissolution and reprecipitation of silica gel result in the reorganization of smaller pores (micropores) into larger pores, i.e. into sharply distributed

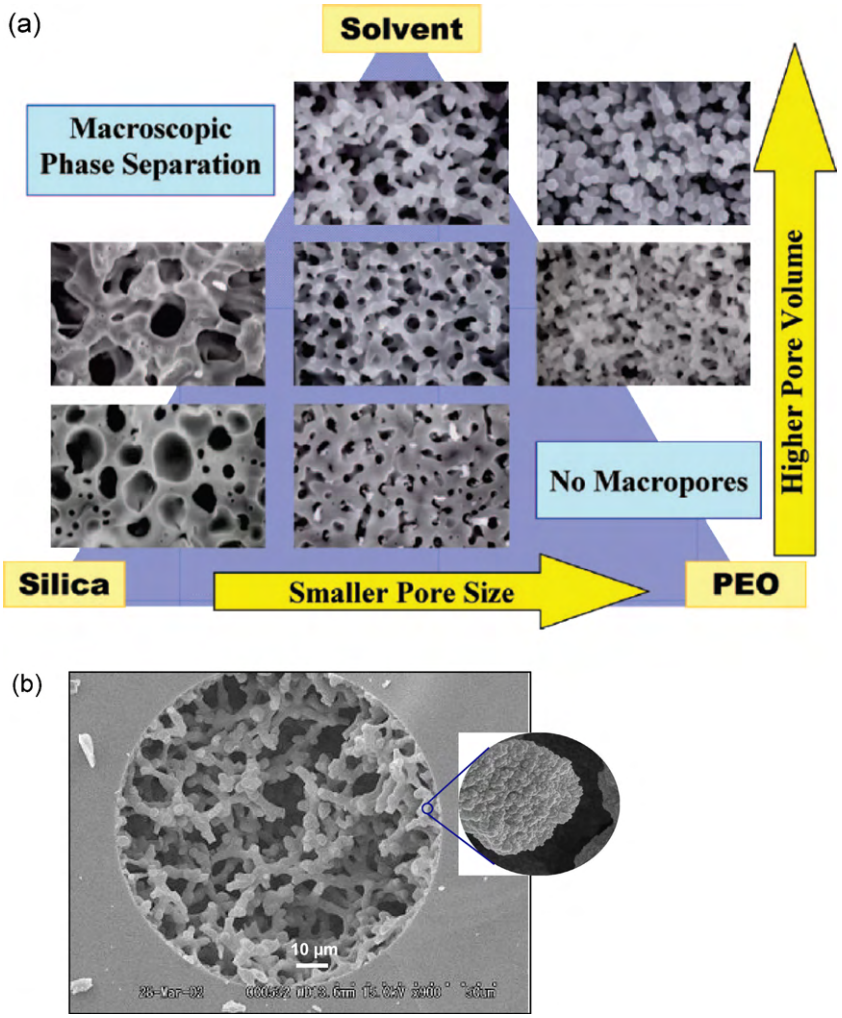

Fig. 16. Morphology of silica monoliths: (a) relationship between the ternary system silica(precursor)/solvent/poly(ethylene oxide) and pore size as well as pore volume of the resultant monolith. (b) Morphology of a monolithic silica capillary as prepared by Merck for Chromolith CapRod Si revealing the spongelike structure and bimodal pore distribution. Reprinted with permission from Ref. [95] and in modified form from Ref. [102].

mesopores (the so-called Ostwald ripening mechanism). After drying steps the typical silica monolith structure and morphology with bimodal pore distribution is obtained (see Fig. 16b).

For capillaries the protocol has been slightly modified to account for the co-condensation and crosslinking of the silica matrix with the fused-silica wall [98]. In most cases, 100 μm i.d. capillaries were employed. Yet, narrower capillaries might be of advantage in terms of attachment of the silica to the fused-silica wall and have therefore been used, e.g. by Zou's group [99–101]. Thereby, the risk for formation of gaps between silica monolith and fused-silica wall is minimized. Modification of the precursor solution by admixing a trimethoxymethylsilane to the commonly employed TMOS has been reported to prevent the formation of voids in fused-silica capillaries [97].

Silica monoliths possess typically a bimodal pore structure consisting of a large number of macropores and a mesoporous silica skeleton with relatively narrow pore size distribution profiles (see Fig. 16b for morphology of a typical silica monolith in a 100 μm ID capillary as prepared by the Nakanishi method [98]) [102]. The macropores with diameters of ~2 μm (or even wider) enable convective bulk mobile phase flow. Along with high total porosities of sometimes >90% they render these materials highly permeable. The mesopores on the other hand provide a large active surface area for chromatographic adsorption, similar to mesoporous silica beads. The thin silica skeleton guarantees short diffusion distances and effective mass transfer. One significant advantage of monolithic silica capillaries over some compressible low-crosslinked organic polymer gel monoliths constitutes their pressure stability which makes them useful for both CEC and nano-HPLC applications [103]. The high permeability of such monolithic silica capillary columns allows their pressure-driven operation with the external pressurization system of common CE instruments [104]. With on-column detection through the silica matrix any extra-column peak broadening effects are definitely eliminated and biases in comparison of CEC and nano-HPLC are avoided. Monolithic silica capillary columns are, moreover, robust and stable which results in enhanced column longevity. Owing to these advantages stationary phases based on monolithic silica materials received a lot of interest over the last years and seem to become the support of choice for small molecule enantiomer separations by CEC.

The major drawback of this type of capillary column is the lengthy and tedious multi-step column preparation. The sol–gel process itself involves multiple steps and the surface functionalization usually requires first an activation of the silica matrix with reactive linkers that enable the bonding of the chiral selector in an additional subsequent step. Pioneering studies in this field on CEC enantiomer separations have been reported by Chen and coworkers [103,105–107], Schurig's group [108] as well as Otsuka and Terabe [109]. A summary on chirally functionalized silica monoliths for enantioselective CEC is given in Table 4.

Table 4
Enantioselective CEC with chiral monolithic silica stationary phases.

Analytes	Chiral selector, activation, and coupling strategy	Sol–gel reaction mixture or silica matrix	Mobile phase (applied voltage)	Maximum efficiency (theor.pl/m)	Ref.
Silica monoliths with post-functionalization					
DNS-amino acids	L-Phe-amide bonded to epoxy-silica monolith	TMOS, PEO (M_r 10,000) in AcOH; ammonia wash, heat treatment (up to 300 °C), epoxy-functionalization	ACN–50 mM NH_4Ac with 0.5 mM $Cu(Ac)_2$ pH 5.5 (70:30, v/v); −300 V/cm	90,000	[105]
DNS-amino acids and hydroxy carboxylic acids like m- and p-hydroxy-mandelic acid (MDA), 3-hydroxy-4-methoxy-MDA, 4-hydroxy-3-methoxy-MDA, 3-(4-hydroxyphenyl)lactic acid, 3-phenyllactic acid, and indole-3-lactic acid	L-Pro-amide bonded to epoxy-silica monolith	TMOS, PEG in 0.01 M AcOH; ammonia wash, heat treatment (up to 300 °C), epoxy-functionalization	ACN–50 mM NH_4Ac with 0.5 mM $Cu(Ac)_2$ pH 6.5 (70:30, v/v); −13.6 kV	17,000	[103]
DNS-amino acids	Cu(II) complexes with L-amino acid amides		pH 5.5, acetonitrile–[50 mM NH_4Ac–0.50 mM Cu(Ac)] (7:3)		[106] [107]
DNS-AAs, benzoin, positional isomers of cresol	β- and γ-CD; on-column modification (covalent bonding *via* a (3-isocyanatopropyl)triethoxysilane linkage)	TMOS, PEG in 0.01 M AcOH; ammonia wash, heat treatment (up to 300 °C)	50 mM MES–Tris/methanol (70/30) buffer at pH 7.0	50,000	[110]
17 Basic drug substances	(*S*)-*N*-(4-allyloxy-3,5-dichlorobenzoyl)-2-amino-3,3-dimethylbutanephosphonic acid; immobilized onto thiol-modified silica monolith	Chromolith Si CapRod, Merck	ACN–MeOH 80:20 (v/v) 25 mM FA, with 12.5 mM AB or 12.5 mM HB or 6.25 mM TEMED		[102]
Phosphinic acid pseudodipeptides	*O*-9-(*tert*-butylcarbamoyl)-quinidine; immobilized onto thiol-modified silica monolith	Chromolith Si CapRod, Merck	ACN/MeOH (50:50, v/v) containing 200 mM acetic acid, 200 mM formic and 4 mM TEA	up to 600,000	[111]
Various chiral bases	trans-(1S,2S)-2-(N-4-allyloxy-3,5-dichlorobenzoyl)amino cyclohexanesulfonic acid; immobilized onto thiol-modified silica monolith	Chromolith Si CapRod, Merck	ACN/MeOH ratios of 100:0, 80:20 and 50:50 (v/v) with AcOH or formic acid (mM) 400–0, 200–200, 0–400	50,000–300,000	[104,112]
2-Aryloxypropionic acid herbicides including inter alia dichlorprop, mecoprop and fenoprop	O-9-(*tert*-butylcarbamoyl)quinidine; immobilized onto thiol-modified silica monolith	Chromolith Si CapRod, Merck	ACN–MeOH (80:20–0:100, v/v) 50–400 mM AcOH; 0.5–4 mM TEA	92,000–43,000	[113]
Amino acid dansyl-derivs., 2-arylpropionic acids and 2-aryloxypropionic acids	(+)-1-Allyl-(5R,8S,10R)-terguride	TMOS, PEG, urea in acidic conditions; post-gelation heat treatment (120 °C for 3 h)	20 mM NH_4OAc (pH 3.6)/ACN (1:1, v/v) pH 3.5–5.5	80 000–100 000	[114]
9 Neutral and six basic compounds	Cellulose tris(3,5-dimethylphenylcarbamate) (CDMPC); on-column modification (coating with 6% solution of SO in acetone)	TMOS/PEG/acetic acid	ACN/phosphate buffer (2 mM) with different pH (40/60; 64/40, v/v) ACN/2 mM NaH_2PO_4–10 mM DEA, pH 9.60 (40/60, v/v), MeOH/5 mM NH_4OAc	230,000–20,000	[101]
Tröger's base, tetrahydropalmatine, pindolol, indapamide, benzoin, praziquantel	Covalent bonding of cellulose tris(3,5-dimethylphenylcarbamate) (CDMPC)	Sol–gel process	40% ACN and 4 mM phosphate buffer or TEA–phosphate buffer at pH 6.8	42,000–7000	[100]

Table 4 (*Continued*)

Analytes	Chiral selector, activation, and coupling strategy	Sol–gel reaction mixture or silica matrix	Mobile phase (applied voltage)	Maximum efficiency (theor.pl/m)	Ref.
Tröger's base; indapamide; benzoin; *trans*-stilbene oxide; oxazepam; pindolol; mebeverine; alprenolol; propranolol; ambucetamide; warfarin	Cellulose tris(3,5-dimethylphenylcarbamate) (CDMPC); CDMPC immobilized by polycondensation of the cellulose deriv. contg. a triethoxysilyl group, or on a vinylized silica monolith through radical copolymn. of the cellulose deriv., which also contained a vinyl group	Sol–gel process	2 mM H_3PO_4 or 10 mM DEA adjusted with 1 M NaOH (pH range 5–9.5)/ACN (60/40, v/v).		[115]
β-Blockers, terbutaline, thalidomide benzoin	Vancomycin; immobilized by reductive amination	Sol–gel process	MeOH/ACN/CH_3COOH/TEA (80:20:0.1:0.1 by volume); 10 mM TEA phosphate buffer containing 20% ACN at pH 6.5	208,000–43,000	[99]
Neutral, acidic and basic racemates (e.g. warfarin, thalidomide)	Norvancomycin (NVC), by a one-step *in situ* derivatization process		Different content of ACN/1% (v/v) TEAA buffer 15:85 (v/v)	180,000	[116,117]
THP, TB	MIP coating by copolymerization with γ-MAPS-modified silica monolith from MAA, EDMA or TRIM and ACN; L-tetrahydropalmatine (L-THP) and (5*S*,11*S*)-(−)-Troeger's base (S-TB) as the imprinted template	TMOS/HCl/PEG	ACN/5–20 mM acetate buffer (75:25–95:5, v/v) (pH 4.0–7.0)		[70]
Silica monoliths with in situ incorporation of chiral selector sites					
D,L-Trp	BSA; *in situ* encapsulation of BSA into silica matrix	TMOS/HCl or TMOS/MTMS/HCl	5, 20, 40 and 50 mM Phosphate buffer (pH 5–8.0)	22,000–9000	[118–120]
DNS-Leu, baclofen	β-CD, *in situ* incorporated	TMOS and an organofunctional silicon alkoxide that contains -CD	0–40% Organic modifier (MeOH, ACN, EtOH) and/100–60% phosphate buffer (50 mM, pH 7.2)	158,000–25,000	[121]

3.2. Surface functionalization of silica monoliths

Silica monoliths are commonly functionalized by virtually the same strategies as employed for particulate silica. Amongst them, adsorptive and covalent bonding of a chiral selector has been reported for the preparation of chirally functionalized silica monoliths for CEC application. Besides, surface imprinting on the silica monolith has been proposed as another route to enantioselective monolithic capillary columns [70].

Adsorptive bonding of chiral selectors avoids complicated multistep immobilization procedures, yet is amenable preferentially for polymeric selectors. Thus, a sol–gel derived silica monolith was statically coated by Schurig's group with Chirasil-β-Dex (Fig. 17a) which represents a chiral polymer prepared by grafting permethyl-β-cyclodextrin to polymethylsiloxane with an octamethylene spacer [108]. Subsequent heat treatment at 120 °C rendered the polymeric selector non-extractable affording a stable immobilized CSP. Various enantiomers were well resolved by CEC with the EOF originating from residual silanols (see Fig. 17b).

Liu et al. proposed a physical adsorption method for the preparation of avidin coated monolithic silica capillary columns [109]. Column fabrication comprised two steps: (i) activation, and (ii) protein coating. Activation was performed by rinsing with 150 mM ammonia solution for 120 min, followed by water for 60 min. In the second step, a 50 μM avidin solution in 10 mM phosphate buffer (pH 5.95) was pumped through the column and then the column was washed with 10 mM phosphate buffer (pH 5.95) for 120 min. The adsorbed amount of avidin was measured by frontal analysis. It was found that the optimized monolithic silica column with physically adsorbed avidin has a surface coverage of about 9.4×10^{-9} mol/m^2, which is in the same order as reported for silica beads with adsorbed and covalently bonded avidin. The surface coverage corresponded to about 14% of the estimated specific surface area of the monolith, which is much lower than that achieved in an open fused-silica tube with the same selector (46%). It was concluded that the reason is inaccessibility of the smaller pores (<5 nm) so that only a small part of the silica monolith surface is available for adsorption. Whatsoever, the physically adsorbed avidin column allowed the enantiomer separation of various acidic and basic chiral compounds in pressure-driven and electrically driven chromatography mode, with electrophoretic migration being the driving force for analyte migration through the column in CEC. EOF turned out to be low under employed conditions (pH ~ 6) due to effective coating of silanols, which in turn prevented the efficient analysis of neutral chiral compounds by CEC. Drifting baselines, though, may be an indication for a slight bleeding of such columns which may be accompanied by a low column stability and system robustness.

Column fabrication by coating of polymeric selectors onto the surface of monolithic capillaries was also the method of choice for polysaccharide selectors [101]. Zou's group [101] followed a

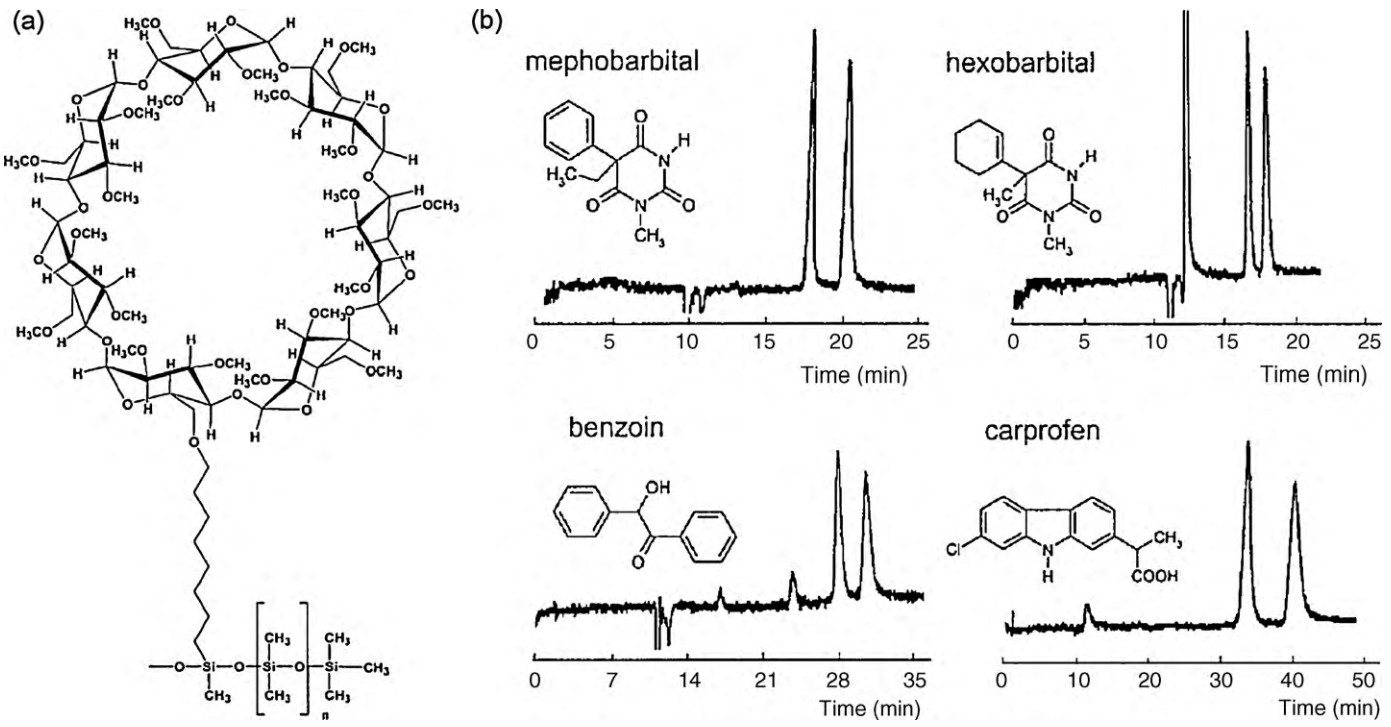

Fig. 17. (a) Structure of Chirasil-β-Dex. (b) Separation of enantiomers on the Chirasil-Dex monolithic column. Conditions: fused-silica capillary column, 25 cm (effective length) × 50 μm i.d.; UV detection was performed at 210 nm. For mephobarbital, hexobarbital and benzoin: mobile phase, MES–Tris buffer (pH 6)/methanol (90/10, v/v); applied field strength, 0.4 kV/cm; samples injection at 3 kV for 4 s; carprofen: mobile phase, MES–Tris buffer (pH 6)/methanol (60/40, v/v); applied field strength, −0.4 kV/cm; sample injection, at −3 kV for 4 s. Reprinted with permission from Ref. [108].

coating procedure for the non-covalent immobilization of cellulose tris(3,5-dimethylphenylcarbamate) (CDMPC) published earlier by Chankvetadze et al. for (capillary-)HPLC application using a solvent evaporation protocol from acetone solution [122]. The capillary was filled with constant pressure for 60 min with a solution of CDMPC in acetone (30, 60, or 90 mg/mL). A drying step first at ambient conditions over 4–7 days and subsequently under vacuum for 2 h at 40 °C followed. The obtained coated polysaccharide capillaries (50 μm i.d.) with 60 mg/mL showed highly efficient separations in CEC for a variety of test compounds, while enantioselectivity was poor for capillaries made with the 30 mg/mL solution. With 90 mg/mL no EOF could be measured in CEC. Up to 240,000 theoretical plates/m have been reported on a 20 cm long optimized monolith prepared from 60 mg/mL CDMPC solution (Fig. 18a). Short-end injection with 10 cm bed length and high voltage (30 kV) provided extremely fast separations (Fig. 18b).

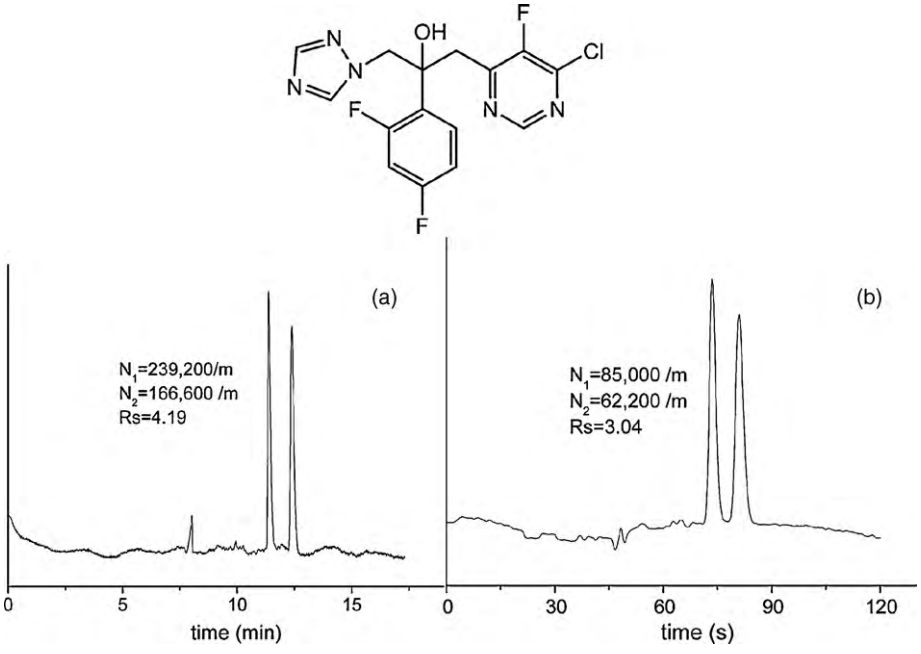

Fig. 18. Electrochromatograms for resolution of the enantiomers of a drug candidate on monolithic silica capillary with coated cellulose tris(3,5-dimethylphenylcarbamate) as chiral selector. Mobile phase: acetonitrile/phosphate buffer (2 mM, pH 6.80) (60:40, v/v). Capillary: 30.2 cm (total length) × 50 μm i.d.; (a) effective length, 20 cm; voltage, 10 kV. (b) Effective length, 10.2 cm; voltage, 30 kV. Reprinted in modified form from Ref. [101].

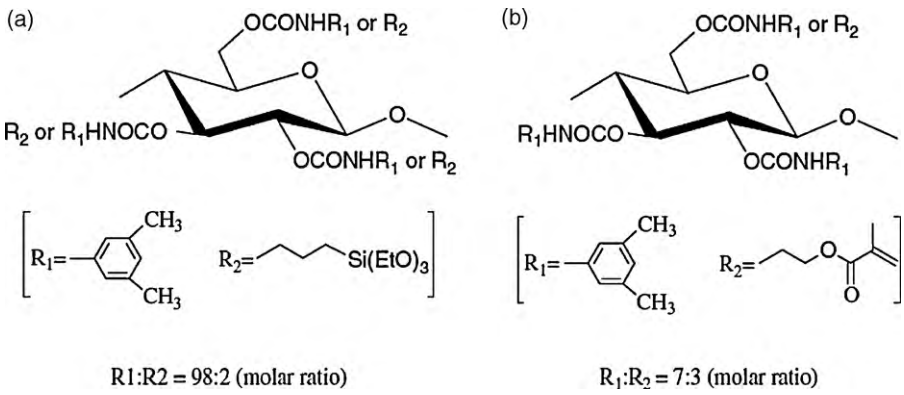

Fig. 19. Cellulose carbamate derivatives containing 3-triethoxysilylpropyl (a) and 2-methacryloyloxyethyl residues for covalent immobilization on silica matrix. Reprinted with permission from Ref. [115].

More recently, silica monoliths with immobilized polysaccharide derivatives were proposed for capillary formats as well [100,115,123]. Zou and coworkers [100] followed a protocol described earlier by Chankvetadze et al. for the fabrication and evaluation of a covalently bonded cellulose tris(3,5-dimethylphenylcarbamate) monolithic silica capillary column [124]. First, cellulose 2,3-bis(3,5-dimethylphenylcarbamate) was coated from acetone solution (60 mg/mL) onto the surface of 3-glycidoxypropyl trimethoxysilane-modified silica monolith. After drying, a 10% solution of BF_3-etherate in dry toluene was filled into the capillary as catalyst to promote the covalent attachment of the residual hydroxyls of the cellulose derivative to the epoxy groups by nucleophilic substitution. After acetone washing and drying, unreacted hydroxyls of the polysaccharide were carbamoylated with 3,5-dimethylphenylisocyanate in pyridine to yield after washing and drying the final covalently linked cellulose tris(3,5-dimethylphenylcarbamate). Although with worse performance than the above described coated analogs, such immobilized polysaccharide monolithic silica capillaries could successfully separate the enantiomers of several test compounds by CEC. Vander Heyden and coworkers proposed two different linking chemistries for the immobilization of cellulose carbamate selectors onto the surface of monolithic silica capillaries [115]. In one approach, the polysaccharide derivative (CDMPC analog) containing a small percentage of triethoxysilylpropyl groups instead of 3,5-dimethylphenyl residues were coated and then immobilized through polycondensation employing a solution of trimethylsilylchloride in water and ethanol (Fig. 19a). In another linking strategy, a certain number of methacrylic groups were introduced in 6-position, coated onto vinyl-modified silica monolith surface and then crosslinked by free radical copolymerization using styrene as comonomer and AIBN as radical initiator (Fig. 19b). The great benefit of such immobilized polysaccharide columns is their ability to use "non-standard solvents" such as THF, dioxane, chloroform, dichloromethane, ethylacetate which may dissolve polysaccharide derivatives and strip off the selector from the surface of coated columns. For CEC, however, this advantage appears to be of less importance since normal-phase conditions making use of such solvents are less favorable for this electrokinetic technique, as they provide less stable current and low EOF.

A serious drawback of all these approaches with coated as well as coated and then immobilized neutral polymeric selectors is their weak EOF. The residual silanols are responsible for EOF generation and they are efficiently covered by the polymeric coating which makes separations, in particular of neutral analytes, with such columns usually slower than in HPLC mode. Moreover, it has also to be pointed out once more that surface functionalization by coating is, of course, not a viable route for low-molecular mass selectors that would be easily desorbed from the column under common CEC run conditions.

For the immobilization of such low-molecular selectors, activation of the silica surface with bifunctional linkers such as (tri)alkoxysilanes with reactive functional groups is required before covalent attachment of the selector. Common activation chemistries involve silanization with 3-glycidoxypropyl silane [99,105,110,113], 3-mercaptopropyl silane [102,104,111,112,114], γ-methacryloyloxypropyl silane [70], and 3-isocyanatopropyl silane [110,116].

In a series of papers Chen et al. immobilized various L-amino acid amides and L-hydroxyproline as chiral selectors onto 3-(glycidoxypropyl)trimethoxysilane-modified silica monoliths *via* nucleophilic attack of amino groups of the amino acid selectors at the epoxy ring [105]. Thus, L-phenylalanine amide (L-PheA) [105], L-proline amide (L-ProA) [103] and L-alanine amide (L-AlaA) [106] modified silica monoliths were obtained. Addition of copper acetate to the running buffer resulted in the formation of ternary mixed Cu^{II}-complexes between chiral selector and solutes (various dansyl-amino acids) which enabled enantioselective ligand-exchange CEC and CLC. In CEC, enantioselectivity on the three different CSPs was found to be in the order L-PheA > L-ProA > L-AlaA using 50–100 mM ammonium acetate buffer (pH 5.5–7.6) containing 70% acetonitrile and 0.5 mM Cu(II)acetate. Later, in another study also hydroxyproline immobilized to 3-(glycidoxypropyl)trimethoxysilane-modified silica monoliths was tested as selector in enantioselective ligand-exchange CEC for dansyl amino acids, free amino acids and some other solutes [107].

In a later study, the same group reported on cyclodextrin-modified silica monoliths [110]. Monolithic silica capillaries were treated with (3-isocyanatopropyl) trimethoxysilane and the resultant 3-isocyanatopropyl-modified silica monoliths were derivatized with β- or γ-cyclodextrin yielding bonded CD-monoliths with stable carbamate linkages [110]. These capillaries afforded enantioselective separations of dansyl amino acids (DNS-AAs) and benzoin. Employing the γ-CD-modified CSP, the enantiomers of tested DNS-AAs could be separated in the negative polarity mode using a mixture of 50 mM MES-Tris (pH 8) and methanol (60:40, v/v) as mobile phase in a counterdirectional separation process in which the cathodic EOF (originating from residual silanols) was outbalanced by self-electrophoretic migration of the anionic solutes. A low residual silanol concentration may be favorable in that case. Overall, the separations were slow, probably because of the counterdirectional setup regarding EOF and electrophoretic migration of the negatively charged solutes as well as strong solute–sorbent interactions driven by inclusion complexation of hydrophobic solutes such as DNS-amino acids under

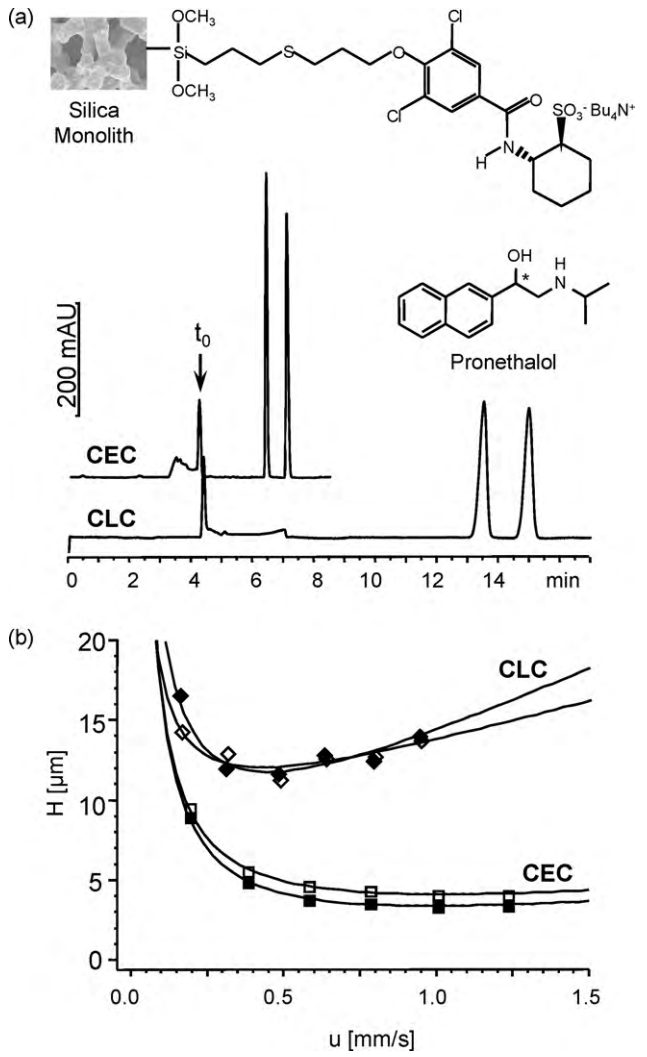

Fig. 20. CEC and CLC enantiomer separations of pronethalol (a) and *H–u* curves of mefloquine (b) on a monolithic strong chiral cation-exchanger CSP. Deconvoluted migration contributions for pronethalol in CEC (a): $\mu_{eo} = 26.7 \pm 0.9 \times 10^{-5}$ cm^2 V^{-1} s^{-1}; $\mu_{ep,eff} = 22.4 \pm 0.6 \times 10^{-5}$ cm^2 V^{-1} s^{-1}; $k_{CLC,1} = 1.96 \pm 0.02$; $k_{CLC,2} = 2.28 \pm 0.02$; $\mu_{CEC,1} = 17.6 \pm 0.4 \times 10^{-5}$ cm^2 V^{-1} s^{-1}; $\mu_{CEC,2} = 15.8 \pm 0.30 \times 10^{-5}$ cm^2 V^{-1} s^{-1}.
Experimental conditions: mobile phase, ACN–MeOH (80:20, v/v) containing 25 mM formic acid and 12.5 mM 2-aminobutanol; applied voltage, 20 kV (a) and 4–24 kV (b), respectively; electrokinetic injection at 10 kV/10 s; capillary temperature, 20 °C; UV detection at 216 nm; capillary dimension, L_{tot} = 33.5 cm, L_{eff} = 25 cm. (b) CLC: applied pressures, 2–12 bar. Reprinted in modified form from Refs. [112] and [104].

employed highly aqueous-based eluents. Only two DNS-AAs were resolved with the β-CD CSP.

Vinyl-group containing chiral selectors can be readily immobilized in a controlled manner and with good yield to thiol-modified silica monoliths by radical addition reaction (thiol click). Thus, (*S*)-*N*-(4-allyloxy-3,5-dichlorobenzoyl)-2-amino-3,3-dimethylbutane phosphonic acid [102] and trans-(1*S*,2*S*)-2-(*N*-4-allyloxy-3,5-dichlorobenzoyl)amino cyclohexanesulfonic acid [104,112] have been covalently bonded to the activated support to yield chiral cation-exchange type monolith capillaries for enantiomer separation of chiral bases (Fig. 20). In these cases, the utilized monolithic capillaries were research samples of the Chromolith Si CapRod from Merck (Fig. 16b). It can be seen from the SEM image that the monolith is well adhering to the fused-silica wall without formation of gaps (Fig. 16b). The skeleton thickness may be assessed to around 1.5–3 μm leading to short diffusion path lengths in the mesopores of the skeleton which is associated with efficient mass transfer. ISEC experiments performed on the 100 μm capillaries in comparison to 4.6 mm i.d. analogs (with 1.9 μm macropore diameters) proved the existence of a bimodal pore distribution with both macropores and mesopores, and revealed that the mesopores had diameters (ca. 16 nm) comparable to those of the commercial 4.6 mm i.d. monolith (13 nm) [102]. The surface concentration of active sulfhydryls on the thiolated-monolith in the capillary was monitored by thiol/disulfide exchange reaction with DPDS and quantitative spectrophotometric measurement of liberated pyridine-2-thione [85,102]. This photometric assay was helpful for the optimization of the surface coverage of the monolith with thiol and selector functionalities, respectively. The developed cation-exchange type monolithic capillary columns showed excellent performance with nonaqueous eluents (acetonitrile–methanol based 80:20, v/v) especially in CEC mode (see Fig. 20). A significant advantage of these ion-exchange CSPs is that the EOF is mainly governed by the ionic selector and maintained under acidic conditions as well. Due to same signs of the charges in solutes and counterions a faster co-directional separation process resulted with same migration directions for analytes and EOF. In spite of electrophoretic contributions, the ion-exchange mechanism is the dominant process in such separations. Thus, effective counterions like 2-aminobutanol or TEMED are favorable to accomplish reasonably fast and highly efficient separations in this SCX-type system [102]. Theoretical plate counts of as low as 3.5 μm, corresponding to about 300,000 theoretical plates per meter, could be obtained by CEC for the amino sulfonic acid-based SCX-type monolithic silica columns (Fig. 20b). The very flat *H*/*u* curves under CEC conditions indicate an efficient mass transfer in such monolithic columns. It is also evident from Fig. 20a that in such cation-exchange CEC systems even for basic solutes highly symmetric peaks may be obtained. In the pressure-driven mode, plate heights were significantly larger (e.g. ~factor of 3). It is also worth mentioning that the sulfonic acid selector turns out to exhibit better efficiencies in comparison to corresponding phosphonic acid selectors for which plate numbers were typically below 90,000 m^{-1} [102]. Desorption kinetics of the cationic solutes from the bivalent phosphonic acid ion-exchange site may be slow under CEC conditions. Due to absence of concentration polarization under employed conditions, individual migration contributions could be derived by deconvolution [104]. For the separation of pronethalol shown in Fig. 20, the corresponding values can be found in the caption of Fig. 20.

Following the same concept, chiral anion-exchange type monolithic capillaries have been proposed, utilizing the same thiol click surface functionalization with either *O*-9-(*tert*-butylcarbamoyl)quinidine (tBuCQD) [111], *O*-9-(*tert*-butylcarbamoyl)quinine (tBuCQN) [113], or (+)-1-allyl-(5*R*,8*S*,10*R*)-terguride [114]. With (weakly) acidic eluents, such weak anion-exchange type monolithic silica capillaries generate anodic EOF due to positive net charge on the surface enabling for a favorable co-directional separation. These capillaries are therefore operated in the negative polarity mode employing nonaqueous polar organic or hydroorganic reversed-phase conditions. Enantiomer and diastereomer separations, respectively, of phosphonic acid pseudodipeptide (*R*,*S*)-H-hPheψ(PO$_2$CH$_2$)-(*R*,*S*)-Phe-OH as *N*-benzyloxycarbonyl-derivative with C-terminal methylester as well as *N*-2,4-dinitrophenyl (DNP) derivatives on tBuCQD-modified silica monolith [111], of various aryloxycarboxylic acids on tBuCQN-modified silica monolith [113], and of dansyl amino acids, aryloxy and aryl carboxylic acids on terguride-derivatized silica monolith [114] were reported. Plate numbers in the order of 80,000–100,000 m^{-1} were commonly observed with these anion-exchange monolithic capillaries employing acetate as counterion [111,113,114], yet sometimes exceeded 100,000 m^{-1} [111]. Messina et al. assessed also the column-to-column repeatability

and found RSD values of around 8% for retention times and 0.2% for separation factors which is quite acceptable considering the multi-step synthesis procedure [114].

Also monolithic silica capillary columns with glycopeptide selectors have been developed and described in the literature [99,116,117]. Zou and coworkers adapted the surface modification strategy described above via reductive amination [99]. Thus, epoxy groups of 3-glycidoxypropylsilane-modified silica monolith capillaries were hydrolyzed with 10 mM HCl for 6 h, washed and the diol surface oxidized with sodium periodate to yield aldehyde groups. Onto the aldehyde groups the vancomycin (with secondary amine anchor) was bonded by reductive amination employing sodium cyanoborohydride as reducing agent. Various β-blockers could be resolved in the polar organic mode with plate numbers between 50,000 and 200,000 m^{-1}, and benzoin and thalidomide in the reversed-phase mode with 50,000–130,000 m^{-1} [99]. In a simplified procedure norvancomycin was immobilized instead of vancomycin, the former having a leucine substituted for a *N*-methylleucine, by a single-step surface modification employing the bifunctional linker silane 3-isocyanatopropyltriethoxysilane [116]. Thus, a reaction mixture was prepared from norvancomycin and 3-isocyanatopropyltriethoxysilane in dry DMF and pyridine (2:1, v/v), heated for 1 h to 70 °C and then introduced into the monolithic capillary column where reaction was allowed to proceed for another 8 h at 70 °C. Norvancomycin is thereby bonded to the silica surface via urea and carbamate linkages, respectively, having a propyl spacer. The obtained capillaries showed useful separations and were later tested for analysis of thalidomide in spiked human urine samples employing on-line preconcentration by hyphenation of octyl and norvancomycin bonded monoliths [117].

Surface-imprinting on silica monoliths was proposed as another approach towards enantioselective monolithic capillaries. Thus, MIP films were prepared from MAA as functional monomer, TRIM or EDMA as crosslinker, for tetrahydropalmatine (THP) and Tröger's base (TB), respectively, as templates with ACN as porogen directly on the surface of γ-methacryloyloxypropyl-modified silica monolith by copolymerization and simultaneous crosslinking in the course of the imprinting process [70]. Although this surface-molecular imprinting process on a silica monolith column was successful, the results were not groundbreaking in terms of efficiency. Performances were not much better than for MIP-type polymethacrylate monoliths. Obviously, polydispersity of binding sites and mass transfer limitations still persisted also in this approach.

3.3. *In situ* preparation of functionalized silica monoliths

Enantioselective silica monoliths are commonly obtained by post-functionalization with chiral selector sites as outlined above. Though, few attempts have also been made to prepare monolithic silica CSPs *in situ* by single-step concepts.

Protein-encapsulation into a sol–gel matrix during its formation constitutes such an *in situ* preparation approach [118,120,125]. The protein (bovine serum albumin, BSA, or ovomucoid) was admixed to a fully or partially hydrolyzed silica precursor, injected into the capillary and the protein finally trapped in the silica network. In the initial study, sucessful separations of Trp could only be obtained with a gel which was made from TMOS and MTMS (methyl trimethoxysilane), but not with gels made from TMOS alone [125]. Column fabrication also involved an aging step of the gel for about 3 days after which the proteins were completely encapsulated in the gel network. Separation factor and efficiency for the first eluted enantiomer of Trp were both satisfactory, yet the second eluted peak revealed a low plate number and a strong tailing.

Various experimental parameters influencing the gelation process such as pH and ionic strength of the buffer, ratio of hydrolyzed sol–gel solution and buffer as well as BSA concentration were investigated in a subsequent paper [118]. It was found that the choice of starting conditions had a major impact on the number of residual silanol groups and hence on EOF. ATR-FT-IR (attenuated total reflection-Fourier transform-infrared) spectroscopy was used to continuously measure the amount of silanol groups during gelation on the one side, and alterations in the conformation of the protein due to encapsulation on the other side. Thus hydrolysis and polycondensation as well as silica network formation was followed by characteristic IR vibrations for the silanol group (indicative for TMOS hydrolysis), for methanol (which is formed by TMOS hydrolysis or condensation), and for the siloxane group (indicating silica matrix formation). It was noticed that the silanol band became weaker while the siloxane band became stronger with progressing gelation time. After 3 days, the relative intensities remained essentially constant which was taken as indication of completion of the process. Since amide I and amide II bands, both characteristic frequencies for protein conformation, were virtually unaltered in the course of the process, it was concluded that conformation and thus biological activity of the encapsulated protein was maintained in the silica gel. Further, dynamic light scattering measurements were performed during the sol–gel process to follow the variation of the diameters of the silica in dependence of time as the colloid formation and sol–gel transition occurred. Maximal diameters of the silica spheres were found around the gelation point and the presence of BSA in the monomer solution had no significant effect (ca. 1.3 μm). Successful CEC enantiomer separations of Trp could be obtained. Later, a similar methodology was employed by encapsulation of BSA in a hybrid sol–gel/organic polymer matrix (with gelatin or chitosan as organic polymer materials) without evident striking advantage in terms of chromatographic performances [119].

More recently, another *in situ* column fabrication approach was proposed. CD-derivatized trialkoxysilane precursors (see Fig. 21a) were synthesized and admixed as co-precursor to the sol made up from TMOS, PEG and acetic acid [121]. The sol–gel process was then allowed to proceed in accordance with the Nakanishi protocol including subsequent solvent exchange and aging steps. The chiral CD selector was thus *in situ* incorporated in the gel matrix. The morphology of the obtained silica material revealed a globular structure with skeletons of about 3.1 μm and throughpores of around 10 μm. Nitrogen adsorption–desorption measurements showed that mesopores with about 6 nm were present in the silica matrix. CEC enantiomer separations could be accomplished for dansyl-leucine and baclofen enantiomers (Fig. 21b) which proved the usefulness of this approach.

3.4. Comparison of silica monoliths with organic polymer monoliths and packed particulate columns

For readers it might of interest to receive information on how the different monolith approaches compare with each other and which one provides the best performance in terms of enantioselectivities and efficiencies.

Information in this regard can be derived from work by Preinerstorfer et al. who reported on CEC characteristics of capillary columns that were based on different supports, viz. monolithic and particulate silica as well as monolithic polymethacrylate, yet always functionalized by thiol-activation followed by radical addition reaction with *N*-(4-allyloxy-3,5-dichlorobenzoyl)-2-amino-3,3-dimethylbutanephosphonic acid [102]. Fig. 22 depicts chromatograms of the CEC separation of *O*-(*tert*-butylcarbamoyl)mefloquine. With all three types of capillaries, i.e. silica monolith (Fig. 22a), packed particulate bed (Fig. 22b) and organic polymethacrylate monolith (Fig. 22c), baseline separations could be achieved. However, the capillary packed with the modified silica beads exhibited the best enantioselectivity

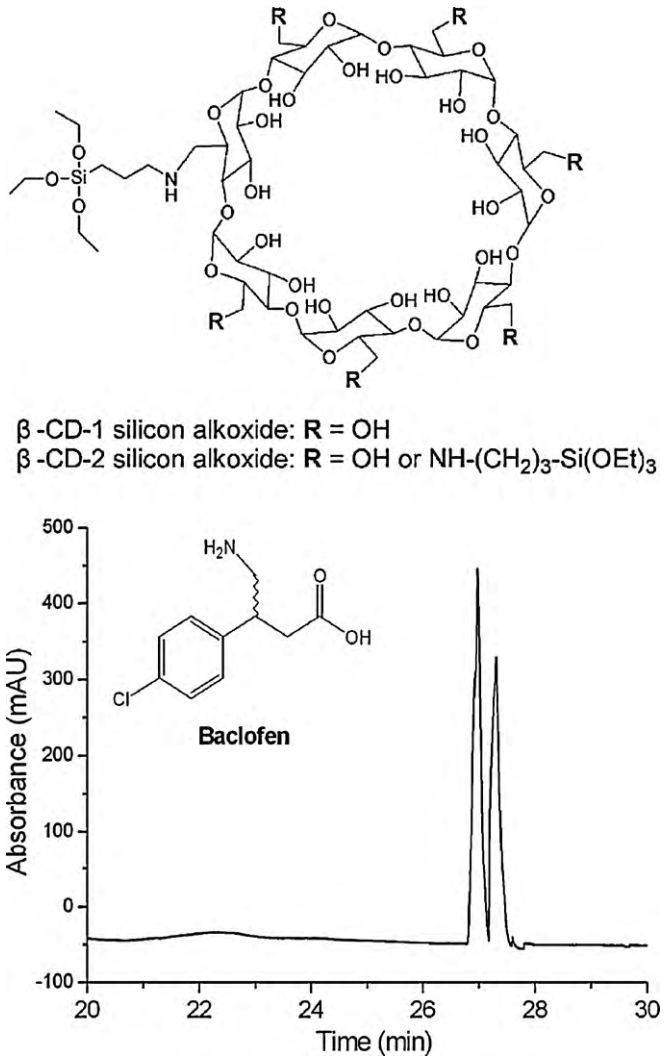

Fig. 21. *In situ* CD-modified chiral silica monoliths: (a) CD-modified trialkoxysilane precursor used for sol–gel process and (b) CEC enantiomer separation of baclofen on β-CD-2 column. *Experimental conditions*: capillary, 25 cm (overall length 33.5 cm) × 75 μm i.d.; detection wavelength, 214 nm; 15 °C; 2 bar pressure support; mobile phase, TEAA buffer (1%, pH 7.0)/acetonitrile (70:30, v/v); applied voltage, 10 kV; sample injection, 10 kV, 5 s. Reprinted with permission from Ref. [121].

and a stronger EOF, although the ionic strength was higher by a factor of two. The 100 Å particles have a large surface area (300 m^2/g). Since the chemical modification of the beads could be carried out in a stirred reaction vessel, the surface could be efficiently covered with selector moieties (about 0.21 mmol selector/g CSP) [126]. This explains the long retention times and higher retention factors. The monolithic silica backbone has mesopores (ca. 16 nm [102]) and thus a larger surface area than the organic polymer monolith support which is devoid of a tailored mesopore structure and has usually surface areas less than 10 m^2/g [36]. However, due to the large total porosity (>90%) the amount of silica in the capillary is limited and therefore the totally available adsorption surface restricted compared to the packed capillary. It is thus no surprise that the retention factors are lower than for the packed column. Moreover, the two-step surface modification may have yielded suboptimal selector coverage which may be another factor for the lower retention and enantioselectivity for the silica monolith capillary. Later experiments with a different selector have confirmed that improvements in selector coverage can bring a further enhancement of enantioselectivities [104]. The organic polymer column obtained by post-modification of a poly(GMA-*co*-HEMA-*co*-EDMA) monolith with the supposedly lowest surface area provided the lowest retention factors and the fastest separation. Shortcomings in terms of homogeneity of the monolithic polymer bed, non-uniform distribution of the selector on the monolith surface and non-equal adsorption–desorption kinetics at chiral and non-chiral adsorption sites may have all contributed to lower theoretical plate numbers as compared to the silica materials.

A similar comparison of capillaries with distinct supports based on glycopeptide selectors (vancomycin and norvancomycin, respectively) are fully in agreement with the above outlined trends and conclusions [116]. Ding and Tang compared the performance of their norvancomycin-derivatized silica monolith capillary with that of earlier reported packed vancomycin-bonded particulate column [127] as well as with vancomycin-derivatized polyacrylamide-type organic polymer monolith [13] (all obtained by post-functionalization with the glycopeptide selector and employing comparable RP-type mobile phase conditions, i.e. acetonitrile–triethylammonium acetate buffer). Resolutions for thalidomide and warfarin were little lower with the silica monolith capillaries compared to the packed column analogs, yet better than for the polyacrylamide monoliths. For example, R_S values for thalidomide enantiomers were reported to reach 3.7 for the norvancomycin-bonded silica monolith column, 13.0 for the vancomycin-bonded particulate (5 μm) packed capillary and 0.6 on vancomycin-derivatized polyacrylamide monolithic capillary column [116].

Overall, considering all factors the silica monolith technology is expected to be the most promising one for CEC enantiomer separation of low-molecular compounds, and the increasing number of papers in this field seems to confirm this assessment.

4. Monoliths made from particles

Monoliths made from particles represent a hybrid form of packed and monolithic columns. Such monoliths have been first proposed for enantioselective CEC by Lin and coworkers in 1996 in the form of capillaries packed with MIP particles that were embedded in a polyacrylamide matrix [128] and later by Chirica and Remcho who employed a similar approach but embedded MIP particles in a silicate matrix [129]. In another early paper Schurig's group proposed the sintering of silica particles of a packed bed over its entire length into a consolidated silica monolith [130]. In a second step, the consolidated silica monolith was derivatized with chiral selector moieties by a subsequent coating of a polymeric selector such as Chirasil-Dex, a dimethylpolysiloxane derivative bearing permethylated β-cyclodextrin moieties. This column showed a good performance in CEC for a number of chiral aromatic compounds. The complexity of column fabrication as well as limitations in terms of column-to-column reproducibility may have been the reasons that such type of monolithic columns disappeared soon thereafter.

Two other concepts, however, attracted more interests. In one (high particle density approach), chiral particles are densely packed into fused-silica capillaries like with common packed column technology and then the packed bed is stabilized by some kind of matrix, usually a sol–gel matrix. Modified silica-based particles possess residual silanols which may participate in the sol–gel process leading to crosslinking by co-condensation. From the SEM images in Fig. 23a it becomes evident that the particles are glued together at their contact points. This technology was promoted by Schurig and coworkers. Capillaries with Chira-Dex-silica (3 or 5 μm) particles that were glued together with a sol–gel matrix were successfully employed for pCEC enantiomer separations of various aromatic solutes and some non-aromatic compounds [131]. The tedious

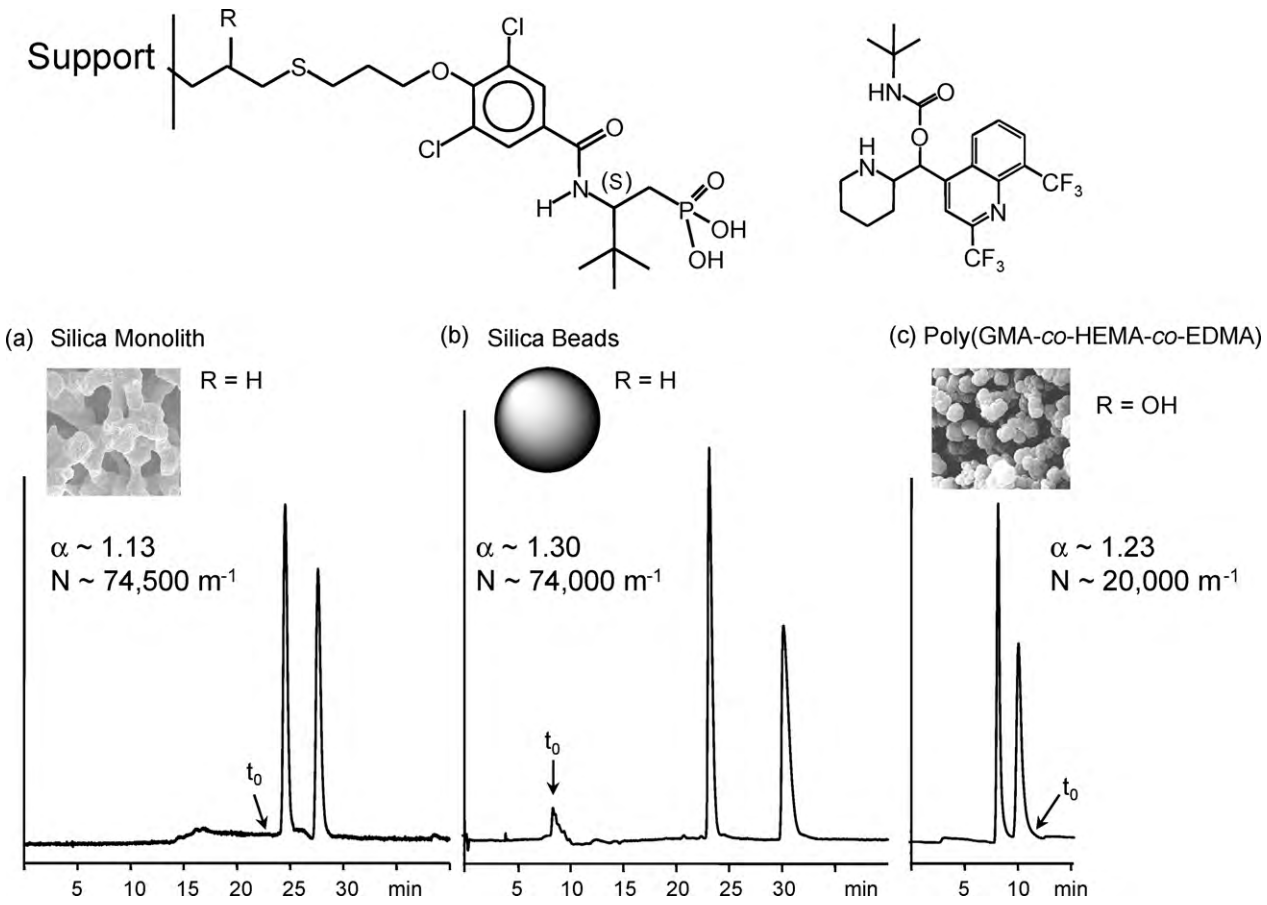

Fig. 22. Comparison of different supports for the CEC separation of O-(*tert*-butylcarbamoyl)mefloquine: (a) monolithic silica, (b) silica particles (100 Å, 3.5 μm), (c) monolithic poly(GMA-*co*-HEMA-*co*-EDMA) (40:40:20, w/w/w), all functionalized with the β-aminophosphonic acid-derived strong cation-exchange moieties. *Experimental conditions*: Mobile phase, acetonitrile–methanol (80:20, v/v) containing 25 mM (a and c) and 50 mM (b) formic acid, and 12.5 mM (a and c) and 25 mM (b) 2-amino-1-butanol; applied voltage, +7 kV (9 μA) (a), +15 kV (15 μA) (b), +12 kV (10 μA) (c). Reprinted in modified form from Ref. [102].

column packing procedure that is involved in this approach, however, makes it less attractive compared to other technologies. If organic polymers are used as embedding matrix or if polymeric particles are embedded in a sol–gel matrix the particles are simply entrapped in the polymer network. Due to entrapment and crosslinking, respectively, the particles are immobilized and the need for frits is alleviated.

In the other concept (low particle density approach), particle-loaded monoliths can be prepared from a 10–25% (w/w) suspension of chiral beads in a sol–gel precursor solution [132] or a reaction mixture containing organic monomers, crosslinker and porogens [133–135]. After introduction of the suspension into the capillary with a syringe, the sol–gel matrix or polymer matrix is formed leading to particle fixation in the bed. As pointed out, the main difference from the approach above is a lower density of particles in the chromatographic bed. Moreover, the particles which are entrapped or encapsulated in the matrix are not only less densely but also less regularly packed as compared to the above "high-density approach" which involves a slurry packing procedure (*cf.* SEM images in Fig. 23). Schmid et al. employed this methodology for embedding various chiral stationary phases (3 μm) including macrocyclic antibiotics and ligand-exchange type CSPs into polyacrylamide- [133,134] (Fig. 23b) or ROMP-type polymer matrix [135] (Fig. 23c). The approach is technically straightforward and more or less generic. On the other hand, irregularities in the chromatographic bed seem to yield somewhat lower efficiencies than obtained with other monolithic column types such as silica monoliths.

It is common to both the low and high density approach that the particles largely dictate the chromatographic properties. They provide the chromatographic selectivity and a high adsorption capacity. The matrix entraps the particles and may contain functionalities which support EOF generation. As the main advantage cumbersome frits are not required like with other monolithic capillary columns. CEC applications of monoliths made from particles are summarized in Table 5.

5. Other approaches with monolithic materials

Organic–inorganic-MIP-type hybrid monolithic materials have been developed by Wang et al. for CEC enantiomer separation [138]. Thus, a room-temperature ionic liquid (RTIL)-mediated nonhydrolytic sol–gel (NHSG) protocol was proposed for the synthesis of molecularly imprinted silica-based hybrid monoliths with zolmitriptan as template for its chiral separation by CEC. The reaction mixture consisted of 3-(methacryloyloxy)propyl trimethoxysilane (γ-MAPS), a functional monomer such as methacrylic acid (MAA), trifluoromethacrylic acid (TFMAA) or cinnamic acid, (*S*)-zolmitriptan as template, RTIL (1-butyl-3-methylimidazolium hexafluorophosphate, $BMIM^+PF_6^-$, or tetrafluoroborate, $BMIM^+BF_4^-$), AIBN as radical initiator, and acetonitrile as solvent and porogen, respectively. The process involved free radical copolymerization of functional monomers and γ-MAPS, and concurrent condensation of methacryloxypropyl trimethoxysilane catalyzed by the acidic functional monomers in the reaction mixture. During polymerization the acidic functional monomers should

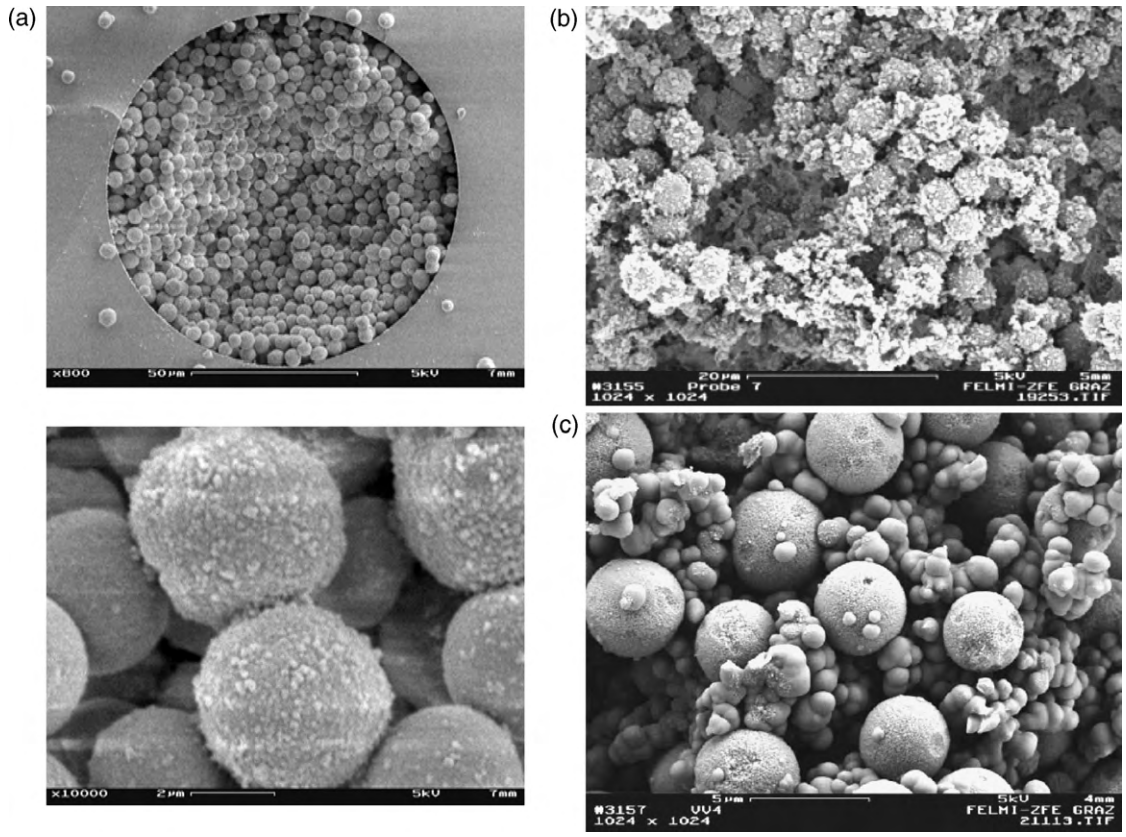

Fig. 23. SEM images of monoliths made from particles: (a) Chira-Dex-silica particle-glued monolith. Magnification, 800× (top) and 10,000× (bottom). (b) Particle-loaded polyacrylamide-based, and (c) ROMP-based monolithic stationary phases. Reprinted from Refs. [131] (a), [133] (b) and [135] (c).

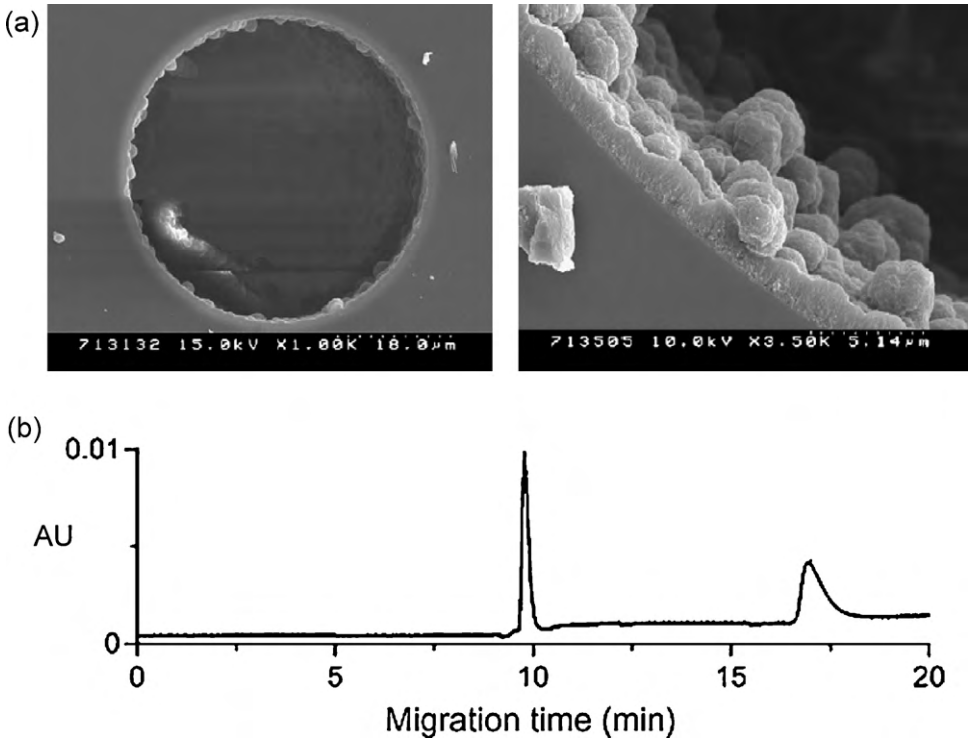

Fig. 24. Porous layer open-tubular (PLOT) column with monolithic (*S*)-ketoprofen MIP layer. (a) Scanning electron micrographs, (b) CEC enantiomer separation. *Experimental conditions in* (*b*): Eluent, acetonitrile-50 mM sodium formate buffer pH 3.5 (70:30, v/v); applied potential, 15 kV; injection, 5 mbar for 4 s. Reprinted with permission from Ref. [141].

Table 5
Enantioselective CEC with monoliths made from particles.

Analytes	Chiral selector or particle type	Matrix	Mobile phase (applied voltage)	Maximum efficiency (theor.pl/m)	Ref.
Barbiturates, benzoin, α-methyl-α-phenylsuccinimide, MTH-proline, mecoprop methyl, fenoxaprop ethyl, carprofen, ibuprofen	Chira-Dex immobilized on sintered silica-packed bed (Na_2CO_3 flush, 380 °C)		MeOH–20 mM MES, pH 6 (30:70, v/v)	68,000	[130]
Phe, Tyr, Phg, Phe-anilide; DNS-Phe, DNS-Leu	L-Phe, L-Phe-anilide directed MIP; DNS-L-Leu	MIP entrapped in polyacrylamide matrix	0.05 M Tris–citric acid (pH 2.5); ACN–CH_3COOH–water (90:5:5, v/v/v); *T*, 60 °C, 350 V/cm		[128,136,137]
DNS-Phe	L-DNS-Phe directed MIP	Entrapped in potassium silicate	ACN–acetate (pH 3.0) (89/20, v/v); 30 kV		[129]
NBD-amino acids	(*S*)-DNB-1-naphthylglycine-silica (5 μm) and (*S*)-*N*-(3,5-dinitrophenylaminocarbonyl)valine-silica (5 μm)	Embedded in sol–gel matrix (TEOS, EtOH, HCl)	ACN–5 mM phosphate buffer (pH 2.5) (70/30, v/v)		[132]
Barbiturates, benzoin, α-methyl-α-phenylsuccinimide, MTH-proline, mecoprop methyl, fenoxaprop ethyl, carprofen, ibuprofen	Chira-Dex-silica	Embedded in sol–gel matrix (TEOS, EtOH, HCl)	MeOH–20 mM MES, pH 6 (30:70, v/v) 25 kV; MeOH–acetate (70/30, 30/70, v/v) 20 kV	100,000	[131]
Amino acids, dipeptides, α-hydroxy acids	Teicoplanin aglycone-silica (3 μm) or ristocetin A-silica (3 μm)	Chiral silica particle entrapped in polymethacrylamide matrix	40%MeOH/20% ACN 40% 15 mM TEAA (pH 4.1)/25 kV		[133]
Amino acids, α-hydroxy acids	L-4-hydroxyproline-modified silica (3 μm)	Chiral silica particle entrapped in polymethacrylamide matrix	50 mM Phosphate solution 0.1 mM Cu(II) pH 4.5		[134]
Glycyl-dipeptides	Teicoplanin aglycone-silica (3 μm)	Chiral silica particle entrapped in NBE/DMN-H6 matrix through ROMP polymerization	50% aq. TEAA solution (0.2% pH 4.1)–50% ACN		[135]

be complexed with the basic zolmitriptan template which was promoted by the non-protic solvent (ACN) and was supposed to lead to the effective formation of chiral recognition sites in the resultant hybrid material. It was found that reaction time of the NHSG process was shorter when stronger acids were used (e.g. 4.5 h for TFMAA, 5 h for cinnamic acid and 6 h for MAA). RTILs have been used to reduce gel shrinkage and also to act as pore templates. In fact, their incorporation in the reaction mixture increased porosity and yielded an improved enantioselectivity for zolmitriptan (α up to 1.34, R_S up to 4.3).

Few studies also reported on the fabrication of MIP-type monolithic PLOT columns. For example, Remcho and coworkers proposed a protocol for the fabrication of thin porous films of highly crosslinked molecularly imprinted polymers anchored to the inner walls of 25 μm i.d. fused-silica capillaries by an *in situ* polymerization process [139]. Polymers imprinted towards *N*-dansyl (*S*)-phenylalanine were directly prepared in vinylized capillaries by copolymerization of methacrylic acid and 2-vinyl pyridine (functional monomers), with crosslinkers such as EDMA or TRIM in presence of template and porogens such as toluene and acetonitrile. The capillary was filled with the polymerization mixture and the polymerization process initiated thermally. The preparation of the capillary column was completed by applying a vacuum at one end of the capillary and a pressure of 0.7 MPa at the other to remove the porogens and promote shrinkage of the polymer matrix to form a thin film. This method of film preparation occasionally resulted in permanent occlusion. Moreover, scanning electron micrographs showed that the coating was not completely uniform. In spite of that reasonable enantioselectivities and a high efficiency of ca. 250,000 plates/m could be achieved for the non-imprinted enantiomer. Unfortunately, the more retained enantiomer showed strong tailing and plate counts as low as ca. 8,000, indicating polydispersity in the recognition sites and slow exchange kinetics.

A similar methodology was recently rediscovered and improved by Zaidi and Cheong [140–143]. For example, (*S*)-ketoprofen [140,141] MIP-type monolithic PLOT columns have been prepared in 50 μm i.d. capillaries by thermal initiated *in situ* polymerization. Unlike to common MIP monolith approaches, the typical monomer mixture composed of template ((*S*)-ketoprofen [140,141] or (*S*)-ofloxacin [143]), methacrylic acid as functional monomer, EDMA as crosslinker dissolved in 9/1 (v/v) acetonitrile/2-propanol representing the porogenic mixture contained also 4-styrenesulfonic acid (4-SSA) as comonomer. 4-SSA was added to form a MIP layer capable of generating a stable and strong electroosmotic flow over the entire pH range. The amount of 4-SSA seemed to have a decisive role on the performance of the monolithic PLOT columns. If the amount was too low, enantiomer separations were

deteriorated. On contrary, if larger quantities had to be copolymerized also the 2-propanol content needed to be increased to dissolve 4-SSA, then the CEC system and baseline became unstable, respectively, and the separation also deteriorated. SEM images revealing the porous monolithic layers are shown in Fig. 24a and a CEC enantiomer separation of ketoprofen in Fig. 24b. Chromatographic resolutions of ketoprofen enantiomers up to 10.5 with theoretical plates of 156,000/m for (*R*)-ketoprofen and 40,000/m for (*S*)-ketoprofen could be accomplished. The approach was later extended to various other templates such as ofloxacin [143], other profens [142] and mandelic acid [142]. Overall, such MIP-type monolithic PLOT columns showed quite promising results for CEC enantiomer separations. Yet, reasonable sample loadability needs yet to be demonstrated under real life analysis conditions.

Also a silica-based PLOT column has been reported. Wang et al. prepared a PLOT column with porous silica layer by sol–gel technology [144]. Onto this monolithic sol–gel layer 2,6-dibutyl-β-cyclodextrin was coated and was explored for open-tubular capillary electrochromatographic enantiomer separation. While such a sol–gel-based PLOT column might be a useful alternative, the non-covalent bonding of the cyclodextrin derivative makes the column less stable.

6. Concluding remarks

Numerous studies continuously demonstrate the better chromatographic performance of CEC over HPLC for enantiomer separation of chiral compounds. Theoretical plate numbers in the range of 50,000–300,000 theoretical plates per meter are routinely obtained and make CEC enantiomer separation a highly efficient alternative to both HPLC and CE. In spite of that this technology remained mainly of academic interest. It is because enantiomer separations by HPLC and CE are highly developed and is routine nowadays. In contrast, for enantioselective CEC capillary columns are not commercially available, CEC methods are not robust enough for high throughput quality control and bioanalysis, and hyphenation to MS is still far from being a trivial and robust technique. On the other hand, CE and upcoming UHPLC or fused-core particle technology are competing technologies that are capable of developing equally efficient enantiomer separations.

Whatsoever, the papers published over the last decade or so, clearly emphasizes that chiral monolithic capillary columns are becoming more and more the preferred column technology for enantioselective CEC. A limited column-to-column reproducibility is often invoked as a problematic issue of monolith technologies. However, with some experience and awareness that all parameters need to be under careful control, it is readily possible to produce monolithic columns with good batch-to-batch reproducibility. Amongst the various monolith technologies monolithic silica capillaries with chiral selectors attached by post-functionalizations or coating of polymeric selectors are somehow outperforming other approaches on gross and may become prevailing which is substantiated by an increasing number of papers on this topic. The surface chemistries known from particles employed in HPLC may be readily transferred, but need to be carefully re-optimized for in-capillary functionalization. Enantioselectivities turned out to be slightly lower compared to packed bed analogs, yet outperform them in terms of column robustness and longevity. Chiral capillary columns obtained by *in situ* prepared organic polymer monoliths have been prepared in a single step with also excellent performance. Yet, tedious optimizations probably prevent the wider use of this technology. Recently, monolithic MIP-type PLOT columns had a renaissance and attracted significant interest. Efficient separations could be accomplished in spite of some inherent limitations of the MIP technology in terms of efficiency for the high-affinity enantiomer. Whether this approach is fit for the purpose of practical applications need yet to be demonstrated.

A future focus will also be the implementation of electrically driven liquid chromatography mode and monolithic approaches to microchip separations [33,34]. Overall, it is safe to assume that enantioselective CEC will remain a research field of significant academic interest and there is a lot of room for further developments in the area of monolithic columns and their implementation to routine analysis.

Definitions and symbols

% T	total acrylamide concentration % T = 100 $(a+b+c)/V$ (a, b, c are the masses of monomer, crosslinker, and comonomers, and V is the volume of the reaction solvent in mL, mostly Tris–boric acid buffer)
% C	percentage (w/w) of crosslinker related to total monomers; % C = $100b/(a+b+c)$
% S	percentage (w/w) of chargeable comonomer (mostly AMPS) related to total monomers; % S = $100\gamma/(\alpha+\beta+\gamma)$ where α, β and γ are the molarities of acrylamide, crosslinker and chargeable comonomer, respectively
$d_{p,mode}$	mode pore diameter

Symbols

k_{CLC}	chromatographic retention factor
L_{eff}	effective capillary length
L_{tot}	total capillary length
μ_{eo}	electroosmotic mobility
$\mu_{ep,eff}$	effective electrophoretic mobility

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References

[1] F. Svec, T.B. Tennikova, Z. Deyl (Eds.), Monolithic materials: preparation, properties and applications, J. Chromatogr. Libr. 67, Elsevier, Amsterdam, 2003.
[2] J. Urban, P. Jandera, Polymethacrylate monolithic columns for capillary liquid chromatography, J. Sep. Sci. 31 (2008) 2521–2540.
[3] E.F. Hilder, F. Svec, J.M.J. Frechet, Development and application of polymeric monolithic stationary phases for capillary electrochromatography, J. Chromatogr. A 1044 (2004) 3–22.
[4] M. Bedair, Z. El Rassi, Recent advances in polymeric monolithic stationary phases for electrochromatography in capillaries and chips, Electrophoresis 25 (2004) 4110–4119.
[5] E. Klodzinska, D. Moravcova, P. Jandera, B. Buszewski, Monolithic continuous beds as a new generation of stationary phase for chromatographic and electro-driven separations, J. Chromatogr. A 1109 (2006) 51–59.
[6] Z. Deyl, F. Svec (Eds.), Capillary electrochromatography, J. Chromatogr. Libr 62, Elsevier, Amsterdam, 2001.
[7] F. Svec, Porous polymer monoliths: amazingly wide variety of techniques enabling their preparation, J. Chromatogr. A 1217 (2010) 902–924.
[8] S. Hjertén, J.-L. Liao, R. Zhang, High-performance liquid chromatography on continuous polymer beds, J. Chromatogr. 473 (1989) 273–275.
[9] T.B. Tennikova, B.G. Belenkii, F. Svec, High-performance membrane chromatography. A novel method of protein separation, J. Liq. Chromatogr. 13 (1990) 63–70.
[10] S. Hjerten, A. Vegvari, T. Srichaiyo, H.-X. Zhang, C. Ericson, D. Eaker, An approach to ideal separation media for (electro)chromatography, J. Capill. Electrophor. 5 (1998) 13–26.
[11] M.G. Schmid, N. Grobuschek, C. Tuscher, G. Gubitz, A. Vegvari, E. Machtejevas, A. Maruska, S. Hjerten, Chiral separation of amino acids by ligand-exchange capillary electrochromatography using continuous beds, Electrophoresis 21 (2000) 3141–3144.
[12] A. Vegvari, A. Foldesi, C. Hetenyi, O. Kocnegarova, M.G. Schmid, V. Kudirkaite, S. Hjerten, A new easy-to-prepare homogeneous continuous electrochromatographic bed for enantiomer recognition, Electrophoresis 21 (2000) 3116–3125.

[13] O. Kornysova, P.K. Owens, A. Maruska, Continuous beds with vancomycin as chiral stationary phase for capillary electrochromatography, Electrophoresis 22 (2001) 3335–3338.
[14] H. Minakuchi, K. Nakanishi, N. Soga, N. Ishizuka, N. Tanaka, Octadecylsilylated porous silica rods as separation media for reversed-phase liquid chromatography, Anal. Chem. 68 (1996) 3498–3501.
[15] Q. Tang, M.L. Lee, Monolithic columns prepared from particles, in: F. Svec, T.B. Tennikova, Z. Deyl (Eds.), Monolithic Materials: Preparation, Properties and Applications, J. Chromatogr. Libr. 62, Elsevier, Amsterdam, 2003, pp. 197–211.
[16] M. Lammerhofer, F. Svec, J.M.J. Frechet, W. Lindner, Separation of enantiomers by capillary electrochromatography, Trends Anal. Chem. 19 (2000) 676–698.
[17] S. Fanali, P. Catarcini, G. Blaschke, B. Chankvetadze, Enantioseparations by capillary electrochromatography, Electrophoresis 22 (2001) 3131–3151.
[18] M. Lämmerhofer, W. Lindner, Capillary electrochromatography, in: F. Svec, T.B. Tennikova, Z. Deyl (Eds.), Monolithic Materials: Preparation, Properties and Applications, Elsevier, Amsterdam, 2003, pp. 489–559.
[19] C. Fujimoto, Enantiomer separation by capillary electrochromatography using fritless packed columns, Anal. Sci. 18 (2002) 19–25.
[20] F. Qin, C. Xie, Z. Yu, L. Kong, M. Ye, H. Zou, Monolithic enantiomer-selective stationary phases for capillary electrochromatography, J. Sep. Sci. 29 (2006) 1332–1343.
[21] D. Wistuba, V. Schurig, Comparison of monolithic approaches for enantioselective capillary electrochromatography involving cyclodextrins, J. Sep. Sci. 29 (2006) 1344–1352.
[22] B. Preinerstorfer, M. Laemmerhofer, Recent accomplishments in the field of enantiomer separation by CEC, Electrophoresis 28 (2007) 2527–2565.
[23] I. Tanret, D. Mangelings, Y. Vander Heyden, Monolithic stationary phases in classic and chiral pharmaceutical analysis with CEC and pCEC, J. Chromatogr. Sci. 47 (2009) 407–417.
[24] A. Guttman, A. Paulus, S. Cohen, N. Grinberg, B.L. Karger, Use of complexing agents for selective separation in high-performance capillary electrophoresis. Chiral resolution via cyclodextrins incorporated within polyacrylamide gel columns, J. Chromatogr. 448 (1988) 41–53.
[25] I.D. Cruzado, G. Vigh, Chiral separations by capillary electrophoresis using cyclodextrin-containing gels, J. Chromatogr. 608 (1992) 421–425.
[26] S. Birnbaum, S. Nilsson, Protein-based capillary affinity gel electrophoresis for the separation of optical isomers, Anal. Chem. 64 (1992) 2872–2874.
[27] M. Teraguchi, M. Ohtake, H. Inoue, A. Yoshida, T. Aoki, T. Kaneko, K. Yamanaka, Preparation of chiral polystyrene monoliths by utilizing W/O emulsion polymerization and their optical resolution ability, J. Polym. Sci. Part A: Polym. Chem. 43 (2005) 2348–2357.
[28] G. Götmar, T. Fornstedt, G. Guiochon, Apparent and true enantioselectivity in enantioseparations, Chirality 12 (2000) 558–564.
[29] M. Lämmerhofer, Chiral recognition by enantioselective liquid chromatography: mechanisms and modern chiral stationary phases, J. Chromatogr. A 1217 (2010) 814–856.
[30] F.M. Sinner, M.R. Buchmeiser, Ring opening metathesis polymerization: access to a new class of functionalized, monolithic stationary phases for liquid chromatography, Angew. Chem. Int. Ed. 39 (2000) 1433–1436.
[31] F. Sinner, M.R. Buchmeiser, A new class of continuous polymer supports prepared by ring-opening metathesis polymerization: a straightforward route to functionalized monoliths, Macromolecules 33 (2000) 5777–5786.
[32] B. Mayr, F. Sinner, M.R. Buchmeiser, Chiral beta-cyclodextrin-based polymer supports prepared via ring-opening metathesis graft-polymerization, J. Chromatogr. A 907 (2001) 47–56.
[33] H.-L. Zeng, H.-F. Li, J.-M. Lin, Chiral separation of dansyl amino acids by PDMS microchip gel monolithic column electrochromatography with γ-cyclodextrin bonded in polyacrylamide, Anal. Chim. Acta 551 (2005) 1–8.
[34] H.-L. Zeng, H. Li, W. Xu, J.-M. Lin, Chiral separation of FITC-labeled amino acids with gel electrochromatography using a polydimethylsiloxane microfluidic device, J. Capill. Electrophor. Microchip Technol. 10 (2007) 19–24.
[35] M.G. Schmid, N. Grobuschek, O. Lecnik, G. Gubitz, A. Vegvari, S. Hjerten, Enantioseparation of hydroxy acids on easy-to-prepare continuous beds for capillary electrochromatography, Electrophoresis 22 (2001) 2616–2619.
[36] M. Laemmerhofer, E.C. Peters, C. Yu, F. Svec, J.M.J. Frechet, W. Lindner, Chiral monolithic columns for enantioselective capillary electrochromatography prepared by copolymerization of a monomer with quinidine functionality. 1. Optimization of polymerization conditions, porous properties, and chemistry of the stationary phase, Anal. Chem. 72 (2000) 4614–4622.
[37] M. Laemmerhofer, F. Svec, J.M.J. Frechet, W. Lindner, Chiral monolithic columns for enantioselective capillary electrochromatography prepared by copolymerization of a monomer with quinidine functionality. 2. Effect of chromatographic conditions on the chiral separations, Anal. Chem. 72 (2000) 4623–4628.
[38] M. Laemmerhofer, E. Tobler, E. Zarbl, W. Lindner, F. Svec, J.M.J. Frechet, Macroporous monolithic chiral stationary phases for capillary electrochromatography: new chiral monomer derived from cinchona alkaloid with enhanced enantioselectivity, Electrophoresis 24 (2003) 2986–2999.
[39] M. Lammerhofer, F. Svec, J.M.J. Frechet, W. Lindner, Monolithic stationary phases for enantioselective capillary electrochromatography, J. Microcol. Sep. 12 (2000) 597–602.
[40] F. Svec, J.M.J. Frechet, Molded rigid monolithic porous polymers: an inexpensive, efficient, and versatile alternative to beads for the design of materials for numerous applications, Ind. Eng. Chem. Res. 38 (1999) 34–48.
[41] E.C. Peters, M. Petro, F. Svec, J.M.J. Frechet, Molded rigid polymer monoliths as separation media for capillary electrochromatography. 1. Fine control of porous properties and surface chemistry, Anal. Chem. 70 (1998) 2288–2295.
[42] C. Fujimoto, Charged polyacrylamide gels for capillary electrochromatographic separations of uncharged, low molecular weight compounds, Anal. Chem. 67 (1995) 2050–2053.
[43] M. Zhang, Z. El Rassi, Capillary electrochromatography with polyacrylamide monolithic stationary phases having bonded dodecyl ligands and sulfonic acid groups: evaluation of column performance with alkyl phenyl ketones and neutral moderately polar pesticides, Electrophoresis 22 (2001) 2593–2599.
[44] C. Yu, F. Svec, J.M.J. Frechet, Towards stationary phases for chromatography on a microchip: molded porous polymer monoliths prepared in capillaries by photoinitiated in situ polymerization as separation media for electrochromatography, Electrophoresis (2000) 120–127.
[45] F. Svec, E.C. Peters, D. Sykora, J.M.J. Frechet, Design of the monolithic polymers used in capillary electrochromatography columns, J. Chromatogr. A 887 (2000) 3–29.
[46] E.C. Peters, K. Lewandowski, M. Petro, F. Svec, J.M.J. Frechet, Chiral electrochromatography with a 'molded' rigid monolithic capillary column, Anal. Commun. 35 (1998) 83–86.
[47] T. Koide, K. Ueno, Enantiomeric separations of cationic and neutral compounds by capillary electrochromatography with charged polyacrylamide gels incorporating chiral selectors, Anal. Sci. 14 (1998) 1021–1023.
[48] T. Koide, K. Ueno, Enantiomeric separations of cationic and neutral compounds by capillary electrochromatography with beta-cyclodextrin-bonded charged polyacrylamide gels, Anal. Sci. 15 (1999) 791–794.
[49] J.-M. Lin, T. Nakagama, K. Uchiyama, T. Hobo, Capillary electrochromatographic separation of amino acid enantiomers using on-column prepared molecularly imprinted polymer, J. Pharmaceut. Biomed. Anal. 15 (1997) 1351–1358.
[50] L. Schweitz, L.I. Andersson, S. Nilsson, Capillary electrochromatography with molecular imprint-based selectivity for enantiomer separation of local anesthetics, J. Chromatogr. A 792 (1997) 401–409.
[51] L. Schweitz, L.I. Andersson, S. Nilsson, Capillary electrochromatography with predetermined selectivity obtained through molecular imprinting, Anal. Chem. 69 (1997) 1179–1183.
[52] M. Laemmerhofer, Chirally-functionalized monolithic materials for stereoselective capillary electrochromatography, Anal. Bioanal. Chem. 382 (2005) 873–877.
[53] T. Koide, K. Ueno, Enantiomeric separations by capillary electrochromatography with charged polyacrylamide gels incorporating chiral selectors, Anal. Sci. 16 (2000) 1065–1070.
[54] T. Koide, K. Ueno, Enantiomeric separations of cationic and neutral compounds by capillary electrochromatography with monolithic chiral stationary phases of beta-cyclodextrin-bonded negatively charged polyacrylamide gels, J. Chromatogr. A 893 (2000) 177–187.
[55] T. Koide, K. Ueno, Enantiomeric separations of acidic and neutral compounds by capillary electrochromatography with beta-cyclodextrin-bonded positively charged polyacrylamide gels, J. High Resolut. Chromatogr. 23 (2000) 59–66.
[56] O. Kornysova, E. Machtejevas, V. Kudirkaite, U. Pyell, A. Maruska, Synthesis and characterization of polyrotaxane-based polymeric continuous beds for capillary electrochromatography, J. Biochem. Biophys. Methods 50 (2002) 217–232.
[57] O. Kornysova, R. Surna, V. Snitka, U. Pyell, A. Maruska, Polyrotaxane approach for synthesis of continuous beds for capillary electrochromatography, J. Chromatogr. A 971 (2002) 225–235.
[58] T. Koide, K. Ueno, Enantiomeric separations of primary amino compounds by capillary electrochromatography with monolithic chiral stationary phases of chiral crown ether-bonded negatively charged polyacrylamide gels, J. Chromatogr. A 909 (2001) 305–315.
[59] O. Lecnik, G. Guebitz, M.G. Schmid, Role of the charge in continuous beds in the chiral separation of hydroxy acids by ligand-exchange capillary electrochromatography, Electrophoresis 24 (2003) 2983–2985.
[60] E. Machtejevas, A. Maruska, A new approach to human serum albumin chiral stationary phase synthesis and its use in capillary liquid chromatography and capillary electrochromatography, J. Sep. Sci. 25 (2002) 1303–1309.
[61] A. Maruska, Capillary electrochromatography, in: F. Svec, T.B. Tennikova, Z. Deyl (Eds.), Monolithic Materials: Preparation, Properties and Applications, J. Chromatogr. Libr. 67, Elsevier, Amsterdam, 2003, pp. 143–172.
[62] L. Schweitz, L.I. Andersson, S. Nilsson, Molecular imprint-based stationary phases for capillary electrochromatography, J. Chromatogr. A 817 (1998) 5–13.
[63] P. Spegel, J. Nilsson, S. Nilsson, Chiral separations by capillary electrochromatography using molecularly imprinted polymers, in: G. Gübitz, M.G. Schmid (Eds.), Chiral Separations, Series: Methods in Molecular Biology, Vol. 243, Humana Press, Totowa, NJ, United States, 2004, pp. 411–424.
[64] L. Schweitz, P. Spegel, S. Nilsson, Molecularly imprinted microparticles for capillary electrochromatographic enantiomer separation of propranolol, Analyst 125 (2000) 1899–1901.
[65] J.M. Lin, T. Nakagama, X.Z. Wu, K. Uchiyama, T. Hobo, Capillary electrochromatographic separation of amino acid enantiomers with molecularly imprinted polymers as chiral recognition agents, Fresenius' J. Anal. Chem. 357 (1997) 130–132.
[66] J.-M. Lin, T. Nakagama, K. Uchiyama, T. Hobo, Capillary electrochromatographic separation of amino acid enantiomers using on-column

prepared molecularly imprinted polymer, J. Pharm. Biomed. Anal. 15 (1997) 1351–1358.
[67] Z.-S. Liu, Y.-L. Xu, H. Wang, C. Yan, R.-Y. Gao, Chiral separation of binaphthol enantiomers on molecularly imprinted polymer monolith by capillary electrochromatography, Anal. Sci. 20 (2004) 673–678.
[68] Y.-L. Xu, Z.-S. Liu, H.-F. Wang, C. Yan, R.-Y. Gao, Chiral recognition ability of an (S)-naproxen-imprinted monolith by capillary electrochromatography, Electrophoresis 26 (2005) 804–811.
[69] Q.-L. Deng, Z.-H. Lun, R.-Y. Gao, L.-H. Zhang, W.-B. Zhang, Y.-K. Zhang, (S)-Ibuprofen-imprinted polymers incorporating gamma-methacryloxypropyltrimethoxysilane for CEC separation of ibuprofen enantiomers, Electrophoresis 27 (2006) 4351–4358.
[70] J. Ou, X. Li, S. Feng, J. Dong, X. Dong, L. Kong, M. Ye, H. Zou, Preparation and evaluation of a molecularly imprinted polymer derivatized silica monolithic column for capillary electrochromatography and capillary liquid chromatography, Anal. Chem. 79 (2007) 639–646.
[71] L. Schweitz, L.I. Andersson, S. Nilsson, Molecular imprinting for chiral separations and drug screening purposes using monolithic stationary phases in CEC, Chromatographia 49 (1999) S93–S94.
[72] L. Schweitz, L.I. Andersson, S. Nilsson, Rapid electrochromatographic enantiomer separations on short molecularly imprinted polymer monoliths, Anal. Chim. Acta 435 (2001) 43–47.
[73] L. Schweitz, L.I. Andersson, S. Nilsson, Molecularly imprinted CEC sorbents: investigations into polymer preparation and electrolyte composition, Analyst 127 (2002) 22–28.
[74] J. Ou, J. Dong, T. Tian, J. Hu, M. Ye, H. Zou, Enantioseparation of tetrahydropalmatine and Troeger's base by molecularly imprinted monolith in capillary electrochromatography, J. Biochem. Biophys. Methods 70 (2007) 71–76.
[75] P. Spegel, L. Schweitz, L.I. Andersson, S. Nilsson, Novel vinylpyridine based cationic MIP monoliths for enantiomer separation in CEC, Chromatographia 69 (2008) 277–285.
[76] M. Li, X. Lin, Z. Xie, Investigation of enantiomer recognition of molecularly imprinted polymeric monoliths in pressurized capillary electrochromatography screening the amino acids and their derivatives, J. Chromatogr. A 1216 (2009) 5320–5326.
[77] T. Rohr, E.F. Hilder, J.J. Donovan, F. Svec, J.M.J. Frechet, Photografting and the control of surface chemistry in three-dimensional porous polymer monoliths, Macromolecules 36 (2003) 1677–1684.
[78] S. Eeltink, E.F. Hilder, L. Geiser, F. Svec, J.M.J. Frechet, G.P. Rozing, P.J. Schoenmakers, W.T. Kok, Controlling the surface chemistry and chromatographic properties of methacrylate-ester-based monolithic capillary columns via photografting, J. Sep. Sci. 30 (2007) 407–413.
[79] F.M. Sinner, M.R. Buchmeiser, Ring-opening metathesis polymerization: access to a new class of functionalized, monolithic stationary phases for liquid chromatography, Angew. Chem. Int. Ed. 39 (2000) 1433–1436.
[80] B.S. Mayr, M.R. Buchmeiser, Chiral beta-cyclodextrin-based polymer supports prepared via ring-opening metathesis graft-polymerization, J. Chromatogr. A 907 (2001) 47–56.
[81] M.R. Buchmeiser, Stationary phases for chromatography prepared by ring opening metathesis polymerization, J. Sep. Sci. 31 (2008) 1907–1922.
[82] X. Dong, R.a. Wu, J. Dong, M. Wu, Y. Zhu, H. Zou, Polyacrylamide-based monolithic capillary column with coating of cellulose tris(3,5-dimethylphenylcarbamate) for enantiomer separation in capillary electrochromatography, Electrophoresis 29 (2008) 919–927.
[83] B. Chankvetadze, Monolithic chiral stationary phases for liquid-phase enantioseparation techniques, J. Sep. Sci. 33 (2010) 305–314.
[84] O. Kornysova, R. Jarmalaviciene, A. Maruska, A simplified synthesis of polymeric nonparticulate stationary phases with macrocyclic antibiotic as chiral selector for capillary electrochromatography, Electrophoresis 25 (2004) 2825–2829.
[85] B. Preinerstorfer, W. Bicker, W. Lindner, M. Lammerhofer, Development of reactive thiol-modified monolithic capillaries and in-column surface functionalization by radical addition of a chromatographic ligand for capillary electrochromatography, J. Chromatogr. A 1044 (2004) 187–199.
[86] B. Preinerstorfer, W. Lindner, M. Laemmerhofer, Polymethacrylate-type monoliths functionalized with chiral amino phosphonic acid-derived strong cation exchange moieties for enantioselective nonaqueous capillary electrochromatography and investigation of the chemical composition of the monolithic polymer, Electrophoresis 26 (2005) 2005–2018.
[87] A. Messina, M. Flieger, F. Bachechi, M. Sinibaldi, Enantioseparation of 2-aryloxypropionic acids on chiral porous monolithic columns by capillary electrochromatography, J. Chromatogr. A 1120 (2006) 69–74.
[88] A. Messina, M. Sinibaldi, CEC enantioseparations on chiral monolithic columns: a study of the stereoselective degradation of (R/S)-dichlorprop [2-(2,4-dichlorophenoxy)propionic acid] in soil, Electrophoresis 28 (2007) 2613–2618.
[89] Y. Tian, C. Zhong, E. Fu, Z. Zeng, Novel beta-cyclodextrin derivative functionalized polymethacrylate-based monolithic columns for enantioselective separation of ibuprofen and naproxen enantiomers in capillary electrochromatography, J. Chromatogr. A 1216 (2009) 1000–1007.
[90] Y. Li, C. Song, L. Zhang, W. Zhang, H. Fu, Fabrication and evaluation of chiral monolithic column modified by β-cyclodextrin derivatives, Talanta 80 (2010) 1378–1384.
[91] M. Guerrouache, M.-C. Millot, B. Carbonnier, Functionalization of macroporous organic polymer monolith based on succinimide ester reactivity for chiral capillary chromatography: a cyclodextrin click approach, Macromol. Rapid Commun. 30 (2009) 109–113.
[92] Y. Tian, C. Zhong, E. Fu, Z. Zeng, Novel β-cyclodextrin derivative functionalized polymethacrylate-based monolithic columns for enantioselective separation of ibuprofen and naproxen enantiomers in capillary electrochromatography, J. Chromatogr. A 1216 (2009) 1000–1007.
[93] G. Ding, A. Tang, Preparation of norvancomycin-bonded chiral silica monolithic column for capillary electrochromatography and its applications, Sepu 24 (2006) 402–406.
[94] A.M. Siouffi, Silica gel-based monoliths prepared by the sol–gel method: facts and figures, J. Chromatogr. A 1000 (2003) 801–818.
[95] K. Nakanishi, N. Tanaka, Sol–gel with phase separation. Hierarchically porous materials optimized for high-performance liquid chromatography separations, Acc. Chem. Res. 40 (2007) 863–873.
[96] K. Nakanishi, N. Soga, Phase separation in silica sol–gel system containing polyacrylic acid. I. Gel formation behavior and effect of solvent composition, J. Non-Cryst. Solids 139 (1992) 1–13.
[97] O. Núñez, K. Nakanishi, N. Tanaka, Preparation of monolithic silica columns for high-performance liquid chromatography, J. Chromatogr. A 1191 (2008) 231–252.
[98] N. Ishizuka, H. Kobayashi, H. Minakuchi, K. Nakanishi, K. Hirao, K. Hosoya, T. Ikegami, N. Tanaka, Monolithic silica columns for high-efficiency separations by high-performance liquid chromatography, J. Chromatogr. A 960 (2002) 85–96.
[99] X. Dong, J. Dong, J. Ou, Y. Zhu, H. Zou, Preparation and evaluation of a vancomycin-immobilized silica monolith as chiral stationary phase for CEC, Electrophoresis 28 (2007) 2606–2612.
[100] X. Dong, R.a. Wu, J. Dong, M. Wu, Y. Zhu, H. Zou, The covalently bonded cellulose tris(3,5-dimethylphenylcarbamate) on a silica monolithic capillary column for enantioseparation in capillary electrochromatography, J. Chromatogr. B 875 (2008) 317–322.
[101] F. Qin, C. Xie, S. Feng, J. Ou, L. Kong, M. Ye, H. Zou, Monolithic silica capillary column with coated cellulose tris(3,5-dimethylphenylcarbamate) for capillary electrochromatographic separation of enantiomers, Electrophoresis 27 (2006) 1050–1059.
[102] B. Preinerstorfer, D. Lubda, W. Lindner, M. Laemmerhofer, Monolithic silica-based capillary column with strong chiral cation-exchange type surface modification for enantioselective non-aqueous capillary electrochromatography, J. Chromatogr. A 1106 (2006) 94–105.
[103] Z. Chen, T. Hobo, Chemically L-prolinamide-modified monolithic silica column for enantiomeric separation of dansyl amino acids and hydroxy acids by capillary electrochromatography and μ-high performance liquid chromatography, Electrophoresis 22 (2001) 3339–3346.
[104] B. Preinerstorfer, M. Laemmerhofer, C.V. Hoffmann, D. Lubda, W. Lindner, Deconvolution of electrokinetic and chromatographic contributions to solute migration in stereoselective ion-exchange capillary electrochromatography on monolithic silica capillary columns, J. Sep. Sci. 31 (2008) 3065–3078.
[105] Z. Chen, T. Hobo, Chemically L-phenylalaninamide-modified monolithic silica column prepared by a sol–gel process for enantioseparation of dansyl amino acids by ligand exchange-capillary electrochromatography, Anal. Chem. 73 (2001) 3348–3357.
[106] Z. Chen, M. Niitsuma, K. Uchiyama, T. Hobo, Comparison of enantioseparations using Cu(II) complexes with L-amino acid amides as chiral selectors or chiral stationary phases by capillary electrophoresis, capillary electrochromatography and micro liquid chromatography, J. Chromatogr. A 990 (2003) 75–82.
[107] Z. Chen, T. Nishiyama, K. Uchiyama, T. Hobo, Electrochromatographic enantioseparation using chiral ligand exchange monolithic so–gel column, Anal. Chim. Acta 501 (2004) 17–23.
[108] J. Kang, D. Wistuba, V. Schurig, A silica monolithic column prepared by the sol–gel process for enantiomeric separation by capillary electrochromatography, Electrophoresis 23 (2002) 1116–1120.
[109] Z. Liu, K. Otsuka, S. Terabe, M. Motokawa, N. Tanaka, Physically adsorbed chiral stationary phase of avidin on monolithic silica column for capillary electrochromatography and capillary liquid chromatography, Electrophoresis 23 (2002) 2973–2981.
[110] Z. Chen, H. Ozawa, K. Uchiyama, T. Hobo, Cyclodextrin-modified monolithic columns for resolving dansyl amino acid enantiomers and positional isomers by capillary electrochromatography, Electrophoresis 24 (2003) 2550–2558.
[111] B. Preinerstorfer, D. Lubda, A. Mucha, P. Kafarski, W. Lindner, M. Lammerhofer, Stereoselective separations of chiral phosphinic acid pseudodipeptides by CEC using silica monoliths modified with an anion-exchange-type chiral selector, Electrophoresis 27 (2006) 4312–4320.
[112] B. Preinerstorfer, C. Hoffmann, D. Lubda, M. Laemmerhofer, W. Lindner, Enantioselective silica-based monoliths modified with a novel aminosulfonic acid-derived strong cation exchanger for electrically driven and pressure-driven capillary chromatography, Electrophoresis 29 (2008) 1626–1637.
[113] S. Buchinger, B. Follrich, M. Laemmerhofer, D. Lubda, W. Lindner, Chirally functionalized anion-exchange type silica monolith for enantiomer separation of 2-aryloxypropionic acid herbicides by non-aqueous capillary electrochromatography, Electrophoresis 30 (2009) 3804–3813.
[114] A. Messina, S. Moroni, M. Flieger, M. Sinibaldi, O. Ursini, CEC enantioseparations of carboxylic acids on silica-based monoliths modified with ergot alkaloid derivative, Electrophoresis 30 (2009) 2890–2896.
[115] C. He, A. Hendrickx, D. Mangelings, J. Smeyers-Verbeke, Y. Vander Heyden, Monolithic silica capillary columns with immobilized cellulose

tris(3,5-dimethylphenylcarbamate) for enantiomer separations in CEC, Electrophoresis 30 (2009) 3796–3803.
[116] G.-S. Ding, A.-N. Tang, Capillary electrochromatographic separation of racemates on a norvancomycin-bonded monolithic column under reversed-phase conditions, J. Chromatogr. A 1208 (2008) 232–238.
[117] A.-N. Tang, X.-N. Wang, G.-S. Ding, X.-P. Yan, On-line preconcentration and enantioseparation of thalidomide racemates by CEC with the hyphenation of octyl and norvancomycin monoliths, Electrophoresis 30 (2009) 682–688.
[118] M. Kato, N. Matsumoto, K. Sakai-Kato, T. Toyo'oka, Investigation of chromatographic performances and binding characteristics of BSA-encapsulated capillary column prepared by the sol–gel method, J. Pharmaceut. Biomed. Anal. 30 (2003) 1845–1850.
[119] M. Kato, H. Saruwatari, K. Sakai-Kato, T. Toyo'oka, Silica sol–gel/organic hybrid material for protein encapsulated column of capillary electrochromatography, J. Chromatogr. A 1044 (2004) 267–270.
[120] K. Sakai-Kato, M. Kato, H. Nakakuki, T. Toyo'oka, Investigation of structure and enantioselectivity of BSA-encapsulated sol–gel columns prepared for capillary electrochromatography, J. Pharmaceut. Biomed. Anal. 31 (2003) 299–309.
[121] M.-L. Hsieh, G.-Y. Li, L.-K. Chau, Y.-S. Hon, Single-step approach to beta-cyclodextrin-bonded silica as monolithic stationary phases for CEC, J. Sep. Sci. 31 (2008) 1819–1827.
[122] B. Chankvetadze, C. Yamamoto, N. Tanaka, K. Nakanishi, Y. Okamoto, High-performance liquid chromatographic enantioseparations on capillary columns containing monolithic silica modified with cellulose tris(3,5-dimethylphenylcarbamate), J. Sep. Sci. 27 (2004) 905–911.
[123] B. Chankvetadze, T. Kubota, T. Ikai, C. Yamamoto, M. Kamigaito, N. Tanaka, K. Nakanishi, Y. Okamoto, High-performance liquid chromatographic enantioseparations on capillary columns containing crosslinked polysaccharide phenylcarbamate derivatives attached to monolithic silica, J. Sep. Sci. 29 (2006) 1988–1995.
[124] B. Chankvetadze, T. Ikai, C. Yamamoto, Y. Okamoto, High-performance liquid chromatographic enantioseparations on monolithic silica columns containing a covalently attached 3,5-dimethylphenylcarbamate derivative of cellulose, J. Chromatogr. A 1042 (2004) 55–60.
[125] M. Kato, K. Sakai-Kato, N. Matsumoto, T. Toyo'oka, A protein-encapsulation technique by the sol–gel method for the preparation of monolithic columns for capillary electrochromatography, Anal. Chem. 74 (2002) 1915–1921.
[126] E. Zarbl, M. Lammerhofer, A. Woschek, F. Hammerschmidt, C. Parenti, G. Cannazza, W. Lindner, Strong versus weak chiral cation exchangers: comparative evaluation for enantiomer separation of chiral bases by non-aqueous CEC, J. Sep. Sci. 25 (2002) 1269–1283.
[127] C. Karlsson, L. Karlsson, D.W. Armstrong, P.K. Owens, Evaluation of a vancomycin chiral stationary phase in capillary electrochromatography using polar organic and reversed-phase modes, Anal. Chem. 72 (2000) 4394–4401.
[128] J.-M. Lin, K. Uchiyama, T. Hobo, Enantiomeric resolution of dansyl amino acids by capillary electrochromatography based on molecular imprinting method, Chromatographia 47 (1996) 625–629.
[129] G. Chirica, V.T. Remcho, Silicate entrapped columns. New columns designed for capillary electrochromatography, Electrophoresis 20 (1999) 50–56.
[130] D. Wistuba, V. Schurig, Enantiomer separation by capillary electrochromatography on a cyclodextrin-modified monolith, Electrophoresis 21 (2000) 3152–3159.
[131] D. Wistuba, L. Banspach, V. Schurig, Enantiomeric separation by capillary electrochromatography using monolithic capillaries with sol–gel-glued cyclodextrin-modified silica particles, Electrophoresis 26 (2005) 2019–2026.
[132] M. Kato, M.T. Dulay, B. Bennett, J.-R. Chen, R.N. Zare, Enantiomeric separation of amino acids and nonprotein amino acids using a particle-loaded monolithic column, Electrophoresis 21 (2000) 3145–3151.
[133] M.G. Schmid, J. Koidl, C. Freigassner, S. Tahed, L. Wojcik, T. Beesley, D.W. Armstrong, G. Guebitz, New particle-loaded monoliths for chiral capillary electrochromatographic separation, Electrophoresis 25 (2004) 3195–3203.
[134] M.G. Schmid, J. Koidl, P. Wank, G. Kargl, H. Zoehrer, G. Guebitz, Enantioseparation by ligand-exchange using particle-loaded monoliths: capillary-LC versus capillary electrochromatography, J. Biochem. Biophys. Methods 70 (2007) 77–85.
[135] C. Gatschelhofer, M.G. Schmid, K. Schreiner, T.R. Pieber, F.M. Sinner, G. Guebitz, Enantioseparation of glycyl-dipeptides by CEC using particle-loaded monoliths prepared by ring-opening metathesis polymerization (ROMP), J. Biochem. Biophys. Methods 69 (2006) 67–77.
[136] J.-M. Lin, T. Nakagama, K. Uchiyama, T. Hobo, Temperature effect on chiral recognition of some amino acids with molecularly imprinted polymer filled capillary electrochromatography, Biomed. Chromatogr. 11 (1997) 298–302.
[137] J.-M. Lin, T. Nakagama, K. Uchiyama, T. Hobo, Enantioseparation of D,L-phenylalanine by molecularly imprinted polymer particles filled capillary electrochromatography, J. Liq. Chromatogr. Relat. Technol. 20 (1997) 1489–1506.
[138] H.-F. Wang, Y.-Z. Zhu, J.-P. Lin, X.-P. Yan, Fabrication of molecularly imprinted hybrid monoliths via a room temperature ionic liquid-mediated nonhydrolytic sol–gel route for chiral separation of zolmitriptan by capillary electrochromatography, Electrophoresis 29 (2008) 952–959.
[139] Z.J. Tan, V.T. Remcho, Molecular imprint polymers as highly selective stationary phases for open tubular liquid chromatography and capillary electrochromatography, Electrophoresis 19 (1998) 2055–2060.
[140] S.A. Zaidi, W.J. Cheong, Robust open tubular layer of S-ketoprofen imprinted polymer for chiral LC separation, J. Sep. Sci. 31 (2008) 2962–2970.
[141] S.A. Zaidi, W.J. Cheong, Preparation of an open-tubular capillary column with a monolithic layer of S-ketoprofen imprinted and 4-styrenesulfonic acid incorporated polymer and its enhanced chiral separation performance in capillary electrochromatography, J. Chromatogr. A 1216 (2009) 2947–2952.
[142] S.A. Zaidi, K.M. Han, D.G. Hwang, W.J. Cheong, Preparation of open tubular molecule imprinted polymer capillary columns with various templates by a generalized procedure and their chiral and non-chiral separation performance in CEC, Electrophoresis 31 (2010) 1019–1028.
[143] S.A. Zaidi, K.M. Han, D.G.H. Sung Soon Kim, W.J. Cheong, Open tubular layer of S-ofloxacin imprinted polymer fabricated in silica capillary for chiral CEC separation, J. Sep. Sci. 32 (2009) 996–1001.
[144] Y. Wang, Z. Zeng, N. Guan, J. Cheng, Sol–gel technique for the preparation of beta-cyclodextrin derivative stationary phase in open-tubular capillary electrochromatography, Electrophoresis 22 (2001) 2167–2172.