Focussing Review

Enantiomeric Separation by CEC Using Chiral Stationary Phases

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Abstract

Capillary electrochromatography (CEC) is now a promising technique for enantiomeric separation, because of the high separation efficiency of the technique. In the last few years, many chiral stationary phases (CSPs) were prepared for CEC and acidic, neutral, and basic enantiomers were separated by these CSPs. These CSPs are classified into three types : (I) open tubular capillary, (II) packed capillary and (III) monolithic capillary. In this review, we evaluate these types of CSPs and compared separation conditions (inner diameter of capillary and mobile phase) and data (theoretical plate number, separation factor and resolution) by these CSPs.

Keywords: capillary electrochromatography, enantiomeric separation, chiral stationary phase

1. Introduction

Capillary electrochromatography (CEC) is a hybrid technique of high performance liquid chromatography (HPLC) and capillary electrophoresis (CE). CEC shows good separation efficiency because it uses electroosmotic flow (EOF) for pumping the mobile phase and can separate both charged and uncharged compounds *via* electrophoresis and chromatographic separation. These principles of CEC could be a main driving force for application in many research fields, including enantiomeric separation. Reviews have been published about CEC and its applications (1-8).

Separation of enantiomers is very important because some enantiomers show completely different biological activities than their optical isomers (9). For example, thalidomide is a sedative drug, but *S*-(-)-thalidomide is teratogenic. Another example : *R*-(+)-limonene smells like orange, but *S*-(-)-limonene smells like lemon. Thus, separation of enantiomers is required to clarify the biological activity of each isomer. However, it is difficult to separate enantiomers, because they show exactly the same chemical and physical properties except for optical rotation. CEC is a suitable separation technique to separate enantiomers, because of its excellent separation efficiency.

Enatiomeric separation by chromatography, including CEC, can be performed in three modes : (I) diastereomer formations, (II) adding chiral selectors to the mobile phase and (III) using chiral stationary phases (CSPs). Reviews and books explain these separation modes in detail (10).

Using CSPs is the most popular mode for enantiomeric separation in HPLC. There are CSPs which are modified with cyclodextrins (CDs), modified CDs, Pirkle type, macrocyclic antibiotics, proteins, cellulose derivatives, etc. These CSPs, well known in HPLC, can also be utilized for packing material for CEC. For chiral mobile phase additive mode, the solubility of chiral selector in the running buffer and the absorbance of chiral selector at the detection wavelength are critical. CSPs do not need to use a chiral selector in the mobile phase and, thus, have no detection problems. Another advantage in CEC is the amount of chiral selector for preparing a separation column, one mg ; this is about 100 times less than that for HPLC. Some chiral selectors are expensive because they are difficult to synthesize. In these cases, enantiomeric separation is a suitable application field of CEC. Three different approaches are used for preparation of separation column in CEC : (I) open tubular capillary, (II) packed capillary and (III) monolithic capillary.

In this review, we mainly introduce some recent progress in the field of enantiomeric separation using packed and monolithic capillary, some important papers about open tubular capillary were also introduced. The kinds of chiral selectors and the parameters affecting the separation on CSPs are summarized on Table 1. We do not discuss molecularly imprinted polymer (MIP) based CSP, because many reviews have already been published (11-13) on this subject.

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Table 1. Chiral selectors and the separation data by CEC.

Chiral selector	Packing material	Capillary I.D.	Packing procedure	Analyte	Mobile phase	Separation parameters	Remarks	Reference
Cyclodextrins and modified cyclodextrins								
β- and γ-cyclodextrin	polyacrylamide gel	75μm I.D.	m-CEC	DNS derivatives (Leu, Ser, Val, Glu, Asp, Met, Thr, norleucine, norvaline, α-amino-*n*-butyric acid, Phe, Trp)	100mM Tris/250mM boric acid (pH 8.3)/methanol=90/10	α=1.12, N=100000/per column(15cm), Rs=6.4 (DNS-Ser)	Concentrarion of cyclodextrin, addition of methanol to the buffer and temperature were examined.	[28]
β-cyclodextrin	5μm particle	50μm I.D.	p-CEC	benzoin, hexobarbital, DNS-Thr, DNP derivatives (α-amino-*n*-butyric acid, norleucine, α-amino-*n*-caprylic acid, methionine sulfone, Met, ethionine, citrulline, Glu)	4mM phosphate, 5% acetonitrile (pH 6.8)	α=1.09, N=30000/column, HETP=7μm, Rs=1.39 (hexobarbital)	Buffer composition, pH, concentration of background electrolyte and organic modifier were studied. Comparisons between the mobile phase additives and packed-tubular systems were made.	[35]
hydroxypropyl-β-cyclodextrin	5μm silica particle	50μm I.D.	p-CEC	chlorthalidone, mianserin	5mM phosphate buffer (pH 6.5), 15% acetonitrile	α=1.30, N=10000, Rs=3.4 (chlorthalidone)	Comparisons between the mobile phase additives and using the stationary phase were made.	[36]
hydroxypropyl-β-cyclodextrin	5μm sulfonated silica particle	100μm I.D.	p-CEC	DNS derivatives (Leu, norleucine, Val, Ser, Thr, Trp, Phe, Met, Glu, Asp), silvex, 2-(2,4-dichlorophenoxy)propionic acid, 2-(4-chloro-2-methylphenoxy)propionic acid, 2-(4-chlorophenoxy)propionic acid, 2-(3-chlorophenoxy)propionic acid, 2-(2-chlorophenoxy)propionic acid	acetonitrile/phosphate buffer (pH5.5)=20/80, 1.6 mM sodium phosphate	α=1.30, Rs=2.05 (DNS-Leu)	Organic modifier content, pH and ionic strength were examined.	[37]
permethylated-β-cyclodextrin	5μm silica particle	100μm I.D.	p-CEC, pressure-assisted CEC	mephobarbital, hexobarbital, pentobarbital, 1-methyl-5-(2-propyl)-5-(*n*-propyl)barbituric acid, 5-ethyl-1-methyl-5-(*n*-propyl)barbituric acid, benzoin, α-methyl-α-phenylsuccinimide, glutethimide, MTH-Pro, methyl mandelate	5mM phosphate buffer (pH 7.0)/methanol=4/1	α=1.31, N=17600/m, Rs=3.00 (mephobarbital)	Type and composition of organic modifiers were studied. Comparisons between micro-HPLC and CEC.	[38]
permethylated-β-cyclodextrin (Chirasil-Dex silica)	5μm silica particle (octamethylene spacer)	100μm I.D.	p-CEC, pressure-assisted CEC	mephobarbital, hexobarbital, glutethimide, 1-methyl-5-(2-propyl)-5-(*n*-propyl)-barbituric acid, 5-ethyl-1-methyl-5-(*n*-propyl)-barbituric acid, benzoin, α-methyl-α-phenylsuccinimide, γ-phenyl-γ-butyrolactone, MTH-Pro, methyl mandelate, 1-(2-naphthyl)ethanol, mecoprop methyl, diclofop methyl, fenoxaprop ethyl	20 mM MES buffer (pH 6.0)/methanol=1/1	α=1.35, N=65900/m, Rs=2.43 (MTH-Pro)	Type and concentration of buffer, amount and nature of organic modifiers were studied. Comparisons between micro-HPLC and CEC.	[20]
permethylated-β-cyclodextrin (Chirasil-Dex silica)	5mm silica particle (octamethylene spacer)	100μm I.D.	m-CEC	mephobarbital, hexobarbital, 1-methyl-5-(2-propyl)-5-(*n*-propyl)barbituric acid, 5-ethyl-1-methyl-5-(*n*-propyl)barbituric acid, benzoin, α-methyl-α-phenylsuccinimide, MTH-Pro, mecoprop methyl, fenoxaprop methyl, carprofen, ibuprofen	20mM MES buffer (pH 6.0)/methanol=7/3	α=1.20, N=60900/m, Rs=3.17 (mephobarbital)	Comparisons capilary LC, pressure-assisted CEC and without pressure-assisted CEC.	[39]
2-hydroxy-3-alloxy-propyl-β-cyclodextrin	polyacrylamide gel(acrylamide, ammonium persulfate, *N*,*N*′-methylenebisacrylamide, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine)	25μm I.D.	m-CEC	hexobarbital, mephobarbital, warfarin, tropicamid, ibuprofen, propranolol, mephenytoin, hydrobenzoin	100mM Tris/150mM boric acid buffer (pH 8.2)	α=1.15, N=560000/m, Rs=1.40 (warfarin)	Effect of 2-hydroxy-3-allyloxy-propyl-β-cyclodextrin on separation.	[40]
polymeric-β-cyclodextrin and carboxymethyl β-cyclodextrin polymer	polyacrylamide gel	75μm I.D.	m-CEC	terbutaline, benzoin	200mM Tris/300mM boric acid buffer (pH 9.0)	α=1.03, N=26000, Rs=1.21 (terbutaline)	Chiral selectors were examined.	[41]

allyl carbamoylated β-cyclodextrin	charged polyacrylamide gel (acrylamide, *N*,*N*′-methylenebisacrylamide, *N*,*N*,*N*′,*N*′-tetramethylethylendiamine, ammonium peroxodisulfate, 2-acrylamido-2-methylpropane sulfonic acid	75μm I.D.	m-CEC	terbutaline, propranolol, benzoin	200mM Tris/300mM boric acid buffer (pH 9.0)	α=1.05, N=67000/m, Rs=1.86 (terbutaline)	Reproducibility was studied.	[42]
allyl carbamoylated β-cyclodextrin	2-acrylamido-2-methylpropane sulfonic acid	75μm I.D.	m-CEC	terbutaline, metaproterenol, isoproterenol, propranolol, pindolol, chlorpheniramine, tryptophan methyl ester, tryptophan ethyl ester, α-methyltryptamine, clenbuterol, 1-(1-naphthalene)ethanol, methyl mandelate, tryptophanol, 1-aminoindan, 1,2,3,4-tetrahydro-1-naphthylamine, 1-(1-naphthyl)ethylamine, primaquine	200mM Tris/300mM boric acid buffer (pH 7.0) containing 10mM of 18-crown-6	α=1.09, N=150000/m, Rs=4.84 (1-(1-naphthyl)ethylamine)	Content of chiral selectors were examined.	[43]
polymeric-β-cyclodextrin	polyacrylamide gel	75μm I.D.	m-CEC	propranolol, chlorpheniramine, 1,2-diphenylethanol, DNS derivatives (Asp, Glu, Ser, Val, Leu, norleucine, Thr, Met, Phe, α-amino-*N*-butylic acid), phenylmercapturic acid, wafarin, mephobarbital	200mM Tris/300mM boric acid buffer (pH 8.1)	α=1.03, N=224000/m, Rs=1.69 (mephobarbital)	Reproducibility and stability of the column were studied. Negatively and positively charged polyacrylamide were used.	[44]
allyl carbamoylated β-cyclodextrin	polyacrylamide gel	75μm I.D.	m-CEC	DNS derivatives(Asp, Glu, Ser, Val, norvaline, Leu, norleucine, Thr, Met, Trp, α-amino-*n*-butyric acid, Phe), phenylmercapturic acid, warfarin, 2-phenoxypropionic acid, *N*-Fmoc-Val, benzoin, 1-(1-naphthalene)ethanol, terbutaline	200mM Tris/300mM boric acid buffer (pH 8.1)	α=1.10, N=151000/m, Rs=4.30 (terbutaline)	Reproducibility and stability of the column were studied.	[45]
Small molecules(amino acids derivatives and Pirkle type)								
(*S*)-naproxen	3μm silica particle	100μm I.D.	p-CEC	5 neutral analyte (DNP derivatives)	25mM MES buffer(pH 6.0)/acetonitrile=1/3.5	α=1.49, N=196000, Rs=8.02 (DNP-Val methyl ester)	Run-to-run, day-to-day and column-to-column reproducibility were studied.	[46]
(3*R*,4*S*)-Whelk-O	3μm silica particle	100μm I.D.	p-CEC	5 neutral analyte (DNP derivatives and other aromatic compounds)	25mM MES buffer(pH 6.0)/acetonitrile=1/3.5	α=3.82, N=200000, Rs=30.95 (*N*-[1-(4-bromophenyl)]-2,2-dimethylpropionamide)		[46]
(3*R*,4*S*)-Whelk-O	3.0μm silica particle	100μm I.D.	p-CEC	more than 30 neutral analytes containing stereogenic center, axe or plane chiral enantiomers	25mM MES buffer(pH 6.0)/acetonitrile=1/2	α=1.55, N=178000, Rs=16.65 (DNP-1-phenylethylamine)	Buffer concentration, modifier amount, temperature, applied voltage and pH were studied. Comparisons between HPLC and CEC.	[47]
(*S*)-*N*-3,5-dinitrobenzoyl-1-naphthylglycine (SUMICHIRAL OA-2500(S))	5μm aminopropyl silica particle	75μm I.D.	p-CEC	NBD derivatives (Ala, Gln, Glu, Ile, Met, Phe, Pro, Ser, Thr, Val, 2,3-diaminopropionic acid, 2-aminobutyric acid, 3-aminobutyric acid)	5mM phosphate buffer (pH2.5)/acetonitrile=3/7	HETP=8.7, Rs=4.01 (NBD-Ala)		[48]

(*S*)-*N*-3,5-dinitrobenzoyl-1-naphthylglycine (SUMICHIRAL OA-2500(S))	5μm aminopropyl silica particle	75μm I.D.	m-CEC	NBD derivatives (Ala, Gln, Glu, Ile, Met, Phe, Pro, Ser, Thr, Val, 2,3-diaminopropionic acid, 2-aminobutyric acid, 3-aminobutyric acid)	5mM phosphate buffer (pH2.5)/acetonitrile=3/7	HETP=14, Rs=4.45 (NBD-Val)	pH and composition of acetonitrile were examined.	[49]
(*S*)-*N*-3,5-dinitrophenylaminocarbonyl-valine (SUMICHIRAL OA-3100)	5μm aminopropyl silica particle	75μm I.D.	m-CEC	NBD derivatives (Ala, Gln, Glu, Met, Pro, Ser, Thr, 2,3-diaminopropionic acid, 2-aminobutyric acid)	5mM phosphate buffer (pH3.0)/acetonitrile=3/7	HETP=56, Rs=1.17 (NBD-Val)		[49]
2-hydroxyethyl methacrylate (*N*-L-valine-3,5-dimethylanilide) carbamate	organic gel (ethylene dimethacrylate, 2-acrylamido-2-methyl-1-propansulfonic acid, butyl or glycidyl methacrylate)	100μm I.D.	m-CEC	*N*-(3,5-dinitrobenzoyl)leucine diallylamide	acetonitrile/5mM phosphate buffer (pH 7)=80/20	N=61000/m, Rs=2.0 (*N*-(3,5-dinitrobenzoyl)leucine diallylamide)	Effect of hydrophilicity was studied.	[50]
N-(2-hydroxy-3-allyloxypropyl)-L-4-hydroxyprolin	organic gel (methacrylamide, piperazine diacrylamide, vinyl sulfonic acid)	75μm I.D.	m-CEC, ligand exchange mode, *in situ* copolymerization	Asn, dopamine, α-methyl-dopamine, α-methylphenylalanine, Tyr, Phe, Ser, Thr, Trp	50mM sodium dihydrogenphsphate and 0.1mM Cu(II) (pH4.6)	α=2.649, Rs=1.721 (α-methyl-dopamine)	Comparisons between capillary LC, pressure-assisted CEC and without pressure-assisted CEC.	[51]
Lys-Tyr	adosorbed capillary wall	25μm I.D.	o-CEC	Tyr, Phe, fenoprofen	10mM Phosphate buffer (pH 7.20)/2-propanol=90/10	Rs=2.0 (fenoprofen), N=560000/m (N.I.)	Run-to-run and day-to-day reproducibility were studied.	[52]
Lys-Ser-Tyr	adosorbed capillary wall	10μm I.D.	o-CEC	Tyr, Phe	10mM Phosphate buffer (pH 6.86)/2-propanol=50/50	Rs=2.0 (Phe), N=390000/m (N.I.)	Run-to-run reproducibility was studied.	[52]
L-Lys	adosorbed capillary wall	10μm I.D.	o-CEC	Phe	10mM Phosphate buffer (pH 6.86)/2-propanol=50/50	Rs=1.5 (Phe), N=590000/m (N.I.)	Run-to-run reproducibility was studied.	[52]
Macrocyclic antibiotics								
vancomycin	5 μm spherical silica gel	100μm I.D.	p-CEC	wafarin, hexobarbital	0.1 % triethylamine acetate (pH 5)/acetonitrile=80/20	α=1.28, N=13300, Rs=2.7 (wafarin)	Effect of mobile phase composition was studied.	[53]
vancomycin	5μm diol silica	75μm I.D.	p-CEC, *in situ* immobilisation	thalidomide, alprenolol, atenolol, bupivacaine, ephedrine, isoprenaline, ketamine, metoprolol, phenylamine, practolol	methanol/acetonitrile/acetic acid/triethylamine=80/20/0.2/0.2	α=2.48, N=115000/m, Rs=2.52 (thalidomide)	Composition of mobile phase (reversed phase and polar organic phase) was studied.	[54]
vancomycin	5μm silica particle	75μm I.D.	p-CEC	pindolol, alprenolol, atenolol, fenoterol, metoprolol, sotalol, propranolol, bupivacaine, labetalol, verapamil, terbutaline, thalidomide, ketamine, warfarin, coumachlor, felodipine, binaphthol	acetonitrile/methanol/triethylamine/acetic acid=20/80/0.1/0.1	N=190000/m, Rs=13.8 (thalidomide)	Organic modifier, organic solvent ratio, ionic strength, pH, temperature and voltage were examined. Aqueous and non-aqueous mode.	[55]
teicoplanin	5μm silica particle	100μm I.D.	p-CEC	tryptophan, DNB-Leu	acetonitrile/water=50/50	N=29000/m, Rs=1.74 (tryptophan)	Composition of mobile phase, temperature and reproducibility were studied.	[56]

teicoplanin	5μm silica particle	75μm I.D.	p-CEC	wafarin, coumachlor, felodipine, 5,5-diphenylhydantoin, tryptophan, 5-(4-methylphenyl)-5-phenylhydantoin, *N*-Z-glutamic acid, 5-(4-hydroxyphenyl)-5-phenylhydantoin, benzoin, terbutaline, ibuprofen, bupivacaine, alprenolol, atenolol, fenoterol, pindolol, sotalol, propanolol, bupivacaine, labetalol, verapamil, metoprolol, phenylpropanolamine, β-hydroxyphenethylamine, thalidomide, ketoprofen, dopa	methanol/acetonitrile/triethylamine/acetic acid =80/20/0.1/0.1	N=136000/m, Rs=3.27 (alprenolol)	Non-aqueuous polar organic mode and reversed-phase mode were compared.	[57]
Proteins							
bovine serum albumin	cross-linked gel (glutaraldehyde)	75μm I.D.	m-CEC	tryptophan	50mM potassium phosphate (pH 7.5)	N=91000, Rs=6.0 (Try)	Capillary affinity gel electrophoresis.	[58]
cellobiohydrolase I, bovine serum albumin	cross-linked gel (glutaraldehyde)	75μm I.D.	m-CEC	atenolol, metoprolol, pindolol, propranolol	50mM potassium phosphate (pH 6.8) + 1% 2-propanol		Effect of sample volume on peak shape.	[59]
cellobiohydrolase I	cross-linked gel (glutaraldehyde)	75μm I.D.	m-CEC	acebutolol, atenolol, metoprolol, pindolol, prenalterol, propranolol	50mM potassium phosphate (pH 6.8) + 1% 2-propanol	N=75000-30000/m, Rs=4.2 (pindolol)	Comparisons between mobile phase additive (cyclodextrin derivatives) separation mode, using chiral stationary phase mode and MIP mode.	[60]
α_1-acid glycoprotein	5μm particle	50μm I.D.	p-CEC	benzoin, hexobarbital, pentobarbital, ifosfamide, cyclophosphamide, metoprolol, oxprenolol, alprenolol, disopyramide, propranolol	5% 1-propanol/5 mM phosphate (pH 6.5)	N=5800, HETP=29mm (benzoin)	pH, electrolyte concentration and concentration of organic solvent are studied.	[61]
human serum albumin	7μm silica particle	50μm I.D.	p-CEC	oxazepam, temazepam, benzoin	4mM phosphate(pH 7.0) + 2% 2-propanol	α=2.4, N=7000/m (temazepam)	Type and concentration of organic modifier are studied. Comparisons between the mobile phase additive mode and using the stationary phase mode.	[62]
bovine serum albumin	chemically bond	25 or 50μm I.D.	o-CEC	DNP derivatives (Ala, Glu, Phe, Pro), lorazepam, oxazepam	50mM Phosphate buffer (pH6.0)	Rs=4.64, N=34000/m (DNP-Ala)	Comparison between OTLC and CEC	[63]
lysozyme	adosorbed capillary wall	10μm I.D.	o-CEC	Trp, PTH-Asp, PTH-Thr, DNS-Leu, mephenytoin	10mM Phosphate buffer (pH 7.20)/2-propanol=90/10	Rs=2.05, N=110000/m (DNS-Leu)	Run-to-run reproducibility was studied.	[52, 64]
cytochrome c	adosorbed capillary wall	25μm I.D.	o-CEC	Tyr, Phe, Trp, chrysanthemic acid, DNS-Leu	10mM Phosphate buffer (pH 6.86)/2-propanol=80/20	Rs=4.1 (DNS-Leu), N=400000/m (N.I.)	Run-to-run and day-to-day reproducibility were studied.	[52]
avidin	adosorbed capillary wall	28 or 50μm I.D.	o-CEC	ketoprofen, flubiprofen, ibuprofen, warfarin, adenochrome semicarbazone, chlormezanone, DNS derivatives (Ser, Met, Thr, Val, norleucine, α-amino-*n*-butyric acid, Trp) abscisic acid, suprofen	10mM Phosphate buffer (pH 5.95)/methanol=85/15	Rs=1.51, N=186600/m (ketoprofen)	Buffer pH, organic modifier, applied voltage and temperature were examined. Run-to-run, day-to-day and column-to column reproducibility were studied.	[65]
Quinine-based anion exchange							
tert. -butyl carbamoyl quinine	5μm silica particle	75 or 100μm I.D.	p-CEC	Fmoc-Leu, DNZ-Leu, DNB-Leu	50mM acetic acid/acetonitrile=20/80 (mixture titrated to pH 6 with triethylamine)	α=2.16, N=122000(DNZ-Leu)	pH, organic modifier, buffer concentration were studied. comparisons between HPLC and CEC.	[66]

tert.-butyl carbamoyl quinine	3μm silica particle	100μm I.D.	p-CEC	DNZ derivatives (Leu, Phe, Pro), Z derivatives (Leu, Phe, Tyr), Fmoc derivatives (Ala, Asn, Trp, Arg, Leu), DNP derivatives (Phe, Lys, α-amino caprylic acid, Phe, Ser), Bz-Leu, Bz-Phe, Ac-Phe, Ac-Trp, dichlorprop, suprofen, flurbiprofen, etodolac, sulfinpyrazone	acetonitrile/methanol=80/20+400 mM acetic acid+4mM teiethylamine	N=106000/m, Rs=6.9 (Fmoc-Leu)	Electrolyte concentration, mobile phase composition and temperature were studied. Nonaqueous mobile phase.	[67]
tert.-butyl carbamoyl quinene	1.5 (nonporous), 3 (porous) or 5μm silica particle	75 or 100μm I.D.	p-CEC, electrokinetic ally packing	DNZ-Leu, Fmoc-Leu, DNB-Leu, 1,1'-binaphthyl-2,2'-diylhydrogenphosphate, Bz-Leu, DNP-Val, Fmoc derivatives(Arg, Leu, Phe, Trp, Asn)	acetonitrile/buffer=80/20 + 150mM 2-(*N*-morpholino)ethanesulfonic acid (pH 6.0)	α=1.14, N=120000/m, Rs=4.32 (DNB-Leu)	Comparisons three differnt types of silica particles. Effect of electric field strength, temperature, pH, buffer type and concentration, organic modifer type and concentration were examined.	[68]
O-[2-(methacryloyloxy)ethylcarbamoyl]-10,11-dihydroquinidine	organic polymer (ethylene dimethacrylate, glycidyl methacrylate or 2-hydroxyethyl methacrylate)	100μm I.D.	m-CEC	DNB-Leu, DNZ-Leu	acetonitrile/methanol=80/20 + 400mM acetic acid + 4mM triethylamine	α=2.48, N=74000/m, Rs=22.57 (DNB-Leu)	Polymerization conditions, monomer and porogen were studied.	[69]
O-[2-(methacryloyloxy)ethylcarbamoyl]-10,11-dihydroquinidine	organic polymer (ethylene dimethacrylate, 2-hydroxyethyl methacrylate)	100μm I.D.	m-CEC	DNP-Val, Fmoc-Leu, DNZ-Leu, DNB-Leu, Bz-Leu Ac-Phe, Fmoc-Val, Z-Phe, DNZ-Phe, DNP-Ser, DNP-Gln, DNP-Leu, 2-(4-chloro-2-methylphenoxy)propionic acid (mecoprop), 2-(2,4,5-trichlorophenoxy)propionic acid (fenoprop)	acetonitrile/methanol=80/20 + 600mM acetic acid + 6mM triethylamine	α=1.21, N=242000/m, Rs=6.28 (DNP-Val)	Effect of pore size of monolith and mobile phase composition on separations were studied.	[70]
Cellulose derivatives								
cellulose tris (3,5-dimethylpheny lcarbamate)	coated capillary (0.025μm thikness)	50μm I.D.	o-CEC	1-(9-anthryl)-2,2,2-trifluoroethanol	40mM phosphate buffer (pH 7)/acetonitrile=60/40		Temperature was examined. Comparisons between HPLC, OTLC and CEC.	[71]
cellulose tris(4-methylbenzoate)	coated capillary (0.025μm thikness)	50μm I.D.	o-CEC	glutethimide, aminoglutethimide, mephobarbital, 1-(1-naphthyl)ethyl alcohol	40mM phosphate buffer (pH 7)/acetonitrile=80/20	α=1.69, N=36700, Rs=2.8 (glutethimide)	Coating thickness and organic modifier were examined. Comparisons between HPLC, OTLC and CEC.	[71]
cellulose tris(3,5-dimethylpheny lcarbamate)	5μm silica particle	100μm I.D.	p-CEC, with and without pressure-assisted CEC	indapamide	20mM sodium citrate(pH 7.0)/acetonitrile=55/45		Comparisons between pressure-driven and electrically-driven CEC.	[72]
cellulose tris(3,5-dimethylpheny lcarbamate)	5 or 7μm macroporous silica particle	100μm I.D.	p-CEC, electrokinetically packing	benzoin, indapamide, *trans*-stilbene oxide, glutethimide, lorazepam, α-1-hydroxyethylnaphthalene	20mM sodium citrate(pH 5)/water/acetonitrile=10/20/70	α=1.21, N=20000/m, Rs=1.26 (glutethimide)	Content of modifier, concentration and pH of the mobile phase were studied. Comparions between capillary-LC and CEC.	[73]
cellulose tris(3,5-dimethylpheny lcarbamate) (Chiralcel OD)	3 or 5μm silica particle	100μm I.D.	p-CEC	pindolol, propranolol, 4-phenyl-2-butanol, benzoin, indapamide, homatropin, wafarin, verapamil, enilconazole, ibuprofen, 3-phenylbutyric acid	50mM phosphate buffer(pH 4.0)/acetonitrile=30/70	α=1.19, N=34000/m, HETP=7.1 (homatropine)	Comparisons between 3μm and 5μm silica particles.	[74]
amylose tris(3,5-dimethylpheny lcarbamate) (Chiralpak AD)	5μm widepore aminopropylsilica particle	100μm I.D.	p-CEC, non-aqueous	thalidomide, 5-hydroxythalidomide, *cis*-5'-hydroxythalidomide	methanol/ethanol =75/25 + 2.5mM ammonium acetate		Comparisons between HPLC, capillary LC and CEC. Preparation of a column packed with mixture of Chiralpak AD, Chiralpak OD and aminopropylsilica.	[75]
amylose tris(3,5-dimethylpheny lcarbamate) (Chiralpak AD)	5μm widepore aminopropylsilica particle	100μm I.D.	p-CEC, non-aqueous	aminoglutethimide, *trans*-stilbene oxide, metomidate, piprozolin	10mM ammonium acetate in ethanol (pH 7.7)	α=3.23, N=51000/m, Rs=1.36 (*trans*-stilbene oxide)	pH and composition of mobile phase were examined. Comparisons between HPLC, capillary LC and CEC.	[76]

cellulose tris(3,5-dimethylpheny lcarbamate) (Chiralcel OD)	5μm widepore aminopropylsil ica particle	100μm I.D.	p-CEC, non-aqueous	piprozolin, glutethimide, etozolin, Troeger's base, indapamide, piprozolin	10mM ammonium acetate in methanol (pH 7.7)	α=2.01, N=24000/m, Rs=1.55 (piprozolin)	pH and composition of mobile phase were examined. Comparisons between HPLC, capillary LC and CEC.	[76]
cellulose tris(4-methylbenzoat e) (Chiralcel OJ)	5μm widepore aminopropylsil ica particle	100μm I.D.	p-CEC, non-aqueous	*trans* -stilbene oxide, econazole, 2,2'-diamino-6,6'-dimethylbiphenyl, glutethimide	10mM ammonium acetate in methanol (pH* 7.7)	α=1.35, N=24000/m, Rs=2.36 (*trans* -stilbene oxide)	pH and composition of mobile phase were examined. Comparisons between HPLC, capillary LC and CEC.	[76]
cellulose tris(3,5-dichlorophenylcarbamate)	5mm aminopropyl silica particle	100μm I.D.	p-CEC, non-aqueous	2-(benzylsulfinyl)benzamide, 2-(benzylsulfinyl)-banzoic acid benzyl ester, etozolin, piprozolin	2.5mM ammonia acetate in methanol (pH* 7.7)	N=213400/m, Rs=1.84 (2-(benzylsulfinyl) benzamide)	Amount of chiral selector was examined. Comparisons capillary LC and CEC.	[77]
Helically chiral organic polymer								
poly-*N*-acryloyl-L-phenylalanine ethylester (Chiraspher)	5μm silica particle	100μm I.D.	p-CEC, with and without pressure-assisted CEC	bendroflumethiazide	50mM NaH_2PO_4 (pH 8.0)/acetonitrile= 60/40		Comparisons between pressure-driven and electrically-driven CEC.	[72]
poly(diphenyl-2-pyridylmethyl methacrylate)	5μm wide-pore aminopropyl silica particle	100μm I.D.	p-CEC, non-aqueous	benzoin acetate, methylbenzoin, Troger's base, *trans* -stilbene oxide	2.5mM ammonium acetate in methanol (pH* 4.5)	N=5400 (methylbenzoin)	Comparisons between HPLC, capillary LC pressure-assisted CEC and without pressure-assisted CEC.	[78]
poly(diphenyl-2-pyridylmethyl methacrylate)	5μm silica particle	100μm I.D.	p-CEC, non-aqueous	benzoin, methylbenzoin, ethylbenzoin, isopropylbenzoin, benzoin acetate, 1,1'-binaphthyl-2,2'-diol, *trans* -stilbene oxide, cyclbutyldianilide carbamate	acetonitrile/water =80/20 + 2.5mM ammonium acetate (pH* 4.5)	α=2.79, N=23000/m, Rs=4.57 (*trans* -stilbene oxide)	Composition and type of mobile phase were studied. Contribution of pressure-driven flow and electrokinetically driven flow was evaluated.	[79]

ABBRIBATION, α: separation factor, Ac: acetyl, Bz: benzoyl, DNB: dinitrobenzoyl, DNP: dinitrophenyl, DNS: dansyl, DNZ: *N*-3,5-dinitrobenzyloxycarbonyl, Fmoc: *N*-9-fluorenylmethoxycarbonyl, HETP: height equivalent to a theoretical plate, MES: 2-(*N*-morpholino)ethanesulfonic acid, MTH: methylthiohydantoin, N: theoretical plate number, NBD: 4-fluoro-7-nitro-2,1,3-benzoxadiazole, N.I.: Not identified, OTLC: open tubular liquid chromatography, *pH: apparent pH, Rs: resolution, Z: benzyloxycarbonyl

2. Type of capillary

2.1 Open tubular capillary

Open tubular capillary is coated by the chiral selectors physically or chemically onto the internal capillary wall and there is no packing material inside the capillary. The first report of enantiomeric separation using open tubular CEC (o-CEC) was described by Mayer and Schurig in 1992 (14). They coated the capillary wall with permethyl-β-CD. The main drawback of o-CEC is low sample loading capacity, due to the low surface area of o-CEC (inner diameter of an o-CEC capillary is less than 50 μm and the thickness of coating is less than 1 μm). By etching the internal wall of the capillary, the surface area increased up to 1,000- fold and reduce the loading problem (15).

2.2 Packed capillary

In packed CEC (p-CEC), the capillary is filled with chiral modified particles, many of which are also used as packing particles for HPLC columns. p-CEC is the most common CEC mode and numerous numbers of commercially available LC packing materials with different selectivities are applicable. Packing particles for HPLC columns, however, suffer an end-capping treatment, which reduces the free silanol groups. The treatment reduces not only interactions between silanol and analyte, but also the EOF, which means separation time is extended. Some specially designed packing particles based on ion-exchange phase, organic polymer, sol-gel etc. have been developed to promote the EOF (16). A serious drawback of p-CEC is the difficulty of frit fabrication (frits prevent packing particles from flowing away). Frit fabrication with a good repeatability, permeability and durability is technically difficult, because the inner diameter of capillary is narrow (100-50 μm) and the frit length is very short (1-2 mm). Furthermore, the frit itself disturbs separation efficiency by encouraging bubble formation or by disturbing the flow profile of the mobile phase at the interface between the packed section and the frit (17-19). Bubbles lead to increase of baseline noise, and sometimes current breaks down which stop EOF. To prevent bubble formation, the separation can be performed under a pressurized condition, in which both inlet and outlet vials are pressurized by gas. The pressurizing system also helps to compensate for the slow EOF (20). Although the system is useful, the frit still deteriorates the separation efficiency. To overcome these frit problems, another mode of CEC, that is, m-CEC was developed.

2.3 Monolithic capillary

m-CEC consists of a single piece of porous solid packing material, without a frit. Gusev (21) defines this "monolithic stationary phases" as "a continuous unitary porous structure prepared by *in situ* polymerization or consolidation inside the column tubing and, if necessary, the surface is functionalized to convert it into a sorbent with the desired chromatographic binding properties." The monolithic structure is fixed to a capillary by chemical or physical interaction, which prevents it from being pushed out from the capillary by EOF or electrophoretic forces. There are two main methods of m-CEC preparation as follows :

(a) The monolithic structure is prepared by co-polymerization of a homogeneous mixture of chiral selector and monomer (acrylamide or methacrylate). After polymerization, chiral recognition is achieved either through (1) molecular recognition of analytes by the chiral selector, or (2) physical recognition of analytes in the cavities remaining throughout the monolithic. The latter, which is the basis for MIP separations, takes advantage of highly selective spatial recognition properties of the cavities, which similar to that of antibodies or receptors.

(b) The monolithic structure is prepared from slurry solution of CSP and monomer. After polymerization, CSP is encapsulated by porous polymer and is fixed within capillary.

These structural features do not cause frit problems. Furthermore, m-CEC is prepared in one step using *in situ* polymerization inside the capillary ; hence, preparation of m-CEC is easier than p-CEC. A serious problem in m-CEC is the limited choices of monomer. The monolithic structure needs to be porous, robust and stable and it also needs to be charged for EOF. Despite this problem, m-CEC is a popular trend in CEC.

There are several new trends in CEC research. One is the use of non-aqueous mobile phases. Many CSPs in HPLC obtained successful enantiomeric separation in non-aqueous mobile phases, such as ethanol or mixtures of hexane and iso-propanol (22, 23). Non-aqueous mobile phases have also been used in CEC. This method is attractive when solubility or stability problems exist in aqueous buffers, but run-to-run reproducibility problems still need to be solved (24). The other trends are hyphenation technology of CEC with mass spectrometry or CEC separation system on a chip. The details of these new trends have been reported in some papers including this special issue (25-27).

3. Chiral selectors

3.1 CDs and modified CDs

CDs and modified CDs are the most widely used compounds as chiral selectors for enantiomeric separation in LC, GC and CE. CDs are cyclic oligosaccharides with truncated cylindrical molecular shapes, and have particular names, α-, β- and γ-CD for those having six, seven and eight glucopyranose units, respectively. Guttman *et al.* reported the first enantiomeric separation using p-CEC with immobilized CDs in 1988 (28). In this study, polyacrylamide gel and β- or γ-CD were packed inside the capillary. These discharged compounds does not cause fast EOF, hence acidic analytes migrate toward the anode by electrophoresis with forming complexes with β- or γ-CD. Dansyl-Ser enantiomers were separated using this technique (α = 1.12, N = 100,000/m).

Some o-CECs were prepared using CDs and modified CDs. These reports compared CEC with other separation methods (GC, HPLC, OTLC, SFC) using the same capillary (29-34). Li and Lloyd reported enantiomeric separations of neutral drugs and amino acid derivatives using p-CEC packed with β-CD modified particles (35). The separation efficiency is better than that of β-CD additives in the mobile phase. Leliévre *et al.* used hydroxypropyl-β-CD as a chiral selector (36). Separation efficiency of p-CEC packed with the chiral selector was compared with that of ODS packed p-CEC with the chiral selector additive in the mobile phase. The latter method achieved higher selectivity and resolution in a shorter analysis time. Zhang *et al.* also used hydroxypropyl-β-CD as a chiral selector for p-CEC (37). They packed particles with sulfate and separated acidic compounds within a short time.

Schurig and co-workers used permethyl-β-CD as a chiral selector for enantiomeric separation of mephobarbital on both p-CEC and m-CEC (20, 38, 39). m-CEC obtained better efficiency than p-CEC. In another example of m-CEC, Végvári *et al.* prepared m-CEC from acrylamide gel containing 2-hydroxy-3-allyloxy-propyl-β-CD as a chiral selector (40). They separated acidic, neutral and basic drugs using m-CEC. Koide and Ueno prepared m-CEC composed of acrylamide gel and allyl carbamoylated β-CD (41-45). Allyl carbamoylated β-CD was trapped inside the gel by chemical or physical interactions. Acidic, neutral and basic drugs were separated enantiomerically by m-CEC and mephobarbital showed a good enantiomeric separation (α = 1.03, N = 224,000/m).

3.2 Small molecules (amino acid derivatives and Pirkle type)

Some amino acid derivatives or drug derivatives are used as chiral selectors in HPLC. These chiral selectors make it easy to reverse the elution order of enantiomers by changing a configuration of the chiral selectors. Wolf *et al.* modified 3 μm silica particles with (*S*)-naproxen and (3 *R*, 4 *S*)-Whelk-O for p-CEC, and separated more than 30 neutral compounds (46, 47). Enantiomeric separation of *N*-[1-(4-bromophenyl)]-2,2-dimethylpropionamide showed good efficiency (Rs = 30.95, N = 200,000/m).

Fluorescently derivatized amino acids and non-protein amino acids were separated by a p-CEC packed with 5 μm aminopropyl silica-gel modified with (*S*)-*N*-3,5-dinitrobenzoyl-1-naphthylglycine (48). Amino acids and non-protein amino acids

were derivatized with the fluorogenic reagent, 4-fluoro-7-nitro-2, 1, 3-benzoxadiazole (NBD-F). Resolution ranged from 1.21 to 8.29, with a plate height from 8.7 to 39 μm. NBD-amino acids were also separated by m-CECs packed with 5 μm aminopropyl silica-gel modified with (*S*)-*N*-3,5-dinitrobenzoyl-1-naphthylglycine and (*S*)-*N*-3,5-dinitrophenylaminocarbonyl-valine (49). These modified particles were fixed within a capillary by porous monolithic structure, which was prepared by sol-gel reaction. The m-CEC prepared by (*S*)-*N*-3,5-dinitrobenzoyl-1-naphthylglycine showed better enantiomeriseparation than that by (*S*)-*N*-3,5-dinitrophenylaminocarbonyl-valine. The other m-CEC was prepared by co-polymerization of 2-hydroxyethyl methacrylate (*N*-L-valine-3, 5-dimethylanilide) carbamate and acrylamide (50). *N*-(3, 5-dinitrobenzoyl) leucine diallylamide enantiomers were separated by the m-CEC with a resolution of 2, and theoretical plate number for the first eluted enantiomer of 61,000/m. Schmid *et al.* prepared m-CEC composed of polyacrylamide gel containing *N*-(2-hydroxy-3-allyloxypropyl)-L-4-hydroxyproline (51). They added copper in the mobile phase and separated amino acids and biological compounds by ion-exchange mode (α-methyl-dopamine : α = 2.65). Liu *et al.* used amino acid (Lys) or peptides (Lys-Tyr and Lys-Ser-Tyr) for chiral selectors for o-CEC (52). These chiral selectors have positively charged and adsorbed onto the capillary wall by flushing a solution containing the chiral selectors into the capillary. These columns separated Phe, Tyr and fenoprofen with the resolution of 1.3 to 2.0.

3.3 Macrocyclic antibiotics

Macrocyclic antibiotics are popular for chiral selectors in HPLC. These selectors have both a binding site (hydrogen bonding, π-π interaction, etc.) and a cavity for forming the host-guest complex. Dermaux used vancomycin as a chiral selector for p-CEC (53). Owens and co-workers also used vancomycin as a chiral selector (54, 55). They separated drugs (β-blocker and nonsteroidal anti-inflammatory drugs) using polar organic and reversed-phase mode. The resolution and theoretical plate number for thalidomide was 13.8 and 190,000, respectively. Another macrocyclic antibiotic, teicoplanin, was also used as a chiral selector for p-CEC. Carter-Finch and Smith separated tryptophan enantiomers ; the HETP of the first eluted enantiomer was 56 μm and the resolution was 1.17 (56). About 30 enantiomers (β-blocker, nonsteroidal anti-inflammatory drugs etc.) were separated by the group of Owens (57). Resolution of tryptophan and alprenolol were 1.74 and 3.27, respectively, and theoretical plate numbers were 29,000/m and 136,000/m.

3.4 Proteins

Proteins are also used as chiral selectors in HPLC. But composition of applicable mobile phase is restricted, because the three-dimensional structures of proteins are dramatically changed by pH or organic modifiers in the mobile phase. Bovine serum albumin and cellobiohydrolase I was used as chiral selectors for m-CEC by Nilsson and coworkers (58-60). They used glutaraldehyde to entrap these proteins inside the capillary. Six β-adrenergic antagonists were separated by m-CEC. Li and Lloyd reported enantiomeric separations of 10 enantiomers using p-CEC packed with 5 μm particles modified with α_1-acid glycoprotein (61). The theoretical plate number of benzoin was low (5,800), because the velocity of mass transfer is slow between protein and enantiomers. They also prepared a p-CEC packed with human serum albumin to separate 3 drugs (62). The theoretical plate numbers of the first eluted enantiomer of temazepam and the separation factor of it were 7,000/m and 2.4, respectively. Bovine serum albumin was used as chiral selector of o-CEC (63). Hofstertter *et al.* bound bovine serum albumin onto the capillary wall chemically and separated dinitrophenyl (DNP)-amino acids and 3-hydroxy-1,4-benzodiazepines. Lysozyme, cytochrome c and avidin were also used as chiral selectors of o-CEC (64, 65). These proteins were adsorbed onto the capillary wall through two kinds of force (electrostatic attraction and hydrophobic interaction). Lysozyme, cytochrome c and avidin column separated 5,5 and 16 enantiomers, respectively. Column-to-column reproducibility of the avidin column (1.1%) was superior to run-to-run reproducibility of it (2.2%) (65).

3.5 Quinine-base anion-exchange

Lindner and co-workers used anion exchange type CSPs modified with *t*-butyl-carbamoyl quinine as a chiral selector (66-70). Quinine has five chiral centers and two basic amino groups : the tertiary quinuclidine group and the aromatic quinoline group. These amino groups have a positive charge at low pH ; thus EOF moves towards the anodes. Anions migrated toward the anode (the same direction of the EOF), hence the anions eluted within a short time. Glutethimide was separated ; its resolution was 4.32 and theoretical plate number of the first eluted enantiomer was 120,000/m with *t*-butyl-carbamoyl quinine as a chiral selector. They also prepared m-CEC by co-polymerizing *O*-[2-(methacryloxyloxy)-ethylcarbamoyl]-10,11-dihydroquinidine and methacrylate (69, 70). *N*-Derivatized aminoacids were separated by m-CEC and resolution of DNP-Val and theoretical plate number of the first eluted enantiomer were 6.28 and 242,000/m, respectively.

3.6 Cellulose derivatives

Cellulose derivatives, amylose-tris (3,5-dimethylphenylcarbamate), cellulose-tris (3,5-dimethylphenylcarbamate) and cellulose

tris (4-methylbenzoate), are some of the most popular chiral selectors in HPLC. CSPs packed with these chiral selectors separated a variety of enantiomers in HPLC. Hence, these chiral selectors have been used for CEC. First paper used the cellulose derivatives as chiral selectos for CEC was reported in 1996. Francotte and Jung coated cellulose-tris (3,5-dimethylphenylcarbamate) and cellulose tris (4-methylbenzoate) onto a capillary wall of o-CEC and separated some enantiomers (71). Cellulose tris (4-methylbenzoate) column separated glutethimide with respolution of 2.8 and theoritical platenumber of 36,700. Krause *et al.* prepared p-CEC packed with 5 μm silica particles modified with cellulose-tris (3,5-dimethylphenylcarbamate) and separated indapamide enantiomers (72). Mayer *et al.* used the same chiral selector for p-CEC and separated glutethimide with a resolution of 1.26 and theoretical plate number of first eluted enantiomer is 20,000/m (73). Otsuka *et al.* used the same chiral selector and compared the effect of packing particle diameter (3 or 5 μm) (74). Blaschke *et al.* prepared p-CEC using amylose-tris (3, 5-dimethylphenylcarbamate), cellulose-tris (3,5-dimethylphenylcarbamate) and cellulose tris (4-methylbenzoate) as chiral selectors (75, 76). Separation efficiencies of these p-CEC are superior to those of HPLC in non-aqueous conditions.

3.7 Helically chiral organic polymer

Helically chiral organic polymers have also been used as chiral selectors for p-CEC. Krause *et al.* used poly-*N*-acryloyl-L-phenylalanineethylester or poly(diphenyl-2-pyridylmethyl methacrylate) as chiral selectors (72, 78, 79). *trans*-Stilbene oxide enantiomers were separated by p-CEC containing poly (diphenyl-2-pyridylmethyl methacrylate) as a chiral selector. Their resolution was 4.57 and theoretical plate number was 23,000/m.

4. Conclusion

Enantiomeric separation by CEC has received considerable attention in recent years. Most reports on CEC showed better separation efficiencies than those in HPLC using similar columns. On the other hand, CEC is still limited with respect to application to the real samples, such as environmental or biological samples. CEC still has problems with column technologies, system stability and column-to-column reproducibility. Further improvements need to be demonstrated and also a wide variety of CSPs for CEC need to become available commercially, before CEC is applied to enantiomeric determination of real samples.

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References

[1] Cikalo, M. G. ; Bartle, K. ; Robson, M. M. ; Myers, P. ; Euerby, M. R. *Analyst* **1998**, *123*, 87 R-102 R.

[2] Fujimoto, C. *Trends Anal. Chem*. **1999**, *18*, 291-301.

[3] Gubitz, G. ; Schmid, M. G. *J. Chromatogr. A* **1997**, *792*, 179-225.

[4] Schurig, V. ; Wistuba, D. *Electrophoresis* **1999**, *20*, 2313-2328.

[5] Gubitz, G. ; Schmid, M.G. ; *Enantiomer* **2000**, *5*, 5-11.

[6] Lämmerhofer, M. ; Svec, F. ; Fréchet, J. M. J. *Trends Anal. Chem*. **2000**, *19*, 676-698.

[7] Wistuba, D. ; Schurig, V. *Electrophoresis* **2000**, *21*, 4136-4158.

[8] Wistuba, D. ; Schurig, V. *J. Chromatogr. A* **2000**, *875*, 255-276.

[9] Subrammanian, G. A Practical Approach to Chiral Separation by Liquid Chromatography ; VCH : Weinheim, 1994 ; Chapter 3.

[10] Ahuja, S. Chiral Separation by Liquid Chromatography ; American Chemical Society : Washington, 1991 ; Chapter 1.

[11] Remcho, V. T. ; Tan, Z. J. *Anal. Chem*. **1999**, *71*, 248 A-255 A.

[12] Takeuchi, T. ; Haginaka, J. *J. Chromatogr. B* **1999**, *728*, 1-20.

[13] Andersson, L. I. *J. Chromatogr. B* **2000**, *745*, 3-13.

[14] Mayer, S. ; Schurig, V. *J. High. Res. Chromatogr*. **1992**, *15*, 129-131

[15] Pesek, J. J. ; Matyska, M. T. *J. Chromatogr. A* **2000**, *887*, 31-41.

[16] Pursch, M. ; Sander, L. C. *J. Chromatogr. A* **2000**, *887*, 313 -326.

[17] Behnke, B. ; Grom, E. ; Bayer, E. *J. Chromatogr. A* **1995**, *716*, 207-213.

[18] Carney, R. A. ; Robson, M. M. ; Bartle, K. D. ; Mayers, P. *J. High Resolut. Chromatogr.* **1999**, *22*, 29-32.

[19] Asiaie, R. ; Huang, X. ; Farnan, D. ; Horvathe, C. *J. Chromatogr. A* **1998**, *806*, 251-263.

[20] Wistuba, D. ; Schurig, V. *Electrophoresis* **1999**, *20*, 2779-2785.

[21] Gusev, I. ; Huang, X. ; Horváth, C. *J. Chromatogr. A* **1999**, *855*, 273-290.

[22] Subrammanian, G. A Practical Approach to Chiral Separation by Liquid Chromatography ; VCH : Weinheim, 1994 ; Chapter 8.

[23] Ahuja, S. Chiral Separation by Liquid Chromatography ; American Chemical Society : Washington, 1991 ; Chapter 3.

[24] Chankvetadze, B. ; Blaschke, G. *Electrophoresis* **2000**, *21*, 4159-4178.

[25] von Brooke, A. ; Nicholson, G. ; Bayer, E. *Electrophoresis* **2001**, *22*, 1251-1266.

[26] Chankvetadze, B. ; Blaschke, G. *J. Chromatogr. A* **2001**, *906*, 309-363.

[27] Uchiyama, K. ; Xu, W ; Hobo, T. *Chromatography* **2001**, *22*, 181-186.

[28] Guttman, A. ; Paulus, A. ; Cohen, A. S. ; Grinberg, N. ; Karger, B. L. ; *J. Chromatogr*. **1988**, *448*, 41-53.

[29] Armstrong, D. W. ; Tang, Y. ; Ward, T. ; Nichols, M. *Anal. Chem*. **1993**, *65*, 1114-1117.

[30] Schurig, V. ; Jung, M. ; Mayer, S. ; Negura, S. ; Fluck, M. ; Jakubetz, H. *Angew. Chem. Int. Ed. Engl*. **1994**, *33*, 2222-2223.

[31] Szeman, J. ; Ganzler, K. *J. Chromatogr. A* **1994**, *668*, 509-517.

[32] Schurig, V. ; Jung, M. ; Mayer, S. ; Fluck, M. ; Negura, S. ; Jakubetz, H. *J. Chromatogr. A* **1995**, *694*, 119-128.

[33] Jakubetz, H. ; Czesla, H. ; Schurig, V. *J. Micro. Sep*. **1997**, *9*, 421-431.

[34] Schurig, V. ; Wistuba, D. *Electrophoresis* **1999**, *20*, 2313-2328.

[35] Li, S. ; Lloyd, D. K. *J. Chromatogr. A* **1994**, *666*, 321-335.

[36] Lelievre, F. ; Yan, C. ; Zare, R. N. ; Gareil, P. *J. Chromatogr. A* **1996**, *723*, 145-156.

[37] Zhang, M. ; EI Rassi, Z. *Electrophoresis* **2000**, *21*, 3135-3140.

[38] Wistuba, D. ; Czesla, H. ; Roeder, M. ; Schurig, V. *J. Chromatogr. A* **1998**, *815*, 183-188.

[39] Wistuba, D. ; Schurig, V. *Electrophoresis* **2000**, *21*, 3152-3159.

[40] Végvári, Á. ; Földesi, A. ; Hetényi, C. ; Kocnegarova, O. ; Schmid, M. G. ; Kudir kaite, V. ; Hjertén, S. *Electrophoresis* **2000**, *21*, 3116-3125.

[41] Koide, K. ; Ueno, K. *Anal. Sci.* **1998**, *14*, 1021-1023.

[42] Koide, K. ; Ueno, K. *Anal. Sci.* **1999**, *15*, 791-794.

[43] Koide, K. ; Ueno, K. *J. Chromatogr A* **2000**, *893*, 177-187

[44] Koide, K. ; Ueno, K. *Anal. Sci.* **2000**, *16*, 1065-1070.

[45] Koide, K. ; Ueno, K. *J. High Resol. Chromatogr* **2000**, *23*, 59-66.

[46] Wolf, C. ; Spence, P. L. ; Pirkle, W. H. ; Derrico, E. M. ; Cavender, D. M. ; Rozing, G. P. *J. Chromatogr. A* **1997**, *782*, 175-179.

[47] Wolf, C. ; Spence, P. L. ; Pirkle, W. H. ; Cavender, D. M. ; Derrico, E. M. *Electrophoresis* **2000**, *21*, 917-924.

[48] Kato, M. ; Dulay, M. T. ; Bennett, B. D. ; Quirino, J. P. ; Zare, R. N. *J. Chromatogr. A* **2001**, *924*, 187-195.

[49] Kato, M. ; Dulay, M. T. ; Bennett, B. D. ; Chen, J. -R. ; Zare, R. N. *Electrophoresis* **2000**, *21*, 3145-3151.

[50] Peters, E. C. ; Lewandowski, K. ; Petro, M. ; Svec, F. ; Fréchet, J. M. J. *Anal. Commun*. **1998**, *32*, 83-86.

[51] Schmid, M. G. ; Grobuschek, N. ; Tuscher, C. ; Gübitz, G. ; Végvári, Á. ; Machtejevas, E. ; Maruska, A. ; Hjertén, S. *Electrophoresis* **2000**, *21*, 3141-3144.

[52] Liu, Z. ; Zou, H. ; Ye, M. ; Ni, J. ; Zhang, Y. *Electrophoresis* **1999**, *20*, 2891-2897.

[53] Dermaux, A. ; Lynen, F. ; Sandra, P. *J. High Resol. Chronatogr*. **1998**, *21*, 575-576.

[54] Wikström, H. ; Svensson, LA. ; Torstensson, A. ; Owens, P. K. *J. Chromatogr. A* **2000**, *869*, 395-409.

[55] Karlsson, C. ; Karlsson, L. ; Armstrong, D.W. ; Owens, P. K. *Anal. Chem*. **2000**, *72*, 4394-4401.

[56] Carter-Finch, A. S. ; Smith, N. W. *J. Chromatogr. A* **1999**, *848*, 375-385.

[57] Karlsson, C. ; Wikström, H. ; Armstrong, D. W. ; Owens, P. K. *J. Chromatogr*. **2000**, *897* , 349-363.

[58] Birnbaum, S. ; Nilsson, S. *Anal. Chem*. **1992**, *64*, 2872-2874.

[59] Ljungberg, H. ; Nilsson, S. *J. Liq. Chromatogr.* **1995**, *18*, 3685-3698.

[60] Nilsson, S. ; Schweitz, L. ; Petersson, M. *Electrophoresis* **1997**, *18*, 884-890.

[61] Li, S. ; Lloyd, D. K. *Anal. Chem*. **1993** *65*, 3684-3690.

[62] Lloyd, D. K. ; Li, S. ; Ryan, P. *J. Chromatogr. A* **1995**, *694*, 285-296.

[63] Hofstetter, H. ; Hofstetter, O. ; Schurig, V. *J. Microcol. Sep*. **1998**, *10*, 287-291.

[64] Liu, Z. ; Zou, H. ; Ni, J. ; Zhang, Y. *Anal. Chim. Acta* **1999**, *378*, 73-76.

[65] Liu, Z. ; Otsuka, K. ; Terabe, S. *J. Sep. Sci.* **2001**, *24*,17-26.

[66] Lämmerhofer, M. ; Lindner, W. *J. Chromatogr. A* **1998**, *829*, 115-125.

[67] Tobler, E. ; Lämmerhofer, M. ; Lindner, W. *J. Chromatogr. A* **2000**, *875*, 341-352.

[68] Lämmerhofer, M. ; Tobler, E. ; Lindner, W. *J. Chromatogr. A* **2000**, *887*, 421-437.

[69] Lämmerhofer, M. ; Peters, E. C. ; Yu, C. ; Svec, F. ; Fréchet, J. M. J. ; Lindner, W. *Anal. Chem.* **2000**, *72*, 4614-4622.

[70] Lämmerhofer, M. ; Svec, F. ; Fréchet, J. M. J. ; Lindner, W. *Anal. Chem*. **2000**, *72*, 4623-4628.

[71] Francotte, E. ; Jung, M. *Chromatographia* **1996**, *42*, 521-527.

[72] Krause, K. ; Girod, M. ; Chankvetadze, B. ; Blaschke, G. *J. Chromatogr. A* **1999**, *837*, 51-63.

[73] Mayer, S. ; Briand, X. ; Francotte, E. *J. Chromatogr. A* **2000**, *875*, 331-339.

[74] Otsuka, K. ; Mikami, C. ; Terabe, S. *J. Chromatogr. A* **2000**,

887, 457-463.

[75] Meyring, M. ; Chankvetadze, B. ; Blaschke, G. *J. Chromatogr. A* **2000**, *876*, 157-167.

[76] Girod, M. ; Chankvetadze, B. ; Blaschke, G. *J. Chromatogr. A* **2000** *887*, 439-455.

[77] Girod, M. ; Chankvetadze, B. ; Okamoto, Y. ; Blaschke, G. *J. Sep. Sci*. **2001** *24*, 27-34.

[78] Krause, K. ; Chankvetadze, B. ; Okamoto, Y. ; Blaschke, G. *Electrophoresis* **1999**, *20*, 2772-2778.

[79] Krause, K. ; Chankvetadze, B. ; Okamoto, Y. ; Blaschke, G. *J. Micro. Sep*. **2000**, *12*, 398-406.