

Chiral Separations

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This fundamental review article covers developments and applications in chiral separations from January 2004 to January 2006 and is restricted to the English language literature. If the numbers of publications appearing during the last several review periods are used as a guide for the acceptance of a technique, it is clear that chiral HPLC is a well-established and widely accepted tool in the analytical laboratory. It is interesting to note that approximately 20 years ago, in a 1987 A-pages review article, Armstrong (1) observed that, "...we are not far from the point when the majority of enantiomeric separations will be considered routine." While the technique clearly has become a well-used tool in the laboratory, it is not far from becoming a classical technique and one that still generates significant interest in the scientific literature. With the tremendous number of publications in this field, a comprehensive review of all published papers is not feasible, and thus, this fundamental review focuses on major developments and trends in the field of chiral separations, as well as representative applications. Several excellent general reviews

covering chiral separations are presented first, followed by an examination of the methods and techniques utilized in each area.

REVIEWS

Capillary Electrophoresis (CE). Capillary electrophoresis remains a popular technique for the enantioseparation of biologically active compounds. Enantiomeric separations by CE employing nonaqueous conditions were reviewed by Laemmerhofer (2), with the focus on solvent effects on chiral recognition and separation mechanism. Riekkola and Siren (3) also reviewed enantioseparations by CE in nonaqueous media. The use of macrocyclic glycopeptides as chiral selectors in CE was reviewed (4), and procedures for their use were described. A review of CE in the chiral separation of nonsteroidal antiinflammatory drugs was presented by Patel et al. (5) with applications including the detection of enantiomeric composition after synthesis, chiral impurity profiles in bulk drugs and formulated products, and metabolite profiles in biological fluids. The chiral analysis of pollutants and their metabolites by CE was reviewed by Hernandez-Borges et al. (6), with emphasis on articles published in the last 10 years. Recent advances in the analysis of small achiral and chiral solutes by CE–mass spectrometry were discussed by Shamsi and Miller (7), with application areas including pharmaceuticals, agrochemicals, carbohydrates, and small peptides. (Additional reviews of chiral separations are listed in Multiple Techniques Reviews.)

High-Performance Liquid Chromatography (HPLC). An overview of enantiomeric separations by HPLC using macrocyclic glycopeptide-based chiral stationary phases (CSPs) was discussed by Xiao and Armstrong (8), including materials, methods, and notes. Roussel et al. (9) reviewed examples in which chiral HPLC was combined with classical methods such as NMR or CD for the on- or off-line detection of the absolute configuration of enantiomers. A review of penicillin G acylase-based CSPs was compiled by Calleri et al. (10), focusing on immobilization methods, materials, and applications in chiral HPLC. An exhaustive compilation of the separation of agrochemical enantiomers by HPLC on commercial CSPs was given by Felix (11).

Gas Chromatography (GC). A review of original research papers from 2001 to 2005 dealing with the applications of chiral GC for direct enantioseparation of optically active compounds was presented (12), with applications grouped by CSP types and fields of interest, including natural products, asymmetric synthesis, environmental contaminants, and compounds important to space science, agricultural, food, flavor, and fragrance industries.

Supercritical Fluid Chromatography (SFC). The use of SFC for the rapid screening and optimization approach for enantioseparations of pharmaceuticals was discussed (13), with a success rate of over 95% for an automated system. The analytical scale

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chiral separations were also adapted for semipreparative work.

Thin-Layer Chromatography (TLC). The achievements in chiral separations by TLC from 2001 to 2005 were reviewed by Siouffi et al. (14), with emphasis on cellulose derivatives and especially microcrystallized cellulose triacetate. Where possible, data were compared to that obtained by HPLC. An overview of the versatility, applicability, and separation mechanisms of TLC enantiomeric separations was discussed by Guenther et al. (15).

Capillary Electrochromatography (CEC). Chiral separations by CEC in nonaqueous media were reviewed by Laemmerhofer (16). The chiral selectors and columns used in highly efficient enantioseparations are thoroughly described. A review of chiral separations by CEC using cyclodextrin CSPs was given by Wistuba et al. (17), including open tubular CEC, packed CEC, and rod-CEC.

Multiple Technique Reviews. The analysis of chiral pollutants using GC, HPLC, CEC, micellar electrokinetic chromatography (MEKC), SFC, and TLC was discussed by Aboul-Enein and Ali (18). The use of electrodriven methods including CE, CEC, and MEKC for the enantioseparation of second-generation antidepressant drugs was reviewed by Mandrioli and Raggi (19). A review of the use of molecularly imprinted polymers (MIPs) for chiral drug separations in HPLC, TLC, SFC, and CEC was compiled by Ansell (20), with another review of MIPs in chiral HPLC and CE written by Turiel and Martin-Esteban (21). Chiral separations in HPLC and CE were reviewed (22), with an overview of direct and indirect separations. Chiral selectors are also discussed, as well as screening techniques and method optimizations. The theory and practice of enantioselective capillary chromatography using metal coordination compounds and modified cyclodextrins as CSPs are discussed by Schurig (23), and a preparative-scale enantioseparation by GC was presented. A review focusing on the advances in CSPs in chiral separations of drugs in HPLC and SFC was discussed by Zhang et al. (24). Novel CSPs for GC and LC were developed and discussed by Oi (25). The use of Marfey's reagent for preparing CSPs for direct enantiomeric resolution was described by Bhushan and Brueckner (26).

CAPILLARY ELECTROPHORESIS

Methods and research in CE continues to be a major area of chiral analytical separations, with cyclodextrin (CD) use as chiral selectors remaining high. By far the most popular chiral selector used in CE remains the cyclodextrins. This is no doubt due to their solubility and stability in aqueous solution, non-UV-absorbing properties, cost, and excellent selectivity offered through the various cyclodextrin derivatives. A discussion and commentary on the feasibility of the separation of enantiomers by CE based solely on equal binding constants between analyte enantiomers and the chiral selector was given by Chankvetadze et al. (27). A screening strategy for the development of enantiomeric separation methods in capillary electrophoresis was developed by Jimidar et al. (28).

Cyclodextrins. A study on the reproducibility of CE enantioresolution with randomly substituted cyclodextrins was reported (29), using 1,1'-binaphthyl-2,2'-diyl hydrogen phosphate as a test solute. The antidepressant mirtazapine and its active demethylated metabolite were enantioseparated by CE using carboxymethyl- β -CD as chiral selector (30), and carboxymethyl- β -CD showed the

highest chiral separation power as chiral selector for the enantiomeric separation of the sunscreen agent 3-(4-methylbenzylidene)-camphor by EKC (31). The addition of methanol was found to enhance the enantioseparation of dimetindene using carboxymethyl- β -CD as chiral selector in CE (32), and the influence of methanol was studied on the enantioresolution of antihistamines again employing carboxymethyl- β -CD as chiral selector (33). Carboxymethyl- β -CD was found to give outstanding enantioresolution of deprenyl-*N*-oxide isomers by CE (34). Succinyl- β -CD was used as chiral selector in the determination of phenylglycidol enantiomers by CE (35).

β -CD was used as chiral selector in the CE separation of dopamino-derived neurotoxins (36) and the enantioseparation of simendan enantiomers (37). The chiral selector 2,6-dimethyl- β -CD was used in the enantioresolution of calcium levofolinate (38). Hydroxypropyl- α -CD and hydroxypropyl- β -CD were found to give good results in the capillary zone electrophoresis (CZE) enantioseparation of *N*-imidazole derivatives (39). The enantioseparation of derivatized serine by CE was accomplished using hydropropyl- β -CD (40).

Sulfated β -CDs were characterized and used in the determination of enantiomeric purity of (1*R*,2*S*)-ephedrine by CZE (41), and the enantioseparation of hydrobenzoin and related compounds by CZE (42). The sulfated β -CDs were also used for the enantioresolution of baclofen (43), *N*-imidazole derivatives (44), rivastigmine in pharmaceuticals (45), tramadol enantiomers and metabolites (46), and new nucleoside analogues related to d4T and acyclovir (47). Control of enantioselectivity of the separation of enantiomers of phenylalanine, tyrosine, and tryptophan was demonstrated using two additives: sulfated- β -CD as the chiral selector and dextran sulfate (48). Sulfated α -CDs were synthesized, characterized, and used as the chiral selector for the CE enantioseparation of nonionic, weak acid, and weak base analytes (49–51). Cyclodextrins were chiral selectors used in the chiral analysis of ephedrine (52) and adrenergic amines (53) in dietary supplements.

Basic compounds were enantioseparated using heptakis(2,3-di-*O*-methyl-6-*O*-sulfo)- β -CD in combination with potassium camphorsulfonate in nonaqueous CE (54), and heptakis(2,3-di-*O*-ethyl-6-*O*-sulfo)- β -CD was used as chiral selector for the determination of salbutamol enantiomers by nonaqueous CE (55). Heptakis(2,3-dihydroxy-6-*O*-sulfo)- β -CD was used in the CZE enantioseparation of hydrobenzoin, and an elution order reversal was noted when β -CD was added to the system (56). The chiral separation of anticholinergic drug enantiomers by nonaqueous CE was achieved using heptakis(2,3-dimethyl-6-sulfo)- β -CD (57). A mixture of sulfobutyl ether- β -CD and dimethyl- β -CD was used as chiral selectors in the validated CE method for the chiral purity of ragaglitazar (58) and the enantioseparation of glitazone compounds (59).

A positively charged quaternary ammonium β -CD derivative was used as the chiral selector in the CE separation of highly negatively charged enantiomers (60). The influence of (hydroxy)-alkylamino substituents on the enantioresolution ability of single-isomer amino- β -CD derivatives in chiral CE was reported (61). The enantioseparation of a mixture of seven carboxybenzylamino acids was achieved by heptakis(6-amino-6-deoxy)- β -CD by CE (62). Mono(6-amino-6-deoxy)- β -CD was used as chiral selector in

the enantiomeric separation of a variety of anionic analytes (63), and mono-6A-butylammonium-6A-deoxy- β -CD tosylate was investigated for the enantioseparation of α -hydroxy acids, carboxylic acids, and ampholytic analytes (64). A 3-amino derivative of β -CD and its Cu(II) complexes were studied as a chiral ligand exchanger for the enantioseparation of tyrosine and phenylalanine (65).

The enantioseparation of anisodamine by CE using carboxymethyl- γ -CD as chiral selector was validated (66), and carboxymethyl- γ -CD chiral selector was also used in the CE enantiomeric determination, validation, and robustness studies for racemic citalopram (67). The chiral separation of labetalol in human plasma was achieved using a new γ -CD derivative as the chiral selector (68).

Antibiotics. The role of the sugar moiety of glycopeptide antibiotics in chiral recognition was investigated with CE using vancomycin and balhimycin as models (69). Balhimycin and its halo analogue bromobalhimycin were thoroughly evaluated as chiral selectors for enantioresolution by CE (70).

Micelles. The effect of several experimental parameters in micellar capillary electrophoresis (MCE) was studied using a system of SDS and hydroxypropyl- β -CD as the chiral selector (71) as the model, with the first reported attempt of a computer simulation of the CD-SDS-MCE system. Enantioseparation of furan derivatives and fused polycycles were performed using CD-modified MCE (72), with the most effective chiral selector found to be γ -CD. The enantiomeric separation of hydrophobic dihydroflavones were performed using hydroxypropyl- γ -CD by micellar CE (73). A combination of cyclodextrins and polymeric surfactants was investigated for chiral separation of three binaphthyl derivatives in MEKC by Warner et al. (74). Cationic vesicles were used as chiral selector for the MEKC enantioseparation of nonsteroidal antiinflammatory drugs (75). The enantioseparation of chiral allenic acids by MEKC using CDs as chiral selectors has been described (76).

The use of CD modified microemulsion electrokinetic chromatography (MEEKC) was applied to enantioresolve (*S*)- and (*R*)-levetiracetam (77). MEEKC with the chiral surfactant dodecoyl-carbonylvaline was used in the investigation of the enantioseparation of 15 different pharmaceutical compounds (78), and the effect of surfactant concentration and buffer selection was discussed. The effect of oil substitution in chiral MEEKC was studied (79). The solutes norephedrine, ephedrine, nadolol, and propranolol were enantioresolved by a MEEKC method using chiral alcohols as cosurfactants (80).

Microchip CE. An effective method to form a protein CSP for fast enantioseparation with electrochemical detection on a microchip was presented by Bi et al. (81), using BSA as the protein and enantioresolving *D*- and *L*-tryptophan. The chiral separation of gemifloxacin using (+)-18-crown-6-tetracarboxylic acid as a chiral selector was performed by microchip electrophoresis (82). The chiral separation of amino acid derivatives by ligand-exchange electrophoresis in a microchannel chip was performed using a Cu(II) complex with *L*-prolinamide as chiral selector (83). Sub-second chiral separations on a microchip using highly sulfated γ -CD as chiral selectors to separate DNS-amino acids (84) achieved a baseline separation in 720 ms.

Miscellaneous. Capillary zone electrophoresis using (+)-18-crown-6-tetracarboxylic acid and CD as chiral selectors was used

to enantioseparate amino acids and some amino acid derivatives including esters and dipeptides (85). Chiral CE using (+)-18-crown-6-tetracarboxylic acid was employed in the complete enantioseparations of eight aromatic amino acids and four alkyl esters of 2-phenylglycine (86), and prediction of enantiomer migration orders by a three-dimensional quantitative structure–property relationship/comparative field analysis model was performed.

An affinity CE method using human serum albumin as chiral selector was employed in the enantioseparation of propranolol (87), and the applicability to quality control of pharmaceuticals was discussed. Human serum albumin was also used as chiral selector in the CE partial-filling method to resolve bupivacaine enantiomers (88).

Cu(II)–prolinamides were used as chiral selector in a ligand-exchange CE method for the chiral resolution of dansyl amino acids (89). Sulfated cyclophoraoes were synthesized and used as novel chiral selectors for the CE enantioseparation of five β -blockers: arterenol, atenolol, isoproterenol, propranolol, and metoprolol (90). A nonaqueous CE method using *O*-(*tert*-butyl-carbamoyl)quinine as chiral selector and detection by FT-IR was used to separate the enantiomers of (*R,S*)-3,5-dinitrobenzoyl leucine (91). A reversible gel based on guanosine was introduced as chiral selector in CE (92), and the enantioseparation of propranolol was achieved.

THIN-LAYER CHROMATOGRAPHY

Though TLC is used less often than other separation techniques in enantioresolution methods, it remains a reliable separation technique. Chiral separations of ibuprofen and propranolol were reported on commercially available TLC plates with detection by densitometry (93, 94), and a TLC method for the enantioseparation of ibuprofen was used in physicochemical studies of the oscillatory instability of profens when stored for long periods in aqueous media (95). The direct separation of the enantiomers of metoprolol tartrate employing the chiral mobile-phase additive *D*-(-)-tartaric acid on impregnated TLC plates was reported by Lucic et al. (96). A method using silica gel plates and (-)-brucine as a chiral selector was reported for the enantioseparation of ibuprofen (97). The TLC enantioseparation of verapamil was achieved using the macrocyclic antibiotic vancomycin as the chiral selector impregnated on silica gel plates (98).

SUPERCritical FLUID CHROMATOGRAPHY AND RELATED TECHNIQUES

Supercritical fluid chromatography and related techniques were employed in the separation of various racemates using a variety of CSPs. SFC and HPLC were used in the enantioseparation of carbobenzyloxy derivatives of amines (99), using polysaccharide and Pirkle-type CSPs. Separation of the enantiomers of three different racemates, neutral *rac*- α -tetralol, acidic *rac*-2-phenylpropionic acid, and basic *rac*-1-phenylethylamine was achieved using subcritical and SFC with two different CSPs, Sumichiral OA-7500 (a derivatized β -cyclodextrin) and Chiralpak AD-H (a derivatized amylose) (100). The Kromasil CHI-TBB column was used in an isocratic subcritical/SFC method for the separation of naproxen enantiomers (101). A reversal of elution order for some 2-substituted propionic acid drugs was observed in SFC on Chiralpak AD when the polar modifier methanol in carbon dioxide was replaced by 2-propanol (102). A Chiralcel OD column was used in the SFC

separation of the enantiomers of 1-phenyl-1-propanol (103). A SFC method using a Chiralpak AD column for the enantiomeric separation of a peroxysome proliferator-activating receptor agonist drug was developed, with the enantiomeric purity determined within 10 min on a 5-cm column (104). A SFC-tandem mass spectrometry method using a Chiralcel OD-H column was developed for the enantioselective detection of propranolol and pindolol in mouse blood by serial sampling (105).

GAS CHROMATOGRAPHY

Chiral resolutions by GC continued to find strong application in various fields, and several novel CSPs were reported. The theory and use of the three-phase model in enantioselective gas-liquid chromatography using a methylated CD/polysiloxane stationary phase was presented (106), and equations derived that accounted for all three partition equilibria for the analyte in the system, including partitioning between the gas mobile phase and both stationary-phase components, the polysilane and cyclodextrin pseudophase.

Applications and Mechanistic Studies. The separation of the enantiomers of *N*-TFA-*O*-alkyl amino acids on 2,3-di-*O*-pentyl-6-*O*-acyl α -, β -, and γ -cyclodextrin stationary phases was studied (107), and mechanistic aspects were reported. Eight 2-substituted ethyl propionate enantiomers were enantioseparated on permethylated and 2,6-dimethyl-3-pentyl β - and γ -CD stationary phases (108). A thermodynamic study on the GC enantioseparation of aromatic alcohols using heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)- β -CD as a CSP was reported by Shitangkoon et al. (109). Chiral GC analysis of cyclopropane derivatives was attained with a baseline separation using a Chirasil- β -dex CSP (110). The enantiomeric separation of methylsulfonyl PCB metabolites in seal blubber, pelican muscle, and human adipose tissues was achieved using a modified CD as CSP (111). A GC/MS method using a chiral capillary column was reported for the separation of the enantiomers of ibuprofen from tablets (112). An optimized chiral GC method for the enantioseparation of ibuprofen, fenoprofen, and ketoprofen methyl esters mixture used heptakis-(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)- β -CD as the CSP (113). Chiral PCBs in food samples were enantioseparated on commercially available chiral capillary β -CD columns by means of heart-cut multidimensional GC and two-dimensional GC (114). The determination of the enantiomeric excess of (+)-chlorofluoroiodomethane was obtained from GC using a chiral modified CD CSP (115). The enantiomeric ratio of 1-octen-3-ol in various species of edible mushrooms was investigated using chiral GC (116). Warfarin was converted into *O*-perfluoroacyl derivatives and the enantiomers separated by chiral GC using poly(dimethylsiloxane) anchored with (*S*)-(-)-1-phenylethylamide (117). The enantiomers of the synthetic pyrethroid insecticides cypermethrin and cyfluthrin were separated by a β -CD-based CSP, BGB-172 (118).

Novel CSPs. The first enantiomeric separations using chiral ionic liquids as stationary phases in GC were reported by Armstrong et al. (119), with separated compounds including alcohols, diols, sulfoxides, epoxides, and acetylated amines. The enantiomers of chiral epoxides were resolved on CSPs of four new CD derivatives: 2,6-di-*O*-benzyl-3-*O*-heptanonyl- β -CD, 2,6-di-*O*-benzyl-3-*O*-octanonyl- β -CD, 2,3-di-*O*-benzyl-6-*O*-heptanonyl- β -CD,

and 2,3-di-*O*-benzyl-6-*O*-octanonyl- β -CD (120). Four CD derivatives with allyl groups or propyl groups on the 3-position of β -CD were synthesized and used as CSPs for capillary GC for the enantioseparation of various racemates, including allethrone acetate, propargyllone acetate, and 2-bromopropionic acid methyl ester (121). Modified linear dextrans, dubbed acylclodextrins, were investigated as CSPs for the separation of racemic *N*-trifluoroacetyl-*O*-alkyl esters of α -amino acids (122). The CSP based on heptakis(2,3-di-*O*-methoxymethyl-6-*O*-*tert*-butyldimethylsilyl)- β -CD was found to be suitable for the enantioseparation of volatiles from various chemical classes, with unusually high separation factors for 2-alkyl esters of short-chain (C2-C6) acids (123). A new class of CD derivatives based on 2,3-di-*O*-methoxymethyl-6-*tert*-butyldimethylsilyl- γ -CD were reported to be suitable for the enantioresolution of a broad spectrum of chiral volatiles from different chemical classes (124). Three peralkylated β -cyclodextrins were coated onto a fused-silica capillary by the sol-gel method and exhibited thermal stability and enantioselectivity (125).

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

Polysaccharide CSPs. With many CSPs available from using in chiral HPLC, the most popular CSPs used during this review period were the polysaccharide CSPs and not surprisingly they also had the largest increase in the number of applications reported. Strategies for method development of chiral separations in normal- or reversed-phase liquid chromatography using polysaccharide-based stationary phases were reported by Matthijs et al. (126). The use of preparative chiral HPLC in a preclinical drug discovery strategy was described (127), with the phases evaluated including Chiralpak AD and AS, Chiralcel OD, OJ, OF, and OB, and Chirobiotic V, R, and T. The effect of the addition of (+) or (-) camphorsulfonic acid to the mobile phase was investigated on the enantioseparations of some basic drugs on a Chiralcel OD column (128). A study of enhanced chiral selectivity by the acidic additives ethanesulfonic acid and *n*-butylamine using a Chiralpak AD column was reported (129), exhibiting significant impact on the chiral selectivity of the amino acid esters. The effect of the Chiralpak AD CSP structural change with mobile-phase modifier on the chiral selectivity was investigated (130). A validated chiral HPLC method for the separation of mebeverine enantiomers was reported using a Chiralcel OD column (131). Chiralcel OD and Chiralpak AS columns were found to be excellent for direct enantioseparation of the fungicide ethaboxam (132). A key intermediate of zolmitriptan in bulk drugs was enantioseparated by Chiralpak AD-H CSP in a validated method (133). The major ibuprofen metabolites in human urine were enantioresolved by a Chiralpak AS column (134), and a Chiralpak AD-RH column was used in the chiral analysis of ibuprofen in urine (135). A set of racemic acidic drugs were enantioseparated on an immobilized Chiralpak IA column and coated Chiralpak AD column, and the separations were compared (136). The chiral HPLC analysis of naringenin was achieved on a Chiralcel OD-RH CSP and was successfully applied to analysis of rat and human urine (137). The HPLC enantioseparation of naringenin and other flavanones was accomplished using Chiralcel OD-H and Chiralpak AS-H columns using various hexane/alcohol mobile phases (138).

Separation of the enantiomers of linezolid was compared on derivatized cellulose and amylose columns, Chiralcel OD and Chiralpak AD, with baseline resolution being achieved only on the Chiralpak AD (139). New aromatase inhibitors containing one chiral center were enantioresolved on cellulose carbamate phase Chiralcel OD-H and cellulose ester phase Chiralcel OJ CSP, with the carbamate phase giving a better separation (140), and preparative enantioseparations were also described (141). Enantioresolution of tetrahydropalmatine was achieved on Chiralcel OJ-H (142). Cellulose and amylose tris(3,5-dimethylphenylcarbamate) CSPs were investigated for the enantioseparation of mirtazapine (143), with the cellulose CSP giving good baseline separations in the normal and polar organic modes. A comparative study between Chiralcel OJ and Chiralcel OD columns was performed on the enantioseparation of imidazole analogues of fluoxetine and miconazole (144). A Chiralpak IA column was used in the chiral separation of a Ca-sensitizing drug (EMD 53986) (145), and the preparative potential of the separation was discussed. Zolmitriptan enantiomers were separated on Chiralpak AD-H CSP (146, 147). A validated chiral HPLC method for the enantiodetermination of rivastigmine hydrogen tartrate was performed on a Chiralcel OD-H column (148). The enantioseparation of N-protected non-protein amino acid esters by chiral HPLC was achieved on a Chiralcel OD CSP (149).

The analytical and semipreparative enantioseparation of al-bendazole sulfoxide was reported on amylose tris(3,5-dimethylphenylcarbamate) CSPs (150). Keilcorin derivatives were enantioresolved using multimodal elution on polysaccharide phases, with Chiralpak AD CSP giving the best performance (151). Chiralpak AD column was used in a method for the simultaneous determination of phenylglycidol enantiomers and cinnamyl alcohol (152). Fluoxetine hydrochloride enantiomers were resolved on a Chiralcel OD column (153). An enantioselective HPLC determination of E-6087, a new COX-2 inhibitor, in human plasma, was validated using a derivatized amylose CSP (154). Cellulose tris(3,5-dimethylphenylcarbamate) was coated on aminopropyl silica to prepare a CSP for the enantiomeric resolution of triazole pesticides (155).

The enantioseparation of *trans*-chlordane, *cis*-chlordane, heptachlor, heptachlor epoxide, and α -hexachlorocyclohexane was investigated on Chiralcel OD, Chiralpak AD, and Chiralcel OJ CSPs (156). The Chiralpak AD CSP was found to give superior performance compared to Chiralcel OD and Whelk-01 for the enantioseparation of new synthetic pyrrolylphenylethanoneamine monoamino oxidase inhibitor compounds (157).

Macrocyclic Antibiotic CSPs. The macrocyclic antibiotics were the second most used CSP during this period separating a wide variety of chiral solutes. The enantioseparation of 17 unnatural β -amino acids was achieved using Chirobiotic T (teicoplanin) and TAG (teicoplanin aglycon) CSPs (158), and the separation efficiencies of the two CSPs were compared. Chirobiotic T and TAG were evaluated for the HPLC chiral resolution of unusual amino acids (159), including unnatural conformationally constrained α -amino acids, Phe and Tyr analogues, and β -amino acids having cycloalkane or cycloalkene skeletons. Separation of the enantiomers of unusual secondary amino acids were investigated on Chirobiotic T and TAG (160), with the better separations obtained by the Chirobiotic TAG column in most cases. A capillary

teicoplanin CSP was used in the chiral separation of amino acids derivatized with 7-fluoro-4-nitrobenzoxadiazole in a LC/tandem mass spectrometry method (161). A comparison of various CSPs was made in the direct HPLC enantioseparation of 1- or 3-methyl-substituted tetrahydroisoquinoline analogues, with the most suitable CSPs determined to be β -CD, vancomycin, and teicoplanin (162). Methods of enantioseparation of bicalutamide and its related compounds were investigated on the following CSPs (163): a cellulose-based Chiralcel OD-H, macrocyclic glycopeptide-based Chirobiotic T, TAG, and R, β -CD-based Cyclobond I 2000SN, and *tert*-butyl carbamate-derivatized quinine-based columns. Chiral HPLC methods for the separation of β -lactams were developed using Chiralcel OD-RH and OD-H and Chirobiotic T and TAG (164). The use of native and derivatized CD-based and macrocyclic glycopeptide-based CSPs for the chiral HPLC separation of pterocarpanes was investigated by Armstrong et al. (165). A comparative study of teicoplanin, teicoplanin aglycon, and methylated teicoplanin aglycon was performed using the lowest free energy relationship model (166).

Thalidomide enantiomers were determined in biological samples by HPLC and a vancomycin CSP (167). Two vancomycin-based CSPs with different chiral selector coverage, Chirobiotic V and V2, were evaluated for enantioresolution of selected drugs including β -blockers and profens (168). A vancomycin CSP was used in a nanoliquid chromatography method for the separation of chlorophenoxy acid herbicides (169). The chiral separation of natural and unnatural amino acid derivatives was achieved by micro-HPLC on a ristocetin A CSP (170). Seventy-one chiral compounds were enantioresolved on teicoplanin, teicoplanin aglycon, ristocetin A, and vancomycin in a study of temperature and enantioseparation (171). A report on transforming chiral LC methodologies into more sensitive LC/electrospray ionization MS without losing enantioselectivity was presented (172), utilizing Chirobiotic T, Chirobiotic V, and Chirobiotic R narrow-bore CSPs. A novel norvancomycin-bonded CSP was synthesized and used to enantioseparate some neutral and basic drugs (173). Two antibody-based CSPs that have opposing stereoselectivity were used in a study of the effect of the mobile phase on enantioseparation of amino acids (174). Hepta-Tyr antibiotic-modified silica CSP was used for the enantioseparation of D,L-loxiglumide by CEC and nano-LC methods (175).

Cyclodextrin CSPs and Mobile-Phase Additives. Cyclodextrins continue to enjoy substantial use as CSPs and mobile-phase additives. A study of the separation of enantiomers of 20 isochromene derivatives was performed on native and derivatized CD CSPs (176), with the most effective columns being used in the reversed-phase mode for the derivatized β -CD columns Cyclobond RSP and Cyclobond DM and the γ -CD Cyclobond II. No enantioseparations were observed in the polar organic mode, and only a few enantiomers separated in the normal-phases mode. The enantiomeric separation of 12 chiral dihydrobenzofurans was achieved on derivatized β -CD CSPs, with the hydroxypropyl- β -CD CSP being the most effective (177), baseline separating 9 of the 12 compounds. The enantioseparation of 16 racemic dihydroflavones was investigated using CD CSPs (178), with the hydroxypropyl- β -CD CSP (Cyclobond RSP) being the most effective, exhibiting enantioselectivity for 14 compounds, and producing baseline separations for 8 compounds in the reversed-phase mode.

Enantioselective semipreparative HPLC separation of PCB metabolites was examined on various β -CD-based columns (179). The enantiomeric purity of novel nucleoside analogues was determined using Cyclobond I 2000 and Cyclobond I 2000 RSP (180).

Two novel CD CSPs, mono-2A-azido-2A-deoxyperphenylcarbamoylated- β -CD and mono-2A-azido-2A-deoxyperacetylated- β -CD, were prepared and used to enantioseparate β -adrenergic blockers, flavanone compounds, benzodiazepinones, and antihistamines, among others (181). A simple method to prepare a β -CD bonded silica stationary phase containing a propylene short spacer was reported (182) and used to enantioseparate dansyl-amino acids.

Protein-Based CSPs. Though not as widely used in previous review periods, the α_1 -acid glycoprotein (AGP) was the column of choice for a variety of methods during this review period. The chiral separation of atropine by HPLC was performed on a AGP CSP (183). Methadone and its major metabolites (EDDP and EMDP) were enantioresolved on AGP by a HPLC-MS method (184). Warfarin enantiomers in human blood plasma were determined on an AGP CSP after liquid/liquid extraction (185). An affinity monolithic column containing human serum albumin was prepared and evaluated in the chiral resolution of warfarin and tryptophan (186).

Miscellaneous CSPs. A strategy to enhance the chiral recognition capacity of polymer-embedded chiral selectors was proposed by Lindner et al. (187), and the copolymerization of a quinine *tert*-butylcarbamate selector monomer with chiral (and achiral) 3,5-dichlorobenzoyl amino acids was discussed. The use of quinine carbamate-based CSPs was extended from the enantioresolution of chiral acids to neutral polar quinazolone derivatives as reported by Lindner et al. (188), and the overall chiral recognition mechanism was investigated. Quinidine carbamate CSPs were used in the chiral separation of 2,2,2-trifluoro-1-(9-anthryl)ethanol (189) and the resolution of phenylpropanol enantiomers (190, 191). A chiral 1,4-dihydropyridinemonocarboxylic acid was enantioresolved on a *tert*-butylcarbamoylquinine anion exchanger CSP (192), as was 2-methoxy-2-(1-naphthyl)propionic acid (193). Selected pyrethroid acids were enantioresolved on cinchona alkaloid-derived CSPs (194).

A CSP covalently bound with chiral pseudo-18-crown-6 ether unit was used in the LC separation of amino compounds in the normal-phase mode (195, 196). A novel CSP based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid was prepared and applied to the resolution of various racemic amines (197, 198). A chiral crown ether column was used to separate amino acid enantiomers by HPLC with inductively coupled plasma carbon emission detection (199).

A HPLC column using *N,S*-dioctyl-*o*-penicillamine as a chiral ligand-exchange phase (Sumichiral OA-5000) was used for the separation of enantiomers of non-protein amino acids (200). A new chiral ligand-exchange stationary phase was synthesized and used in the enantioresolution of DL-selenomethionine enantiomers (201).

A new (*S*)-biotin-bonded silica gel CSP was prepared for HPLC and was used in the enantioresolution of racemic amino acid derivatives (202). Two new CSPs based on cholic acid bearing 2-naphthylcarbamate and 3,5-dinitrophenylcarbamate moieties were prepared (203), and investigation of the enantiodiscrimination

demonstrated that the enantioselectivity was dependent on the position of the hydroxyl group on the cholestanic backbone. A biostable L-RNA aptamer CSP was prepared (204) and used to resolve racemates of approximately a dozen related compounds (205). Poly(*trans*-1,2-cyclohexandiylbisacrylamide) CSPs were synthesized and could be used in the normal-phase mode, polar organic mode, and with halogenated solvents (206). The columns showed very high sample capacities and could be used for preparative and semipreparative enantiomeric separations. CSPs made of proline peptides were prepared and found to have broad chiral selectivity comparable to that of the Whelk O2 column (207). Enantioresolution by reversed-phased HPLC with hydrophobic phases made of chiral cationic surfactants was reported by Kurata et al. (208).

A thermodynamic study of 13 2-, 3-, and 4-alkoxyphenylcarbamamic acid 2-methoxy-1-[(4-methylpiperazino)methyl]ethyl ester enantiomers separated on a (*S,S*)-Whelk-O1 HPLC CSP was reported by Dungalova et al. (209). Enantioresolution of diarylmethanols, diarylmethyl pivalates, and diarylmethyl acetates was performed with the (*S,S*)-Whelk-O1 CSP (210), and evidence was given in support of a chiral recognition model for the enantioresolution. Direct chiral resolution of fungicide benalaxyl was achieved by a (*R,R*)-Whelk-O1 CSP (211), and the chiral recognition mechanism was discussed. The direct enantioresolution of racemic β -blockers was investigated on α -Burke 2 CSP and Pirkle 1J CSP (212). Synthetic pyrethroid insecticides were enantioresolved on a Sumichiral OA-2500-I CSP (213). Six new CSPs prepared from 4- or 2-chloro-3,5-dinitrobenzoic acid, L-alanine, and different π -donor aromatic units were used in the enantioresolution of 13 racemic dihydropyrimidonic analytes (214).

There were a number of investigations of MIPs in this review period. A comparison of the thermodynamic properties of particulate and monolithic columns of MIP for the purpose of enantiomeric separations was performed by Kim and Guiochon (215). Other thermodynamic studies on the effect of the nature of the organic modifier in chiral chromatography on MIPs (216) and the concentration of the organic modifier were published (217). A comparison of particle size and flow rate optimization using one-monomer MIPs versus traditional MIPs was investigated by Simon et al. (218). A MIP CSP was used in the screening of oxazepine indole enantiomers (219), and the mechanism was scrutinized.

A simulated moving columns technique for chiral HPLC was described (220), in which two or three short chiral HPLC columns were connected in series and unresolved enantiomers recycled through the columns until resolution was obtained. The chiral separation of the β -blocker drug nadolol was achieved by zone simulated moving bed chromatography (221). An optimal standing-wave design of nonlinear simulated moving bed systems for enantioresolutions was developed (222).

CAPILLARY ELECTROCHROMATOGRAPHY

The use of CEC remained popular during this review period, with a variety of CSPs used to resolve enantiomers. The differences exhibited by normal- and reversed-phase versions of the polysaccharide CSPs Chiracel OD and OJ, and Chiralpak AD and AS in CEC were investigated. When using different background electrolytes and different mobile-phase compositions, the enantio-

separations of two basic, two acidic, a bifunctional, and a neutral compound were examined (223). An evaluation of CDs modified with dichloro-, dimethyl-, and chloromethylphenylcarbamate groups for CEC CSPs was made by Fanali et al. (224). Pressurized CEC and capillary HPLC were used in the enantioresolution of promethazine, carteolol, celiprolol, and albuterol with vancomycin as the CSP (225). In addition, the effects of pressure and electric field strength on efficiency, resolution, and capacity factor in pressurized CEC was reported. Fritless particle-loaded monoliths for CEC were prepared (226) using teicoplanin aglycon as the chiral selector bonded to 3- μm silica to enantioseparate chiral α -hydroxy acids. Novel enantioselective strong cation exchanger (SCX) monolithic capillary columns were prepared for the enantioseparation of chiral bases by nonaqueous and aqueous CEC (227). Mefloquine and its *tert*-butylcarbamate derivative were used as test compounds, and the enantioselectivity of the novel SCX phase was comparable to that of packed silica-based capillaries. Other novel SCX-type CSPs based on β -amino sulfonic acid-terminated dipeptide derivatives immobilized on silica particles were used for the enantiomeric separations of chiral bases by nonaqueous CEC (228). A chemically bonded cellulose tris(3,5-dimethyl)phenylcarbamate CSP was prepared and used with aqueous mobile phases for the separation of neutral, acidic, and basic enantiomers (229). An open-tubular CEC method using a polyelectrolyte multilayer coating consisting of a polypeptide and polymeric dipeptide surfactant was evaluated for chiral separations, and the coating was found to be stable and yielded excellent reproducibility (230). A positively charged cellulose derivative CSP was prepared by a copolymerization reaction for chiral CEC in nonaqueous conditions (231). The chiral separation of binaphthol enantiomers on molecularly imprinted polymer monolith by CEC was reported, and the influence of several parameters on the column permeability was studied (232). A CEC method on an achiral stationary phase with the chiral selector hydroxypropyl- β -CD in the mobile phase was used to enantioresolve chlorthalidone (233). Quantification limits in chiral CEC using Chirobiotic V as the CSP were investigated and reported that quantification limits improved 10-fold by utilizing peak compression (234).

MISCELLANEOUS TECHNIQUES

A method for the enantioresolution of gemifloxacin by countercurrent chromatography was developed using (+)-18-crown-6-tetracarboxylic acid (235), and high-speed countercurrent chromatography was used in the enantioseparation of chlorpheniramine using carboxymethyl- β -CD (236).

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