CYCLODETRINS IN CHROMATOGRAPHY. PART 2. MISCELLANEOUS CHROMATOGRAPHIC METHODS.

Gyula Oros[a], Tibor Cserháti[a] and Mária Szőgyi[a]

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The objectives of the reviews are the collection, concise description, comparison and evaluation of the various chromatographic technologies except liquid chromatography using natural and modified cyclodextrins for the increase the seperation capacity of various chromatographic separation systems.

*Corresponding author
E-Mail: szogyim@t-online.hu
[a] Research Center for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary

Introduction

Chromatographic procedures were developed and successfully employed for the separation of a high number of organic and inorganic compounds present at trace level in complicated accompanying matrices. The capacity of chromatographic separation technologies can be increased by the modification of both the stationary and the mobile phase of the system. Because of their specific adsorption character cyclodextrins (CD) and cyclodextrin derivatives (CDs) have been frequently applied for the improvement of the separation parameters (mainly chiral separation capacity) of various chromatographic methods. The chiral separation capacity of cyclodextrins has been frequently exploited for the separation of enantiomers with markedly different biological activity employing CDs. CDs and CD derivatives can be equally used as additives of stationary phase or modifier of the mobile phase. The number of studies dealing with the application of CDs and CD derivatives for the increase of the separation capacity of chromatographic systems increased considerably. Because of their versatility CDs have found application in many special branch of chromatography such as liquid chromatography (LC), gas chromatography (GC), size exclusion chromatography (SEC), gel permeation chromatography (GPC), and electrically driven separation methods (CE, CZE), and ultra performance liquid chromatography (UPLC).

Application of cyclodextrins in gas chromatography/mass spectrometry

The newest results in the application of chiral capillary gas chromatography (GC) have been recently reviewed, the various phase development methodologies were shortly discussed and the efficacy of chiral GC and chiral HPLC were compared. Samples for the application of chiral GC are provided[1].

A rapid total analytical system (TAS) was developed for the detection of the autheticity of fruit-flavoured foods and beverages. The method combined headspace solid phase microextraction (HS-SPME) with enantioselective GC-MS (Es-GC-MS). The results were evaluated by multivariate mathemathical statistical methods such as principal component analysis (PCA) and hierarchical cluster analysis (HCA). Peach, coconut, apricot, raspberry, strawberry and melon were included in the experiments. Cyclodextrin derivatives served as chiral selectors, GC analyses were carried out on both normal and narrow bore columns. It was established that the analysis time of TAS method in markedly lower than that of the conventional analytical procedure.[2]

The concentration and enantiomeric distribution of linalool (3,7-dimethyl-1,6-octadien-3-ol) was determined in the raw and roasted cocoa beans (seeds of *Theobroma cacao*). Samples of cocoa beans, commercial products, cocoa powders and chocolates were included in the investigations. Enantioselective separations were achieved by using multidimensional gas chromatography, heptakis(2,3-di-O-methyl-6-O-tert-butyldimethylsilyl)-β-CD was the chiral stationary phase. Pre-concentration of the analyte was carried out by simultaneous steam distillation-extraction at pH 7.

The results indicated that the technological processes exert a negligible effect on the original enantiomeric distribution of linalool.[3]

Various GC techniques were developed and applied for the enantiomeric separation of DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane and its derivatives. The methods included heart-cut multidimensional GC (MDGC), and comprehensive two dimensional gas chromatography (GC x GC).

Chiral separations were carried out on a β-CD-based column. The measurements indicated that both method can be successfully employed for the enantiomeric separation of DDT and metabolites.[4]

HS-SPME followed with enantioselective GC/MS was applied for the analysis of enantiomeric and non-enantiomeric distribution of monoterpenes in the headspace of *Juniperus communis L*. and *Juniperus oxycedrus* needles and berries. The measurements indicated that the results obtained with the traditional hydrodistillation and HS-SPME techniques are commensurable. It was established that the

concentration of the monoterpenes in needles and berries of *J. Communis* were sabinene (19–30 %), α-pinene (12–24 %), β-myrcene (9–20 %). *J. oxycedrus* contained mainly α-pinene (85–92 %). The investigations revealed that the distribution of monoterpenes depended consideraly on both the type of analyte (needles or berries) and on its origine.[5]

In the last years a considerable number of studies were published dealing with the recent achievements in the synthesis of new mobile and stationary phases suitable for the enantiomeric separation of a wide variety of racemic compounds using electrophoretic techniques. An excellent review has been recently published enumerating and critically evaluating chiral selectors such as cyclic and linear oligo- and polysaccharides, branched polysaccharides, polymeric and monomeric surfactants, macrocyclic and other antibiotics, crown ethers etc. The advantages and disadvantages of the application of single chiral selector and dual-selector systems are also discussed in detail. The pharmaceutical and biomedical applications of the new methods have also been discussed in detail.[6]

The separation of enantiomers of norephedrine (NEP) by CE has been investigated in detail. The enantiomer migration order (EMO) was determined in the presence of various CD derivatives such as α-, β-CD, heptakis(2,3-di-O-acetyl-6-O-sulfo)-β-CD (HDAS-β-CD) and heptakis(2,3-di-O-methyl-6-O-sulfo-β-CD (HDMS-β-CD). It was concluded from the results of CE and MNR measurements that the complex formation of NEP different with different CDs and CDs derivatives.[7]

The extractability and possible toxicity of polycyclic aromatic hydrocarbons (PAHs) in river sediments was investigated in detail, the correlation between the extractabiliy of sediment contaminants, the chemodynamic properties of each extraction method and the resulting toxicity. Sediment samples were treated with various extraction procedures such as Soxhlet extraction with acetone (SOX), membrane dialysis extraction (MDE) with n-hexane. Ultrasonic extraction with acetone (USE), and extraction with (2-hydroxypropyl)-β-cyclodextrin, (HBCD). The PAH concentration of extract was determined with gas-chromatography/mass spectrometry. It was established that the extracting efficacy of SOX and MDE methods were comparable. HBCD technique extracted only 3.4 % of the total PAH content. It was further found that the efficacy of SOX and MDE were similar. USE showed activity between SOX and MDE. It was concluded from the results that these measurements may promote the better understanding of extraction procedures.[8]

Nanogels were preparated from cyclodextrins (γ-CD) or hydroxypropyl-β-cyclodextrin) (HP-β-CD) at a constant ratio of 20 %, w/w and their capacity as nanogels for drug delivery was investigated. The measurements indicated that the addition of ethyleneglycol diglicydil ether (EDGE) was important for the formation of nanogel. Infrared analysis (IR), transmission electron microscopy (TEM), dynamic light scattering (DLS) may help the exact determination of the cross-linking degree, size and size distribution of nanogels. Addition of hydroxypropyl methylcellulose (HPMC) influenced also the formation of nanogels.[9]

Heart-cut multidimensional GC (heart-cut MDGC) carried out on three capilllary columns based on β-cyclodextrin were employed for the simultaneous enantiomeric separation of polychlorinated biphenyls (PCBs).and metylsulfonyl metabolites of PCBs ($MeSO_2$-PCBs). The measurements established that the enantiomer separation capacity of columns shows considerable differences. The method has been successfully employed for the analysis of two fish oil and one cow liver samples.[10]

A new β-CD modified hyperbranched carbosilane GC stationary phase was developed by substituting the –OH group of β-CD by hyperbranched carbosilane. The preliminary investigations indicated that the new stationary phase can be successfully applied for the separation benzenes, acrylates, ketones and alkylchlorides.[11]

Mixed GC selectors were prepared by binding a single L-valine diamide moiety to permethylated β-CD. It was established that the enantiomer separation capacity of the new stationary phases is commensurable with those of commercial preparations. The enhanced enantioselectivity of the mixed electrodes was explained by the better chiral correspondance of the analytes.[12]

Chiral gas chromatography and HPLC were employed for the study the distribution and organoleptic impact of ethyl 2-hydroxy-4-methylpentanoate (ethyl DL-leucate) enantiomers in wine. GC measuremements were carried out using γ-CD in the stationary phase. It was established that white wines contained only the R form, whereas red wines contained both enantiomers. The ratio of R/S markedly depended on the aging. The R/S ratio was about 95:5, the average total concentration was 400 μg L^{-1}. The olfactory treshold for R and S enantiomer was different.[13]

New controlled release systems were developed for the delivery of essential oils. The interaction of CDs and β-cyclodextrin polymers with linalool and camphor in the essential oil of Lavandula angustifolia was investigated in detail. GC measurements were carried out by static headspace GC (SH-GC). Multiple headspace extraction method (MHE) was employed for the study of the application possibility of a new preparation controlled release system.[14]

The role of CDs and CD derivatives in the resolution of chiral natural compounds was reviewed. Besides CDs other chiral selectors such as crown ether, macrocyclic antibiotics, cellulose, have found application in the enantiomeric separation of various bioactive compounds. Thus, the optical isomers of ephedrine show different amphetamine-like stimulus effects, while (S)(α-ionone (woody-like taste) and (R)(-)-α-ionone (violet taste) show different olfactory characteristics. CDs and various CD derivatives have been successfully employed for the chiral separation of a considerable number of chiral natural compounds such as flavanones, alkaloids, lignans, coumarins, terpenoids, amino acids, peptides, etc. CDs have also been applied to enhance the separation efficacy of miscellaneous chromatographic technologies (GC, HPLC, CE, CZE).[15]

Volatile organic compounds (VOCs) were investigated in air samples using cyclodextrin-silica hybrid solid phase. The new samplers have been successfully employed for the

determination of benzene, toluene, ethylbenzene, o-xylene, m-xylene, and p-xylene (BTEX) in air. The recoveries were 89±4 %; 90±6 %; 91±2 %; 87.0±0.9 %; 88±4 %; and 88±4 %, respectively. The investigations indicated that the results obtained with the new method comparable with those obtained by the reference method.[16]

A new procedure was developed for the preconcentration and analysis of eight phenolic compounds in environmental water samples. SPE hyphenated with liquid-phase microextraction (LPME) based on solid organic drop combined with GC-MS was employed for the separation and quantitative determination of analytes. The purification of the samples was obtained by using a column containing 60 mg of β-CD-bonded silica particles as stationary phase. The optimal conditions of the procedure were: LOD = 0.002 – 0.04 μg L^{-1} (S/N=3); LOQ = 0.007 – 0.15 μg L^{-1} (S/N = 10); RSD = ≤ 9.5 %. The recoveries were over 79 %.[17]

Enantioselective GC-MS was employed for the investigation of volatiles and aroma-active compounds in honey bush (Cyclopia subternata). Samples were obtained by high-capacity headspace sample enrichment probe (SEP). GC-MS found total 183 compounds (103 terpenoids, 56 %). Samples were also analysed by gas chromatography olfactometry (GC-O). According to the GC-O assessors some compounds sowed typical honey bush like aroma: (6E,8Z)-megastigma-4,6,8-trien-3-one; 6E,8E-megastigma-4,6,8-triene-3-one; 10-epi-γ-eudesmol; epi-α-murolol; and epi-α-cadinol.[18]

A new CE technique was developed for the analysis of pantoprazole enantiomers employing sulfobutyl-β-cyclodextrin (SBA-β-CD) as chiral additive. The parameters of the method were optimized: the best separation was obtained by using a buffer of 50 mM borax – 150 mM phosphate, pH 6.5, 20 mg mL^{-1} SE-β-CD, and 10 kV voltage. The limit of detection (LOD) and limit of quantitation (LOQ) for R-(+)-pantoprazole were 0.9 and 2.5 μg mL^{-1}, respectively. It was established that the method is suitable for the analysis of a minimum limit of 0.1 % (w/w) of R-enantiomer in pantoprazole samples.[19]

Chiral separation of cathinone derivatives was obtained by applying various CD derivatives as chiral additives. A new and easy to carry out capillary zone electrophoresis (CZE) method was developed for the enantioseparation of 19 cathinone derivatives.CDs included in the investigations were: β- and γ-cyclodextrin, carboxymethyl-β-CD, 2-hydroxypropyl-β-CD, and sulfated-β-CD. Background electrolyte (BGE) using for the optimal separation of analytes consisted of 20 mg mL^{-1} sulfated-β-CD in 50 mM ammonium acetate buffer pH 4.5 containing 10 % v/v acetonitrile. Separations were carried out at 40 ^{0}C with a separation voltage of 20 kV.[20]

A novel CZE technology was developed for the separation and quantitative determination of flavonoids in traditional Chinese medicines. The optimal condition were: background electrolyte of 20 mM borax (pH 7.5), 6 mM β-CD and 20 % (v/v) acetonitrile. Injection time was 65 s. Detection limits for five flavonoids were between 15 – 30 ng/mL. The method was successfully applied for the analysis of traditional Chinese medicine real samples[21].

It was found that neutral and acidic monosaccharide components in *Ganoderma lucidum* polysaccharide can be easily labeled with 2,3-naphtalenediamine and the saccharide-naphimidazole (NAIM) derivatives can be analysed by CE using borate buffer. Sulfated-α-CD can be used as chiral selector. The method was proposed for the analysis of natural carbohydrates by CE.[22]

HPLC and electronic microscopy was employed for the study of the influence of HP-β-CD-PLGA nanoparticles on the bioavailability and the penetration of puerarin. The measurements indicated that the complex formation of puerarin with HP-β-CD-PLGA enhance the therapeutic effect on brain ischemia-reperfusion injury in rats. The drug release kinetics and nanoparticle degradation in phosphate buffered saline (PBS) was investigated in detail. It was concluded from the data that complex formation decreased the infraction volume, therefore, the complex formation is potentially applicable for the brain injura induced by ischemic-reperfusion.[23]

Stir bar sorptive extraction (SBSE) followed by HPLC-UV detection was used for the analysis of estrogens in pork and chicken samples. Poly(dimethylsiloxane)(PDMS)/β-cyclodextrin(β-CD)/divinylbenzene-coated stir bar was prepared by the sol gel technique and used for the SBSE followed with HPLC-UV. The enrichment factor was 19 – 51-fold. The relative standard deviations ranged from 6.0 % to 9.7 %. It was stated that the method is simple, sensitive, and selective and can be successfully applied for the determination of estrogens in pork and chicken samples.[24]

The interaction of the phosphatidyl ethanolamine (PE) of *Heliobacter pilori* with free cholesterol (FC), cholesterol ester (CE), 2,6-di-O-methyl-β-cyclodextrin (dM-β-CD) was established. GC/MS and LC/MS analyses established that the composition of PEs produced by *Heliobacter pilori* and *Escherichia coli* shows marked differences. It was concluded from the results that PE is a key kandidate of nonesterified steroid-binding lipids in *H. pilori*.[25]

Methyl-β-cyclodextrin (MCD) was employed for the extraction and purification of prenylated proteins. It was established that MCD can be successfully employed as a selective for such exptraction procedures. The enzyme was further purified by one-step anion-exchange column chromatography and affinity column chromatography. The measurements suggested that MCD can be easily applied as a useful compound for selective extraction and purification of prenylated periferial membrane proteins from the cytoplasmic surface of biological membranes.[26]

Supercritical fluid chromatography (SFC) has also found application in the chromatographic analysis of various organic and inorganic compounds. Cationic β-cyclodextrin perphenylcarbamoylated derivatives were chemically bonded into vinylized silica. The product was applied as chiral stationary phase in SFC. The enantiomeric separation capacity of the new stationary phase was demonstrated on 14 racemates including flavanones, thiazides, and amino acid derivatives. It was concluded from the data that aromatic cationic moiety on β-CD enhanced the enantiomeric separation capacity compared with aliphatic cationic moiety. It was further established that the presence of acid additives results in lower retention time but can improve chiral resolution.[27]

The application of SFC for the enantiomeric separation, for the simultaneous chiral and achiral separation, impurity control and direct scaling has been recently reviewed. The advantages of SFC technique (fast analysis speed, wide polarity compatibility, lower cost of the mobile phase and high column efficiency) make SFC a method of choice for the analysis of chiral and achiral analytes.[28]

A sliding graft copolymer with grafted (polyethylene glycol) side chains by the „grafting onto" strategy was prepared. The end product contained PEG and CDs, and was characterized by ^{1}H NMR, Fourier-transformed infrared and gel permeation chromatography.[29]

High molecular weight polyrotaxanes (PRs) were prepared from poly(ethylene glycol) and α-CD. The end product was investigated by ^{1}H NMR spectroscopy, wide angle X-ray diffraction and gel-permeation chromatography.[30]

Size-exclusion chromatography was employed for the study of the size stabilization of Au nanoparticles in the presence of salt and organic solvents. The results indicated that 3α-amino-3α-deoxy-(2αS,3αS)-β-cyclodextrin is a good stabilizer for the Au nanoparticles.[31]

The antimicrobial effect of isothiocyanate (AIT) complexed with α- and β-cyclodextrins was investigated. Solid phase microextraction followed by gas chromatography was employed for the determination of the concentration of AIT in samples. The measurements indicated that the inclusion complexes showed more pronounced antimicrobial effect than the uncomplexed compounds. The method was proposed for the antimicrobial treatment of fresh-cut vegetable products.[32]

The enantiomeric separation capacity of some β-dextrin-based chiral stationary phases was compared using structurally different analytes as model compounds (coumarins, dansyl amino acids and propionic acid derivatives). The separation capacity of β-cyclodextrin, (R,S)-2-hydroxypropyl-β-cyclodextrin, and permethyl-β-cyclodextrin-based CPs were compared. Measurements were carried out in reversed phase separation mode, the mobile phase consisted of 0.1 % triethylammonium phosphate (pH 3.5)/MeOH. It was found that the best enantiomeric separations can be achieved on permethyl-β-cyclodextrin stationary phase.[33]

The inclusion complexes of warfarin enantiomers with permethylated monoamino-β-cyclodextrin (PMMABCD) was investigated with CE and ^{1}H NMR spectroscopy. It was established that the pH of the mobile phase exert a marked impact of the mobility of the complex[34].

The inclusion complex formation of isomeric monoterpenes, camphene and fenchene with β-cyclodextrin was employed for facilitate their separation. It was established that the analytes can be easily separated using re-crystallization. The structure of the inclusion complexes was investigated by ^{1}H NMR and X-ray diffraction.[35]

The application of fully or partially sub-2 μm porous silica material as column packing has been recently discussed. Its application in liquid chromatography, ultra high speed chromatography and capillary electrochromatography (CEC) is discussed in detail.[36]

CE and proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) were employed for the separation of the enantiomers of sibutramine applying cyclodextrin (CD) derivatives as chiral selectors. The method of separation included 50 mM of phosphate buffer of pH 3.0 with 10 mM of methyl-β-cyclodextrin (M-β-CD);0.05 % of LOD and 0.2 % of LOQ. The method was validated and applied successfully for the separation of sibutramine enantiomers in commercial preparations.[37]

Bodypy-F1-labeled glycophosphingolipids were separated using capillary elctrophoresis and laser- induced fluorescence detection. Analytes were prepared by acylation using the N-hydroxysuccinimide ester of Bodypy-F1. Micellar electrokinetik capillary chromatography was employed for the separation of analytes. The measurements indicated that the separation capacity of TRIS/CHES/SDS/α-cyclodextrin buffer was better than that of the traditional borate/deoxycholate/methyl-β-cyclodextrin buffer. The theoretical plate number ranged from 640.000 to 741.000. The LOD was approximately 3 pM, the analysis time was lower than 5 min.[38]

A new validated capillary electrophoresis method was developed and successfully applied for the separation of dapoxetine enantiomers. Dapoxetine, a serotonin transporter inhibitor is employed for the treatment of premature ejaculation. CZE measurements were carried out in uncoated fused-silica capillary. Preliminary investigations indicated that randomly methylated γ-cyclodextrin was the best chiral selector. The optimal parameters of the enantioseparation were: 15 ^{0}C; +15 kV; 70 mM acetate; 20 v/v MetOH; pH, 4.5; and 3 mM methylated γ-CD. The optimal resolution was 7.01.

The validation parameters of the method such as repeatability, linearity range, LOD, LOQ, accuracy and robusness were also determined[39].

The enantiomeric impurities of armodafinil was investigated with a new CE method using sulfobutyl –ether-β-cyclodextrin as chiral selector. The best separation 3.3 was obtained by employing 20 mM phoshate buffer (pH 7.5). The concentration of the chiral selector was 20 mM. The LOD and LOQ of (S)-modafinil were 1.25 μg mL^{-1} and 2.50 μg mL^{-1}, respectively. It was established that the method display good selectivity, repeatabiliy, linearity and accuracy, and can be applied for the investigation of the enantiomeric impurity of armodafinil in bulk samples[40].

A CE method was developed using electrokinetic injection to simplify the dissolution testing of amoxicillin capsules. The electroforetic parameters were: 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES) buffer (pH 7.1; 200 mM containing 30 % acetonitrile and 10 % β-CD. Samples were injected employing electrokinetic injection (5 kV, for 100 s). Separations were achieved at positive polarity mode of 25 kV at 30 ^{0}C. The validation parameters such as linearity, precision, accuracy, selectivity, and sensitivity of the method were also determined.[41]

CE was employed for the enantiomeric separation of five β-antagonists using carboxymethyl-β-cyclodextrins as chiral-selector. The impact of various chromatographic parameters on the efficacy of the chiral separation was investigated in detail (the type and concentration of chiral selector, pH, composition of the background electrolyte, capillary temperature, applied voltage, and the length of capillary). The results indicated that the type and concentration of chiral selector as well as the pH exert the highest impact on the enantioselectivity. The optimal separation parameters were: 50 mM phosphoric acid containing 10 mM CM-β-CD at pH 3.5 in a capillary of 48.5 cm x 75 pM (40 cm effective length). The temperature of the uncoated capillary was 15 ^{0}C, the applied voltage was 20 kV.[42]

Micellar electrokinetic chromatography was employed for the separation of the immunosupressive drug cyclosporin A (CyA) from its impurities (cyclosporin H) and isocyclosporin). The best separation was obtained by 50 mM sodium dodecyl sulfate (SDS) in 51 mM tetraborate buffer (pH 9.2) suplemented with 15 mM TM-β-cyclodextrin (heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin TM-β-CD. The temperature of the capillary was 30 ^{0}C, the running voltage 22 kV. Analyses were carried out in a fused silica capillary 50 μm i.d. x 64.5 cm total length, 56 cm to the detector. The method was validated for linearity, sensitivity, accuracy and precision. It was stated that the method can be used for the analysis of commercial available pharmaceuticals (gelatin capsules).[43]

New CD derivatives were synhesized and their enantiomeric separation capacity was assessed and compared. CD derivatives were prepared by anchoring various alkyl chain spaced imidazolium and ammonium sidearm onto the CD primary ring. The measurements indicated that these derivatives can be applied as selectors in the enantiomeric separation of amino acids and acidic racemated in aqueous capillary electrophoresis.[44]

Enantioselective capillary electrophoresis-laser-induced fluorescence (CE-LIF) technology was developed and successfully applied for the determination of D-ser in cellulose matrices. The method included derivatization with FITC followed with CE-LIF. Separations were carried out in borate buffer (80 mM, pH 9.3). The enantioselectivity (D-Serine, L-Serine) was 1.03, the resolution (R-s), 1.37. Linearity ranged from 0.025 to 100.00 mM. The method was employed for the separation of D-and L serine in various cell lines.[45]

The charge state distribution of randomly sulfated cyclodextrins (CDs) and single isomer cyclodetrins was investigated employing hydrophilic intercation liquid chromatography (HILIC). Analyses were carried out on cross-linked diol phase and on unbonded silica stationary phase. Sulfated cyclodextrins with different charge states were separated from each other. Randomly sulfated CDs showed wide charge and regioisomer distribution while HILIC investigations indicated the presence of a single species.[46]

A field-amplified sample stacking (FASS) method combined with capillary zone electrophoresis (CZE) was employed for the separation and quantitative determination of ambroxol hydrochloride in human plasma. Preconcentration was carried out with liquid-liquid extraction. Analytes were separated in a fused-silica capillary (31.2 cm x 75 μm), applied voltage was 15 kV. BGE consisted of 6.25 mM borate – 25 mM phosphate (pH 3.0) containing 1 mM β-cyclodextrin. Analytes were detected at 210 nm. The following validation parameters of the method were determined: stability, specificity, linearity, lower limit of quantitation, accuracy, precision, extraction recovery, and robustness. The caibration was linear between 2 – 500 ng mL^{-1}. The intra- and interday precisions of the lower limit of quantitation (LLOQ) were 9.61 and 11.80 %, respectively. The technique was used for the pharmacological study of ambroxol hydrochloride tables in 12 healthy volunteers.[47]

A capillary zone electrophoresis method was developed for the analysis of lavonoids in traditional Chinese medicines. The separation capacity of the technique was markedly enhanced by adding β-cyclodextrin to the background electrolyte. The optimal conditions for the separation were: 20 mM borax, (pH 7.5), 6 mM β-cyclodextrin and 20 % v/v acetonitrile, injection time 65 s. Detection limits for the analytes ranged 15-30 ng mL^{-1}. It was established that the technique can be applied for the investigation of traditional Chinese medicin real samples.[48]

It was found that the separation on microchip shod marked advantageous characters such as high efficacy, increased throughput, reduced quantitites of hazardous materials, cost saving, relatively easy instrumentation, improved portability, etc. Micellar electrokinetic chromatography (MEKC) carried out on polydimethylsiloxane microchip was employed for the separation and quantitative determination of three phenolic xenoestrogens such as octylphenol (OP), 4-nonylphenol (4-NP), bisphenol A BPA). Xenoestrogens were detected with amperometric method and were baseline separated in 55 s. Borate running buffer (8.0) contained sodium dodecyl sulfate and β-cyclodextrin. The linear range for OP, 4-NP, and BPA are 20-1.000, 15-1.000 and 20-1.000 μg L^{-1}. The detection limits were 5.0, 4.0, and 3.0 μg L^{-1}, respectively. The recoveries were between 90.2 and 109.4 %.[49]

The capacity of three methods, affinity capillary electrophoresis mass spectrometry (ACE-MS), affinity capillary electrophoresis UV detection (ACE-UV) and direct infusion mass spectrometry (DIMS) were compared for the determination of the affinity of some bioactive compounds with β-cyclodextrin. Compounds included in the investigations were: ibuprofen, s-flurbiprofen, diclofenac, phenylbutazone, naproxen, folic acid, resveratrol, and 4,4'-propane-1,3-diyl)dibenzoic acid. It was stated that the ACE-MS method is suitable to interact, separate, and rapidly scan for the simultaneous affinity of multiple interacting pairs.[50]

A chiral capillary electrophoretic method was developed for the analysis of dl-penicillamine. The cost effective neutral β-cyclodextrin was employed as chiral selector. The baseline separation of dl-penicillamine was achieved in the pH range of 2.0 – pH 10. The range or linear calibration curves were: 8.568.56 x 102 μg mL^{-1} (pH 4.5), and 8.561 x 103 μg mL^{-1} (pH 4.5; pH 7.4 and pH 9.7). The correlation coefficients were in each case 0.999. The limit of detections

were 2.58 μg mL^{-1} in acidic and neutral conditions and 1.40 μg mL^{-1} in alkaline conditions. Recoveries varied from 93.1 to 105 %.

It was suggested that the method can be employed for the investigation of other chiral amines or amino acids.[51]

A new cationic cyclodextrin, mono-6(A)-(3-methoxypropan-1-ammonium)-6(A)-β-cyclodextrin was synthesized and applied as chiral selector in CE. The new cyclodextrin derivative has three recognition sites such as β-CD, ammonium cation and methoxy group on the sidearm to contribute three corresponding driving forces (inclusion complexation, electrostatic interaction and hydrogen bonding). It was established that the new CD derivative shows excellent enantioseparation capacity for a wide range of acidic and ampholytic racemates. It was assumed that the method can be applied for the synthesis of new host-guest complexes for practical application.[52]

A new method was developed to enhance the sensitivity of enantiomeric capillary electrophoresis. The method applied large-volume stacking with electroosmotic flow pump. It was established that the sensitivity of the method can be enhanced by adding cyclodextrin to the mobile phase. It was found that the addition of CD to the system increased considerably the enantiomeric separation capacity of the CE method employed. It was further found that the procedure can be used for the investigation of real samples containing a large amount of unnecessary background salt.[53]

The application of 1-adamantanecarboxylate for the desorption of elutes retained on a CD bonded silica was investigated in detail. Solid phase extraction followed with micellar electrokinetic chromatography was employed for the analysis of 4-tert-butylphenol and 2,2-di(4-hydroxyphenyl)propane. It was found that the desorption capacity of 1-adamantanecorboxylate was higher than that of traditional desorption agents.[54]

A new positively charged monolithic stationary phase was developed and applied for the chiral separation of acidic compounds. The new monolithic stationary phase was prepared by incorporating vinylbenzyl trimethylammonium (VBTA) as a positively charged achiral co-monomer to glycydyl methacrylate-β-cyclodextrin (GMA/β-CD). The characteristics of the monomer were investigated by scanning electron microscopy, optical microscopy, pressure drop/flow-rate curves and nitrogen adsorption analysis. The system was optimized, and the separation of 41 pairs of structurally different anionic chiral analytes was obtained. It was established that the separation capacity of the new monolithic was superior to that of similar traditional monolithic stationary phases. It was further established that the combination of the new monolitic column with capillary electrochromatography-mass spectrometry enhanced considerably the enantiomeric separation efficacy of the system. It was further found that the application of triple quadrupole MS system further increased the performance of the CEC-MS method.[55]

Chemometric techniques were employed for the optimization of the efficacy of cyclodextrin-modified micellar electrokinetic chromatography using head column field amplified sample stacking for the analysis of the acid metabolites of the lipoxygenase pathways in human polymorphonuclear leukocytes. The following compounds were included in the investigation: leukotriene B-4, 6-trans-12-epi-leukotriene B-4, 5(S)-hydroxy-6-trans-8,11,14-cis-eicosatetraenoic acid, 12(S)-hydroxy-6-trans-8,11,14-cis-eicosatetraenoic acid, and 15(S)-hydroxy-6-trans-8,11,14-cis-eicosatetraenoic acid. The optimum MEC conditions were: 80 mM sodium borate buffer, pH 10.7, containing 16.6 mM sodium dodecil sulfate and 15 mM α-cyclodextrin. The separation voltage was 12.5 kV, the column temperature 23^0C. The limits of quantification ranged from 30 to 50 ng mL^{-1}, and the limits of detections were between 10 and 17 ng mL^{-1}. It was stated that the method is suitable for the determination of various arachidonic acid metabolites produced by cells and can be applied for the investigation of lipoxygenase inhibitors.[56]

High performance capillary electrophoresis HPCE) was employed for the separation and quantitative determination of free amino acids during fermentation of Bacillus subtilis. Before measurements amino acidc were derivatized with phenylisothiocianate. The optimal analytical system consisted of 30 mM phosphate and 3 mM β-cyclodextrin at pH 7.0. The separation voltage was 20 kV, the detection wavelength 254 nm. It was established that the separation capacity of the method was comensurable with those obtained with the traditional methods using ninhydrin.[57]

The including complex formation between cyclodextrins and polyphenols was investigated by capillary electrokinetic chromatography. Polyphenols included in the experiments were trans-resveratrol, astilbin, taxifolin, ferulic acid and syringic acid. The binding constants were calculated based on the effective electrophoretic mobility of guest molecules. The results indicated that the complex formation depends considerably on the sterical correspondence between the host and guest molecules. It was further established that the complexation procedure is enthalpy-controlled and Van der Waals force and release of high-enthalpy water molecules play a marked role in the formation of inclusion complexes.[58]

Chiral separation and determination of excitory amino acids was carried out using CE followed laser induced fluorescence detection. Analytes were derivatized with 4-fluoro-7-nitro-2,1,3-benzoxadiazol and the enantiomers were separated by CE. The enantiomeric separation capacity was enhanced by adding two CD derivatives to the background electrolite. It was found that the system separated both Asp and glutamated enantiomers. The LOD was 17 and 9 nM, respectively, the LOQ was 50 nM for both analites. It was concluded from the data that the method is suitable for the analysis of excitatory amino acids in brain samples.[59]

The analysis of antimalaries has also been reviewed. The review discuss the capillary electrophoresis methods with special emphasis on chiral separation methods. A considerable number of chiral selectors were employed for the enantiomeric separation of antimalarials such as oligosaccharides (cyclodextrins, oligomaltoclextrins), neutral (amylose, dextrin and dextran) and charged (chondroitin sulfate, heparin, dextransulfate), polysaccharides, and proteins. However, in some cases micellar electrokinetic capillary chromatography or non aqueous CE can be applied for the enantiomeric separation

of antimalarials without using chiral selector in the mobile phase. Moreover, the review discuss the quantitative application of CE in the analysis of antimalarias in biological and food matrices.[60]

Cyclodextrin modified CE was employed for the separation and quantitative determination of hydroxy acids in cosmetics. The method was developed by chemometric techniques using phosphate concentration, surfactant concentration and methanol percentages as variables. The optimal analytical conditions were: running buffer of 150 mM phosphate solution (pH 7) containing 0.5 mM CTAB, 3 mM γ-CD, 25 % methanol, 20 s sample injection, and UV detection at 200 nm at 0.5 psi, separation voltage was -0.1V kV. The temperature was 25^{0}C. The LOD (S/N = 3) was 625 nM both salicylic acid and mandelic acid. The correlation coefficient was over 0.998, the RSD and relative error were lower than 9.21 %. The method was successfully applied to several commercial cosmetic products.[61]

Microwave-assisted extraction followed with capillary electrophoresis was employed for the determination of eight isoquinoline alkaloids in Chelidonium majus L. Both the parameters of MAE and CE were optimized: optimal MAE extraction was carried out at 60^{0}C, 5 min, extracting agent being methanol:water:HCL in ratios of 90:10:0.5 v/v/v. Optimal composition of BGE consisted of 500 mM Tris-H3PO4 bufffer (pH 2.5) containing 50 % methanol and 2 mM HP-β-cyclodextrin. It was stated that the solvent consumption and analysis time of the new method is superior comparing with the traditional methods.[62]

Capillary electrophoresis was employed for the separation of repaglinide enantiomers in pharmaceutical formulations using 2,6-di-O-methyl-β-cyclodextrin (DM-β-CD) as chiral selector. The optimal conditions of the analysis were: UV detection at 243 nm, separation voltage 20 kV, BGE 1.25 % (w/v) in 20 mM sodium phosphate (pH 2.5). The linear caibration curve was 12.5 – 400 μg mL^{-1}. LOD was 100 ng mL^{-1}, the intra-day and inter-day precisions were 2.8 ad 3.2 %, respectively. Recoveries ranged from 97.7 to 100.9 %. It was established that the method is fast and convenient and can be used for the determination of repaglinide enantiomers in quality control of pharmaceutical product.[63]

The enantioseparation of lipoic acid an antioxidant in dietary supplements was obtained by using CE and trimethyl-β-cyclodextrin as chiral selector. Analyses were carried out in sulfonated capillary with the effective voltage of +18 kV and at the detection wavelegth at 200 nm. The best separation was achieved with BGE 100 mM phosphate buffer (pH 7.0) containing 8 mM trimethyl-β-cyclodextrin. Analyses were carried out at 20^{0}C. It was established that the method is suitable for the determination of lipoic acid in dietary supplements.[64]

A RP-HPLC procedure was developed for the separation and quantitative determination of resibufogenin and cinofufagin. The infuence of the nature of organic solvent and cyclodextrins, the concentration of γ-CD, the temperature of the separation on the separation efficacy of the system was investigated in detail. It was assumed that the influence of CD on the retention may be due to the formation of inclusion complexes between the analytes and the cyclodextrin. It was found that CD forms 1:1 inclusion complexes with the analytes resulting in modified retention behaviour. The investigations indicated that the complex formation is spontaneous, exotherm, and enthalpy driven. It was further found that the method can be applied for the analysis of resibufogenin and cinobufagin in different Chansu (Bufonis venenum) samples.[65]

It was found that neutral and acidic monosaccharides are readiliy labeled with 2,3-naphtalenediamine and the resulting saccharide-naphthimidazole derivatives (NAIM) can be separated by CE in borate buffer. Enantiomeric separations were obtained by using sulfated-α-cyclodextrin as chiral selector in phosphate buffer. The method allowed the simultaneous determination of absolut configuration and sugar composition in the mucilage polysaccharide of the medicinal herb Dendrobium huoshanense. It was also established that heparin disaccharides can be successfully derived with the method, however, heparin derivatives with the same degree of sulfation cannot be separated by CE.[66]

A capillary electrophoretic techniqe was developed and applied for the separation of flavonoids by β-cyclodextrin modified CE. The optimal separation conditions were: background electrolite 20 mM borax (pH 7.5) containing 6 mM β-cyclodextrin and 20 % acetonitrile. Injection time was 65 s. Detection limit for five flavonoids ranged 15-30 ng mL^{-1}. It was found that the procedure can be successfully employed for the determination of flavonoids in traditional Chinese medicine real samples.[67]

The chiral selectors applied recently in HPLC and CE have been reiewed and the importance of chiral separations in human healthy care, biophysics, biochemistry and other up to date field of scientific research is emphasized. The review lists the most important chiral selectors and chiral stationary phases reported in the last years.[68]

Rotational electromagnetic stirring, rotating evaporation, differential scanning calorimetry, fourier-transform infrared spectroscopy, X-ray powder diffractometry, scanning electron microscopy and HPLC methods were employed for the preparation and investigation of the quercetin/hydroxypropyl-β-cyclodextrin 1:1 inclusion complex. The objectives of the investigations were the increase of the water solubility of quercetin. The investigations revealed that the complex formation improved six times the water solubility of quercetin.[69]

A cyclodextrin-modified capillary electrophoretic method was developed for the enantiomeric separation of cathinone derivatives. The influence of various components of the CE separation system was investigated in detail. Native-β-CD, carboxymethyl-β-CD, hydroxypropyl-β-CD, sulfated-β-CD were included in the investigation. The results indicated that the best enantioselectivity can be obtained by using negatively charged sulfated-β-CD as chiral selector. The optimal CE conditions were: 20 mg mL^{-1} sulfated-β-CD in 50 mM ammonium acetate buffer (pH 4.5) containing 10 % ACN at a cassette temperature of 40 ^{0}C, and at 20 kV. It was established that a set of 19 cathinone derivatives (except methedrone) were enantioseparated by the CE method.[70]

Pantoprazole enantiomers were separated using CE and sulfobutylether-β-cyclodextrin (SBE-β-CD) chiral additive. The procedure was optimized and the best separation system

was applied for the analyses. BGE consisted of 50 mM borax-150 mM phosphate (pH 6.5), 20 mg mL^{-1} SBE-β-CD, and 10 kV applied voltage. The LOD and LOQ were for R-(+)-pantoprazole 0.9 and 2.9 μg mL^{-1}, respectively. It was stated that the method can be applied for the determination a minimum limit of 0.1 % (w/w) of R-enantiomer in-S-pantoprazole bulk samples.[71]

Countercurrent chromatography has been employed for the separation of the three main α-acids present in the extract of commercially available hops (*Humulus lupulus L.*). The extract contain individual isomerized α-acid (co-, n-, and ad-) in addition to cis/trans diastereomers for each congener. The first step of separation was carried out using hexane and aqueous buffer. The second step was suitable for the separation of cis/trans diastereomers and applied quaternary solvent system. It was established that the presence of β-cyclodextrin enhanced the separation capacity of the system. It was further established that the purity of the end products (individual α-acids, iso α-acids, individual tetrahydro isomerized α-acids) was in each case over 95 %). It was found that the composition of the mobil phases, pH and buffer-to-sample ratio influence considerably the efficacy of the separation.[72]

The influence of α-, β-, and γ-cyclodextrins on the CE migration time of 11 guanidine/imidazoline derivatives, and imidazoline receptor ligands was investigated in detail. The data were evaluated by performed quantitative structure-mobility relationship (QSMR) method. The calculations indicated that the QSMR calculation method can be successfully employed as an initial screening predictive tool for CE migration behaviour of other related guanidine/imidazoline derivatives in the presence of native cyclodextrins.[73]

The recent developments in the synthesis and application of new cyclodextrin derivatives has also been reviewed, and their application in chromatographic separation processes has been discussed. The new results obtained in liquid chromatography (LC), capillary electrochromatography (CEC), gas chromatography and supercritical fluid chromatography (SFC). The use of open tubular CEC (OT-CEC), paked bed-CEC (P-CEC), pseudostationary phase CEC (PSP-CEC) has been discussed in detail.[74]

Electromembrane extraction followed by cyclodextrin-modified capillary electrophoresis was employed for the preconcentration and quantitative analysis of trimipramine (TPM) enantiomers in biological matrices. TPM enantiomers migrated through a thin layer of 2-nitrophenyl octyl ether (NPOE). Response surface technology (RSM) was applied for the optimization of different variables. The optimal conditions were NPOE as supported liquid membrane, inter-electrode distance of 5 mm, stirring rate 1000 rpm, 51 V, potential difference, 34 min extraction time, acceptor phase pH 1.0 and donor phase pH 4.5. BGE consisted of 100 mM phosphate buffer (pH 2.0) containing 10 mM α-CD as chiral selector. The applied voltage was 18 kV, the temperature 20 ^{0}C. The range of quantitation was 20 - 500 ng mL^{-1}. The intra- and interday RSDs (n=6) were <6 % for both enantiomers. LOQ and LOD were 20 and 7 ng mL^{-1}, respectively. It was stated that the procedure can be applied for the determination of the concentration of TPM enantiomers in plasma and urine samples without any pre-treatment.[75]

Achiral ionic liquid, DM-β-CD and TM-β-CD were employed for the enantioseparation of three β-blockers such as PIN, OX and PRO. Good separation was achieved by using dual CDs containing DM-b-CD and TM-β-CD. The investigations indicated that under optimal conditions the detection limit of the enantiomer pairs ranged from 0.10 to 0.65 nM. It was further established that the procedure can be successfully applied for the determination of this class of β-blockers in spiked usine samples with acceptable recoveries.[76]

A novel stacking method of repetitive large volume sample injection followed with sweeping micellar electrokinetic chromatography (MEKC) was employed for the determination of androgenic steroids in urine. Testosterone (T), epitestosterone (E), and epitestosterone glucuronide (EG) were included in the experiments. A phosphate buffer was filled into an uncoated fused silica capillary, the samples were injected into the capillary at 10 psi for 20 s then stacked at -10 kV for 1 min using phosphate buffer containing SDS. Injecting and stacking steps were repeated five times. Separation was carried out at -20 kV, the buffer contained methanol, SDS and (2-hydroxypropyl)-β-cyclodextrin. Analytes were detected at 254 nm. The linearity range was 5 – 200 ng mL^{-1} for T; 20 – 200 ng mL^{-1} for E; 0.5 – 500 ng mL^{-1} for EG. LODs were 1.0 ng mL^{-1} for T; 5.0 ng mL^{-1} for E; and 200.0 pg mL^{-1} for EG. RSD values in intra-day (n=3) and inter-day measurements (n=5) were below 10.0 %. It was stated that the method can be applied for monitoring of doping by sportsmen.[77]

Cyclodextrin-modified micellar electrokinetic chromatography was employed for the chiral separation of the four stereoisomers of vinpocetine in separation time of 9.5 min and resolution of 1.043.87, 2-hydroxy-β-cyclodextrin was applied as chiral selector. Enantiomer separation was carried out in buffer of 40 mM HP-β-CD in 50 mM phosphate buffer containing 40 mM SDS. The separation temperature was 25 ^{0}C, the separation voltage 25 kV.[78]

Fat soluble isoquinoline enantiomers were separated by employing β-cyclodextrin-modified micellar capillary electrokinetic chromatography. The enantioselective separation of 1-phenyl-R,S-tetrahydroisoquinoline (ER,ES). Electrochemical detection (EC) was applied for the determination of analytes. An effectual micellar suspension of 35 ml L^{-1} phosphate buffer saline (pH 7.85) containing 30 mM sodium desoxycholate, 20 mM β-CD and 20 % (v/v) acetonitrile formed the running buffer. Analytes were baseline separated in 12 min, separation voltage was 20 kV. The RSD (=5) of migration time and peak areas are 2.3 % (ER), 2.7 % (ES), 2.0 % (ER) %, 3.5 % (ES). LODs were 0.5 μmol L^{-1} for ER and 0.2 μg L^{-1} for ES. The method has been successfully applied for the determination of ER from ES in synthetic drug intermediate.[79]

A double junction interface was employed for the reservation of separation efficacy and for the alleviation of ion suppression from sulfated β-cyclodextrin (S-β-CD) in electrokinetic chromatography-electrospray ionization-mass spectrometry. The good separation capacity of the novel system was verified in the analysis of dihydroxyphenylalanin and methyldihydroxyphenylalanine. Enantiomers were separated either (counter-migration mode;

0.1 % S-β-CD) or carrier mode (2 % S-β-CD). It was found that no ion suppression was observed during the analysis, and the sensitivity of the method was improved considerably.[80]

Chemically bonded cationic β-cyclodextrin derivatives were employed as chiral stationary phase for the enantioseparation of aromatic compounds and pharmaceuticals. Vinylene-functionalyzed cationic β-cyclodextrins were co-polymerized with vinylzed silica in the presence of conjugated monomers. The chemically immobilized cationic β-cyclodextrins were employed as chiral stationary phases in packed column supercritical fluid chromatography. The good separation characteristics of the new chiral stationary phases was verified.[81]

Abbreviations

ACE-MS = affinity capillary electrophoresis mass spectrometry
ACN = acetonitrile
AIT = isothiocyanate
BGE = bacground electrolyte
BPA = bisphenol A
CD-CSPs = cyclodextrin chiral stationary phases
CEC = capillary electrochromatography
CE-LIF = capillary electrophoresis-laser induced fluorescence
CZE = capillary zone electrophoresis
DIMS = direct infusion mass spectrometry
EDGE = ethylenegycol diglicidyl ether
DLS = dynamic light scattering
EKC = electrokinetic chromatography
EMO = enantiomeric migration order
E = epitestosterone
EG = epitestosterone glucuronide
Es-GC-MS = enantioselective GC-MS
FASS = field-amplified sample stacking
FC = free cholesterol
GCxGC=comprehensive two dimensional gas chromatography
GC/MS = gas chromatography/mass spectrometry
GMA = glycidyl methacrylate
HCA = hierarchical cluster analysis
H-DAS-β-CD= heptakis(2,3-di-O-acetyl-6-O-sulfo)-β-CD
H-DMS-β-CD = heptakis(2,3-di-O-methyl-6-sulfo)-β-CD
HEPES=hydroxypropyl-β-2-[4-(2-hydroxyethyl)pipera-zin-1-yl]ethanesulfonic acid
HRE = heat reflux extraction
HILIC = hydrophic interaction liquid chromatography
HPCE = high performance capillary electrophoresis
HPMC = hydroxypropyl methylcellulose
HRE = heat reflux extraction
HSA = human serum albumin
HS-SPME = headspace solid phase microextraction
LD = liquid desorption
LLE = liquid-liquid extraction
LOD = limit of detection
LLOQ = lowest limit of quantitation
LOQ = limit of quantitation
LVSEP = large volume sample stacking
MAE = microwave-assisted extraction
MD = in vivo microdialysis sampling
MDGC = heart-cut multidimensional gas cromatography
MDE = membrane dialysis extraction
MDMA = 3,4-methylenedioxymethamphetamine
MHE = multiple headspace extraction
MEKC = micellar electrokinetic chromatography
MAE = microwave-assisted extraction
NAIM = saccharide-naphthimidazole derivatives
NEP = norephedrine
4-NP = 4-nonylphenol
NPOE = 2-nitrophenyl octyl ether
OP = octylphenol
OT-CEC = open tubular CEC
PCA = principal component analysis
p-CEC = packed-bed CEC
PE = phosphatidylethanolamine
PEG = polyethylene glycol
PMMABCD = permethylated momoamino-β-cyclodextrin
psp-CEC = pseudostationary CEC
QSMR = quantitative structure-mobility relationship
RSM = response surface methodology
SBE-β-CD = sulfobutylether-β-cyclodextrin
SBSE = stir bar sorptive extraction
SDS = sodium dodecyl sulfate
SEP = high capacity headspace sample enrichment probe
SFC = supercritical fluid chromatography
SOX = Soxhlet extraction with acetone
SH-GC = static headspace gas chromatography
SBE-β-CD = sulfobutylether-β-cyclodextrin
TAS = total analysis system
TEM = transmission electron microscopy
T = testosterone
TFC = turbulent-flow chromatography
TPM = trimipramine
U-PLS = unfolded-partial least squares regression
USE = ultrasonic extraction
VOCs = volatile organic compounds
VBTA = vinylbenzyl trimethylammonium

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