INTRODUCTION

Chiral separation has become not only a central theme in the research of chirality but also an important task in the field of analytical chemistry in the past few decades. In most cases, as for a chiral compound only one of the enantiomers (eutomer) is responsible for the desired activity, while the other enantiomer (distomer) may exhibit no therapeutic value and may potentially cause unsuspected adverse effects. Consequently, the development of methods for enantiometric separation has become very important for drug quality control, pharmacodynamic and pharmacokinetic studies as well as toxicological investigations.

Enantioseparation can be performed by the indirect or direct method. The indirect method involves the derivatization of the enantiomers with a stereochemically pure agent to form diastereomers, which can be subsequently separated in an achiral system. The direct enantioseparation is based on the formation of transient diastereomeric complexes between the enantiomers and a chiral selector. The analytical methods so far employed for chiral separation include high performance liquid chromatography (HPLC), gas chromatography (GC), thin layer chromatography (TLC), supercritical fluid chromatography (SFC), capillary electrophoresis (CE), etc.

Several CE modes such as capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MEKC), nonaqueous capillary electrophoresis (NACE), affinity electrokinetic chromatography (AEKC), capillary isotachophoresis (CITP) and capillary gel electrophoresis (CGE) have been used for chiral separation. In addition to CZE, MEKC and NACE have exhibited their characteristics and advantages in enantioseparation. To date, various sorts of chiral selectors have been developed in CE, including cyclodextrins (CDs) and derivatives, antibiotics, polysaccharides, chiral surfactants, crown ethers, proteins, chiral metal ion complexes and so on. Conclusively, all these advantages make CE a powerful tool for chiral separation. Some reviews [1-3] have summarized chiral separation principles and their applications using CE techniques.

Keywords: Review, antibiotics, polysaccharides, Enantioseparation, Capillary electrophoresis, Advances

ABSTRACT: This review gives an overview of the use of antibiotics and polysaccharides as chiral selectors in the field of enantioseparation by capillary electrophoresis (CE). Antibiotics and polysaccharides are two important types of chiral selectors in CE, and have been utilized successfully in enantioseparation of various compounds including pharmaceuticals, biochemicals, agrochemicals, fine chemicals, etc. In this review, recent advances covering literature published from January 2006 to April 2010 in chiral CE separation with antibiotics and polysaccharides are summarized. These developments focus on the introductions of new chiral selectors, investigations in different CE modes containing micellar electrokinetic chromatography and nonaqueous capillary electrophoresis, studies on separation mechanisms and improvements in separation methods.
The aim of this review is to give an overview of the use of antibiotics and polysaccharides as chiral selectors in CE, and to focus on the major developments of CE enantioseparation with antibiotics and polysaccharides covering literature published from January 2006 to April 2010. These developments consist of the introductions of new chiral selectors, investigations in different CE modes, studies on separation mechanisms and improvements in separation methods.

Use of antibiotics
Since Armstrong and co-workers demonstrated that rifamycin B and vancomycin were useful chiral selectors, antibiotics containing macrolyclic antibiotics (glycopeptides, ansamycins and macrolides) and non-macrolyclic antibiotics (aminoglycosides and lincosamides) have proved to be a powerful class of chiral selectors for CE. All these antibiotics possess several characteristics that allow them to interact with analytes and serve as chiral selectors. They have a number of stereogenic centres and functional groups, allowing them to have multiple interactions with chiral molecules. Electrostatic or charge-charge, dipole–dipole, π–π, hydrogen bonding and steric repulsion are assumed to be the interactions responsible for chiral recognition. Among these antibiotics, glycopeptides are the most important group for enantioseparation of lots of chiral compounds in CE. This group mainly consists of vancomycin and its analogues [such as A828436, LYS07599, teicoplanin and its analogue MDL 63 246, ristocetin A, actaplanin A, A35512B, avoparcin, balhimycin, eremomycin, etc.]. Since glycopeptides have adsorption on the capillary wall and strong UV absorption, coated capillaries and partial filling-counter current methods have been applied to overcome these problems. Rifamycins B and SV belonging to macrolyclic antibiotics of ansamycins type, have been successfully used for enantioseparation of cationic and anionic compounds, respectively. Moreover, aminoglycosides include fradiomycin sulfate, kanamycin sulfate and streptomycin sulfate have been utilized as CE chiral selectors. Comprehensive descriptions of the properties of these antibiotics above mentioned and their applications to the chiral separation of a broad series of compounds are given in several reviews (4-6) between 1997 and 2001. Macrolides containing erythromycin and its derivatives as well as lincosamides (clindamycin phosphate) are two newer types of antibiotics, which have been used successfully for enantioseparation of some drug enantiomers. In order to keep track of antibiotic selectors in CE, some recent advances concerning references published from January 2006 to April 2010 are summarized below.

Gao et al. (7) described baseline separation of three racemic dimethyl diphenyl dicarboxylate analogues by CE with vancomycin. They used a polyacationic electrolyte hexadimethrine bromide to produce a positively charged coating, which minimized the adsorption of vancomycin on the capillary wall and shortened the separation time by reversing the EOF. Wang et al. (8) achieved an improvement in separation performance using vancomycin by dynamic coating the capillary wall with poly(dimethylacrylamide) to minimize the adsorption of vancomycin. FMOC amino acids derivatives, ketoprofen and fenoprofen were baseline separated within 4.2 min used the partial filling technique and short-end-injection. Maier et al. (9) employed an electrokinetic partial filling technique (EK-PFT) and the counter-current separation mode with contactless conductivity detection for enantioresolution of lactic acid with vancomycin in CE. EK-PFT allowed removal of the chloride counterions from vancomycin, which otherwise deteriorated sensitivity of conductivity detection, and thus increase in enantioresolution was obtained. Prokhorova et al. (10, 11) introduced glycopeptide antibiotic eremomycin as a novel chiral selector for CE enantioseparations of five profens (flurbiprofen, fenoprofen, ibuprofen, indoprofen, and ketoprofen), and then used coupled chitosan-coated capillary to shorten analysis time and enhance separation efficiency. Jiang et al. (12) employed balhimycin and its dehaloanalogue dechlorobalhimycin for enantioresolution of 11 dansyl amino acids and six 2-arylpropionic acids by CE. They observed that enantioresolution capability of balhimycin was clearly higher than dechlorobalhimycin, and suggested that chlorine substituents of balhimycin played a major role in the enantioresolution of these analytes. Then they (13) also separated a series of N-benzyolylated derivatives of four amino acids (leucine, alanine, methionine and threonine) using balhimycin, bromobalhimycin and dechlorobalhimycin as chiral selectors. They thought the possible secondary interactions, such as hydrophobic interaction, steric hindrance and π–π interaction, could play an important role in enantiorecognition process depended on the structure of the analytes. The structures of balhimycin, bromobalhimycin and dechlorobalhimycin are shown in Figure 1. Dai et al. (14) prepared β-CD-derivatized erthyromycin (β-CD-EM) as a novel CE chiral selector by covalently linking the CD with EM. As shown in Figure 2, β-CD-EM exhibited better enantioselectivity.
towards chlorpheniramine and salsolinol compared with single β-CD and EM. Chen et al. (15) studied the enantiomeric separation of propranolol and duloxetine by NACE in methanol-based medium using erythromycin lactobionate (see its structure in Figure 3) as chiral selector. The effects of organic solvent type, capillary temperature, pH and composition of the BGE, erythromycin lactobionate concentration and running voltage were investigated.

In this report, antibiotic was first used as chiral selector in NACE. Electropherograms of the chiral separations of propranolol and duloxetine are shown in Figure 4. Chen et al. (16) introduced a novel CE chiral selector clindamycin phosphate (see its structure in Figure 5) belonging to the group of lincosamides for enantioseparation of some basic drugs consisting of nefopam, propranolol, tryptophan, chlorphenamine, citalopram, tryptophan methyl ester, metoprolol and atenolol. This work first employed lincosamide antibiotics as chiral selector. Then they (17) also investigated the enantioseparation capability of clindamycin phosphate towards these basic drugs by MEKC using SDS as the surfactant, propanol as the organic additive, and phosphate as the BGE.

It was found that the enantioseparation (e.g. resolution, selectivity factor, and peak shape) of the studied drugs was improved in this MEKC system. Electropherograms of the enantioseparations of nefopam, propranolol, tryptophan and chlorphenamine are shown in Figure 6.

Use of polysaccharides

Polysaccharides have been demonstrated to exhibit prominent enantioselective properties toward plenteous chiral compounds (18–20). They have very low absorbance in the UV region, which is beneficial for high detection sensitivity. Additionally, varying structures and functional groups of polysaccharides provide a range of enantioselectivity in CE and many of them are water-soluble. A series of polysaccharides have been used successfully in CE, and they can be mainly divided into two types containing electrically neutral and ionic polysaccharides. As described by Hiroyuki Nishi, polysaccharides have been applied to the CE chiral separation in two modes. One is CZE with a neutral polysaccharide, and the other is AEKC with an ionic polysaccharide. Electrically neutral polysaccharides mainly consist of dextrins (dextrin, maltodextrin and amylose), dextran, laminaran, pullulan, methyl cellulose, hydroxypropyl cellulose, glycogen, etc. Ionic polysaccharides including natural, synthetic or modified ones, mainly consist of heparin, chondroitin sulfates, lambda-carrageenan, pectins, glycosaminoglycans, colominic acid, dextrin sulfate, dextrin sulfopropyl ether, diethylaminoethyl dextran, pentosan polysulfate, oversulfated galactosaminoglycans, desulfated chondroitin sulfate C, carboxymethyl dextran, carboxymethyl mylase, carboxymethyl cellulose, sulfated cyclophorases, N-(3-sulfo, 3-carboxy)-propionylchitosan, etc. To keep track of polysaccharide selectors in CE, some recent advances concerned with references published from January 2006 to April 2010 are summarized below.
Kwon et al. (21) introduced cyclic $\beta$-(1$\rightarrow$3),$(1\rightarrow6)$-glucans produced by Bradyrhizobium japonicum as novel chiral selector in CE for the enantiomeric separation of some flavanones such as eriodictyol, homoeriodictyol, hesperetin, naringenin and isosakuranetin. The cyclic $\beta$-(1$\rightarrow$3),$(1\rightarrow6)$-glucans consisting of 10-13 glucose residues linked by $\beta$-(1$\rightarrow$3) and $\beta$-(1$\rightarrow$6) glycosidic bonds were isolated by hot ethanol extraction and purified by size exclusion and anion exchange chromatography. Moreover, the chiral discrimination of catechin was investigated in the presence of this novel selector as chiral shift reagent by $^{13}$C-NMR (22). Kwon et al. (23, 24) also isolated and purified succinoglycan monomers (M1, M2, and M3, see their structures in Figure 7) by the degree of succinylation, and used them as chiral selectors for enantiomeric separation of some flavanones as well as chiral shift reagents with $^{13}$C-NMR spectroscopy for chiral discrimination of catechin. Succinoglycan, a shinorhizobial exopolysaccharide produced by Shinorhizobium meliloti, is composed of an octasaccharide subunit. Electropherograms of the chiral separation of catechin using M2 and M3 are shown in Figure 8. Wei et al. (25) discovered the helical- and

![Figure 7. Structures of succinoglycan monomers (reprinted with permission from (24)).](image)

![Figure 8. CE separation of catechin in the presence of (A) 60mM succinoglycan monomer (M2), (B) 30mM succinoglycan monomer (M3). Conditions: 100mM borate buffer pH 8.8; applied voltage, 25kV; positive polarity at the inlet, 5kPa pressure for 4s; temperature, 20°C; detection at 280 ± 10 nm. Reprinted with permission from (24).](image)
ahelical-dependent chiral recognition mechanisms when amylose was used as a chiral selector in CE.

In the range of chiral compounds tested (ibuprofen, primaquine, trihexyphenidyl, cetirizine, sulconazole, econazole, diitiazem, and norflouxetine), the helical structures were necessary for the recognitions of the enantiomers with linear and flexible molecular structure and molecular size less than 0.78 nm. Ahehical structures alone could recognize some gauche enantiomers and the existence of helical structure had no effect on the chiral separation of them. Then they [26] also used partial-filling and semi-permanent coating techniques with high concentration of amylose solutions as separation medium for the enantioseparations of primaquine, trihexyphenidyl, sulconazole and cetirizine.

This method was applied to separation and determination of trihexyphenidyl enantiomers in rabbit blood. Zhu et al. [27] used polygalacturonic acid as a chiral selector in CE for the separation of enantiomers of some basic drugs including nefopam, Chlorpheniramine and ketoconazole, and they found that the choices of running buffer pH and concentration of chiral selector were important for the improvement of enantioselectivity.

Chen et al. [28] introduced electrically neutral glycogen (see its structure in Figure 9), belonging to the class of branched polysaccharides, as a novel chiral selector in CE. Among the tested compounds, the enantiomers of ibuprofen which is an acidic drug were successfully recognized by 3.0 percent w/v glycogen with 90mM Tris-H3PO4 buffer (pH 7.0), as seen in Figure 10. The enantiomers of basic drugs such as citalopram, cetirizine and nefopam were also baseline-resolved with 50mM Tris-H3PO4 buffer (pH 3.0) containing 3.0 percent glycogen. This study first utilized branched polysaccharides as chiral selector. Chu et al. [29] employed dextran sulfate as chiral selector for enantioseparation of two antiparkinsonian drugs including rotigotine and trihexyphenidyl by electokinetic chromatography (EKC).

The enantioseparation was performed under normal and reversed polarity modes, and reversed enantiomer migration order was achieved under two modes. In the optimal conditions, rotigotine and trihexyphenidyl enantiomers were enantioresolved in 40 min with the resolution of 2.0 and 5.8, respectively.

CONCLUSION

To date, a series of chiral selectors consisting of CDs and derivatives, antibiotics, polysaccharides, chiral surfactants, crown ethers, proteins, etc. have been utilized successfully in CE. Among these chiral selectors, antibiotics and polysaccharides have been demonstrated to possess excellent enantioselective properties toward plenteous racemic compounds.

In this review, we have given an overview of the use of antibiotics and polysaccharides as chiral selectors in CE, and put emphasis on the major advances of CE enantioseparation with antibiotics and polysaccharides covering literature published from January 2006 to April 2010. The number of publications on the use of antibiotics and polysaccharides in chiral CE separation reflects the continuously growing importance of this field.

ACKNOWLEDGEMENTS

This work was supported by the Project of National Natural Science Foundation of China (Nos: 81072610 and 81001422) and the Key Project of Chinese Ministry of Education (No.: 109085).

REFERENCES AND NOTES

29. B. Chu, Q. Feng et al., Chromatographia, 70, pp. 817-824 (2009).