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Review

Recent advances in pharmaceutical applications of chiral capillary electrophoresis

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Abstract

This review article summarizes developments and applications of chiral capillary electrophoresis (CE) in the pharmaceutical field published from January 2004 to June 2005. Due to the tremendous number of publications, this article is aimed to focus on major developments in chiral separations and some selected applications rather than to provide a descriptive overview of all published papers. Valuable information is also collected from several excellent reviews published during this period. Developments are classified according to CE modes, namely capillary zone electrophoresis (CZE), micellar electrokinetic capillary chromatography (MEKC), microemulsion electrokinetic chromatography (MEEKC). In the CZE section, different types of chiral selectors including cyclodextrins, oligo- and polysaccharides, crown ethers, macrocyclic antibiotics, ligand exchange systems and proteins are described. Nonaqueous capillary electrophoresis is also included in this section. Coupling CE to MS is discussed in a separate part, followed by a summary of selected pharmaceutical applications of enantioselective CE. Finally, some conclusions are drawn and prospects of CE in chiral analysis are also drafted.

Keywords: Capillary electrophoresis; Chiral; Overview

Contents

Abbreviations: CE-β-CD, carboxyethyl-β-CD; CM-β/γ-CD, carboxymethyl-β/γ-CD; CMC, critical micellar concentration; DDCV, dodecoxycarbonylvaline; DM-β-CD, dimethyl-β-CD; DS, substitution degree; EOF, electroosmotic flow; HDAS, heptakis(2,3-di-acetyl-6-sulfato)-β-CD; HDMS, heptakis(2,3-di-methyl-6-sulfato)-β-CD; HP-β-CD, hydroxypropyl-β-CD; HSA, human serum albumin; LIF, laser-induced fluorescence; MEEKC, microemulsion electrokinetic chromatography; NACE, nonaqueous capillary electrophoresis; QA-β-CD, quaternary ammonium β-CD; RS-CDs, randomly substituted CDs; SBE, sulfobutylether-β-CDs; SDC, sodium deoxycholate; SI-CDs, single-isomer cyclodextrins; TM-β-CD, heptakis(2,3,6-tri-*O*-methyl)-β-CD

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1. Introduction

During the last decade, there has been a dramatic increase in number of studies on chiral separation. The two most important analytical techniques used in chiral separations still are LC and CE, followed by gas chromatography (GC) and capillary electrochromatography (CEC). Compared to other techniques, CE has several advantages: the high resolving power, low consumption of sample, solvent and chiral selector, as well as its high flexibility in choosing and changing types of selectors. Advances in chiral CE and CEC were reviewed in a paper by Gübitz and Schmid [1]. In another review paper on chiral separations by LC, SFC and CE, Armstrong and co-authors have summarized the basic principles, structural features and usefulness of most types of chiral selectors used by these three analytical techniques [2]. Enantioselective CE was also discussed in another paper, where recent progress in all techniques of chiral separation was reviewed, covering publications until January 2004 [3]. The current paper covers the publication period of January 2004–June 2005. During this period, no major development in basic chiral separation principles was observed. However, some new chiral selectors were developed. These chiral selector developments are summarized in different sections, depending on the CE mode that they are classified in. Applications are normally discussed together with the corresponding type of chiral selector used. However, in the last part of the review, they are also classified according to application subject types, i.e. starting material impurity testing, quantitation in pharmaceutical preparations, or in biological samples.

2. Capillary zone electrophoresis (CZE)

2.1. Cyclodextrin-mediated capillary electrophoresis

Until this moment, cyclodextrins (CDs) still remain the most important group of chiral selectors in CZE, in terms of popularity and diversity. Among the applications found during this reported period, almost half used CDs. The main reason is that CDs present very high chiral resolving capacity. Another advantage is the diversified choice of more than 50 different neutral or charged CD derivatives offered to the users. And new derivatives continue to come on the market.

2.1.1. Neutral cyclodextrins

A new neutral CD derivative 2-*O*-acetonyl-2-*O*-hydroxypropyl-β-CD (AHP-β-CD) was synthesized by Lin et al. [4]. It is a mixture of isomers with an average degree of substitution (DS) of around 1.0 for the acetonyl group and 3.8 for the hydroxypropyl group. The effectiveness of AHP-β-CD as a chiral selector was tested on 6 acidic and 16 basic drugs, and compared to three existing CDs: β-CD, dimethyl-β-CD (DM-β-CD, DS ∼ 1.8), and hydroxypropyl-β-CD (HP-β-CD, DS ∼ 4.0), both synthesized in their laboratory according to literature. On most tested compounds, AHP-β-CD offered better enantioselectivity. Another new neutral CD, 2-*O*-(2-hydroxybutyl)-β-CD (HB-β-CD) was introduced very recently by the same research group [5]. Two HB-β-CDs with DS ∼ 3.0 and DS ∼ 4.0 were tested, both showing better chiral selectivity when compared to underivatized β-CD and HP-β-CD (DS ∼ 4.0), a classic neutral derivative which has similar structure. Comparing the two HB-β-CDs, the derivative with lower DS (3.0) induced better enantioresolution than the one with high DS.

Despite the emergence of new CD derivatives, the conventional ones were still used very often. The use of the CDs with longest tradition, the natural cyclodextrins β-CD and γ-CD, was reported in several quantitative methods summarized in Table 1 [6–9]. Especially in [10], β-CD and γ-CD were used in Hadamard transform capillary electrophoresis (HTCE) for the separation of glutamic acid enantiomers by a CE–LIF method, with the analyte being labelled by a fluorescent agent. The special point of this method is the multiple sample injection followed by data processing by Hadamard transformation. With Hadamard transformation, the noise in the background

Table 1
Chiral CE applications in enantiomeric purity determination

Analytes	LOD and LOQ	Detection	Selector(s)	Notes	Reference
Simendan	5.0 μg/ml (1.0%)[a] and 2.0 μg/ml (0.4%)	UV 380 nm	12 mM β-CD	D-simendan is minor form	[6]
Rivastigmin	0.7 μg/ml (0.05%) and 2.3 μg/ml (0.15%)	UV 214 nm	7.5 mM β-CD	R-rivastigmin is minor form	[7]
Lisuride	0.2 μg/ml (0.02%) and 0.5 μg/ml (0.05%)	UV 230 nm	20 mM γ-CD	D-lisuride is minor form	[9]
Two organic disulfates	0.3 μg/ml (0.1%), LOQ not mentioned	UV 200 nm	5 mM QA-β-CD	Highly negatively charged analytes	[20]
A novel antianginal agent	1 μg/ml (1.0%), LOQ not mentioned	UV 214 nm	10 mM HP-γ-CD + 10 mM CM-β-CD	Enantiomer + two other stereoisomers as impurities	[43]

[a] Percentage of minor form against the main form.

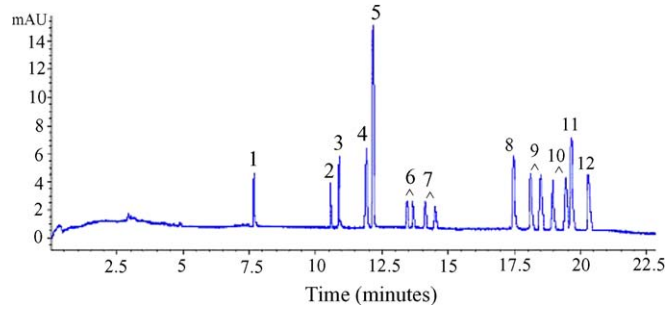

Fig. 1. Chiral separation of 12 amphetamine and piperazine compounds: (1) 1-benzylpiperazine, (2) phenethylamine, (3) 1,4-dibenzylpiperazine, (4) 3-chloroaniline, (5) 1-[2-methoxyphenyl]-piperazine, (6) D,L-amphetamine, (7) D,L-methamphetamine, (8) 1-[4-methoxyphenyl]-piperazine, (9) D,L-3,4-methylenedioxyamphetamine, (10) D,L-methylenedioxymethamphetamine, (11) 1-[3-trifluoromethylphenyl]-piperazine, and (12) 1-[3-chlorophenyl]-piperizane. Reprinted, with permission, from the Journal of Forensic Sciences, vol. 50, no. 2, copyright ASTM International, 100 Barr Harbor Drive, West Conshohocken, PA 19428 [11].

signal is reduced, thus sensitivity of the method is remarkably enhanced.

Among the neutral CDs, randomly substituted HP-β-CD remains one of the most common ones. Bishop et al. [11] described a chiral CE method using 20 mM HP-β-CD (DS ∼ 4.9) in sodium phosphate buffer pH 2.8. This method could separate all peaks of six piperazine-like compounds and four chiral amphetamine-like compounds (Fig. 1). Quantitation of the six piperazine compounds was also performed with this method, on urine samples with liquid–liquid and solid phase extraction (SPE) and synthesized samples of piperazine. In another publication by de Pablos et al., HP-β-CD even showed the best selectivity among 13 different neutral and anionic CD derivatives towards etodolac enantiomers [12]. Being aware of the reproducibility problem of randomly substituted CDs (RS-CDs), HP-β-CD from three different suppliers were tested at different concentrations. HP-β-CD from Fluka (DS ∼ 4.2), showing the best resolution at all concentrations was chosen. A 20 mM HP-β-CD in 100 mM phosphate buffer pH 7 represented the final conditions to be validated for enantioselective quantitation of etodolac in commercial formulations (Table 2). As summarized in Table 3, determination of D- and L-lactic acid in plasma was also done by enantioselective CE-UV using HP-β-CD (DS ∼ 7) at a very high concentration of 220 mM in a Tris–phosphate buffer (150 mM, pH 7.0) [13].

Methylated CDs, with long tradition similar to hydroxypropylated CDs, also gained a certain interest from chiral CE users. A chiral quantitative method for omeprazole in pharmaceutical preparations was developed and validated with randomly substituted methyl-β-CD [14] (Table 2). Meanwhile in another study, the feasibility of validating a chiral separation method with RS-CDs was investigated [15]. In this study, the reproducibility of resolution obtained with some single-isomers, heptakis(2,3,6-tri-*O*-methyl)-β-CD (TM-β-CD) (also used in [16]), heptakis(2,6-di-*O*-methyl)-β-CD and heptakis(2,3-di-*O*-acetyl)-β-CD was compared to that obtained from the corresponding randomly methylated and acetylated CDs. The variation of enantioselectivity between batches, even of the same manufacturer, was quite remarkable, and did not show any clear relation with the DS of the molecule. It was therefore also concluded that a simple characterization of DS is not enough to define a CD derivative, and validation of a method could be very difficult in the cases of such "undefined" RS-CDs.

The influence of some organic solvents (methanol, ethanol, acetonitrile, isopropanol) on enantioresolution of dimethindene maleate was investigated [17]. In this study, methanol enhanced the resolution of dimethindene enantiomers by carboxymethyl-β-CD (CM-β-CD, DS ∼ 3) at a concentration lower than the optimal concentration. This is in contrast to the model developed by Wren and Rowe [18]. To elucidate this phenomenon, other CDs (β-CD, HP-β-CD, carboxyethyl-β-CD and succinyl-β-CD) [17] and other antihistamines were also investigated, but the addition of organic solvent resulted in different effects on the enantioresolution, depending not only on the type of solvent, but also the type of chiral selector and the structure of the analytes [19].

2.1.2. Charged cyclodextrins

Charged CDs often have higher resolution capacity than neutral CDs. With charged CDs, electrostatic forces play an important role in selector–selectand interactions. Therefore, chiral selectors carrying opposite charge to that of the analytes are very commonly used. For example, two highly negatively charged organic disulfates were separated with positively charged quaternary ammonium β-CD (QA-β-CD) in a study by Liu et al. [20], whereas anionic highly sulfated β-CD was used by Lipka et al. to separate four basic antiviral agents [21]. Since a majority of drugs are basic compounds, negatively charged CDs are used much more often than positively charged CDs, and the number of anionic CDs synthesized is therefore also much higher than that of the cationic CDs. However, it is also known that not only the neutral and oppositely charged analytes, but also analytes with the same charge as the selectors could be well resolved.

Table 2
Chiral CE quantitation of pharmaceuticals in preparations

Analytes	Type of matrix	Chiral selector(s)	Notes	Reference
Etodolac	Commercial tablets	20 mM HP-β-CD	UV 225 nm (reference 360 nm)	[12]
Omeprazole	Commercial capsules	30 mM methyl-β-CD	Method validated with randomly substituted CDs	[14]
Citalopram	Commercial tablets	1.5 mg/ml CM-γ-CD	Fully validated method, including robustness study	[29]
Pheniramine	Granulated powder	2.5 mg/ml CE-β-CD		[30]
Bupivacaine	Injectable solutions	160 μM HSA	Partial-filling technique and dynamic coating	[76]
Propranolol	Pills, injectable solutions	100 μM HSA	Partial-filling technique and dynamic coating	[77]
Sertraline	Bulk drug, tablets and capsules	20 mM HP-β-CD + 30 mM SDC	Combined CD-MEKC, detection UV 210 nm	[91]

Table 3
Chiral CE analysis of biological samples

Analytes	Type of matrix	LOD	Chiral selector(s)	Sam. preparation	Detection	Reference
DL-lactic acid	Human and rat plasma	20 and 15 μM (D- and L-)	220 mM HP-β-CD	Liquid extraction	UV 195 nm	[13]
RS-ibuprofen	Human serum Human urine	0.05 μg/ml (both forms) 0.25 μg/ml (both forms)	0.05 mM TM-β-CD	SPE	UV 220 nm	[16]
RS-mirtazapine, RS-*N*-desmethylmirtazapine	Human plasma	0.2 μg/ml (3 ng/ml with SPE preconcentration)	0.24% CM-β-CD	SPE	UV 205 nm	[28]
D-serine[a]	Rat neural samples	0.1 μM D-serine	20 mM HP-γ-CD + 15% D-(+)-glucose	Direct injection	LIF[a]	[59]
D-serine[a]	Neural samples (*Aplysia*)	30 nM D-serine	30 mM β-CD + 60 mM SDC	Direct injection	LIF[a]	[90]
RS-salbutamol	Urine samples	125 ng/ml	15 mM HDAS	SPE	UV 230 nm	[84]
RS-methamphetamine	Urine samples	10 ng/ml	0.85 mM HDAS	Direct injection	CE-MS	[115]

[a] Derivatized with naphthalene-2,3-dicarboxaldehyde for fluorescence detection.

The crucial factor in this case is the counter-current mobility of the selectors and the contribution of electroosmotic flow (EOF) [22]. In the normal polarity mode, anionic CDs would therefore provide better enantioresolution than the neutral or cationic CDs. This might also explain the popularity of anionic CDs.

2.1.2.1. Anionic cyclodextrins. The most commonly used anionic CDs are those possessing sulfate, sulfonate and carboxylate substitutions. Succinyl and phosphoryl derivatives are less often used. A new single-isomer succinyl derivative of β-CD, 6-*O*-succinyl-β-CD, was synthesized, characterized and tested as chiral selector for several catecholamines, but enantioresolution was only obtained for terbutaline racemate [23]. The resolving power of the new CD was not compared to any existing CD derivatives.

Most common sulfonated CDs are still sulfobutylether-β-CDs (SBE, with DS ∼ 4 and DS ∼ 7) [24,25]. Within the carboxylated CDs, carboxymethyl-β-CD (CM-β-CD) [26–28], carboxymethyl-γ-CD (CM-γ-CD) [29] and carboxyethyl-β-CD (CE-β-CD) [30] were used most often. In the meantime a new carboxylated derivative was developed, 6-*O*-carboxymethyl-2,3-di-*O*-methyl-β-CD (CDM-β-CD, DS ∼ 1). It was synthesized and made commercially available by Culha et al. [31]. The resolving power of CDM-β-CD was compared to two existing negatively charged CDs, heptakis(2,3-di-*O*-methyl-6-*O*-sulfo)-β-CD and CM-β-CD in separating eight different positional isomers of dihydroxynaphthalene. Although a real chiral separation screening was not performed, chiral separation of (±)-1,1′-bi-2-naphthol was observed with this new derivative.

Concerning the sulfated derivatives, randomly sulfated-β-CD (S-β-CD) continued to be commonly used both for separating basic [32] or neutral compounds [33,34], despite the known problem with batch-to-batch variability. This problem with the S-β-CD has been addressed for a long time already. In order to avoid this problem, a new family of CD derivatives, single-isomer sulfated derivatives, was developed by Vigh and co-workers in 1997. His research group has been working continuously on this topic, and until now, they have synthesized 11 members of this family. Three of these members were introduced in the period of 2004–2005, including two α-CD derivatives: hexakis(6-sulfato)-α-CD [35], hexakis(2,3-dimethyl-6-sulfato)-α-CD [36] and one β-CD derivative, heptakis(2-methyl-3-acetyl-6-sulfato)-β-CD [37], which is the first member of the family carrying nonidentical substituents at all C2, C3 and C6 positions. Apart from the known batch-to-batch repeatability, another advantage of these single-isomer CDs is the better solubility in organic solvents compared to the randomly sulfated CDs. The single-isomer sulfated CDs, especially the two members that have been developed first, heptakis(2,3-di-acetyl-6-sulfato)-β-CD (HDAS) and heptakis(2,3-di-methyl-6-sulfato)-β-CD (HDMS) have become the most important CD family in nonaqueous capillary electrophoresis (NACE), as will be discussed in another section in this paper.

2.1.2.2. Cationic cyclodextrins. A new positively charged CD, 2-*O*-(2-aminoethyl-imino-propyl)-*O*-hydroxypropyl-β-CD (2-AIPHP-β-CD), was synthesized by the same research group as the two new neutral CDs mentioned in the previous part, with DS ∼ 1 for the 2-aminoethyl-imino-propyl group, and DS ∼ 3.8 for the hydroxypropyl group [38]. This CD was tested as chiral selector for enantiomers of some acidic compounds. Compared to three classic CDs tested (β-CD, HP-β-CD, and DM-β-CD), 2-AIPHP-β-CD showed higher resolution ability. However, similar to other cationic CDs, a polyacrylamide coated capillary was needed in this study to avoid CDs sticking onto capillary walls.

2.1.3. Dual cyclodextrin systems

During this period, there was no inventional study on the dual CD systems. A dual CD system is usually a combination of a neutral and a charged CD, or two oppositely charged CDs. This system offers a solution when a single CD could not baseline resolve the enantiomers. A conventional neutral CD derivative, for instance β-CD [39–41], HP-β-CD [42,43], or DM-β-CD [44] was very often chosen as a supplementary selector for an expensive charged derivative.

2.1.4. Mechanism of chiral recognition by CDs

Although the basic mechanism of interactions between CDs and the selectands in chiral CE is already known, there are still many unanswered questions, especially on how selector–selectand binding behaviour is related to enantioresolution in CE. Even when the two enantiomers have the same

binding constants with a selector, enantioseparation is still possible if the diastereomeric complexes have different mobilities [45].

In the past 2 years, there were a number of articles published in this field. CE itself is a very useful technique in studying the enantiorecognition mechanism. CE is often used to determine the CD–analyte binding constants [46–48] and binding ratios [24,49]. It allows observations of enantioselective effects in CD–analyte interactions, which is not feasible by other techniques. However, CE does not give direct information on chemical and structural mechanism of the interactions. This requires the use of other techniques as X-ray crystallography [50], circular dichroism [51,52], fluorescence [51,52] and the most important one, nuclear magnetic resonance (NMR) spectroscopy [50–53]. More detailed discussion on the mechanism of chiral recognition by CDs can be found in two recent reviews published in 2004 by Chankvetadze [54] and Dodziuk et al. [55].

2.2. Oligo- and polysaccharides

Apart from cyclodextrins, many other linear and cyclic oligo- and polysaccharides were also presented as chiral selectors (e.g. monosaccharides as D-glucose, D-mannose, or polysaccharides as dextrins, dextrans, and many others). We only report here on the newly synthesized oligo- and polysaccharides published in the past 2 years. Park et al. synthesized highly sulfated cyclosophoraoses [56], the sulfated derivatives of chiral unbranched cyclic β-(1 → 2)-D-glucans, and successfully used them to separate five basic chiral drugs. These derivatives exhibited higher resolution than the original cyclosophoraoses. Another new charged selector, *N*-(3-sulfo, 3-carboxy)-propionylchitosan, was studied by Budanova et al. [57]. This polysaccharide appeared to have a mechanism of recognition different from the other charged polysaccharides. Other examples of the use of conventional polysaccharides [58] and saccharides combined with CD [59] were also reported.

2.3. Crown ethers

Crown ethers have been used for a long time as chiral additive. However, until this moment, (+)-(18-crown-6)-tetracarboxylic acid (18C6H4) is still the only chiral crown ether. In an interesting paper by Cho et al., 18C6H4 was used as chiral selector in the separation of gemifloxacin enantiomers in the urine by coupled-channel microchip electrophoresis [60]. It is known that metal ions and salts in biological samples like urine can bind to 18C6H4 and ruin the chiral separation. With the two-channel microchip, these ions and salts in the urine sample were removed to the waste by the first separation channel, so that they could not harm the enantioseparation by 18C6H4 in the second separation channel.

Two new 18C6 diaza derivatives were investigated as chiral selectors by the group of Vander Heyden and Massart [61,62]. Although these derivatives did not show any chiral selectivity towards the investigated analytes, they could serve well as an additive to improve the chiral resolution in combination with cyclodextrins. One of the two diaza derivatives was also used by Leonard et al. in a method separating eight diastereoisomers of a new human immunodeficiency virus protease inhibitor [63]. Although no chiral separation was obtained, resolution of the diastereomeric separation was improved with the addition of the crown ether derivative.

2.4. Macrocyclic antibiotics

Four groups of antibiotics have been used as chiral selector in capillary electrophoresis: glycopeptides, polypeptides, ansamycins and aminoglycosides. Recently, another group of antibiotics, the macrolides, was investigated as chiral selector [64,65]. The feasibility of erythromycin as chiral selector in CE was reported in [64], where chiral separations were obtained on four pairs of enantiomers. In contrast to these results, in [65], a systematic screening of erythromycin and 6 derivatives on 21 different chiral components led to a negative conclusion on chiral resolving capacity of these macrolides.

A novel glycopeptide antibiotic, balhimycin, was investigated as chiral selector in CE [66]. In this study, the enantioresolution ability of balhimycin and its analogue bromobalhimycin was evaluated on 16 acidic racemates. Higher resolution was observed with balhimycin and bromobalhimycin compared to vancomycin. Balhimycin has a structure very similar to vancomycin (Fig. 2). They differ mostly in the attachment sites of the sugar moieties to the aglycon. In a mechanistic study on the enantioselectivity by vancomycin and balhimycin [67], Kang et al. suggested that the dimerization properties of the glycopeptides influence the enantioselectivity. Glycopeptides with higher dimer stability probably offer better chiral recognition, e.g. balhimycin, having a dimerization constant 78 times higher than vancomycin. In general, macrocyclic antibiotics are no longer one of the most common groups of chiral selectors in CE.

2.5. Ligand-exchange capillary electrophoresis

Ligand-exchange capillary electrophoresis (LE-CE) has always been an important technique in chiral analysis of amino acids. LE-CE is based on the interchange of analyte and ligand around the sphere of a central ion. Most of the time, the central ion is a metal ion. Cu(II) is used very often in different complexes: Cu(II)-L-ornithine [68], Cu(II)-L-prolinamide [69,70]. Zheng et al. compared the use of different metals as central ion in the complex with alanine [71]. In this paper, they combined two mechanisms of chiral recognition, chelate formation and CD inclusion, by adding CDs to the LE-CE buffer. However, a better way to obtain a cooperative effect is to use CD as ligand, for example Cu(II)-3-amino-β-CD in the study by Cucinotta et al. [72] or Cu(II)-6^A-(2-aminoethylamino)-6^A-deoxy-β-CD proposed by Wu et al. [73]. In a work published by Kodama et al. [74], borate was used, for the first time, as the central ion to complex with (*S*)-3-amino-1,2-propanediol. Different from the metal chelates formed with LE-CE using central metal ions, the borate complexes are mono-cyclic and di-cyclic complexes formed by reversible covalent binding of

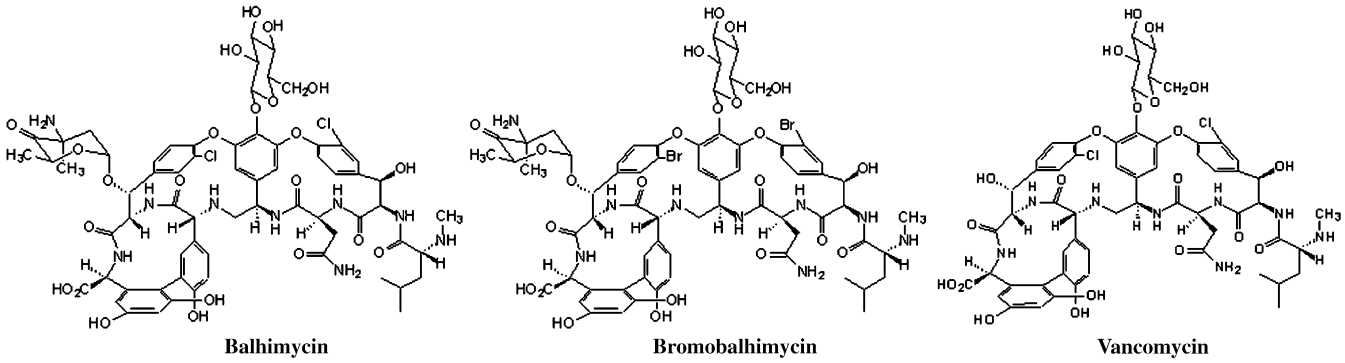

Fig. 2. Chemical structure of balhimycin, bromobalhimycin and vancomycin (from left to right). Reprint with permission from [66].

borate ion to 1,2- or 1,3-diol groups, in this case, to the 1,2-diol of (*S*)-3-amino-1,2-propanediol and the 1,3-diol of the chiral compound DL-pantothenic acid. The asymmetric center formed in the di-cyclic (spirocyclic) complexes is the factor that results in enantioselectivity.

2.6. Proteins

Proteins can be implemented in several ways in chiral CE. The simplest way is to dissolve them in the background electrolyte (BGE). Some examples of human and porcine serum albumin as chiral additive to the BGE using the partial-filling technique can be found [75–77]. Proteins can also be covalently bound to silica materials in CE, to the gel matrix in capillary gel electrophoresis (CGE), or to the inner surface of a coated capillary. Another simple way besides the covalent coating is the dynamic coating of the capillary wall. Bo et al. proposed a novel phospholipids-lysozyme coating for chiral separation [78]. Thanks to the natural bilayer structure, phospholipids will create a membrane in which lysozyme can permeate. This allows better adsorption of lysozyme on the capillary surface, while maintaining the possibility to clean up and regenerate the coating easily.

2.7. Nonaqueous capillary electrophoresis (NACE)

An extensive review on chiral separations by NACE was published early 2005 [79]. In this paper, Lämmerhofer discussed mainly the solvent types and effects, and chiral selectors used in NACE, together with several applications. Most of these chiral applications used HDMS [80–83] and HDAS [84] as chiral selector. As already mentioned in Section 2.1.2, HDAS and HDMS are not only CDs with the longest tradition, but also the most famous CDs in the family of single-isomer sulfated CDs developed by Vigh's group. The solvent used in these applications is methanol [80–84], except in [85], where acetonitrile–water (50:50) was chosen as the solvent for HDAS. The use of acetonitrile alone as the solvent for NACE was only described very recently [86]. In this paper, Vigh and co-workers presented the synthesis and use of HDAS tetraammonium salt that possesses higher solubility in acetonitrile than the normal sodium salt.

3. Micellar electrokinetic capillary chromatography (MEKC)

Micellar electrokinetic capillary chromatography (MEKC) is a mode of electromigration technique, in which a neutral and/or ionic surfactant is added to the BGE at a concentration above the critical micellar concentration (CMC) to form a micellar pseudo-stationary phase in the solution. The distribution of analytes between this micellar pseudo-stationary phase and the surrounding aqueous phase is the principle of separation by MEKC. In MEKC, chiral separation can be obtained by two modes (a) using chiral surfactants or (b) adding other chiral agents to the achiral micellar buffer. The latter mode is most of the time a combination of micelles and cyclodextrins, or CD modified MEKC (CD-MEKC). In CD-MEKC, a CD derivative is combined with a classic achiral surfactant, as sodium dodecyl sulfate (SDS) [87–89]. But CD can also be combined with a chiral surfactant, inducing better resolution than when either of the single chiral agents is used. Some applications will be mentioned later when the corresponding chiral surfactants are discussed.

Chiral surfactants include natural surfactants, monomeric synthetic surfactants and polymeric surfactants. Natural surfactants as bile salts, digitonins and saponins are still used, for example CD-MEKC with sodium deoxycholate [90,91]. Monomeric surfactants did not gain much interest from researchers, except from a new tendency: the chiral surfactants forming vesicles. Vesicles are bigger in size compared to the normal micelles (Fig. 3), and provide a wider migration window. The first enantioseparation by vesicles in MEKC was reported in 2003 by Mohanty and Dey [92]. This research group published further investigations with the same vesicle-forming surfactant, sodium *N*-(4-*n*-dodecyloxybenzoyl)-L-valinate [93] and with a new cationic vesicle system formed by (1*R*,2*S*)-(–)-*N*-dodecyl-*N*-methylephedrinium bromide [94]. Given the good enantioseparation demonstrated in these methods, the vesicles promise to be good chiral resolving systems, especially for the highly hydrophobic chiral analytes.

Overall, most interest in chiral MEKC was recently drawn towards the polymeric surfactants. They are high-molecular-mass surfactants which are formed by covalently binding small surfactant molecules into a single molecule micelle by polymer-

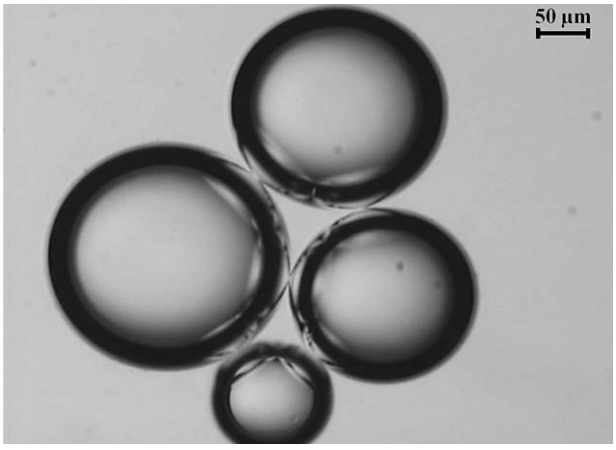

Fig. 3. Light micrograph of vesicles formed by 2 mM aqueous solution of sodium *N*-(4-*n*-dodecyloxybenzoyl)-L-valinate [95] – reproduced by permission of The Royal Society of Chemistry.

ization, and therefore also called molecular micelles. Molecular micelles are actually more stable, more rigid than the conventional micelles. Since CMC is zero in principle, polymeric surfactants can be used at lower concentrations, enabling the use of other additives. However, the rigidity of molecular micelles allows slower mass transfer of the analytes between the pseudo-stationary phase and aqueous phase, resulting in worse resolution compared to the conventional micelles, in general. More discussions on the advantages and disadvantages of MEKC using molecular micelles can be found in a recent review paper [95]. We will now focus on the chiral polymeric surfactants.

The first family of chiral molecular micelles is single-amino acid based, the polysodium *N*-undecenoyl-L-amino acid derivatives, which was developed by Warner's group [96]. Different single-amino acid-based derivatives were further synthesized in the same laboratory. Recently in 2004, they synthesized a co-polymerized surfactant from a water soluble achiral surfactant and a chiral amino acid-based surfactant, in order to enhance the water solubility, which is relatively low for this group of surfactants [97]. Other studies published in the same year on this group of surfactants include CD-MEKC applications [98] and a study in which it was found that minimizing polydispersity of the polymeric surfactant is important for a better enantioselectivity [99].

The second group of chiral molecular micelles consists of the polymerized dipeptide derivatives, i.e. polysodium *N*-undecenoyl-L-dipeptides derivatives, introduced by Shamshi et al. The development and applications of these dipeptide polymers as chiral selector were recently summarized in a review paper [100]. A study on the effect of temperature on chiral recognition by a dipeptide-based surfactant, polysodium *N*-undecenoyl-LL-leucyl-leucinate (poly-LL-SULL) was also published by Warner's group [101].

In 2003, Rizvi and Shamshi [102] introduced a new family of chiral polymeric surfactants, the polymeric alkenoxy amino acid surfactants (Fig. 4). The synthesis of two new surfactants, polysodium *N*-undecenoxy carbonyl-leucinate (poly-L-SUCL) and polysodium *N*-undecenoxy carbonyl-isoleucinate (poly-L-

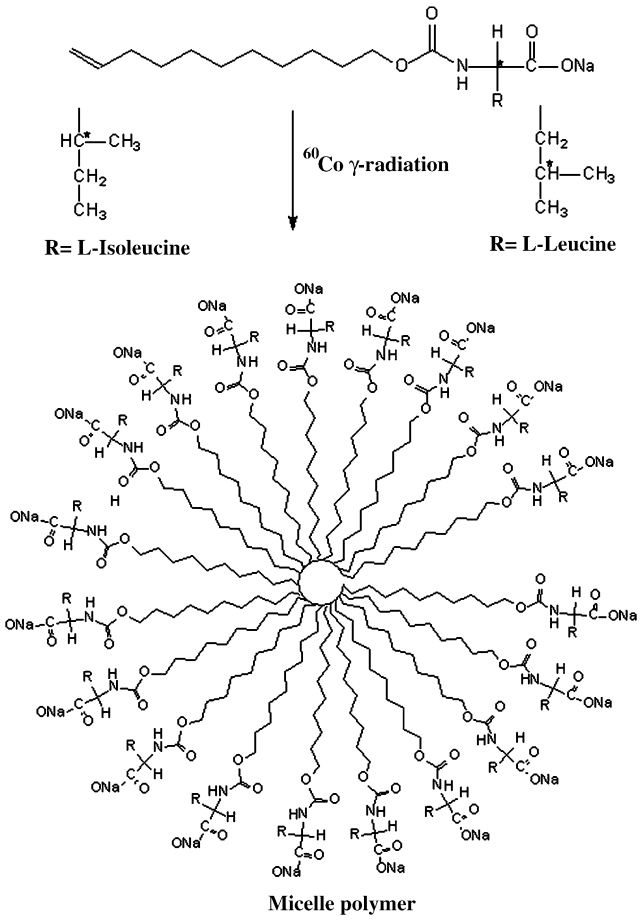

Fig. 4. Structure of monomer and micelle polymer of alkenoxy surfactants. Reprint with permission from [106].

SUCIL) was described, and their enantioseparation capacity was demonstrated in several papers on different groups of analytes [103,104]. Especially in a paper published this year, Shamshi and co-workers presented an MEKC-ESI-MS application using poly-L-SUCL as chiral selector for the separation of eight pairs of chiral β-blockers [105]. Poly-L-SUCL was compared to the monomeric L-SUCL with the same concentration in this application, and proved to be more efficient, providing higher resolution, lower separation current, and higher detection sensitivity than the monomeric surfactant (Fig. 5).

4. Microemulsion electrokinetic chromatography (MEEKC)

Microemulsion electrokinetic chromatography (MEEKC) is very often compared to MEKC, due to their similarity in using surfactant aggregates to form a pseudo-stationary phase. However, the structure of the key elements in these separation modes, namely micelles and microemulsions, are different. While micelles are spherical particles forming only from surfactant molecule(s), microemulsions need a water-immiscible oil as the core of the surfactant formed nanodroplets, and a co-surfactant, usually short chain alcohols, as the acting surface of the droplets. The oil droplets in microemulsions allow much

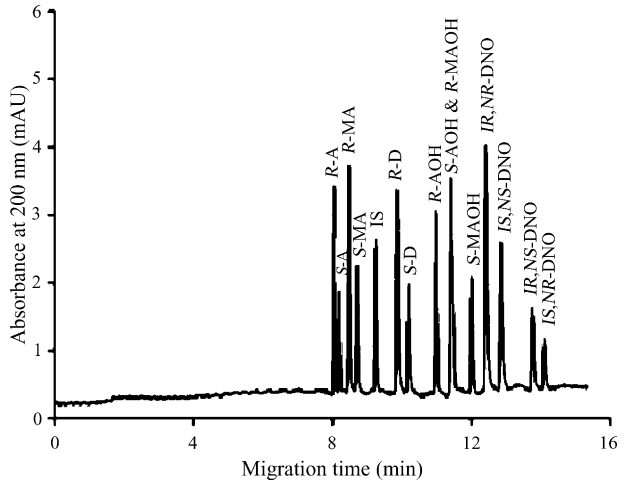

Fig. 5. Separation of deprenyl, amphetamine and methamphetamine enantiomers and their N-oxidation metabolites by FMO1 and FMO3. Reprinted with permission from [126].

higher and faster mass transfer of analytes than the more rigid micelles, and partitioning of the analytes between the oil droplets and the aqueous phase can be mediated by the co-surfactants, types of core oil, temperature, etc. There are much more possibilities to fine-tune a MEEKC method to obtain better resolution than with a MEKC method. Thus, it is not surprising to find a large number of publications on MEEKC development in recent years, summarized in a recent review paper [106]. But surprisingly, MEEKC chiral applications are not large in number. The year 2004 was almost the starting year for chiral MEEKC. Before that, there were only two articles published in 1993 [107] and 2002 [108], respectively.

A chiral separation by MEEKC can be obtained in different ways, using different components of the microemulsion as chiral resolving agent: oil containing chiral agents [107], chiral monomeric surfactant [108] alone and in combination with cyclodextrins [109] and more recently, chiral polymeric surfactant [110] and chiral alcohols as co-surfactant [111]. Examples for each type of component as chiral selector will be discussed below in more detail.

With respect to chiral MEEKC using surfactants, the effects of different variables on enantioresolution were investigated in several recent papers by Mertzman and Foley [108,109,112–114]. The chiral microemulsion used in these publications was based on (i) a chiral surfactant, dodecoxycarbonylvaline (DDCV), which was used for the first time in MEEKC in 2002 [108], (ii) a hydrophobic liquid, ethyl acetate, as the core and (iii) 1-butanol as co-surfactant. Ethyl acetate was chosen after an investigation was done on different types, i.e. ethyl acetate, methyl acetate, methyl propionate and methyl formate [112]. This investigation was aimed to determine the effect of hydrophobic liquid type on enantioselectivity, but the type turned out to be a less important factor than the oil concentration. The effect of chiral surfactant DDCV concentration was also discussed in another study [113]. By increasing DDCV concentration, the average enantioresolution (of 11 chiral compounds) was significantly increased, and the optimum concentration was 4% (m/v). In the same study, the buffer type, a variable often taken for granted, was proved quite important. Other factors like the incorporation of CDs as HP-β-CD or S-β-CD [109], and the effect of temperature were also investigated [114].

Huie and co-workers, the first group to report a chiral MEEKC method using a lipophilic chiral selector (2*R*,3*R*)-di-*n*-butyl tartrate [107], also presented for the first time the use of chiral alcohols as chiral selector in MEEKC [111]. With *n*-octane as core oil and SDS as achiral surfactant, they created chiral microemulsions with different chiral 2-alkanols with the alkyl chain containing 4–7 carbons. Except for *R*-(–)-2-butanol, the use of *R*-(–)-2-pentanol, *R*-(–)-2-hexanol or *R*-(–)-2-heptanol as chiral co-surfactant resulted in partial or baseline enantioseparation of most tested solutes, i.e. norephedrine, ephedrine, nadolol and propranolol.

Shamsi's group contributed another option for chiral MEEKC with a polymeric amino acid-based surfactant, polysodium *N*-undecenoyl-D-valinate (poly-D-SUV) [110]. In this study, they investigated the feasibility of poly-D-SUV as chiral agent in MEEKC with two approaches. In the first approach, the polymeric micelle poly-D-SUV was used as an emulsifier to dynamically coat the oil droplet, thus a microemulsion was formed by mixing four components: poly-D-SUV as surfactant, 1-butanol as co-surfactant, *n*-heptane as oil core, and aqueous buffer. In the second approach, the monomers of D-SUV were polymerized in the presence of *n*-heptane (fixed concentration) and 1-butanol (varied concentration), followed by removal of residual *n*-heptane and 1-butanol by evaporation and freeze drying processes. The obtained microemulsion polymer was then dissolved in buffer solution to form microemulsions. The results of these two MEEKC approaches on enantiomers of binaphthyl derivatives, anionic barbiturates and cationic paveroline were compared to solvent-modified MEKC (using poly-D-SUV as surfactant and *n*-heptane or 1-butanol as solvent). It was concluded that chiral MEEKC by both previously mentioned approaches induced equal or better results compared to MEKC. Conclusions from this study share a common point with other chiral MEEKC studies, that MEEKC offers a very promising land in the field of chiral analysis that still needs to be explored in the coming period.

5. Capillary electrophoresis-mass spectrometry (CE-MS)

MS is a very powerful technique that enables not only very sensitive analysis but also structural information that cannot be achieved by other detection tools coupled to CE. However, the use of chiral CE-MS is still quite limited compared to achiral CE-MS, which has been used quite extensively. The main disadvantage of chiral CE-MS is the interference caused by nonvolatile chiral selectors entering the MS. This can be avoided by using special chiral selectors as counter-current charged CD derivatives [25,115] or macrocyclic antibiotics; or micelle polymers [105] or macrocycles. Special techniques can also help to overcome this limitation, namely coated capillaries to suppress the EOF [116]; coupled capillaries combined

with voltage switching[1]; or most frequently, the partial-filling technique. A review published in 2004 on CE-MS analysis of small achiral and chiral solutes discussed in full detail most chiral CE-MS publications from 2002 up to 2004 [117]. Only few more papers have been published after that. Simo et al. developed a method using the conventional β-CD as chiral selector for the enantioselective determination of amino acids in foods, in which the capillary was coated with a copolymer ethylpyrrolidine methacrylate-*N*,*N*-dimethylacrylamide to eliminate the EOF [116]. Meanwhile negatively charged HDAS in positive polarity mode, which is the counter-current mode that can totally eliminate the possibility of CDs entering the MS, was used in another method separating six pairs of enantiomers, i.e. methamphetamine, amphetamine, dimethylamphetamine, ephedrine, norephedrine and methylephedrine [115]. Shamsi and co-workers, continuing their previous studies with molecular micelles in chiral CE-MS, proposed another polymeric surfactant, poly-L-SUCL [105]. This particular publication was also discussed in more detail in Section 3 (MEKC).

6. Pharmaceutical applications

Numerous review papers have summarized the applications of chiral CE in different groups of analytes, including pharmaceutical compounds. A review on CE analysis of pharmaceuticals was published recently, in which chiral CE applications were also summarized [118]. In an overview of CE analysis of small molecules, Altria and Elder also discussed in detail some chiral CE applications on pharmaceutical compounds [119]. Stereoselective peptide analysis and enantioselective analysis of 2-propionic acid nonsteroidal anti-inflammatory drugs were also reviewed in [120] and [121], respectively.

Specific applications of CCE in drug analysis are summarized in Table 1 for enantiomeric purity determination, in Table 2 for enantioselective analysis of pharmaceutical preparations, and in Table 3 for enantioselective analysis of biological samples.

Apart from that, some special applications should also be mentioned. The use of human serum albumin (HSA) as chiral selector is beneficial not only for chiral analysis of drugs. CCE can also be used to investigate conformational changes of HSA by investigating the effects of these changes on the chiral resolution of mexiletine [122]. This study provides a fast and simple method to reveal the conformation characteristics of proteins. For example, it was found that conformational change of HSA by ultrasound and temperature higher than 70 °C is irreversible, while changes resulting from pH changes (2.0–11.0) and temperature changes below 60 °C are reversible. In the study by Dubsky et al., chiral CE provides a novel approach for determination of all rate constants involved in the interconversion of enantiomers during the separation [123]. With this method, one can understand the processes taking place in an enantioseparation system where enantiomers undergo interconversion, and not only in CZE, but also in LC using a chiral stationary phase. Different from the previous studies that usually determined apparent rate constants of the interconversion taking place at the same time in both free form and bound forms of the compound, this approach enabled to distinguish which part of interconversion takes place in which form. Another noteworthy application was the high-throughput chiral separation demonstrated on a 96-capillary system, with α-, β-, γ-HSCD as chiral selector [124]. In the field of in vitro metabolism study, there are still very few enantioselective studies using CE. Bortocan and Bonato developed a chiral analytical method for enantioselective determination of primaquine, and applied this method to study metabolism of primaquine by rat liver mitochondrial fraction [125]. An interesting study of the N-oxidation of deprenyl, amphetamine, methamphetamine by flavin monooxygenase (FMO1 and FMO3) demonstrated the feasibility of chiral CE in studying stereoselective metabolism of drugs [126]. The enantioselectivity of substrate preference as well as stereoselective formation of a new chiral center after oxidation was assessed in this study.

7. Conclusions

Capillary electrophoresis has been a very important technique in chiral analysis for quite a long time. General drawbacks of CE towards its competitive technique, LC, are mainly low sensitivity and transfer of CE methods is not always straightforward in view of high uncertainty in quantitative analysis. However, thanks to the high resolution capacity and versatility, which is much higher for chiral CE than chiral LC, CE is still a very important technique in chiral analysis. A huge number of chiral selectors have been synthesized, characterized, tested and routinely used. The number of chiral selectors is still increasing, giving not only a more diversified choice for users, but also better enantioresolution, more reproducible results, higher solubility in solvents, or higher compatibility in coupling to MS. These developments will all help to enhance the advantages and overcome the disadvantages of chiral CE. Chiral methods by CE-LIF or hyphenated CE-MS can be considered as efforts to improve the sensitivity of chiral CE. On the other hand, the development of "reproducible" selectors as single-isomer CDs or molecular micelles improve the reproducibility of chiral CE methods. It is also worthwhile to mention the efforts to elucidate chiral recognition mechanisms by CE, including the interpretation of the relation of chiral resolution with binding behaviour and binding forces. Answers to all the remaining open questions on chiral recognition will enable to systemically choose chiral selector type and concentration, and to determine other factors influencing the separation as well. In conclusion, all these tendencies will open new prospects for chiral CE in the pharmaceutical analytical development.

[1] Coupled capillaries combined with voltage switching: this set-up consists of two capillaries joined by a connection vial connected to the electrode: the first capillary for chiral separation, and the second one that is not filled with chiral selector, for transferring separated analytes into the MS. By appropriate voltage switching, the migration of chiral selector into the ion source can be avoided.

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