ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
27 (2002) 373–399

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Review

Selected fundamental aspects of chiral electromigration techniques and their application to pharmaceutical and biomedical analysis

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Received 17 July 2001; received in revised form 24 July 2001; accepted 24 July 2001

Dedicated to Professor Dr Gottfried Blaschke on the occasion of his 65th birthday

Abstract

While capillary electrophoresis has been established as a major enantioseparation technique within the last decade, the potential of capillary electrochromatography is still studied extensively. This review summarizes recent applications of electromigration techniques with regard to the enantioseparation of chiral drugs. The first part discusses the general aspects of migration models and the enantiomer migration order. The application of capillary electrophoresis to chiral pharmaceutical analysis considers recent literature on: (1) chiral resolutions of non-racemic mixtures of enantiomers for the development of assays and the determination of the stereochemical purity of the drugs, (2) chiral separations of compounds in pharmaceutical formulations and products, and (3) enantioseparations of drugs in biological samples. A shorter section devoted to chiral electrochromatography discusses some fundamental aspects as well as the application to the chiral analysis of drugs including bioanalysis.

Keywords: Enantiomer separation; Drugs; Migration models; Enantiomer migration order; Bioanalysis

Abbreviations: CE, capillary electrophoresis; CEC, capillary electrochromatography; ESI, electrospray ionization; DI, direct injection; LIF, laser-induced fluorescence; LLE, liquid–liquid extraction; LOD, limit of detection; LOQ, limit of quantitation; MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight; MEKC, micellar electrokinetic chromatography; NOE, nuclear Overhauser effect; OT CEC, open tubular capillary electrochromatography; 1D-ROESY, one dimensional rotating frame nuclear Overhauser exchange spectroscopy; SPE, solid phase extraction; α-CD, α-cyclodextrin; β-CD, β-cyclodextrin; γ-CD, γ-cyclodextrin; CM-β-CD, carboxymethyl-β-cyclodextrin; DM-β-CD, heptakis-(2,6-dimethyl)-β-cyclodextrin; HDAS-β-CD, heptakis-(2,3-diacetyl-6-sulfato)-β-cyclodextrin; HP-β-CD, hydroxypropyl-β-cyclodextrin; M-β-CD, methyl-β-cyclodextrin; SBE-β-CD, sulfobutylether-β-cyclodextrin; Su-β-CD, succinyl-β-cyclodextrin; TM-β-CD, heptakis-(2,3,6-trimethyl)-β-cyclodextrin; HTAB, hexadecyltrimethyl ammonium bromide; SDS, sodium dodecylsulfate.

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0731-7085/02/$ - see front matter
PII: S0731-7085(01)00653-7

1. Introduction

The utmost importance of the stereochemistry of compounds with regard to their interaction with biological targets is generally recognized. Stereoisomers often differ substantially in their pharmacological, toxicological or pharmacokinetic profiles. This fact has also influenced the requirements of the regulatory authorities towards chiral compounds. As a consequence, the determination of the stereochemical composition and/or the stereochemical purity of a compound is an important issue for the pharmaceutical, chemical and cosmetic industry as well as the biological and environmental analyses.

Specifically the chromatographic techniques had a great impact on the determination of the stereochemical purity of compounds. The first analytical scale chromatographic enantioseparation was reported by Gil-Av in 1966 [1] and a little over 30 years ago Professor Gottfried Blaschke, to whom this volume has been dedicated, published his first paper on chromatographic enantioseparations [2]. Since then, analytical methods have improved substantially and powerful techniques for the separation of stereoisomers have been developed specifically by gas chromatography (GC) and high performance liquid chromatography (HPLC). Within the last two decades, techniques employing the electrophoretic mobility of charged compounds and/or the electroosmotic flow generated at the liquid–solid interface upon application of an electric field as the force driving the analytes through the separation medium have been used for the separation of enantiomers: capillary electrophoresis (CE) and more recently capillary electrochromatography (CEC). Especially, CE has rapidly emerged as a powerful technique due to its high resolution and flexibility. The practical applicability of CEC, which may be regarded as a hybrid technique between CE and HPLC is currently investigated by many researchers.

In electromigration techniques, as in 'pure' chromatographic systems, the separation of diastereomers may be achieved in an achiral environment due to differences in their physico-chemical properties. The resolution of enantiomers 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 analyte enantiomers and an optically pure chiral selector. The selector may be part of the liquid phase of the system or may be immobilized to a support.

The first direct enantiomer separation by CE was reported by Gassman and coworkers in 1985 [3]. Within the last few years, a large number of review papers and a monograph [4] have been published on chiral separations by electromigration techniques. Besides general reviews on chiral CE [5–11] and CEC [11–14], recent papers have been dedicated to the enantioresolution of specific groups of analytes such as amino acids and peptides [15] or pharmaceuticals [16,17], drug analysis in biological media [10,18], the use of specific chiral selectors such as cyclodextrins (CDs) [19–21], macrocyclic antibiotics [22,23], proteins [24] or chiral micelles [25] and the application of non-aqueous buffers [26–28].

The present review will focus on mechanisms underlying the chiral recognition process between the selector and the analyte enantiomers that have not been extensively discussed on a regular basis in the above cited papers or where new findings have emerged within the last few years. In addition, recent developments in the enantioseparation of drugs in pharmaceutical preparations of biological media will be summarized.

2. Selected general aspects of chiral analysis

According to the IUPAC terminology, chirality is defined as the geometric property of a rigid object of being non-superimposable on its mirror image [29]. Enantiomers are chiral molecules related as mirror images while diastereomers are stereoisomers not related as mirror images. The chiral purity of compounds is characterized by the enantiomeric excess (ee) or the diastereomeric excess (de). Enantiomeric excess (synonyms are

enantiomer excess or enantiomeric purity) is defined as the proportion of one enantiomer in a given mixture of both enantiomers [29]:

$$\text{ee} = \frac{|\chi_R - \chi_S|}{(\chi_R + \chi_S)} \times 100\ [\%] \quad (1)$$

where χ_R and χ_S are the mole or weight fraction of the *R*- and *S*-enantiomers, respectively. The enantiomeric excess may be expressed in percent as obtained by Eq. (1) or as a decimal number. The diastereomeric excess (diastereomer excess) is defined by analogy with enantiomer excess. However, the term is not applicable if more than two diastereomers are present in the mixture.

In contrast to the synthesis of compounds where the enantiomeric or diastereomeric excess characterizes the quality of a synthetic procedure, a stereochemical impurity is usually quantified in analytical chemistry. Thus, the enantiomeric impurity (ei) may be defined. It is typically expressed in percent. Assuming that a compound with *R*-configuration contains an impurity of its *S*-enantiomer the enantiomeric impurity can be determined according to Eq. (2) [30]:

$$\text{ei} = \frac{\chi_S}{(\chi_R + \chi_S)} \times 100 = \frac{A_S}{(A_R + A_S)} \times 100\ [\%] \quad (2)$$

A_S and A_R are the areas of the peaks of the enantiomers. Again, a diastereomeric impurity can be defined by analogy. As a stereochemical impurity in a compound supplied as an enantiomer (or diastereomer) is usually small, i.e. $A_R \gg A_S$, Eq. (2) may be simplified without a large error to:

$$\text{ei} \approx \frac{A_S}{A_R} \times 100\ [\%] \quad (3)$$

The quantitation of compounds can be based on response factors obtained by calibration curves. However, as pure standards of stereoisomer impurities are often not available enantiomeric and diastereomeric excess or the respective impurity are obtained by the normalization method and reported as percent of the area ratio according to Eqs. (2) and (3). As there is no linear relationship in CE between the sample concentration and peak height due to peak broadening effects at high sample concentrations [4,30], peak areas are used for quantitative CE analysis in the majority of cases described. It is generally known that compounds including separated enantiomers migrate with different mobilities resulting in different residence times in the detector. This effect may be more pronounced in the case of diastereomers compared to enantiomers. The difference in residence time is usually corrected by the software of commercial CE instruments by division of each peak with the corresponding migration time. However, it has been shown that the complexation of enantiomers with a chiral selector may result in different detector responses of the transient diastereomeric complexes [31]. One should also keep in mind that many chiral separations reported in the literature apply to the resolution of the racemate and not to a mixture of the enantiomers with one of them present at the 0.1% level. Many of these conditions may be well suited when equal amounts of both enantiomers are analyzed but may not be applicable if one of the enantiomers is present in large excess.

3. Chiral capillary electrophoresis

Capillary electrophoresis has developed to a powerful technique for analytical enantioseparations within the last decade. This is primarily based on the high flexibility of the technique with regard to the separation conditions and analytes as well as the low consumption of chemicals and solvents. It is now recognized by most researchers in this field that the migration principle, i.e. the driving forces moving the analytes through the separation capillary, is based on electrophoretic mechanisms. In contrast, a chiral separation is based on enantioselective interactions between the analyte enantiomers and a chiral selector and is, therefore, a chromatographic separation principle [11,20]. The fact that the selector is in the same phase as the analytes in CE and not part of a stationary phase that is immiscible with the mobile phase as found in chromatography does not represent a conceptional difference between both techniques. The chiral selector in CE is also called pseudophase as it is not a physically different phase and may also possess electrophoretic mobil-

ity. Thus, enantioseparations in CE may be summarized under the term 'capillary electrokinetic chromatography' which was introduced by Terabe et al. [32].

Enantioseparations in capillary zone electrophoresis (CZE) can be performed by the indirect method upon derivatization with a stereochemically pure agent to form diastereomers which are subsequently separated in an achiral system. The direct enantioseparation is based on the formation of transient diastereomeric complexes between the analyte enantiomers and an optically pure chiral selector. Addition of a chiral selector to the background electrolyte is the most popular technique in chiral CZE. Numerous selectors have been investigated and several reviews have focused on a specific group of selectors. Among others, these include native CDs as well as neutral and charged derivatives [19–21], oligo- and polysaccharides [33], a chiral crown ether [7,34], macrocyclic glycopeptide antibiotics [22,23], proteins [24] and synthetic cyclopeptides [35,36], as well as chiral surfactants derived from steroids, amino acids, tartaric acid or glycosides [7,25]. In addition, ligand exchange [37] and chiral ion-pairing reagents [38,39] have been used for enantioseparations. Despite efforts to rationalize the use of certain selectors and the experimental conditions that are most suited for a given enantioseparation including the migration behavior, most selections are still based on 'trial and error' or the experience of the researchers for a group of compounds and selectors. Some selected aspects understanding and modeling the migration behavior and interactions between the selectors and analytes will be discussed below.

3.1. Migration models

Several groups have developed mathematical models for a quantitative treatment of the effect of the experimental variables on the migration behavior of the analytes in a chiral separation system. Most studies in chiral CE have been performed with a chiral selector dissolved in the background electrolyte. In such a system, a separation is based on the formation of transient diastereomeric complexes between the enantiomers R and S and the chiral complexing agent C. The reactions are characterized by the complexation constants K_R and K_S, respectively, assuming the formation of a 1:1 complex between the enantiomers and the chiral selector:

$$R + \mathrm{C} \leftrightarrow R\mathrm{C} \quad K_R = \frac{[R\mathrm{C}]}{[R][\mathrm{C}]}$$

$$S + \mathrm{C} \leftrightarrow S\mathrm{C} \quad K_S = \frac{[S\mathrm{C}]}{[S][\mathrm{C}]} \tag{4}$$

Generally, the effective mobility of an analyte μ_{eff}, i.e. the apparent mobility corrected for the mobility of the EOF, is the sum of the electrophoretic mobilities of all different species in which the analyte may exist weighted by the mole fraction φ of the respective species:

$$\mu_{\mathrm{eff}} = \sum_n \varphi^n \times \mu^n \tag{5}$$

n is the number of the species present under the experimental conditions. Assuming that for a given chiral separation the enantiomers exist only in a complexed and non-complexed form Eq. (5) becomes for the R-enantiomer:

$$\mu_{\mathrm{eff}}^R = f \times \mu_{\mathrm{f}} + (1 - f)\mu_{\mathrm{cplx}}^R \tag{6}$$

where μ_{f} is the mobility of the free enantiomer, μ_{cplx}^R is the mobility of the analyte–selector complex and f is the fraction of the non-complexed species. Considering the complexation constant and the concentration of the selector, the effective mobility of the R-enantiomer may be expressed as:

$$\mu_{\mathrm{eff}}^R = \frac{\mu_{\mathrm{f}} + \mu_{\mathrm{cplx}}^R K_R[\mathrm{C}]}{1 + K_R[\mathrm{C}]} \tag{7}$$

Equations analogous to Eqs. (6) and (7) may also be written for the S-enantiomer. For enantioresolutions by CE the effective mobilities of the enantiomers have to be different, i.e. $\mu_{\mathrm{eff}}^R \neq \mu_{\mathrm{eff}}^S$. This occurs due to: (1) a difference in the complex formation constants ($K_R \neq K_S$); and (2) a difference in the mobility of the enantiomer–selector complexes ($\mu_{\mathrm{cplx}}^R \neq \mu_{\mathrm{cplx}}^S$). In addition, the mobilities of the free enantiomer and the enantiomer–selector complex must be different ($\mu_{\mathrm{f}} \neq \mu_{\mathrm{cplx}}$). The complexation constants reflect the enantiose-

lective recognition by the chiral selector and are the basis for enantioseparations in most cases. The different mobilities of the transient diastereomeric complexes can be explained by a difference in either the shape of the complexes, such as the 'better fit' of one enantiomer may result in a smaller complex. In addition, the diastereomeric complexes may differ in their pK_a values, which translate into different overall charges and, thus, different mobilities of the complexes. It has recently been demonstrated by Rizzi and Kremser that in the pH region close to the pK_a values of racemic dansylated amino acids complexation by CDs led to a significant shift of the pK_a values of the enantiomers [40]. The two enantioselective principles in CE, complexation constant and complex mobility, may cooperate in terms of improving a separation or counteract each other leading to a deterioration of the enantioresolution or no separation may be observed under the experimental conditions.

Several models have been proposed to describe and predict the influence of the main parameters of a CE separation such as the concentration of the chiral selector, pH, electroosmotic flow, nature of the background electrolyte co-ion and organic solvent additives. Most models assume a 1:1 complexation between the enantiomers and the chiral complexation agent. The analyte as well as the chiral selector may be neutral, anionic, cationic or zwitterionic. In addition to buffer additives (other than the chiral selector) and the nature of the capillary wall (coated or uncoated), which may both affect the EOF, the charge of the solute and the chiral selector determine the mechanism and direction of the migration in CE. Therefore, the nature of the chiral additive contributes not only to the separation selectivity but also to the migration direction and magnitude.

The models may be divided into 'mobility difference models' where a separation is expressed as the difference of the effective mobilities of the enantiomers and 'selectivity models' that use the separation selectivity α expressed as the ratio between either the effective mobilities of the enantiomers or the complexation constants as a measure of the separation. The initial mobility difference model was developed by Wren and Rowe [41–44] relating the effective electrophoretic mobility to the complexation constant and the concentration of the chiral selector. In its most general form it may be expressed as:

$$\Delta\mu = \mu^R_{\text{eff}} - \mu^S_{\text{eff}} = \frac{\mu_f + \mu^R_{\text{cplx}} K_R[\text{C}]}{1 + K_R[\text{C}]} - \frac{\mu_f + \mu^S_{\text{cplx}} K_S[\text{C}]}{1 + K_S[\text{C}]} \quad (8)$$

where μ^R_{eff} and μ^S_{eff} are the effective mobilities of the R- and S-enantiomers, K_R and K_S are the binding constants between the chiral selector and the enantiomers, μ_f is the mobility of the free analyte, μ^R_{cplx} and μ^S_{cplx} are the mobilities of the complexed R- and S-enantiomers and [C] is the concentration of the chiral selector. The equation may be rearranged to:

$$\Delta\mu = \frac{(K_R(\mu^R_{\text{cplx}} - \mu_f) - K_S(\mu^S_{\text{clpx}} - \mu_f) + K_R K_S(\mu^R_{\text{cplx}} - \mu^S_{\text{cplx}})[\text{C}])[\text{C}]}{(1 + K_R[\text{C}])(1 + K_S[\text{C}])} \quad (9)$$

At high concentrations of the chiral selector $\Delta\mu$ converges to $\mu^R_{\text{cplx}} - \mu^S_{\text{cplx}}$. Assuming equal mobilities of the transient diastereomeric complexes between the chiral selector and the R- and S-enantiomers, i.e. $\mu^R_{\text{cplx}} = \mu^S_{\text{cplx}} = \mu_{\text{cplx}}$, Eq. (9) may be transformed to:

$$\Delta\mu = \frac{[\text{C}](\mu_f - \mu_{\text{cplx}})(K_R - K_S)}{1 + [\text{C}](K_R + K_S) + K_R K_S[\text{C}]^2} \quad (10)$$

Penn et al. extended this treatment in order to express the resolution as a function of the selector concentration and thereby optimize the separation [45]. Sänger-van de Griend et al. further developed the model to the case where one analyte has two or more complexation sites for the selector and can form multiple complexes [46]. If a compound has two complexation sites, three complexes differing in their complexation constants and complex mobilities have to be considered. If a compound has three complexation sites the number of complexes increases to seven. Although not applied to chiral electrophoresis, Bowser and Chen have also developed equations for a quantitative description of the migration behavior of analytes considering multiple binding equilibria [47–49].

Vigh and coworkers accounted in their migration model in a series of papers [50–53] for the protonation equilibrium of an analyte including the effects of competing binding equilibria of dissociated and non-dissociated analytes. For the *R*-enantiomer of a weak acidic compound the effective mobility, μ_{eff}^{R}, can be expressed as [50]:

$$\mu_{\text{eff}}^{R} = \frac{\mu_{-}^{0} + \mu_{\text{RCD}-}^{0} K_{\text{RCD}-}[\text{C}]}{1 + K_{\text{RCD}-}[\text{C}] + \dfrac{[\text{H}_3\text{O}^+]}{K_{\text{a}}}(1 + K_{\text{HRCD}}[\text{C}])} \tag{11}$$

μ_{-}^{0} is the mobility of the fully deprotonated free analyte, $\mu_{\text{RCD}-}^{0}$ is the mobility of the complex between the fully deprotonated species and the chiral selector, K_{a} is the dissociation constant of the analyte, $K_{\text{RCD}-}$ and K_{HRCD} are the complexation constants of the dissociated and non-dissociated species, $[\text{H}_3\text{O}^+]$ is the hydronium ion concentration of the buffer and [C] is the concentration of the chiral selector. An analogous equation may be written for the *S*-enantiomer. As a selectivity model the effective separation selectivity α, the ratio between the effective mobilities of the *R*- and *S*-enantiomers, has been used to characterize the enantioresolution. Assuming identical dissociation constants for the two enantiomers $\alpha_{R/S}$ may be expressed as:

$$\alpha_{R/S} = \frac{1 + \dfrac{\mu_{\text{RCD}-}^{0}}{\mu_{-}^{0}} K_{\text{RCD}-}[\text{C}]}{1 + \dfrac{\mu_{\text{SCD}-}^{0}}{\mu_{-}^{0}} K_{\text{SCD}-}[\text{C}]} \times \frac{1 + K_{\text{SCD}-}[\text{C}] + \dfrac{[\text{H}_3\text{O}^+]}{K_{\text{a}}}(1 + K_{\text{HSCD}}[\text{C}])}{1 + K_{\text{RCD}-}[\text{C}] + \dfrac{[\text{H}_3\text{O}^+]}{K_{\text{a}}}(1 + K_{\text{HRCD}}[\text{C}])} \tag{12}$$

Depending on the fact whether only the non-dissociated species forms a complex with the chiral selector or the dissociated form or both, the dissociated and the non-dissociated species form complexes, the separations can be divided into 'desionoselective', 'ionoselective' and 'duoselective' separations [53] which partially simplifies Eq. (12). Depending on the *K* and μ values of the enantiomers different migration order is possible. Similar equations were also developed for weak bases [51]. While the authors used the separation selectivity α defined as the ratio of the effective mobilities of the *R*- and *S*-enantiomers as a measure of the separation one may certainly write a mobility difference equation analogous to Eq. (9) incorporating the protonation equilibrium as described in Eqs. (11) and (12).

Subsequently, Vigh and coworker developed the chiral charged resolving agent migration model (CHARM) [54] for the permanently charged CDs developed by the same group. The effective mobility of the *R*-enantiomer is given by the equation:

$$\mu_{\text{eff}}^{R} = \frac{\mu_{\text{f}} + \mu_{\text{RCD}} K_{\text{RCD}}[\text{CD}] + K[\text{H}_3\text{O}^+](\mu_{\text{HR}} + \mu_{\text{HRCD}} K_{\text{HRCD}}[\text{CD}])}{1 + K_{\text{RCD}}[\text{CD}] + K[\text{H}_3\text{O}^+](1 + K_{\text{HRCD}}[\text{CD}])} \tag{13}$$

Again, the selectivity as the ratio of the effective mobilities of the *R*- and *S*-enantiomers was applied in this model. Depending on the dissociation behavior of the analytes the model was subdivided in forms for non-electrolytes, strong electrolytes and weak electrolytes. The migration order of the enantiomers depends on the *K* and μ values.

Recently, the model was extended [55] introducing the terms binding selectivity *b* Eq. (14), size selectivity *s* Eq. (15) representing the mobility ratio of the transient diastereomeric complexes and parameter *a* Eq. (16) indicating the effect of the complexation on the analyte mobility:

$$b = \frac{K_{\text{RCD}}}{K_{\text{SCD}}} \tag{14}$$

$$s = \frac{\mu_{\text{RCD}}^{0}}{\mu_{\text{SCD}}^{0}} \tag{15}$$

$$a = \frac{\mu_{\text{RCD}}^{0}}{\mu^{0}} \tag{16}$$

Introduction of the variable *X* as $X = K_{\text{SCD}}[\text{CD}]$ led to the most general form of the CHARM model [55]:

$$\alpha = \frac{1 + bsaX}{1 + aX} \times \frac{1 + X}{1 + bX} \quad (17)$$

While the CHARM model was developed using negatively charged single isomer CDs it may certainly be applied to positively charged CDs as well as charged selectors of any class.

Essentially, both the mobility difference model by Wren using the difference of the effective mobilities and the selectivity model by Vigh employing the ratio of the effective mobilities are quite comparable. Analysis of Eqs. (14)–(16) with Eq. (9) Eq. (10) reveals that coefficient b is closely related to the $(K_R - K_S)$ term in Eq. (10) while terms s and a may be compared to $(\mu^R_{\text{cplx}} - \mu^S_{\text{cplx}})$ and $(\mu_f - \mu_{\text{cplx}})$, respectively.

Yang et al. have recently introduced a model describing the behavior between a (negatively) charged selector and basic analytes using term $\Delta\mu^{\text{eff}}_{\text{DL}}/\bar{\mu}^{\text{eff}}_{\text{DL}}$ as an expression of chiral selectivity [56]. This term is related to the general equation for the resolution R_S in CE:

$$R_S = \frac{\sqrt{N}}{4} \frac{\Delta\mu_{\text{DL}}}{\bar{\mu}_{\text{DL}}} \quad (18)$$

where $\Delta\mu_{\text{DL}}$ is the mobility difference between the individual D- and L-enantiomers $(\mu_D - \mu_L)$ and $\bar{\mu}_{\text{DL}}$ is the average of the mobilities, $(1/2)(\mu_D + \mu_L)$. The models consider the protonation equilibrium of the analyte but assumes that the interaction between the uncharged analyte and the charged selector is much weaker than the complexation of the protonated analyte by the selector and may, therefore, be neglected. Moreover, identical protonation constants of the analyte enantiomers and identical mobilities of the selector–analyte complexes are included. In the most general form, the model is given by the equation:

$$\frac{\Delta\mu^{\text{eff}}_{\text{DL}}}{\bar{\mu}^{\text{eff}}_{\text{DL}}} = \frac{\left(\frac{K_A[H^+]}{1 + K_A[H^+]}\mu^0_{\text{HA}} - \mu^0_{\text{HAS}}\right)(K_{\text{HLS}} - K_{\text{HDS}})[S]}{\mu^0_{\text{HA}} + \frac{1}{2}(K_{\text{HDS}} - K_{\text{HLS}})\left(\frac{K_A[H^+]}{1 + K_A[H^+]}\mu^0_{\text{HA}} + \mu^0_{\text{HAS}}\right)[S] + \frac{K_{\text{HDS}}K_{\text{HLS}}K_A}{1 + K_A[H^+]}\mu^0_{\text{HAS}}[H^+][S]^2} \quad (19)$$

μ^0_{HA} is the mobility of the free analyte, μ^0_{HAS} is the mobility of the analyte–selector complex, K_{HDS} and K_{HLS} are the complexation constants of the protonated D- and L-enantiomers, respectively, K_A is the protonation constant, $[H^+]$ is the hydronium ion concentration of the buffer and [S] is the concentration of the chiral selector. The model was used to describe the influence of the complexation constants, the pH and the selector concentration on the chiral selectivity. Specifically, it was noted that at pH values that are lower than 2 units than the pK_a value of the analytes the buffer pH does not significantly affect the selectivity. Moreover, chiral selectivity will become 0 if [S] = 0 or if [S] is very large. The migration order depends on the complexation constants. Thus, if $K_{\text{HDS}} > K_{\text{HLS}}$, $\Delta\mu^{\text{eff}}_{\text{DL}}/\bar{\mu}^{\text{eff}}_{\text{DL}} > 0$, the D-enantiomer migrates before the L-enantiomer while for $K_{\text{HDS}} < K_{\text{HLS}}$, $\Delta\mu^{\text{eff}}_{\text{DL}}/\bar{\mu}^{\text{eff}}_{\text{DL}} < 0$, the enantiomer migration order is reversed. The larger the difference between the complexation constants the better is the chiral selectivity.

Zhu et al. [57], Surapaneni et al. [58] and Lelievre et al. [59] have developed mathematical models for chiral separation systems utilizing two chiral selectors. All models can be used for the description of the separation systems if the limiting conditions are correctly defined. For example, none of the models considered the protonation equilibrium of the analytes and all experiments were performed at low pH values where the EOF is negligible. Nevertheless, all models help to understand and rationalize the effects observed in the various experiments. Although not applied to enantioseparations, Chen's group has also derived an equation for the migration behavior of analytes considering complexation equilibria in the presence of charged and neutral (chiral) additives [60].

Zhu et al. employed a mobility difference model with two uncharged complexation agents and permanently charged analytes [57]. The enantiomers bind only to either of the selectors and 1:1 complexation occurs. Thus, the quantitative description is similar to the equations developed by Chen's group considering multiple binding equi-

libria of analytes [47–49]. Depending on the enantioselectivity of the individual CDs towards the analyte enantiomers the elution order may be changed by a variation of the concentration ratio of the two CDs.Surapaneni et al. described the enantioseparation of neutral analytes using uncharged CD derivatives in combination with a (negatively) charged CD [58]. The enantiomers migrate only when associated with the charged CD. The selectivity α as the ratio of the apparent electrophoretic mobilities of the enantiomers may be expressed as:

$$\alpha = \frac{\mu_{\mathrm{RICD}}}{\mu_{\mathrm{SICD}}} \times \frac{K_{\mathrm{RICD}}}{K_{\mathrm{SICD}}} \times \frac{1 + K_{\mathrm{SICD}}[\mathrm{ICD}] + K_{\mathrm{SNCD}}[\mathrm{NCD}]}{1 + K_{\mathrm{RICD}}[\mathrm{ICD}] + K_{\mathrm{RNCD}}[\mathrm{NCD}]} \quad (20)$$

μ_{RICD} and μ_{SICD} are the apparent mobilities of the complexes between the ionized selector and the *R*- and *S*-enantiomers, respectively, K_{RICD} and K_{SICD} and K_{RNCD} and K_{SNCD} are the complexation constants between the *R*- and *S*-enantiomers and the ionized (ICD) and neutral (NCD) selectors, and [ICD] and [NCD] are the concentrations of the selectors. As the complexation constants of the ionized CD for the *R*-enantiomer are in the numerator in the second term of Eq. (20) and in the denominator in the third term, the selectivity can be optimized by the neutral CD when $K_{\mathrm{SNCD}} > K_{\mathrm{RNCD}}$ at low concentrations of the ionized CD. Because the complexation constants of the enantiomers with the charged CD always exceeded the constants with the neutral CDs by a factor of 3–25 the contribution of the neutral CD became negligible at high concentrations of the ionic selector as $K_{\mathrm{SICD}}[\mathrm{ICD}]/K_{\mathrm{SNCD}}[\mathrm{NCD}] \gg 1$ and $K_{\mathrm{RICD}}[\mathrm{ICD}]/K_{\mathrm{RNCD}}[\mathrm{NCD}] \gg 1$.

In their dual selector system Lelievre et al. defined the separation by the intrinsic selectivity α as the ratio of the complexation constants [59]. Assuming 1:1 complexes as well as the absence of mixed complexes a system containing the two selectors X and Y is described for two analytes A and B by:

$$\alpha = \frac{K_{\mathrm{Y}}^{\mathrm{B}}}{K_{\mathrm{Y}}^{\mathrm{A}}} \times \frac{1 + K_{\mathrm{X}}^{\mathrm{A}}[\mathrm{X}]}{1 + K_{\mathrm{X}}^{\mathrm{B}}[\mathrm{X}]} \quad (21)$$

$K_{\mathrm{Y}}^{\mathrm{A}}$, $K_{\mathrm{Y}}^{\mathrm{B}}$, $K_{\mathrm{X}}^{\mathrm{A}}$ and $K_{\mathrm{X}}^{\mathrm{B}}$ are the complexation constants of the analytes A and B with the respective selector X and Y and [X] is the concentration of selector X. At high selector concentrations when the amount of free analyte is negligible Eq. (21) can be simplified to $\alpha = \alpha_{\mathrm{Y}}/\alpha_{\mathrm{X}}$. Thus, the selectors cooperate with respect to an enantioseparation only if the affinity order for the enantiomers is opposite. Moreover, if one of the selectors does not exhibit enantioselectivity the system is independent of the concentration of the respective selector. The model was applied to the resolution of arylpropionic acid enantiomers at low pH using a neutral CD derivative exhibiting enantioselectivity towards the analytes and a (positively) charged CD showing no significant enantioselectivity, which was used as a carrier for the enantiomers towards the detector. The analytes were fully protonated and, thus, uncharged. Due to the low buffer pH the EOF was negligible.

Despite several useful applications of dual systems, separations employing only one chiral selector are preferred by most groups due to their better predictability and easier control of the individual parameters. In addition, the latter systems are also easier described by mathematical models. Compared with the situation where two selectors have to be considered, fewer assumptions have to be made for single selector systems. Moreover, no dual system so far has considered the protonation equilibrium of the analytes.

3.2. Enantiomer migration order

The enantiomer migration order is an important issue in CE. Modern synthetic procedures yield compounds with high enantiomeric purity. Thus, only small amounts of enantiomeric impurities may be present. In addition, the regulatory agencies require detection limits for impurities in new chemical entities at the $\leq 0.1\%$ level according to the ICH guideline 2QA [61]. These regulations may also apply to chiral drugs. Therefore, sensitive methods that allow to detect and determine increasingly small amounts of enantiomeric and diastereomeric impurities are required. In contrast to chromatographic techniques peak tailing or peak fronting is a common phenomenon in CE due to peak dispersion as a result of sample overload. For the detection of minute amounts of

chiral impurities high amounts of samples will be commonly injected onto the capillary leading to peak dispersion. Thus, it may be feasible to determine a minor impurity in front of a tailing peak of the main component while it may be more suitable to elute an impurity after a large fronting peak. Most chiral selectors (CDs, macrocyclic antibiotics, proteins, etc.) are not available in both enantiomeric forms so that a change of the migration order of the analyte enantiomers cannot be accomplished by a change of the configuration of the selector. Thus, mechanisms for a reversal of the enantiomer migration order are of great interest.

Based on Eq. (10), reversal of the migration order is observed when the algebraic sign of $\Delta\mu$, i.e. $(\mu_{\text{eff}}^{R} - \mu_{\text{eff}}^{S})$, or of the terms $(K_R - K_S)$ or $(\mu_{\text{f}} - \mu_{\text{cplx}})$ is reversed. The term of the binding constants depends primarily on the nature of the chiral selector but may also be affected by the charge of the analyte. The algebraic sign of the mobility terms can be changed by affecting the effective mobility of the analyte or of the chiral selector or both of them, for example, by a variation of the pH of the background electrolyte, the selector concentration or the direction or magnitude of the EOF. A change of the migration order can also be achieved by affecting the mobilities of the transient diastereomeric complexes between the selector and the analyte enantiomers. Essentially, the same conclusions can be derived from the selectivity models developed by Vigh's group as expressed in Eqs. (12), (14)–(17) except that the ratio of the complexation constants and mobilities are used in these models. Thus, opposite migration order is indicated when $\alpha < 1$ or $\alpha > 1$. The models have also been used to predict a reversal of the enantiomer migration order based on the concentration of the chiral selector and the buffer pH [50,51,53,54]. Summarizing, the enantiomer migration order can be affected by:

1. the binding strength of the analytes by the selector (expressed as complexation constants);
2. the direction and magnitude of the mobility of the free analyte;
3. the direction and magnitude of the mobility of the chiral selector;
4. the direction and magnitude of the mobility of the enantiomer–selector complexes;
5. the direction and magnitude of the EOF;
6. the concentration of the chiral selector.

Different techniques for obtaining opposite migration order of enantiomers in chiral CE have been reported. The literature up to 1997 has been summarized in a review paper [62]. Reversal of the migration order can be achieved due to (1) opposite chiral recognition when using different CDs [63–67] or chiral micelles with opposite configuration [3,68]. Opposite migration order has been observed for CD derivatives with identical substituents but differing in their degree of substitution [69]. (2) Another technique applies a combination of chiral selectors [57,70–73]. This mechanism can only be employed when the selectors possess opposite recognition ability of the enantiomers. Both complexing agents can be dissolved in the background electrolyte [57] or one of them may be attached to the capillary wall [72,73]. Reversal of the migration order can be achieved by (3) a variation of the concentration of the selector [40,74,75] caused by an increasing influence of the complex mobility compared to the mobility of the free enantiomer. Further techniques exploit (4) the pH-dependent mobility of chiral selectors by using a charged and an uncharged CD which possesses the same chiral recognition towards an analyte [65] or by varying the electrophoretic mobility of a charged CD by changing the pH of the run buffer while at the same time not affecting the charge of the analyte [64,65]. (5) The pH-dependent mobility of the chiral solute can result in an opposite migration order of the analyte enantiomers when it can be analyzed as cationic or anionic species [40,76,77]. (6) Exploiting the carrier ability of a charged selector and reversing the polarity of the applied voltage can also reverse the enantiomer migration order [78–81]. Furthermore, the migration order can be altered (7) by a change, i.e. elimination or reversal of the EOF [64,82]. (8) Reversal has also been observed upon addition of achiral micelles or organic solvents to a chiral separation system [83–86]. This has also been found in ligand exchange based CE separations of D,L-Phe in the presence of the copper(II) complex of 4-hydroxyproline upon addition of increasing concentrations of sodium dodecylsulfate (SDS) [87]. Recently, the pH-dependent reversal of the migra-

tion order has been described as an additional mechanism. Using β-CD as chiral selector in the presence of urea, the LL-enantiomer of the dipeptides Ala–Phe and Leu–Phe migrated faster than the respective DD-enantiomers at pH 2.7 whereas the DD forms migrated faster at pH 3.5. This behavior was explained by differences in the binding constants between the peptides and β-CD at the respective pH values [88]. Interestingly, the DL- and LD-isomers of the dipeptides did not change the migration order. A similar behavior observed for the tripeptide enantiomers Gly–β-L-Asp–D-$PheNH_2$ and Gly–β-D-Asp–L-$PheNH_2$ and carboxymethyl-β-cyclodextrin (CM-β-CD) as chiral selector using a polyacrylamide-coated capillary [80,89] is shown in Fig. 1. At pH 3.60 the DL-enantiomer was complexed stronger by CM-β-CD than the LD-enantiomer, resulting in the migration order DL before LD. In contrast, at pH 5.25 the LD-isomer was complexed preferentially leading to the migration order LD before DL. The chiral recognition ability of CM-β-CD towards Gly–β-L-Asp–L-$PheNH_2$ and Gly–β-D-Asp–D-$PheNH_2$ or towards the corresponding α-Asp tripeptides was not affected by an increase of the buffer pH [89]. A detailed study on the separation ability of neutral and charged cyclodextrins towards a series of dipeptides and tripeptides revealed that the pH-dependent reversal of the migration order is dependent on the amino acid sequence of the peptides as well as the structure of the CD derivative. Thus, the behavior was observed for several peptides in the presence of the neutral derivatives β-CD, heptakis-(2,6-dimethyl)-β-cyclodextrin (DM-β-CD) and heptakis-(2,3,6-trimethyl)-β-cyclodextrin (TM-β-CD) or the chargeable compounds CM-β-CD and succinyl-β-cyclodextrin (Su-β-CD) [67,90]. In contrast this was not observed using permanently charged sulfated and sulfonated CD derivatives [91]. Although specifically recognized for peptides, analysis of the literature revealed that a pH-dependent reversal of the migration order was also observed for the enantioseparation of dansylated Phe and Trp using hydroxypropyl-β-cyclodextrin (HP-β-CD) as the selector [40] although this was not addressed in the paper. Moreover, opposite chiral selectivity of HP-β-CD at pH 2.75 and 6.0 expressed as the complexation constants was also reported for dansylated Phe by Wren [92].

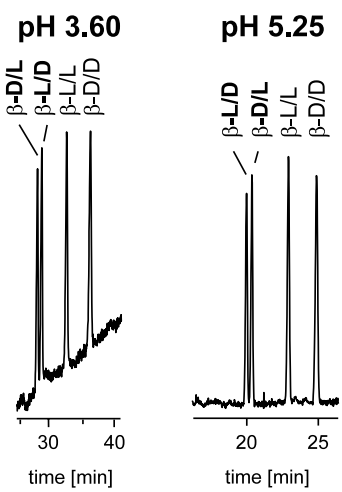

Fig. 1. pH-Dependent reversal of the migration order of the tripeptide enantiomers Gly–β-L-Asp–D-$PheNH_2$ and Gly–β-D-Asp–L-$PheNH_2$ in the presence of CM-β-CD (adapted from Ref. [89]).

Detailed investigations revealed that a pH-dependent reversal of the migration order may not only be due to an opposite chiral recognition of the peptide enantiomers by the CDs but also due to an increasing influence of the complex mobility [90,93]. For example, using β-CD as chiral selector the DD-enantiomer of Ala–Tyr migrated before the LL-enantiomer at pH 2.5 while the LL-isomer migrated before the DD-epimer at pH 3.5. At both pH values the chiral recognition of the CD towards is identical, i.e. the DD-enantiomer is complexed stronger than the LL-epimer. Apparent complexation constants of 173 ± 13 and 110 ± 11 M^{-1} were determined for D-Ala–D-Tyr at pH 2.5 and 3.5, respectively, while the corresponding values for the LL-stereoisomer were 125 ± 16 and 82 ± 11 M^{-1} [93]. Therefore, the reversed migration order cannot be explained by opposite chiral recognition as in the studies reported in Refs. [88,89]. However, while the mobilities of the diastereomeric complexes between the CD and the LL- and DD-enantiomers were essentially identical at pH 2.5 (9.5 ± 0.1 and 9.6 ± 0.1 $cm^2\,V^{-1}\,s^{-1}$) the mobilities differed at pH 3.5. The stronger complexed DD-isomer displayed the

higher mobility (4.3 ± 0.1 $cm^2 V^{-1} s^{-1}$) compared to the LL-epimer (3.8 ± 0.1 $cm^2 V^{-1} s^{-1}$) which counteracted the stronger complexation constant leading to the faster effective migration of the DD-enantiomer at pH 3.5.

Rizzi and Kremser have demonstrated for dansylated Phe and Trp that complexation by HP-β-CD leads to a shift of the protonation equilibrium of the enantiomers in the range of $pH = pK_a \pm 2$ [40]. This caused different charge densities of the resulting diastereomeric complexes, which resulted in different complex mobilities. The increasing contribution of the complex mobilities counteracted the enantioselectivity of the CD, i.e. the magnitude of the complexation constants, resulting in a reversal of the enantiomer migration order upon increasing the CD concentrations. The effects were not observed outside the above mentioned pH range where the complexes did not differ in their mobilities. This complexation-induced pK_a shift might also explain the effects observed for the peptide stereoisomers as different mobilities of the diastereomeric complexes were only observed at pH values close to the pK_a of the carboxyl groups. Despite the fact that the complexation-induced pK_a shift still has to be demonstrated for the peptides, the above cited papers together with other studies [53,94] clearly demonstrate the influence and the importance of the complex mobility on the observed effective mobilities of CD–analyte complexes in CE enantioseparations.

3.3. Analyte–selector interactions and complex structure

Enantioselective interactions between the analyte enantiomers and the selector are the basis for any chiral separation. The transient complexation of the enantiomers may be mediated via van der Waals interactions, hydrogen bonding, dipole–dipole interactions and electrostatic interactions in the case of charged analytes and selectors. Except for CDs, the stoichiometry and the structure of the complexes has not been investigated in great detail. This may be due to the fact that CDs are relatively simple molecules and offer limited interaction sites compared to selectors such a peptides or proteins.

Methods for the determination of the complex stoichiometry and complex structure have been reviewed in detail recently [11] and will, therefore, be only briefly addressed. Techniques for the determination of the complexation constants including the mathematical treatment will not be discussed as there are a number of excellent reviews in the literature [95–98].

Information on the stoichiometry of the complexes may be obtained by UV-, NMR- and mass spectrometry. UV–Vis spectrometry is a simple solution technique that may be applied for studying non-covalent intermolecular interactions. The experimental data are treated according to the continuous variation plot (Job's plot) [99,100]. As the technique does not show different signals for diastereomeric complexes, pure stereoisomers are required [101]. In contrast, NMR spectroscopy provides, in principle, two sets of signals for non-covalent diastereomeric complexes between the selector and the analyte enantiomers [102]. Thus, NMR allows the application of racemic samples or non-racemic mixtures of the enantiomers. Complexation-induced chemical shifts are recorded and analogous to UV spectroscopy a continuous variation plot is constructed. However, as shown in several studies, the resulting plots obtained by both techniques must be interpreted carefully if one does not observe a sharp maximum [11,100,103–105]. In this case multiple complexes may be present. However, in most published studies CD–analyte complexes with a 1:1 stoichiometry were by far the dominant species. The complex stoichiometry may also be derived from the m/z ratio in the mass spectra obtained by soft ionization mass spectrometric techniques such as electrospray ionization (ESI) or matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) [11,104,106,107]. This information has to be analyzed carefully, due to the possible formation of false adducts [108].

The structures of CD–analyte complexes can be investigated in solution by NMR [102] and in the solid state by X-ray crystallography [109]. In NMR, the chemical shift pattern, line shape analysis or nuclear Overhauser effects (NOE) can provide information on the structure of the complexes. Several studies have illustrated the useful-

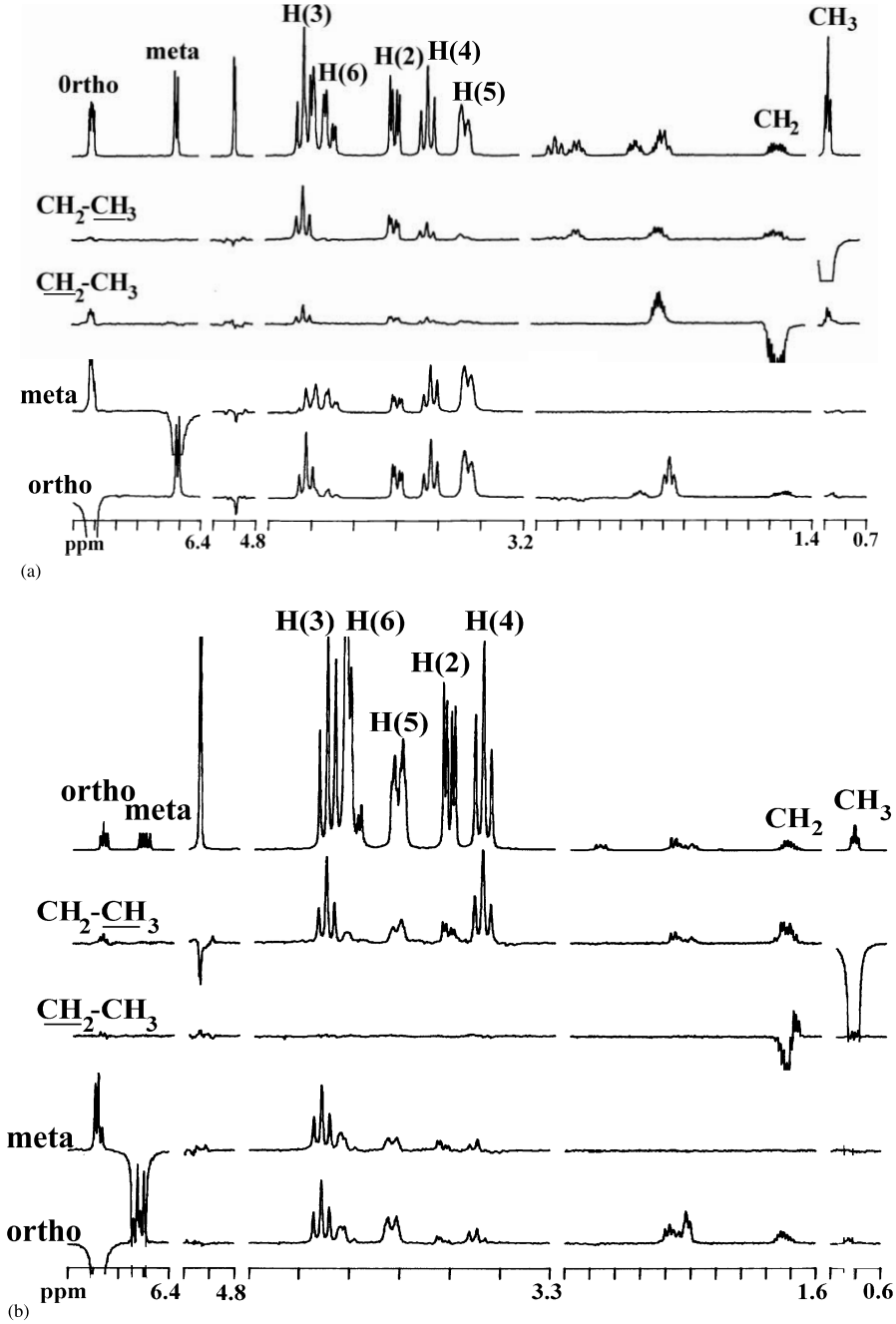

Fig. 2. 1D-ROESY spectra of aminogluthetimide and 2 equivalents of (a) β-CD and (b) γ-CD (reproduced with permission from Ref. [107]).

ness of NMR for studying complexes relevant to CE [11,103–107]. One interesting example is shown in Figs. 2 and 3 [107]. β-CD and γ-CD displayed opposite chiral recognition towards the enantiomers of aminogluthetimide. One dimensional rotating frame nuclear Overhauser

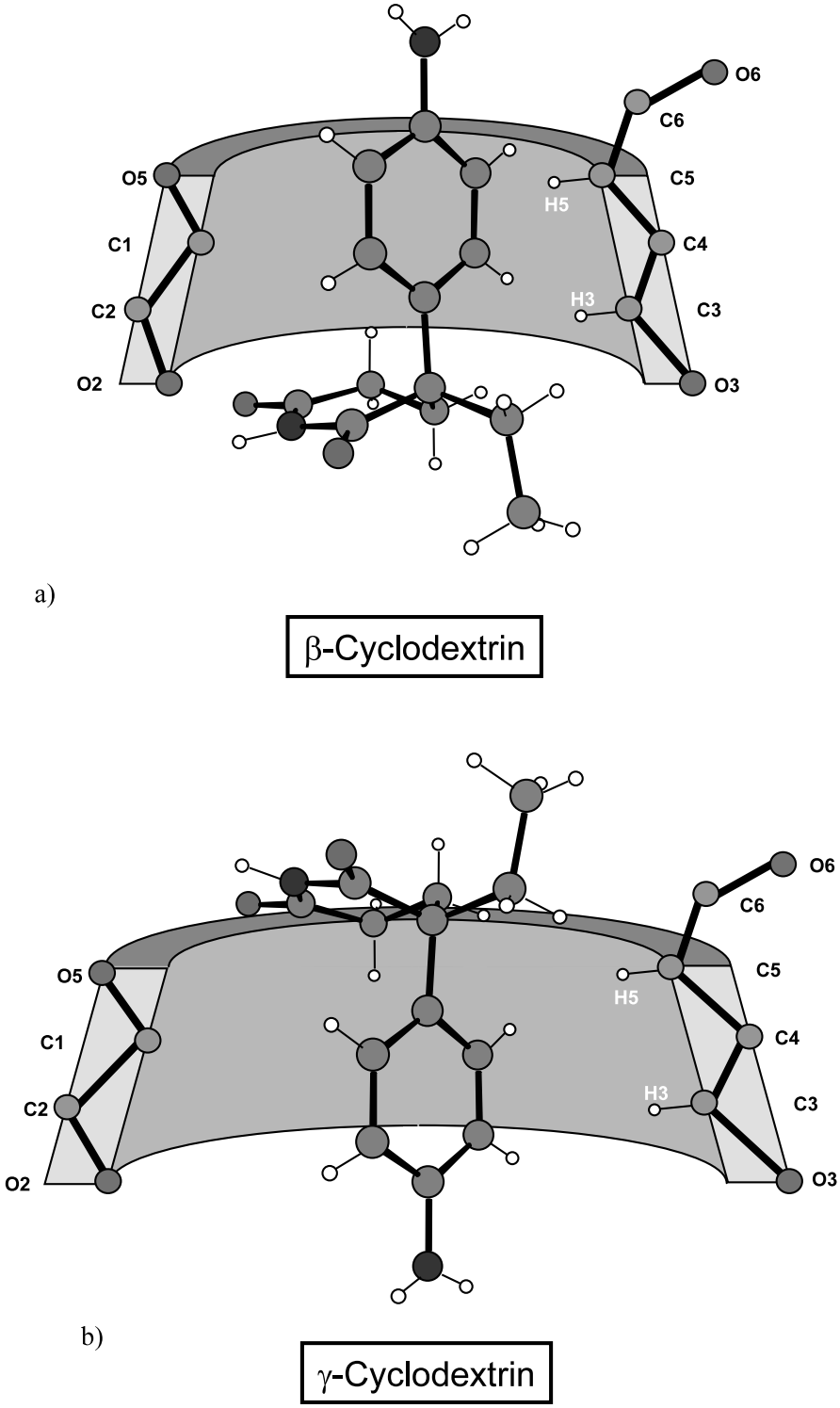

Fig. 3. Theoretical structures of the complexes between aminogluthetimide and (a) β-CD and (b) γ-CD derived from 1D-ROESY spectra (reproduced with permission from Ref. [107]).

exchange spectroscopy (1D-ROESY) demonstrated strong intermolecular interactions effects for the H-3 and H-5 protons of β-CD upon saturation of the aromatic *ortho* protons of aminogluthetimide while only a minor effect was observed upon irradiation of the *meta* protons (Fig. 2a). The latter resulted in a strong effect on the H-6 protons. This suggested the inclusion of the *p*-aminophenyl moiety of aminogluthetimide into the CD cavity entering from the wider secondary side as depicted in Fig. 3a. In contrast, when irradiating the *meta* protons in the complex with γ-CD instead of the *ortho* protons, a weaker effect was observed for the H-5 protons while the effect was basically unchanged for the H-3 protons (Fig. 2b). These data suggest a structure as shown in Fig. 3b with the *p*-aminophenyl moiety entering from the narrower primary side of the γ-CD cavity. However, NOE observed for additional protons in both complexes indicate that the structures shown in Fig. 3 may not be the only possible structures for the respective complexes or that rather complex supramolecular aggregates may be formed.

Only a few studies have used X-ray crystallography of CD complexes with respect to CE separations [103,104]. This may be partly related to the fact that crystallography is a solid-state technique that may not adequately represent the situation in solution. Moreover, monocrystals suitable for analysis may not be easily obtained. Nevertheless, one of the published examples indicated that (+)-brompheniramine formed a 1:2 complex with β-CD in the crystals, a complex stoichiometry that had also been suspected based on NMR experiments [104].

3.4. Recent pharmaceutical applications of chiral capillary electrophoresis

The extraordinary success of CE as an analytical technique in general and specifically for enantioseparations has also prompted the introduction of CE into the US Pharmacopeia [110] and into the European Pharmacopoeia [111]. Chiral CE separations of pharmaceutical drugs may be divided into the following categories: (1) enantioresolution of racemic compounds in systematic studies on a class of compounds or on certain chiral selector(s) where the drugs served as model compounds rather than solutions of 'real' analytical problems in pharmaceutical sciences, (2) chiral resolutions of 'artificial', non-racemic mixtures of enantiomers for the development of assays and the determination of the stereochemical purity of compounds, (3) chiral separations of drugs in pharmaceutical formulations and products, and (4) enantioseparations of drugs in biological samples. Only the latter three categories will be discussed in this review. The use of CE in pharmaceutical analysis including chiral separations with regard to the literature up to 1997 has been comprehensively summarized by Altria in a monograph [112]. In addition, due to the fact that aspects of chiral pharmaceutical analysis have been covered in several of the above cited reviews [4–11,15–28,30] only publications dating 1999 and later will be discussed below.

3.4.1. Enantioseparations of non-racemic mixtures of drug enantiomers

Due to the high flexibility and high resolution of CE, various assays have been developed for the determination of the stereochemical purity of drugs [30] following stereoselective synthesis or the resolution of racemates by crystallization. For this purpose, artificial non-racemic mixtures of the enantiomers have been prepared and used for assay development. In most cases the limit of detection (LOD) for the minor enantiomer was in the 0.1% range or below.

Mardones et al. developed a CE method for the enantioseparation of carnitine upon on-line derivatization with 9-fluorenylmethyl chloroformate in a flow system [113]. The separation was performed in fused-silica capillaries using DM-β-CD as chiral selector in a triethanolamine/sodium dihydrogenphosphate buffer, pH 4.3. Under optimized conditions, the LOD for the D-enantiomer in L-carnitine was 1%.

Nussbaum investigated the chiral analysis of a proprietary pharmaceutical compound using a triethanolamine/phosphoric acid buffer, pH 2.6, and a dual CD system consisting of DM-β-CD and randomly sulfated β-CD [114]. The impact of the total CD concentration, ionic strength of the

buffer and temperature was studied. The LOD of the minor enantiomer was between 0.1 and 0.05%. Thiamphenicol analogs were resolved by HP-β-CD with an LOD of 0.88% of the 2*S*,3*S*-isomer in a sample of the 2*R*,3*R*-enantiomer [115].

Using the so-called complete filling technique, the chiral purity of *S*-ropivacaine was assessed in an interlaboratory study [116]. The background electrolyte was sodium phosphate, pH 3.0, and randomly substituted methyl-β-cyclodextrin (M-β-CD) was the chiral selector. A LOD of 0.1% of the minor *R*-enantiomer was determined while the limit of quantitation (LOQ) was 0.25%. A LOD of *R*-propranolol in *S*-propranolol was found to be 0.1 in the presence of a dual CD system consisting of CM-β-CD and DM-β-CD in a Tris/phosphoric acid buffer, pH 3.0 [117].

Sarac et al. evaluated several CDs for the chiral separation of DOPA and carbidopa [118]. A high R_S value of 15.6 within an analysis time of about 20 min was obtained for DOPA in the presence 20 mM heptakis-(2,3-diacetyl-6-sulfato)-β-cyclodextrin (HDAS-β-CD) in a sodium phosphate buffer, pH 2.5. Chiral assays for L-DOPA and L-carbidopa were developed using HDAS-β-CD and CM-β-CD, respectively. By adding 0.1–1.5% racemate to samples of L-DOPA and L-carbidopa, a LOD of 0.05% of D-DOPA was determined and a LOD of 0.03% D-carbidopa, while the LOQ was 0.17 and 0.10%, respectively.

Zhou et al. employed randomly sulfated β-CD to the chiral analysis of a spiro-benzofuranoquinazoline-pyridinedione studying the influence of the concentration of the chiral selector, buffer pH, type and concentration of the buffer, organic modifier, temperature, and applied voltage [119]. The optimized assays were validated. The LOD and LOQ of the minor enantiomer were 0.1 and 0.3%, respectively.

A robust method for assaying the chiral purity of the anti-thrombotic agent SB-214857-A was developed by Camilleri's group [120].The method used a lithium phosphate buffer, pH 3.0, containing M-β-CD as chiral selector and hydroxyethylcellulose as a dynamic capillary coating agent to further suppress the EOF. The LOD for the distomer SB-214856-A was 0.05%. As the specification limit of the distomer with a previously used chiral HPLC method was only 0.5%, the CE assay was chosen as the primary method for the determination of the enantiomeric purity of SB-214857-A and included in the regulatory submission files.

The enantiomers of the antidepressant drug sertaline hydrochloride and synthetic analogs were achieved by CD-modified micellar electrokinetic chromatography (MEKC) in a sodium borate buffer, pH 9.0, containing 50 mM sodium cholate, 15 mM sulfated β-CD and 15 mM HP-β-CD [121]. The method allowed the quantitation of 0.1% of impurities in bulk ware.

3.4.2. Enantioseparations of drugs in pharmaceutical preparations

CE may also be used for the analysis of drugs including stereoisomers in pharmaceutical formulations and products. However, these contain a matrix that may interfere with CE analysis. For example, several cellulose derivatives tend to adsorb to the capillary wall affecting the EOF and by this the reproducibility of the assay. This may be the reason why fewer publications on chiral analysis of formulations have appeared compared to the analysis of pure compounds. Examples prior to 1999 have been summarized in Ref. [10]. A recent chiral CE assay was developed for the enantioseparation of the celiprolol enantiomers using sulfated β-CD as chiral selector and central composite design for method optimization [122]. The method showed good validation data with respect to precision accuracy and linearity and was found suitable for the determination of the celiprolol enantiomers in tablets.

Another paper describes the development of a CE method for the enantioseparation of atropine in the presence of sulfated β-CD as chiral selector also using central composite experimental design for assay optimization [123]. Under optimized conditions, the separation was achieved in less than 5 min. The method was applied to the analysis of atropine in an ophthalmic solution. In addition, the assay was suitable to evaluate the enantiomeric purity of (−)-hyoscyamine in plant extracts upon liquid extraction by sonication or percolation and supercritical fluid extraction. It was demonstrated that supercritical fluid extrac-

tion induced less racemization than classical liquid–solid extraction procedures.

3.4.3. Enantioseparations of drugs in biological samples

As for other chiral applications, CE has proved to be a suitable technique for the bioanalysis of drugs. Despite the fact that biological samples are quite complex mixtures and the low concentration sensitivity of CE compared to HPLC, an increasing number of researchers have used CE for bioanalytical purposes. So far, the compounds have been analyzed in body fluids, hair and microsomal preparations for studying the enantioselective pharmacokinetics or metabolism of drugs or in clinical and forensic toxicology for the analysis of illicit and banned substances. The literature prior to 1999 has been reviewed in Refs. [10,18,124]. Recent examples dating 1999 and later are summarized in Table 1 and some selected applications will be discussed in more detail below.

Except for one paper investigating the separation of diastereomers [141], the analysis of drug enantiomers is the primary focus of the research. Although a large variety of chiral selectors is available for the enantioseparation of compounds [4–11,19–25], most assays are based on the use of native or derivatized CDs. Only one paper reports the use of a linear polysaccharide, the glycosaminoglycan dermatan sulfate [145]. Dual systems combining a neutral and a charged CD were also used [125,136]. CD-modified MEKC employed SDS [141,142] or sodium deoxycholate [140]. Hexadecyltrimethyl ammonium bromide (HTAB) was used as buffer additive in the analysis of rogletimide [144]. The cationic detergent reduced the adsorption of endogenous substances in serum by the silanol groups on the wall by covering the negatively charged silica wall of a capillary, forming a net positive charge, thereby reducing protein adsorption as well as adsorption of the analyte to the wall. Other buffer additives include the polyethyleneglycol PEG 6000 [143] and the organic modifiers methanol [132,145], 1-propanol [142] or 2-propanol [137,141]. Most chiral assays were performed in untreated fused-silica capillaries. Polyvinyl alcohol-coated capillaries were employed for the enantioseparations of celiprolol [122] and tramadol and metabolites [150].

UV is the most common method of detection of analytes. The use of a Z-shaped detector cell for increasing the assay sensitivity has been reported [135]. Laser-induced fluorescence (LIF) was applied to the analysis of ofloxacin and metabolites [139] and following derivatization to the determination of baclofen [129]. In addition, off-line [142] and on-line [132,150] mass spectrometry detections have been employed. The partial filling technique was used in the latter cases to avoid the appearance of the CDs in the ion source of the mass spectrometer. Experimental design for optimization of the separation conditions [122,145] has been applied.

In order to avoid matrix interferences and for analyte concentration extraction, procedures including liquid–liquid extraction (LLE) and solid phase extraction (SPE) were frequently employed. However, there are also examples of the direct injection (DI) of urine [138,139,141] and plasma following deproteinization with organic solvents [128,129,141]. Petersson et al. investigated the applicability of on-line SPE for the achiral and chiral analysis of terbutaline in plasma [146]. They employed a self-constructed 'enrichment capillary' obtained by incorporation of a short plug of a restricted access material, alkyl-diol silica, into the capillary. Using a sequence of washing steps by applying pressure to the inlet or outlet end of the capillary the adsorption and desorption of the analyte to the sorbent material prior to the analysis was accomplished. Plasma proteins adsorbed to the capillary wall could be removed by washing with alkaline SDS solution. However, partial clogging of the enrichment capillary was noted after repeated injections of plasma.

Several groups have developed chiral assays to study the urinary excretion of amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine (Ecstasy) and analogs. Although the run buffers differ slightly in their compositions, the principal difference between the methods is the use of different CDs, i.e. HP-β-CD [127], CM-β-CD [126] and a dual system of β-CD and CM-β-CD [125]. The assays were applied to the toxicological analysis of the urinary excretion of

Table 1
Recent examples of stereoisomer separations of drugs in biological fluids by CE

Analyte	Matrix	Selector (concentration)	Run buffer	Extraction [a]	Application/comment	Reference
Amphetamine and analogs, epinephrine, norepinephrine (18 drugs)	Urine	β-CD (3 mM) and DM-β-CD (10 mM)	Tris/phosphate, pH 2.5	LLE	Samples from patients and drug abusers	[125]
Amphetamine, methamphetamine	Urine	CM-β-CD (10 mM)	Sodium phosphate, pH 2.5	LLE	Samples from rats and humans, distinction between use of selegeline and illicit amphetamines	[126]
Amphetamine, methamphetamine, ecstasy and analogs	Urine	HP-β-CD (8.3 mM)	Potassium phosphate, pH 2.5	LLE	Samples from patients and drug abusers, distinction between use of selegeline and illicit amphetamines	[127]
Atropine	Serum	TM-β-CD (40 mM)	Tris/phosphate, pH 2.8	DI	Spiked sample	[128]
Baclofen	Plasma	α-CD (7 mM)	Sodium borate, pH 9.5	DI	Spiked sample	[129]
Carvedilol	Serum	HP-β-CD (10 mM)	Sodium phosphate, pH 2.5	LLE	Spiked samples	[130]
Celiprolol	Urine	Sulfated β-CD (3 mM)	Sodium acetate, pH 4.0	LLE	Urinary excretion	[122]
Ciprofibrate, ciprofibrate glucuronide	Urine	γ-CD (7.5 mM)	Sodium phosphate, pH 6.0	SPE	Urinary excretion, stereoselective metabolism	[131]
Clenbuterol	Plasma	DM-β-CD (40 mM)	Ammonium acetate, pH 2.5, 20% methanol	SPE	Spiked samples	[132]
Disopyramide, mono-*N*-dealkyldisopyramide	Plasma	Sulfated β-CD (0.2%, w/v)	Sodium acetate, pH 5.0	LLE	Stereoselective pharmacokinetics	[133]
Etoclopride, sulpiride	Serum	Sulfated β-CD (2%, w/v)	Sodium citrate, pH 2.9	SPE	Spiked samples	[134]
Fluoxetine, norfluoxetine	Serum, plasma	DM-β-CD (0.5 mg ml^{-1}) and phosphated γ-CD (0.6 mg ml^{-1})	Sodium phosphate, pH 2.5	LLE	Patient samples, stereoselective metabolism	[135]
Methadone and metabolite	Urine	HP-β-CD (8.3 mM)	Potassium phosphate, pH 2.5	LLE	Samples from patients and drug abusers	[127]
Methaqualone	Urine	HP-β-CD (8 mM)	Sodium phosphate, pH 2.5	LLE	Urinary excretion	[136]
Metoprolol and metabolites	Urine	CM-β-CD (10 mM)	Sodium acetate, pH 4.0, 5% 2-propanol	LLE	Urinary excretion, stereoselective metabolism	[137]
Ofloxacin	Urine	Sulfated β-CD (0.35 mM)	Sodium phosphate, pH 2.5	DI	Urinary excretion	[138]
Ofloxacin and metabolites	Urine	SBE-β-CD (0.65 mg ml^{-1})	Triethylamine/phosphate, pH 2.0	DI	Urinary excretion, stereoselective metabolism	[139]
Praziquantel, *trans*-4-hydroxypraziquantel	Plasma	Sulfated β-CD (2%, w/v)	Sodium borate, pH 10, 20 mM sodium deoxycholate	LLE	Patient samples, stereoselective metabolism	[140]

Table 1 (*Continued*)

Analyte	Matrix	Selector (concentration)	Run buffer	Extraction [a]	Application/comment	Reference
Quinine, quinidine and metabolites	Plasma, urine, saliva	β-CD (15 mM)	Sodium phosphate, pH 2.5	LLE	Patient and volunteer samples	[141]
		–	Sodium borate, pH 9.4, 75 mM SDS, 7% 2-propanol	DI	Patient and volunteer samples	[141]
Racemorphan	Urine	β-CD (10 mM)	Sodium borate, pH 9.4, 50 mM SDS, 20% 1-propanol	SPE	Volunteer sample	[142]
Reduced haloperidol	Plasma	DM-β-CD (10 mM)	Tris/phosphate, pH 2.5, 10% PEG 6000 (20 mg ml^{-1})	LLE	Patient samples, in vivo metabolism of haloperidol	[143]
Rogletimide	Serum	α-CD (60 mM)	Sodium phosphate/sodium borate, pH 2.5, 30 nM HTAB	SPE	Spiked samples	[144]
Salbutamol	Urine	Dermatan sulfate (1.75%)	Tris/citric acid, pH 5.3, 5% methanol	SPE	Spiked samples	[145]
Terbutaline	Plasma	DM-β-CD (15 mM)	Potassium phosphate, pH 6.4	On-line SPE	Spiked samples	[146]
Thiopentone and pentobarbitone	Plasma	HP-γ-CD (5 mM)	Sodium phosphate, pH 8.5	LLE	Patient samples, in vivo metabolism	[147]
Tramadol and metabolites	Urine	CM-β-CD (5 mM)	Tris/phosphate, pH 2.5	LLE	Urinary excretion, stereoselective metabolism	[148]
Tramadol and metabolites	Urine	CM-β-CD (30 mg ml^{-1})	Sodium borate, pH 10.1	LLE	Urinary excretion, stereoselective metabolism	[149]
Tramadol and metabolites	Plasma	SBE-β-CD (2.5 mg ml^{-1})	Ammonium acetate, pH 4.0	LLE	Volunteer sample	[150]
Venlafaxine, *O*-desmethylvenlafaxine	Plasma	Phosphated γ-CD (20 mg ml^{-1})	Tris/phosphate, pH 2.5	LLE	Patient samples, stereoselective metabolism	[151]

[a] LLE: liquid–liquid extraction; SPE: solid phase extraction; DI: direct injection.

enantiomers of the amphetamine analogs upon illicit use. In addition, the metabolic conversion of the monoamine oxidase inhibitor selegiline to *R*-(−)-amphetamine and *R*-(−)-methamphetamine was studied. Ramseier et al. also investigated the use of commercial fluorescence polarization immunoassay reagents in a capillary zone electrophoresis immunoassay indicating a stereoselectivity with respect to the cross reactivity of the enantiomers of methamphetamine with the amphetamine reagents [127]. However, for unequivocal identification of the enantiomers and, thus, a differentiation between the use of illicit drugs or selegiline as therapeutic agent, the chiral CE assay employing CDs is necessary. The method was also applied to the analysis of the methadone enantiomers in urine [127].

The chiral analysis of the analgesic drug tramadol and its demethylated metabolites in urine was studied using CM-β-CD as chiral selector in an acidic buffer [148] or in a basic background electrolyte [149]. Both groups demonstrated the stereoselective metabolism of the drug upon oral administration of a tablet containing the racemate to a healthy volunteer. Using ESI-MS detection, tramadol and its active metabolite *O*-demethyltramadol could also be analyzed in plasma [150]. Sulfobutylether-β-cyclodextrin (SBE-β-CD) was the chiral selector and the partial filling technique was applied in order to avoid contamination of the ion source of the mass spectrometer by the CD.

Rudaz et al. investigated the enantiomeric composition of the antidepressant drug venlafaxine and its active metabolite *O*-desmethylvenlafaxine in patient serum using phosphated γ-CD as chiral selector in an acidic phosphate buffer [151]. Interindividual differences in the metabolism were observed. For about half of the patients, higher concentrations of the metabolite compared to the parent drug were found. In the majority of the cases, non-racemic mixtures of the compounds were observed indicating a stereoselective metabolism.

Zaugg and Thormann separated the rotamers of methaqualone in a phosphate buffer, pH 2.5, using HP-β-CD as the chiral selector [136]. The assay was applied to investigate the urinary excretion of the methaqualone enantiomers upon oral administration of a tablet of the racemic drug. Although metabolites were not analyzed, the results indicated a stereoselective metabolism of methaqualone.

An interesting study by Zaugg and Thormann investigated the separation and determination of the diastereomers quinine and quinidine, their hydroderivatives, hydroquinine and hydroquinidine, which are common impurities of the drugs, and the quinidine metabolites 3-hydroxyquinidine and quinidine-*N*-oxide by CE in body fluids [141]. While the diastereomeric pairs quinine and quinidine and hydroquinine and hydroquinidine could not be separated by CZE at acidic, neutral or alkaline pH employing just buffers, they could be resolved upon addition of 15 mM β-CD. Separation was also possible under MEKC conditions using SDS in a borate buffer, pH 9.4, containing 7% 2-propanol as the organic modifier. The addition of 2-propanol proved to be unequivocal for the separation of the compounds. The quinidine metabolites were also well separated from the other compounds in both systems. Due to pH differences in the fluorescence properties of the compounds LIF detection in the CZE method proved to be approximately 17-fold more sensitive compared to the MEKC method. Both assays were applied to the analysis of urine and plasma samples of patients under quinidine therapy. While LLE was applied to the samples analyzed by CZE, (DI) of plasma and urine was performed in the MEKC assay. In addition, the applicability of reagents from commercial fluorescence polarization assays for the therapeutic drug monitoring of quinidine was investigated for MEKC- and CZE-based immunoassays in this study [141]. Both methods allowed rapid screening of body fluids for the presence of quinidine at lower ppb levels. The assay showed stereospecificity for quinidine as no cross-reactivity was observed for the diastereomeric quinine.

4. Chiral capillary electrochromatography

4.1. Selected fundamental aspects of capillary electrochromatography

Capillary electrochromatography is considered a

hybrid technique between HPLC and CE that combines the high peak efficiency which is characteristic of electrically driven separations with the high separation selectivity of multivariate chiral stationary phases (CSP) available in HPLC [12–14,152]. Theoretical and practical aspects of chiral CEC have also been summarized [152,153]. Therefore, only a few selected topics will be briefly addressed. The driving force in CEC is the EOF generated upon application of the electric field along the capillary as a consequence of the charged surface of the capillary and/or the packing material. The advantage of electrokinetically driven techniques compared to pressure driven systems is the fact that the EOF has a plug like profile and is independent of the particle size and geometry of the packing material [154–156].

Besides studies in wall coated capillaries, the majority of the reported studies used fused-silica capillaries packed with silica gel based material of organic polymers carrying a charge. Due to the greater number of ionizable groups and the larger surface area, the contribution of the packing material to the EOF will be more significant compared to the capillary wall. Using silica-based packing materials, the EOF will be directed to the cathode except for aminopropyl-silica which generates an anodic EOF [157–161]. The preparation of a sulfonated silica support with a strong cathodic EOF was also reported [162]. Polymer-based packing material can be manufactured incorporating sulfonate or quaternary ammonium groups for EOF generation.

Besides the utmost importance for a chiral separation a chiral selector may also affect the EOF. Selectors such as quinine-derivatives [163–167], macrocyclic antibiotics [168–171] or poly(-diphenyl-2-pyridylmethylmethacrylate) [158,160] contain chargeable groups which may cause an EOF depending on the buffer pH. This 'selector-generated' EOF can be co-directional or counter-directional compared to the 'support-generated' EOF. In contrast, neutral chiral selectors may shield the ionizable or ionized groups of the support and, thus, reduce the EOF, an effect that increases with increasing concentrations of the chiral selector on the support material [159]. A high loading of a selector may be necessary for achieving good enantioseparations when the selector does not possess high enantiorecognition towards the analytes.

The properties of the mobile phase also influence the EOF. Apparently, pH is the most important variable but composition, nature and concentration of the electrolytes, ionic strength, viscosity, etc. of the mobile phase also affect the magnitude of the EOF in addition to effects on sample diffusion, mass transfer kinetics, Joule heating and most importantly selector–analyte interactions. The effects of the composition of the mobile phase and the organic or inorganic electrolyte additives on chiral separations have not yet been studied in great detail. A preliminary study on the effect of different electrolytes in methanol on enantioseparations using polysaccharide stationary phases has been published [172].

Recently, the importance of the pore size of the packing material on electrochromatographic separations has been realized in achiral [173] as well as chiral analyses [153,157,161,163]. Increasing the pore size resulted in higher peak efficiencies and reduction of the height of the theoretical plates. This was explained by the generation of an intraparticle flow in the supports with wider pores, which significantly reduces the stagnant layer of the mobile phase in the pores resulting in improved mass transfer kinetics [157].

4.2. Chiral separations by capillary electrochromatography

Enantioseparations by CEC were performed in wall-coated open tubular capillaries as pioneered by Mayer and Schurig in 1992 [174] and recently summarized by the authors of Refs. [12,13]. In these capillaries the chiral selector is covalently bound or coated on the inner surface of the capillary. However, majority of the studies have used capillaries that were packed capillaries which

Table 2
Recent examples of enantioseparations in CEC

Analyte	Chiral selector	Type of CEC column [a]	Reference
β-Adrenergic blocking agents	Sulfated β-CD	Dynamically modified anion exchange packing	[178]
	Poly-β-CD/CM-β-CD	Organic monolith	[179]
	Vancomycin	Bound to silica gel, packed column	[168–170]
	Teicoplanin	Bound to silica gel, packed column	[171]
Barbiturates	M-β-CD	OT CEC	[180]
	M-β-CD	Sintered silica monolith	[181]
Benzodiazepines	β-CD	OT CEC	[182]
Non-steroidal antiinflammatory drugs	M-β-CD	OT CEC	[180]
	Avidin	OT CEC	[183]
	Vancomycin	Bound to silica gel, packed column	[169]
	Teicoplanin	Bound to silica gel, packed column	[171]
Aminogluthetimide	Cellulose tris(3,5-dimethylphenylcarbamate), amylose tris(3,5-dimethylphenylcarbamate), cellulose tris(4-methylbenzoic acid ester)	Coated on silica gel, packed column	[159,161]
	Amylose tris(*S*-ethylphenylcarbamate)	Coated on silica gel, packed column	[153]
Atropine	Sulfated β-CD	Dynamically modified anion exchange packing	[178]
Bendroflumethiazide	Poly(*N*-acryloyl-(*S*)-phenylalanine ethylester)	Coated on silica gel, packed column	[184]
Bupivacaine	*S*-Ropivacaine	Molecular imprinted monolithic polymer	[185]
Etozolin	Cellulose tris(3,5-dimethylphenylcarbamate), amylose tris(3,5-dimethylphenylcarbamate), cellulose tris(4-methylbenzoic acid ester)	Coated on silica gel, packed column	[159,161]
Gluthetimide	Cellulose tris(3,5-dimethylphenylcarbamate), amylose tris(3,5-dimethylphenylcarbamate), cellulose tris(4-methylbenzoic acid ester)	Coated on silica gel, packed column	[159,161,186]
Labetalol	Vancomycin	Bound to silica gel, packed column	[169]
	Teicoplanin	Bound to silica gel, packed column	[171]
Mepivacaine	*S*-Ropivacaine	Molecular imprinted monolithic polymer	[185]
Felodipine	Teicoplanin	Bound to silica gel, packed column	[171]
Metomidate	Cellulose tris(3,5-dimethylphenylcarbamate), amylose tris(3,5-dimethylphenylcarbamate), cellulose tris(4-methylbenzoic acid ester)	Coated on silica gel, packed column	[159,161]
	Amylose tris(*S*-ethylphenylcarbamate)	Coated on silica gel, packed column	[153]
Metoprolol	*R*-Propranolol	Molecular imprinted monolithic polymer	[187]
Praziquantel	Sulfated β-CD	Dynamically modified anion exchange packing	[178]

Table 2 (*Continued*)

Analyte	Chiral selector	Type of CEC column [a]	Reference
Ropivacaine	*S*-Ropivacaine	Molecular imprinted monolithic polymer	[185]
Terbutaline	Vancomycin	Bound to silica gel, packed column	[169,170]
	Teicoplanin	Bound to silica gel, packed column	[171]
Thalidomide and metabolites	Vancomycin	Bound to silica gel, packed column	[169]
	Cellulose tris(3,5-dimethylphenylcarbamate), amylose tris(3,5-dimethylphenylcarbamate), cellulose tris(4-methylbenzoic acid ester)	Coated on silica gel, packed column	[175]
Venlafaxine and metabolites	Vancomycin	Bound to silica gel, packed column	[168,194]
Verapamil	Sulfated β-CD	Dynamically modified anion exchange packing	[178]
	Vancomycin	Packed column	[169,170]
	Teicoplanin	Bound to silica gel, packed column	[171]
	Cellulose tris(3,5-dimethylphenylcarbamate)	Coated on silica gel, packed column	[188]
Warfarin	Poly-β-CD	Organic monolith	[179]
	Allylcarbamoyl-β-CD	Organic monolith	[189]
	Teicoplanin	Bound to silica gel, packed column	[171]
	Vancomycin	Packed column	[169,170]
	Cellulose tris(3,5-dimethylphenylcarbamate)	Coated on silica gel, packed column	[188]
Amino acids	Sulfated β-CD	Dynamically modified anion exchange packing	[178]
	4-Hydroxyproline derivative	Organic monolith	[190]
Derivatized amino acids	Poly-β-CD	Organic monolith	[179]
	Allylcarbamoyl-β-CD	Organic monolith	[189]
	(*S*)-*N*-3,5-Dinitrobenzoyl-1-napthylglycine	Silica-based monolith	[191]
	(*S*)-*N*-3,5-Dinitrophenylaminocarbonylvaline	Silica-based monolith	[191]
	Avidin	OT CEC	[183]
	Quinidine derivative	Organic monolith	[167,168]
	Quinine derivative	Coated on silica gel, packed column	[164,165]
	(+)-Naphthylethylamine	OT CEC	[182]

were obtained either by packing with chiral stationary phases or by in situ polymerization procedures yielding monolithic chiral phases. Many chiral stationary phases previously used for enantioseparations by HPLC have also been successfully applied to enantioresolutions in CEC [12–14,152,153] including polysaccharide derivatives, cyclodextrins and derivatives, Pirkle-type stationary phases, proteins, macrocyclic antibiotics, chiral acrylamides and methacrylates, as well as ligand exchange phases. Recent examples (dating 1999 and later) where pharmaceutical drugs or amino acid derivatives served as model compounds to study the enantioseparation ability of the chiral stationary phases are summarized in Table 2. Most separations were carried out in aqueous/organic buffers, though an increasing number of studies employed non-aqueous background electrolytes [157–161,164,166–168,172, 175,176]. In addition, the use of CDs [177] or

chiral ion-pairing reagents [166] in combination with an achiral stationary phase has been reported.

4.3. Application to pharmaceuticals and biological samples

Due to the relative novelty of CEC, chiral separations of drug compounds have focused on the separation principles and the influence of the operative parameters on the separations as briefly described above rather than on the application to real pharmaceutical chiral analysis. Thus, only few reports have appeared to date. A method for the determination of the chiral purity of the enantiomers of metoprolol on a teicoplanin stationary phase was developed and validated [192]. The LOD of the respective minor enantiomer was 0.09%. The enantioseparation of thalidomide and its hydroxylated metabolites was achieved on Chiralpak AD material [175]. The in vitro biotransformation of *R*-(+)-thalidomide was studied [193]. Moreover, an assay for the simultaneous chiral analysis of venlafaxine and its main metabolite *O*-desmethylvenlafaxine on a vancomycin stationary phase was validated and applied to the analysis of patient plasma samples indicating a stereoselective metabolism of the drug [194].

5. Concluding remarks

CE and CEC are both miniaturized separation techniques that are also well suited for enantioseparations. Especially CE has grown to be the most often applied method for the analysis of enantiomers. Along with the high flexibility maybe the greatest advantage of CE is the presence of freely mobile selector(s). This allows, for example, the counter-directional migration of analyte and selector increasing the separation selectivity, the possibility of using a large excess of the selector as well as the combination of selectors. As a result, very effective enantioseparations can be achieved in CE. In addition, there are several examples in the literature describing chiral separations that could not be performed by HPLC but that were easily accomplished by CE using the same selector as in HPLC. As demonstrated by the above cited examples, CE is also well suited for the chiral analysis of drugs in bulk material, pharmaceutical formulations and body fluids.

In contrast, CEC has not yet really matured to a competitive technique. This may partially be due to the fact that column fabrication is still somewhat tedious and not always reproducible. Moreover, the miniaturization of HPLC (microLC) led to a significant reduction of the use of chiral stationary phase, organic solvents and sample amounts reducing the immediate need for another miniaturized separation technique. However, most stationary phases that have been applied to enantioseparations in CEC are identical with those used in HPLC. Thus, as stationary phases specifically designed for CEC will emerge and, in addition, we will further understand the basic and specific features of CEC the impact of CEC in separation sciences will grow. At present it is difficult to predict the true potential of CEC. Most likely the technique will never be as versatile and flexible as CE. However, there will certainly be applications where CEC will have advantages over CE and also HPLC and microLC.

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