Laura Sánchez-Hernández
Antonio Luis Crego
María Luisa Marina
Carmen García-Ruiz

Departamento de Química Analítica,
Facultad de Química,
Universidad de Alcalá,
Alcalá de Henares,
Madrid, Spain

Received July 20, 2007
Revised September 19, 2007
Accepted September 20, 2007

Review

Sensitive chiral analysis by CE: An update

A general view of the different strategies used in the last years to enhance the detection sensitivity in chiral analysis by CE is provided in this article. With this purpose and in order to update the previous review by García-Ruiz *et al.*, the articles appeared on this subject from January 2005 to March 2007 are considered. Three were the main strategies employed to increase the detection sensitivity in chiral analysis by CE: (i) the use of off-line sample treatment techniques, (ii) the employment of in-capillary preconcentration techniques based on electrophoretic principles, and (iii) the use of alternative detection systems to the widely employed on-column UV–Vis absorption detection. Combinations of two or three of the above-mentioned strategies gave rise to adequate concentration detection limits up to 10^{-10} M enabling enantiomer analysis in a variety of real samples including complex biological matrices.

Keywords:
CE / Chiral analysis / Detection sensitivity / In-capillary preconcentration
DOI 10.1002/elps.200700531

1 Introduction

Due to the different biological activity that the enantiomers of a chiral compound may have, chiral analysis has nowadays an increasing interest in a variety of disciplines such as pharmaceutical, environmental, or food analysis, among others. The individual determination of the enantiomers of a chiral compound may require a high sensitivity for a great number of applications. Thus, the analysis of low concentrated and limited amounts of biological samples, the analysis of environmental samples where analytes are usually present at trace level or the determination of food components, ingredients, or residues are examples of this kind of applications. Moreover, the determination of enantiomeric impurities in drugs can require sensitive analytical methodologies due to the low percentages of the impurity that should be determined according to the ICH guidelines (for impurity contents higher than 0.05% the impurities have to be reported) [1].

Since CE has shown in the last years a great potential to achieve chiral separations, the development of analytical methodologies enabling the sensitive determination of enantiomers has been the aim of a considerable number of articles. The use of at least one chiral selector in the separation media is necessary to enable a chiral separation by CE being CDs the favorite chiral selectors employed. Although there are some authors that consider as CZE the separation mode in CE when neutral CDs are used, we will consider in this review that when CDs are employed as chiral selectors, and independently of their nature, the separation mode is EKC. In fact, the enantiomeric discrimination in EKC is produced by a chromatographic mechanism where interactions are established between each one of the enantiomers and the chiral selector [2–4]. Although much less used, CEC and NACE can also be employed for chiral analysis. CEC is a hybrid technique between CE and HPLC characterized by a high separation efficiency due to the plug profile of the mobile phase driven by the EOF. A wide range of HPLC chiral stationary phases (CSPs) transferable to CEC or monolithic columns can be used to provide adequate enantioselectivity. Moreover, an important aspect from the point of view of the sensitivity and selectivity in chiral analysis is the easy coupling of CEC to MS detection, when the chiral selector in CEC is immobilized [5]. On the other hand, the

Correspondence: Dr. Carmen García-Ruiz, Departamento de Química Analítica, Facultad de Química, Universidad de Alcalá, Ctra. Madrid-Barcelona Km. 33.600, E-28871 Alcalá de Henares, Madrid, Spain
E-mail: carmen.gruiz@uah.es
Fax: +34-91-8854971

Abbreviations: CBI, cyanobenz[*f*]isoindole; **CSP,** chiral stationary phase; **DAS-β-CD,** heptakis(2,6-diacethyl-6-sulfato)-β-CD; **DIM,** dimethindene; **DIO,** dioxopromethazine; **ECL,** electrochemiluminescence; **FASS,** field-amplified sample stacking; **HS-β-CD,** highly sulfated-β-CD; **HS-γ-CD,** highly sulfated-γ-CD; **HDAS-β-CD**, heptakis(2,3-di-*O*-acetyl-6-*O*-sulfo)-β-CD; **LE,** leading electrolyte; **LLE,** liquid–liquid extraction; **MA,** methamphetamine; **MDA,** methylenedioxyamphetamine; **MDMA,** methylenedioxymethamphetamine; **MTD,** methadone; **NAC,** *N*-acetyl L-cysteine; **NE,** norephedrine; **OPA,** orthophthalaldehyde; **PHM,** pheniramine; **poly-L-SUCL,** poly(sodium *N*-undecenoxy carbonyl-L-leucinate); **poly-LL-SUCLV,** poly (sodium *N*-undecenoxycarbonyl-LL-leucyl-valinate); **SPCD,** sample preconcentration with chemical derivatization; **TEA,** triethylamine; **TE,** terminating electrolyte; **TMD,** tramadol

© 2008 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

aqueous buffer employed in CE is replaced in NACE by an organic solvent containing an electrolyte. The use of a solvent instead of an aqueous buffer has provided additional selectivities to those obtained in aqueous CE systems and may be advantageous for the hyphenation of this separation mode with MS, if volatile solvents are used [6].

A variety of strategies have been employed in CE in order to obtain the detection sensitivity needed for a given application. These strategies included the use of off-line or on-line sample treatment techniques, sample preconcentration in the capillary using techniques based on electrophoretic principles, and/or the use of alternative detection systems to the UV–Vis absorption detection [7]. In addition, other strategies such as partial filling of the capillary or the use of CDs with countercurrent migration can be necessary, if MS detection is employed when chiral selectors are in the separation media. These strategies avoid the introduction of these compounds into the MS detector which causes a damage in electrospray efficiency and increases background noise decreasing the sensitivity of detection.

Some reviews have been published in the last years covering the chiral separation of drugs [8–10] or pollutants and their metabolites [11]. The aim of this review is to provide a general view of the different strategies that have been used in the last years in order to enhance the sensitivity of detection in chiral analysis by CE with application to the analysis of drugs or biological, environmental, or food samples. Articles appeared from the publication of the previous review by García-Ruiz and Marina [7] have been considered covering the period of time from January 2005 to March 2007.

2 Enhancement of the sensitivity in chiral analysis by CE

An important aspect to take into account in CE is the very low volumes injected in the system (in the nL range) that imply that the detection of a concentration 10^{-5} M leads to the detection of $\sim10^{-14}$ mol of analyte. For this reason, all those works where at least one strategy has been employed to improve the detection sensitivity in chiral analysis by CE enabling the determination of enantiomers at least at molar concentrations of 10^{-5} have been included in this review.

Next sections will describe the different approaches used in the last years to enhance the detection sensitivity in chiral analysis by CE: (i) off-line sample treatment techniques, (ii) in-capillary preconcentration techniques, and (iii) alternative detection systems to the widely employed on-column UV–Vis absorption detection.

2.1 Off-line sample treatment techniques in sensitive chiral analysis by CE

Table 1 groups the different sample treatments employed in the period of time reviewed in this article prior to chiral analysis by CE. Analytes, samples, separation buffer, detection system, and detection limits (LODs) obtained are also given in this table. Sample treatments can be aimed to eliminate some components of the sample matrix in addition to enhance the detection sensitivity through sample preconcentration. SPE, liquid–liquid extraction (LLE), solid-phase microextraction (SPME), or microdialysis were used. In all cases, off-line strategies were used.

SPE and LLE were the main extractive techniques employed. Preconcentration by both techniques implies the reconstitution of the residue obtained after sample treatment in a volume smaller than the initial sample volume. Therefore, the improvement in the concentration sensitivity will depend on the sample volume available. However, preconcentration possibilities by SPE are usually better than by LLE, because the volume factor (sample volume/residue volume) is more favorable in SPE than in LLE.

SPE is one of the most popular and widely used extractive techniques used for liquid samples due to its high selectivity and also preconcentration possibilities. SPE was only employed as sample treatment to isolate chiral drugs in biological samples as urine [12, 13, 15, 17] and to selectively preconcentrate chiral herbicides in spiked water samples [14, 16]. Most of these works were performed with UV detection [12–14, 17] achieving LODs in the 10^{-8}–10^{-7} M range. These LODs were improved by one or two orders of magnitude using SPE as sample treatment depending on the sample volume available (preconcentration factors ranging from 3 to 250 were achieved). However, other detection systems such as ESI-MS were also used enabling to detect up to 3×10^{-8} and 6×10^{-8} M for each salbutamol enantiomer [15]. In this case, the SPE step enabled an enrichment of the initial sample concentration of four times. The best LODs (2×10^{-9} M) were reached using fluorescence detection for the analysis of glufosinate enantiomers [16]. The high sensitivity achieved in the latter work was due to the combination of an SPE step with an in-capillary preconcentration strategy.

Classical LLE was also employed for the extraction and preconcentration of chiral compounds prior to CE chiral analysis. Thus, Table 1 shows the use of LLE for the determination of drugs in biological samples as plasma [20–23], human serum [17, 19], human urine [17], and a microsomal fraction of liver homogenates [18]. Preconcentration factors up to 21 were achieved by this extraction procedure. After this sample treatment step, LODs from 10^{-6} to 3×10^{-8} M were reported when UV detection was used [17–20] and LODs ranging form 3×10^{-6} to 4×10^{-9} M were reached with ESI-MS detection [21, 22]. As example, Schappler *et al.* [22] assessed two approaches to enhance the sensitivity in CE. The former consisted of a protein precipitation using ACN followed by hydrodynamic injection of the supernatant. The second was the combination of LLE, which produces a sample cleanup and enrichment minimizing any matrix effect, with electrokinetic injection. In spite of the fact that the former method was rapidly achieved with minimal

© 2008 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Table 1. Off-line sample treatment techniques employed for the enhancement of the sensitivity in chiral analysis by CE

Sample treatment	Analyte and sample	Separation buffer	Detection	LOD (M)	Ref.
SPE	Lorazepam in human urine	6 mM borate/10 mM phosphate (pH 9.1) + 60 mM HP-β-CD + 75 mM SDS	UV-200 nm	$<2\times10^{-5}$	[12]
SPE	CIT, DCIT, DDCIT, CIT-NO, and CIT-PA in human urine	20 mM phosphate (pH 5) + 0.2% CM-γ-CD + 0.05% HPMC	UV-205 nm	6×10^{-8}–3×10^{-7}	[13]
SPE	Malathion in spiked tap water	25 mM Tris (pH 7.0) + 20 mM CM-β-CD	UV-230 nm	6×10^{-7}	[14]
SPE	Salbutamol in human urine	10 mM ammonium formate (acidified with 0.75 M formic acid) + 15 mM HDAS-β-CD	MS	3×10^{-8}, 6×10^{-8}	[15]
SPE	DNS-DL-Glufosinate in spiked river water	2 mM phosphate (pH 6.5) + 17 mM γ-CD	Fluorescence (λ_{exc} = 327 nm, λ_{em} = 557 nm)	2×10^{-9}	[16]
SPE/LLE	Ibuprofen in human serum and urine	200 mM orthophosphoric acid + 200 mM triethanolamine (pH 5.0) + 50 mM TM-β-CD	UV-220 nm	$\sim2\times10^{-7}$ (serum), $\sim10^{-6}$ (urine)	[17]
LLE	Hydroxychloroquine and its metabolites (DCQ, DHCQ, BDCQ) in microsomal fraction of liver homogenates	100 mM Tris/phosphate (pH 9.0) + 1% HS-β-CD + 30 mg/mL HP-β-CD	UV-220 nm	$\sim10^{-7}$	[18]
LLE	Propafenone in human serum	100 mM phosphate (pH 2.0) + 0.6% HS-β-CD.	UV-195 nm	$\sim3\times10^{-8}$	[19]
LLE	Ketamine and norketamine in equine plasma	50 mM Tris (pH 2.5) + 10 mg/mL HS-β-CD	UV-195 nm	$\sim4\times10^{-8}$	[20]
LLE	Amphetamine derivatives (A, MA, MDA, MDMA, MDEA, E, NE) in plasma	20 mM ammonium formate (pH 2.5) + 0.15% HS-γ-CD	MS	$\sim7\times10^{-7}$–3×10^{-6}	[21]
LLE	Amphetamine derivates (A, MA, MDA, MDMA, MDEA, TMD, MTD) in human plasma	20 mM ammonium formate (pH 2.5) + 0.15% HS-γ-CD	MS	4×10^{-9}	[22]
LLE	Disopyramide in spiked plasma sample	40 mM acetate (pH 4.5) + 3 mg/mL HS-β-CD	ECL	$\sim10^{-7}$	[23]
SPME	(1*R*,2*S*)-Ephedrine, (1*R*,2*R*)-pseudoephedrine, (1*S*,2*S*)-pseudoephedrine in water and human urine	150 mM phosphate (pH 2.5) + 17.5 mM β-CD	UV-192 nm	2–3×10^{-8}	[24]

A, amphetamine; BDCQ, bisdesethylchloroquine; CIT, citalopram; CIT-NO, citalopram *N*-oxide; CIT-PA, citalopram propionic acid; CM-γ-CD, carboxymethyl-γ-CD; DCIT, demethylcitalopram; DCQ, desethylchloroquine; DDCIT, didemethylcitalopram; DHCQ, desethylhydroxy-chloroquine; DNS, dansyl choride; E, ephedrine; HDAS-β-CD, heptakis(2,3-di-*O*-acetyl-6-*O*-sulfo)-β-CD; HP-β-CD, 2-hydroxypropyl-β-CD; HPMC, hydroxypropylmethylcellulose; MDEA, methylenedioxyethylamphetamine; NDA, naphthalene-2,3-dicarboxaldehyde; TM-β-CD, heptakis 2,3,6-tri-*O*-methyl-β-CD.

sample manipulation, a concentration factor of 1000-fold was obtained with the second one although it was more time consuming. Finally, the MS signal suppression effect was investigated on the complex matrices of biological samples (plasma) with a conventional CE-ESI-MS setup. Suppression occurred in the migration window of analytes of interest in conventional analysis. However, when LLE was employed, no matrix effects were evidenced leading to the conclusion that this sample preparation method remains of utmost interest for the analysis of biological samples by CE-MS. The combination of LLE-electrokinetic injection in CE-MS enabled to obtain the best LODs ($\sim10^{-9}$ M) indicated in Table 1, and this was for amphetamine derivatives in human plasma. Figure 1 shows the sensitive determination of seven am-

© 2008 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

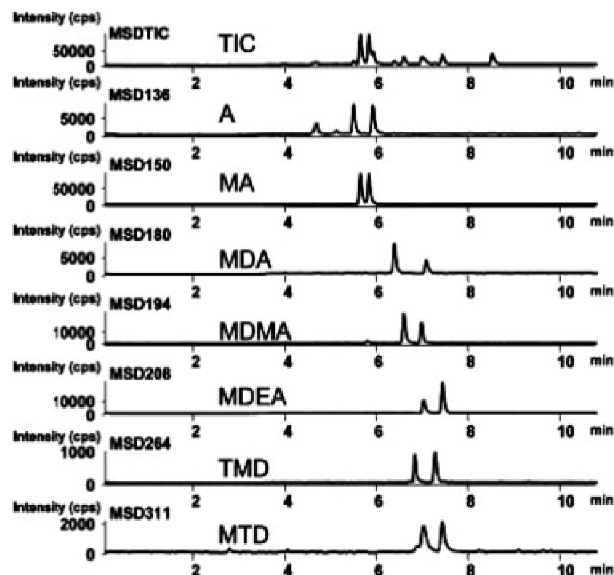

Figure 1. Chiral analysis of a plasma sample spiked with 0.5 ppb of each enantiomer of seven different amphetamine derivatives, after LLE and electrokinetic injection in CE-ESI-MS. Electrophoretic conditions: fused-silica capillary, 75 cm total length and 50 μm id; separation buffer, 20 mM ammonium formate (pH 2.5) containing 0.15% HS-γ-CD; separation temperature, 25°C; applied voltage, 25 kV; electrokinetic injection, 10 kV × 10 s. MS conditions: sheath liquid, isopropanol/H_2O (50:50 v/v) containing 0.5% formic acid; syringe flow rate, 3 mL/min. ESI capillary at 4.5 kV. The nebulizing pressure and the drying gas flow rate were set at 4 psi and 4 L/min, respectively. Gas temperature, 200°C; fragmentor 70 V. Identification of peaks: A, amphetamine; MA, methamphetamine; MDA, methylenedioxyamphetamine; MDMA, methylenedioxymethamphetamine; MDEA, methylenedioxyethylamphetamine; TMD, tramadol; MTD, methadone (reprinted from ref. [22] with permission).

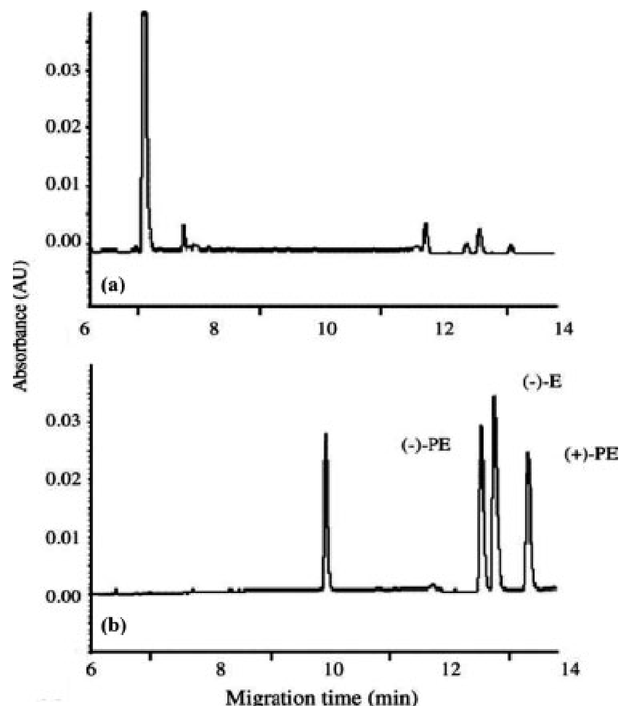

Figure 2. Separation of ephedrine derivatives in a spiked urine sample (a) directly injected in CE (concentration of each analyte: 5.00 μg/mL), and (b) after off-line SPME (concentration of each analyte: 0.25 μg/mL) followed by CE. Electrophoretic conditions: fused-silica capillary, 60.5 cm total length and 75 μm id; separation buffer, 150 mM phosphate buffer (pH 2.5) containing 17.5 mM β-CD; separation temperature, 20°C; applied voltage, 25 kV; electrokinetic injection, 7 kV × 10 s. UV detection at 192 nm. Identification of peaks: (−)-PE, (1*R*,2*R*)-pseudoephedrine; (−)-E, (1*R*,2*S*)-ephedrine; (+)-PE, (1*S*,2*S*)-ephedrine (reprinted from [24] with permission).

phetamine derivatives in a plasma sample spiked with 0.5 ppb of each enantiomer after LLE and electrokinetic injection in CE-ESI-MS.

On the other hand, Fang *et al.* [24] proposed an SPME sample treatment previous to the injection into a CE system with UV detection as a chiral sensitive method for doping control of ephedrine derivatives. Although this extractive technique is mainly used in GC where compounds are desorbed from the fiber by applying high temperatures, in this work the analytes (ephedrine derivatives) were extracted from 5 mL of the liquid samples. This was carried out by exposing the coated fiber end to the headspace of the vial for an appropriate time and then desorbing the analytes in 80 μL of back-extraction solvent containing an optimized concentration of ACN. Figure 2 shows a ~160-fold intensity improvement after SPME of ephedrine derivatives from an urine sample. Interestingly, SPME not only enhanced the sensitivity of detection by concentrating the sample, also supplied the possibility to employ an in-capillary sample preconcentration strategy providing an appropriate sample matrix to be injected in the CE system.

2.2 In-capillary preconcentration techniques based on electrophoretic principles in chiral analysis by CE

Another way to increase the sensitivity in chiral CE is by using in-capillary preconcentration techniques based on electrophoretic principles. Table 2 summarizes the main characteristics of works where two different in-capillary preconcentration strategies were used. On one hand, classical in-capillary preconcentration techniques, such as ITP, stacking, and/or sweeping were employed, and on the other hand, in-capillary sample preconcentration with chemical derivatization (SPCD) is included in this table as an innovative strategy. A brief description of the different preconcentration techniques will be made previously to indicate the applications performed by CE in the chiral field.

In ITP, ionic analytes are concentrated and separated on the basis of their electrophoretic mobilities using a discontinuous buffer system (leading electrolyte (LE) and tailing electrolyte (TE). This technique may be performed in the same capillary where the electrophoretic separation is

© 2008 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Table 2. In-capillary preconcentration techniques employed for the enhancement of the sensitivity in chiral analysis by CE

Precon-centration technique	Analyte and sample	Separation buffer	Detection	LOD (M)	Ref.
cITP	Antihistaminic drugs (PHM, DIM, DIO) in urine	25 mM acetic acid (pH 3.2–4.5) + 2.5 mg/mL (PHM, DIM) and 5.5 mg/mL (DIO) CE-β-CD	UV-265 and 240 nm	4×10^{-9}–2×10^{-8}, 10^{-8}	[25]
cITP	Drugs standards (*S*-alprenolol, *S*-atenolol, *R*-propranolol, *R*-salbutamol, *S*-terbutaline)	LE: 160 mM acetate (pD 4.7) + 10 mM β-CD TE: 160 mM acetic acid (pH 2.4) + 10 mM β-CD	NMR-600 MHz	$<2.5\times10^{-4}$	[26]
tITP FASS	*R,S*-Timolol in standard	40 mM KOH in methanol/ethanol (40:60 v/v) + 100 mM (+)-KPA	UV-220	4×10^{-6} 5×10^{-6}	[27]
FASS	(1*R*,2*S*)-Ephedrine, (1*R*,2*R*)-pseudoephedrine, (1*S*,2*S*)-pseudoephedrine in water and human urine	150 mM phosphate (pH 2.5) + 17.5 mM β-CD	UV-192 nm	2–3×10^{-8}	[24]
LVSS	DNS-DL-glufosinate in spiked river water	2.0 mM phosphate (pH 6.5) + 17 mM γ-CD	Fluorescence (λ_{exc} = 327 nm, λ_{em} = 557 nm)	2×10^{-9}	[16]
LVSS + sweeping	CBI-Ser, CBI-Glu in biological samples	25 mM phosphate (pH 2.0) + 2% HS-β-CD	LIF (λ_{exc} = 420 nm)	2×10^{-10}, 3×10^{-10}	[28]
Sweeping	Lorazepam in human urine	6 mM borate/10 mM phosphate (pH 9.1) + 60 mM HP-β-CD + 75 mM SDS	UV-200 nm	$<2\times10^{-5}$	[12]
In-capillary SPCD	OPA/NAC-amino acids (Ala, Glu) in *E. coli* bacterial culture	140 mM borate (pH 9.5) + 1 mM β-CD	UV-340 nm	4×10^{-7}, 6×10^{-7}	[29]
In-capillary SPCD	OPA/NAC-muramic and diaminopimelic acids in *E. coli* bacterial culture	140 mM borate (pH 9.5)	UV-214 and 340 nm	2×10^{-6}, 2×10^{-7}	[30]

CE-β-CD, carboxyethyl-β-CD; DIKGA, di-*O*-isopropylidene-2-keto-L-gulonic acid; DIM, dimethindene; DIO, dioxopromethazine; KPA, ketopinic acid; PHM, pheniramine.

achieved, which is denominated transient ITP (tITP), or in two different capillaries, which is named capillary ITP (cITP). Table 2 shows that cITP was used as preconcentration technique to determine different antihistaminic drugs in a biological sample as urine [25] and to detect several drugs in standard solutions by CE-NMR [26]. Likewise, this table also shows the use of tITP for the preconcentration of *R,S*-timolol [27]. Thus, Mikus *et al.* [25] proposed a highly sensitive cITP-EKC method combining two coupled capillaries: in the first capillary, ITP was carried out to achieve a preseparation and a sample preconcentration, and in the second capillary, where the sample was transferred on-line, the EKC separation occurred. These experiments showed favorable conditions for the separation and determination of traces (ng/mL) of antihistaminic drugs (dioxopromethazine (DIO), dimethindene (DIM), and pheniramine (PHM)) enantiomers present in urine samples diluted with water. By using UV detection, LODs achieved in this work were from 4×10^{-9} to 2×10^{-8} M depending on the compound analyzed. Another example of the use of cITP sample stacking process is the separation and concentration of analytes for NMR measurements [26]. It is important to emphasize that the coupling of cITP to NMR enabled to detect small amounts (~2 nmol) of the basic drugs studied, but the concentrations detected (~70 mM) were far away of a sensitive detection. Finally, a rapid analytical method by using tITP for the determination of the enantiomeric impurity of *S*-timolol was developed by Hedeland *et al.* [27]. In this method, the combination of 1*S*,4*R*-(+)-ketopinic acid (KPA) as chiral selector in NACE and preconcentration by tITP provided LODs as low as 0.2% of *R*-timolol in *S*-timolol samples.

With respect to stacking preconcentration, it is produced because ions migrate electrophoretically through a low-conductivity sample matrix into a high-conductivity buffer solution and they are focused in a thick zone between the boundaries of both solutions. Most stacking modes use

© 2008 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

hydrodynamic injection of large-volume sample being named large-volume sample stacking (LVSS). However, when electrokinetic injection is used to focus the analytes of the sample, the strategy is denominated field-amplified sample stacking (FASS). As shown in Table 2, highly sensitive chiral methods were developed using this preconcentration technique based on the stacking of the analytes in narrow bands previously to the electrophoretic separation.

The possibility of preconcentration by FASS was evaluated and compared with tITP by Hedeland *et al.* [27]. FASS was performed using an electrokinetic injection of 8 kV for 10 s. Similar LODs, in the 10^{-6} M range, were obtained by both in-capillary preconcentration techniques. In addition, FASS was applied for in-capillary sample concentration of ephedrine derivatives in water and urine samples [24]. In this work, a study of the performance of the FASS procedure (7 kV for 10 s) using the samples prepared in water enabled to obtain a concentration factor of up to 80-fold compared with the conventional electrokinetic sample injection (7 kV for 10 s), where the samples were dissolved in the separation buffer. On the other hand, the concentration enrichment achieved by LVSS allowed the analysis of traces of the enantiomers of the herbicide glufosinate in spiked river water (model sample) [16]. The combination of this in-capillary preconcentration strategy with SPE enabled to obtain LODs for D- and L-glufosinate enantiomers as low as 2×10^{-9} M. Moreover, Kirschner *et al.* [28] obtained a very high sensitivity with a CE-LIF method involving a combination of LVSS and sweeping which is a preconcentration technique enabling a significant increase in the detection sensitivity for those analytes with a high solute-pseudostationary phase association constant [31]. The combined preconcentration mechanism proposed by Kirschner *et al.* is illustrated in Fig. 3A. First, LVSS involves a combination of field-amplified stacking and pH-mediated stacking. Then, sweeping using the anionic highly sulfated-β-CD (HS-β-CD) as pseudophase interacting with the analytes was performed. A solution containing the analytes was injected filling 1/3 of the capillary. Then, reverse polarity was applied and the anionic cyanobenz[*f*]isoindole (CBI)–amino acids migrated toward the pH junction at the outlet side of the injection plug, where they were substantially neutralized and stacked by the low pH buffer. The migration of the analyte anions produced a field-amplified stacking in the dilute water. Simultaneously, EOF began to pump water out of the capillary and moved the stacked analytes band toward the inlet. During this period, most electrolytes stayed within the capillary, since the current was near zero or a few microamperes. Finally, once the analytes were nearly ejected from the capillary, the current increased as the HS-β-CD migrated rapidly through the stacked band of the analyte, sweeping it to the outlet. Interestingly, LODs up to 10^{-10} M for the baseline resolved CBI–amino acid enantiomers were reached by this way. This preconcentration technique was also applied to more complex mixtures of amino acids without loss of resolution, as it can be seen in Fig. 3B. Another example of sweeping is the sensitive determination of lorazepam in human urine, a drug used for the treatment of anxiety and with sedative and hypnotic properties. In this work, the sweeping of the sample was achieved with a buffer containing SDS micelles and a

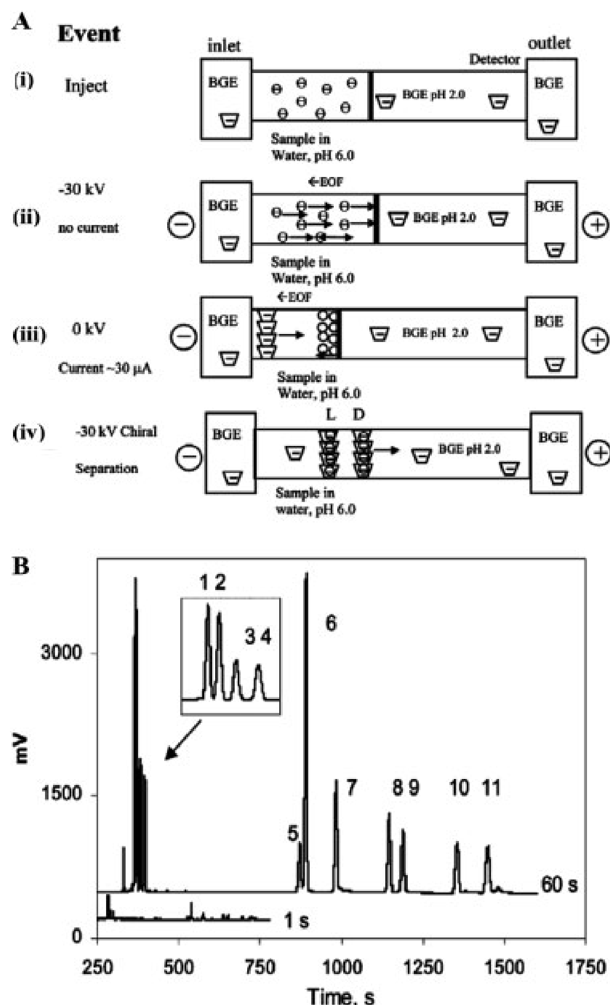

Figure 3. (A) Schematic diagram of the stacking/sweeping: (i) hydrodynamic injection of large volume (1/3 of the capillary) of the CBI–amino acids in water at pH 6.0; (ii) migration toward the pH junction at the outlet side of the injection plug of the anionic CBI–amino acids; (iii) pumping water out of the capillary and movement of the stacked analyte band toward the inlet by the EOF; (iv) sweeping of the HS-β-CD through the stacked band of analyte. (B) Electropherogram showing the potential of stacking/sweeping-EKC combination to enantioseparation of a complex sample of CBI–amino acids (~0.5 μM each). Electrophoretic conditions: fused-silica capillary, 70 cm total length (45 cm detector length) and 25 μm id; separation buffer, 25 mM phosphate buffer (pH 2.0) containing 2% HS-β-CD; applied voltage, −30 kV; hydrodynamic injection, 380 mbar for 180 s. LIF detection with λ_{exc} at 420 nm. Peak identification: 1, CBI-D-arginine (Arg); 2, CBI-L-Arg; 3, CBI-D-histidine (His); 4, CBI-L-His; 5, CBI-glycine (Gly); 6, CBI-L-tyrosine (Tyr); 7, CBI-L-glutamine (Glu); 8, CBI-D-Ser; 9, CBI-L-Ser; 10, CBI-L-Glu; 11, CBI-D-Glu (adapted from ref. [28] with permission).

© 2008 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

neutral CD as chiral selector [12]. Sweeping was performed by preparing the sample in a matrix without micelles but otherwise similar to the running buffer, and prolonging the sample zone by applying longer injection times. In this way, when voltage was applied, micelles sweep the analyte in a narrow band producing a sample preconcentration.

Table 2 also includes two works where a single-step method that combines in-capillary SPCD was developed for the enantioselective determination of amino acids [29] and the bacterial biobacters muramic acid and diaminopimelic acid [30] in *Escherichia coli* bacterial culture using CE with UV detection.

The effective separation of eight amino acid enantiomers using in-capillary derivatization with orthophthalaldehyde (OPA)/*N*-acetyl L-cysteine (NAC) was performed [29]. In comparison with conventional CE, SPCD provided a 40-fold improvement in concentration sensitivity and permitted shorter total analysis times maintaining chiral resolution due to the lower average mobility of zwitterionic amino acids in the weakly acidic sample plug. Figure 4A shows the general principle of SPCD in CE for the separation of the enantiomers of the two amino acids, alanine (Ala) and glutamic acid (Glu). Briefly, the injection sequence was carried out by first rinsing the capillary with borate buffer that served as an optimal alkaline medium for analytes and OPA and NAC as derivatizing agents. A multiple sample hydrodynamic injection sequence was performed consisting of short concentrated plugs of NAC and OPA reagents positioned in between a long plug of dilute sample. OPA was injected at the back end of the sample plug because it is neutral and comigrates with the EOF, unlike the anionic chiral NAC coreagent that migrates with a slower apparent mobility. The sample was injected with low pressure dissolved in phosphate buffer to reduce the local electrophoretic mobility of weakly ionic metabolites in the sample to induce electrokinetic focusing. Consecutively, OPA and NAC zones comigrated and preconcentration sample zones resulted in the formation of diastereomeric isoindole adducts. Afterward, enantiomeric resolution of the adducts was achieved along with increasing band separation of all species. Although the separation of the diastereomers of the amino acids was possible without chiral additives (Fig. 4B), the addition of 1 mM β-CD allowed the resolution of the last amino acid without deterioring sample enrichment properties, but decreasing resolution of the two first migrating amino acids (Fig. 4C). The SPCD method developed integrated sample enrichment with chemical labeling steps directly within a single capillary during electromigration improving the concentration sensitivity but retaining high-resolution chiral separations. LODs of 2×10^{-7} M for muramic acid, and 2×10^{-6} M for diaminopimelic acid, were achieved by this method. Nevertheless, in this case, the use of increasing concentrations of β-CD as a neutral chiral selector to the run buffer was unsuccessful for further improving of diaminopimelic acid resolution [30].

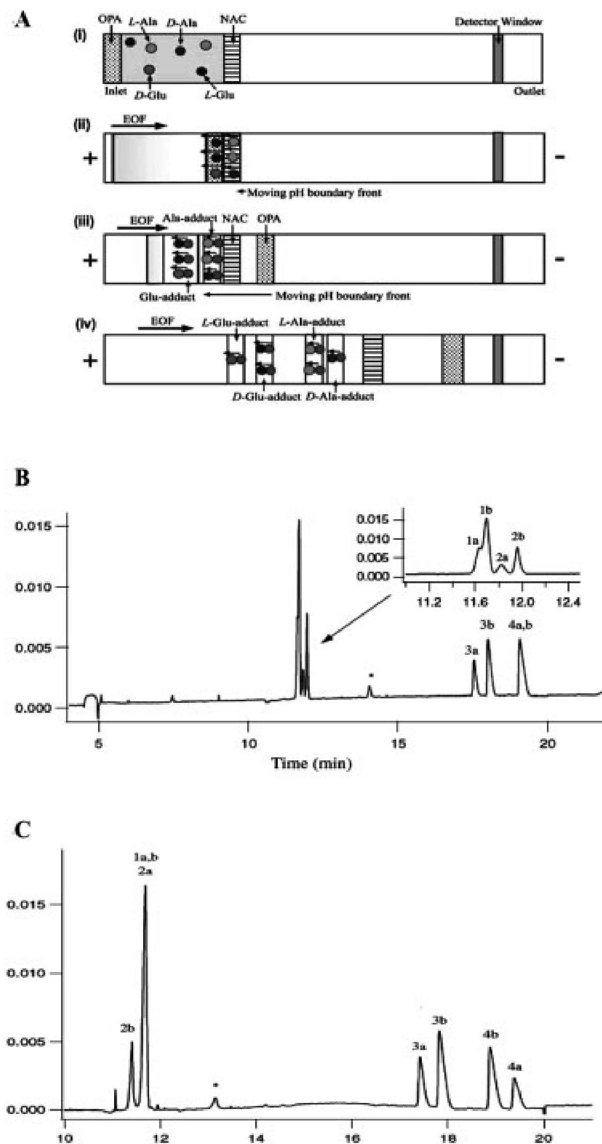

Figure 4. (A) General principle of SPCD–CE for single-step enantioselective analysis of submicromolar levels of amino acids: (i) multiple hydrodynamic injection sequence; (ii) on-line sample preconcentration; (iii) in-capillary chemical labeling by zone passing of OPA/NAC; (iv) chiral separation of diastereomeric amino acid adducts formed. (B) Electropherogram of eight amino acid enantiomers using in-capillary OPA/NAC derivatization of amino acid adducts by SPCD–CE. (C) Electropherogram demonstrating the use of 1 mM β-CD as a chiral additive to enhance the enantioselectivity by dynamic inclusion complexation of amino acid adducts by SPCD–CE. Electrophoretic conditions: fused-silica capillary, 65 cm total length and 50 μm id; separation buffer, 140 mM borate buffer (pH 9.5) containing 1 mM β-CD for Fig. (C); separation temperature, 25°C; applied voltage, 25 kV; hydrodynamic injection, 35 mbar × 100 s; UV detection at 340 nm. Sample solutions contained 25 and 50 μM of the D- and L-amino acids, respectively. Analyte peak numbers correspond to amino acid–isoindole adducts: 1a, D-Ser; 1b, L-Ser; 2a, D-Ala; 2b, L-Ala; 3a, D-Glu; 3b, L-Glu; 4a, D-Asp; 4b, L-Asp; *, OPA hydrolysis products (adapted from ref. [29] with permission).

© 2008 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

2.3 Alternative detection systems to on-column UV–Vis absorption detection for the enhancement of the sensitivity in chiral analysis by CE

UV–Vis absorbance detection is the first option to be considered in CE given its interesting features such as commercial availability, simplicity, versatility, relatively low cost, and frequent use as universal detection technique because many organics can be detected at 195–210 nm. This detector produces LODs in CE corresponding to a few femtomoles of analyte (at subpicomole levels), *i.e.*, high mass sensitivity. However, due to the need of small volumes employed in CE to avoid peak broadening that decrease the efficiency of the separation (pL to nL volumes), such sensitivity is in the micromolar range, *i.e.*, appears modest when expressed in terms of concentration (LODs ranging from 10^{-5} to 10^{-7} M depending upon the analyte being analyzed). These LODs are clearly insufficient to solve many analytical problems. One option to overcome the poor concentration sensitivity obtained in CE is the selection of low-UV wavelengths (190–205 nm), where this detection exhibits the best sensitivity, possibility used in several works summarized in Table 1.

Another option to improve detection sensitivity in CE is the use of alternative detection systems. During the period of time reviewed in this article (see Table 3), LIF and MS detection systems were mainly employed. LIF system is configured to make the detection directly on the separation capillary (on-column detection), such as UV–Vis absorption detection, but the second system is connected to the end of the capillary (end-column detection). In addition, electrochemiluminescence (ECL) was also employed.

2.3.1 On-column detection in chiral analysis by CE

LIF detection is one of the most sensitive on-column detection systems currently available in which it is possible to use several lasers, the common argon laser (458 and 488 nm), the He-Cd laser (440 nm), or the blue diode laser (420 nm). Since only a few chiral compounds possess native fluorescence, derivatization procedures are usually required to detect other nonfluorescent analytes. LIF was used for the chiral determination of photosensitizers [32], drugs in human plasma [33], and anticancer agents in drugs [34] achieving LODs around 10^{-8} M. However, the main application of this detection system in CE was the analysis of amino acid enantiomers in standards [35], foods [36], and biological samples [28, 37–42]. In all cases, the methods involved precapillary chiral derivatization of the amino acids with different derivatizing reagents, FITC [35, 36], 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F) [37], CBI [28, 38–41], or fluorescamin (FA) [42].

Chiral and sensitive analysis of amino acids is a remarkable methodology that can provide important information on adulteration and quality of food products [49]. For example, analysis of chiral amino acids in vinegars was shown to be a powerful method to detect different adulterations including the detection of synthetic vinegars [50]. Thus, several D-amino acids were detected and quantified in vinegars by MEKC-LIF, observing interesting differences in their L- and D-amino acid profiles and contents [36]. LODs lower than ~20 nM were achieved using FITC as derivatizing reagent. This fluorescent label has been frequently chosen since its excitation wavelength matches the 488 nm light of the argon laser, and the derivatives are easily formed and generate strong fluorescence signals [51].

Several improved methods were developed for the sensitive determination of D-serine (Ser) in neural samples [28, 37–39]. This molecule is a primary endogenous amino acid that binds to the glycine site of *N*-methyl-D-aspartate (NMDA) receptor involved in a variety of physiological functions and disorders including memory, learning, pain, and ischemia. The sensitive detection of this amino acid is essential, because it is at low concentration in biological samples. Thus, two different EKC methods with LIF detection for the chiral separation of D/L-Ser were developed and applied to detect D-Ser in higher vertebrates, such as rats, achieving LODs about 10^{-7} M [37, 38]. In one of the methods, a saccharide [D-(+)-glucose] was added to the separation buffer in order to enhance the chiral recognition of 2-hydroxypropyl-γ-CD (HP-γ-CD) [38]. On the other hand, a procedure for the determination of D-Ser in squirrel brain was proposed to achieve a very high sensitivity (LODs up to 10^{-10} M) [28]. In this case, the process involved two preconcentration techniques (stacking and sweeping), as has previously been commented. By this method, in addition to L-Ser, appreciable levels of D-Ser, L-aspartate (Asp), and L-glutamate (Glu) were observed in microdialysate from the hippocampus of artic ground squirrels, while D-Asp and D-Glu were below the LOD. The enantioseparations were accomplished with HS-β-CD as chiral selector at low pH and reverse polarity.

D-Ser was also detected in invertebrates [38, 39]. Thus, Zhao *et al.* [39] studied the contents of D-Ser in *Aplysia californica*, a sea mollusc widely used as neuronal model. The separation of CBI-D/L-Ser enantiomer was achieved by using a dual chiral selector system consisting of β-CD and chiral micelles formed by DOC. For the first time, peaks corresponding to L-Ser and D-Ser were well identified in *Aplysia* ganglian homogenates. It was noticed that while the levels of L-Ser were similar, D-Ser levels varied substantially from animal to animal. Interestingly, D-Ser was not detected in single neurons isolated from *Aplysia* ganglia due to D-Ser might perhaps not occur in neurons of *Aplysia* or the contents in single neurons were too low to be detected. This lack of sensitivity was reported also in other work of the same authors [38].

D-Asp was also detected in the central nervous system of *A. californica* [40, 41]. There are some indications that D-Asp is a neuromodulator, hormone, or even a precursor for the endogenous synthesis of the NMDA receptor. Miao *et al.* [40] demonstrated an approach for the quantitative investigation

© 2008 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Table 3. Alternative detection systems to on-column UV–Vis absorption detection employed for sensitive chiral analysis by CE

Detection System	CE Mode	Analyte and sample	Separation buffer	LOD (M)	Ref.
LIF (λ_{exc} = 422/488 nm, λ_{em} = 690 nm)	CD-MEKC	Porphyrin and phthalocyanines in standards	200 mM borate (pH 9.2) + 10 mM HP-β-CD + 10 mM SDS	$<3\times10^{-8}$	[32]
LIF (λ_{exc} = 442 nm, λ_{em} = 500 nm)	CD-EKC	CBI-Baclofen in human plasma	50 mM borate (pH 9.5) + 2% HS-β-CD	5×10^{-8}	[33]
LIF (λ_{exc} = 320 nm, λ_{em} = 380–600 nm)	CD-EKC	Homocamptothecin derivatives in drugs	75/25 mM phosphate (pH 2.5) + 7.5% w/v HS-β-CD/2.5% w/v HS-β-CD	$\sim10^{-8}$	[34]
LIF (λ_{exc} = 488 nm, λ_{em} = 520 nm)	EKC	FITC-amino acids (Glu, Pro) in standards	50 mM acetate (pH 6.1) + 1.25 mM vancomycin	$<10^{-5}$	[35]
LIF (λ_{exc} = 488 nm, λ_{em} = 520 nm)	CD-MEKC	FITC-amino acids (Arg, Pro, GABA, Ala, Glu, Asp) in vinegars	100 mM borate (pH 9.7) + 20 mM β-CD + 30 mM SDS	$\sim2\times10^{-8}$	[36]
LIF (λ_{exc} = 457.9 nm)	CD-EKC	NBD-F-D-Ser in rat brain	100 mM borate (pH 10.0) + 40 mM HP-β-CD	3×10^{-7}	[37]
LIF (λ_{exc} = 457.9 nm)	CD-EKC	CBI-D-Ser in rat brain and mollusc neurons	60 mM borate (pH 10.0) + 2 M urea + 15% D-glucose + 20 mM HP-γ-CD	10^{-7}	[38]
LIF (λ_{exc} = 420 nm)	CD-EKC	CBI-Ser, CBI-Glu in hippocampus of squirrel	25 mM phosphate (pH 2.0) + 2% HS-β-CD	$\sim10^{-10}$	[28]
LIF (λ_{exc} = 457.9 nm)	CD-MEKC	CBI-D-Ser in mollusc neurons	100 mM borate (pH 9.5) + 30 mM β-CD + 60 mM DOC	3×10^{-8}	[39]
LIF (λ_{exc} = 457.9 nm)	CD-MEKC	CBI-D-Asp in mollusc neurons	50 mM borate (pH 9.4) + 20 mM β-CD + 50 mM SDS	5×10^{-10}	[40]
LIF (λ_{exc} = 457.9 nm)	CD-MEKC	CBI-D-Asp in mollusc neurons	50 mM borate (pH 9.4) + 20 mM β-CD + 50 mM SDS	$<5\times10^{-6}$	[41]
LIF (λ_{exc} = 457.9/488 nm)	CD-MEKC	FA-D-amino acid-containing neuropeptides in mollusc neurons	50 mM borate (pH 9.4) + 20 mM γ-CD + 50 mM SDS	8×10^{-8}	[42]
Fluorescence (λ_{exc} = 327 nm, λ_{em} = 557 nm)	CD-EKC	DNS-DL-Glufosinate in spiked river water	2.0 mM phosphate (pH 6.5) + 17 mM γ-CD	2×10^{-9}	[16]
MS	CD-EKC	Amphetamine derivatives (A, MA, MDA, MDMA, MDEA, E, NE) in plasma	20 mM ammonium formate (pH 2.5) + 0.15% HS-γ-CD	$\sim7\times10^{-7}$–3×10^{-6}	[21]
MS	CD-EKC	Amphetamine derivates (A, MA, MDA, MDMA, MDEA, TMD, MTD) in plasma	20 mM ammonium formate (pH 2.5) + 0.15% HS-γ-CD	4×10^{-9}	[22]
MS	CD-EKC	MA, AP, DMA, E, NE, ME in human urine	1 M formic acid (pH 1.7) + 0.85 mM DAS-β-CD	$\sim6\times10^{-8}$–10^{-7}	[43]
MS	MEKC	β-Blockers (Ate, Met, Pin, Oxp, Alp, Pro, Car, Tal) in standards	25 mM ammonium acetate + 25 mM TEA (pH 8.0) + 15 mM poly-L-SUCL	9×10^{-7}–7×10^{-6}	[44]
MS	MEKC	Lorazepam, oxazepam, and nefopam in standards	25 mM ammonium acetate (pH 8.0) + 15% ACN + 15 mM poly-L-SUL	$\sim6\times10^{-6}$	[45]
		Lorazepam and oxazepam in standards	25 mM ammonium acetate (pH 8.5) + 15 mM poly-LL-SUCLV		

© 2008 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Table 3. Continued

Detection System	CE Mode	Analyte and sample	Separation buffer	LOD (M)	Ref.
MS	MEKC	Pseudoephedrine in human urine	15 mM TEA + 15 mM ammonium acetate (pH 2.0) + 20% ACN + 35 mM poly-L-SUCLS	$\sim 2 \times 10^{-6}$	[46]
MS	CD-NACE	Salbutamol in human urine	10 mM ammonium formate (0.75 M formic acid) + 15 mM HDAS-β-CD	3×10^{-8}–6×10^{-8}	[15]
MS	CEC	β-Blockers (atenolol, metoprolol, pindolol, oxprenolol, alprenolol, propranolol, carteolol, talinolol)	Mobile phase: MeOH/ACN/HOAc /TEA (70:30:1.6:0.2 by volume) PCC: 3 μm vancomycin CSP	$\sim 3 \times 10^{-8}$	[47]
MS	CEC	Warfarin and β-blockers (atenolol, metoprolol, oxprenolol, propranolol)	Mobile phase: MeOH/ACN/HOAc/TEA (70:30:1.6:0.2 by volume) PCC: 3 μm vancomycin CSP	$\sim 3 \times 10^{-8}$	[48]
ECL	CD-EKC	Disopyramide in spiked plasma sample	40 mM acetate (pH 4.5) + 3 mg/mL HS-β-CD	8×10^{-8}–10^{-7}	[23]

DMA, dimethylamphetamine; HP-γ-CD, 2-hydroxypropyl-γ-CD; HS-γ-CD; highly sulfated-γ-CD; KCN, cyanide; ME, methylephedrine; *m*-HBA, *m*-hydroxybenzoic acid; OTC, open tubular column; PCC, packed capillary column; poly-L-SUCL, poly(sodium *N*-undecenoxy carbonyl-L-leucinate); poly-L-SUL, poly(sodium *N*-undecenoyl-L-leucinate).

of the biochemical composition of subcellular regions of single neurons. In this method, the analyte was derivatized with naphthalene-2,3-dicarboxaldehyde (NDA) previous to CD-MEKC analysis with LIF detection. The excellent LOD of this system (5×10^{-10} M) and the relatively high concentration of D-Asp in some regions of the central nervous system allowed the dilution of tissue samples with buffer while maintaining an adequate S/N ratio. The results further demonstrated that D-Asp was present in the processes of individual neurons and morphologically distinct regions of the same neuron exhibited similar ratios of D-Asp demonstrating that D-Asp was not restricted to the nucleus of neurons from *A. californica* (see Fig. 5).

However, although CE with LIF detection has demonstrated to be a powerful tool for the analysis of samples ranging from tissue extracts to single cells, one challenge for the chiral CE analysis of complex biological samples is the accurate peak identification in complex electropherograms, as often matching a migration time between an analyte and the corresponding standard may be insufficient to confirm the peak's identity. Thus, since MS may not be sensitive enough to determine the identity of a metabolite in samples from individual cells or subcellular compartments, Miao *et al.* [41] reported a method to confirm the D-Asp signal identity which combines single-step immunoprecipitation using a specific D-Asp antibody and CD-MEKC-LIF analysis described in [40]. The addition of anti-D-Asp serum to the sample resulted in the disappearance of the D-Asp peak from the electropherogram, thus validating the D-Asp peak assignment in electropherograms obtained from biological tissues and single cells of *A. californica*.

Finally, conventional fluorescence was also used for the determination of glufosinate enantiomers in a model sample of river water spiked with this herbicide [16]. Interestingly, LODs reported in this work were as good as those generally obtained by LIF detection, because two preconcentration strategies (SPE and stacking) were used as has previously been stated.

2.3.2 End-column detection in chiral analysis by CE

CE coupled to MS detection has become a powerful analytical tool that allows combining the separation speed, high resolving power, and minimum sample consumption of CE with the selectivity and structural information provided by MS. This hyphenation enables to obtain information on the solute's molecular weight and structure and may enhance the sensitivity of certain compounds depending on their characteristics (proton affinity, absorptivity, *etc*.). In the last 10 years, there were many significant developments in CE-MS instrumentation and applications that have made CE-MS a competitive tool [9, 52].

An important aspect to be considered when a chiral analysis is going to be performed by CE-MS is the separation mode because chiral selectors are necessary. They are generally nonvolatile molecules, whose introduction into the MS

© 2008 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

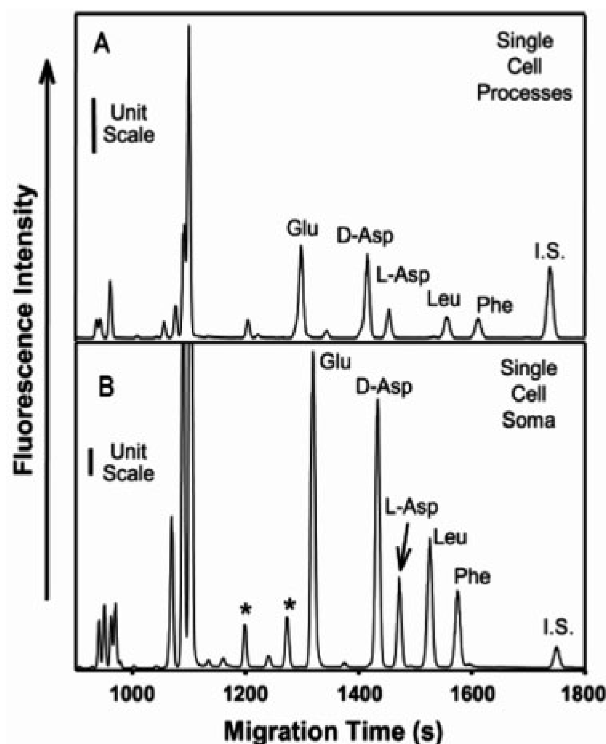

Figure 5. Detection of D-Asp in the processes of sensory neurons from *A. californica*. Electropherograms of D-Asp in (A) the processes and (B) the soma from a single pleural sensory neuron. Electrophoretic conditions: fused-silica capillary, 65–75 cm total length and 50 μm id; separation buffer, 50 mM borate buffer and 50 mM SDS (pH 9.4) containing 20 mM β-CD; applied voltage, 21 kV; electrokinetic injection, 5 kV × 3 s; LIF detection with λ_{exc} at 457.9 nm. Peak identities: Glu, glutamate, with a comigrating compound; D-Asp, D-aspartate; L-Asp, L-aspartate; Leu, leucine; Phe, phenylalanine; IS, internal standard, L-cysteic acid; *, unidentified peaks (reprinted from ref. [40] with permission).

system should be avoided because they damage electrospray efficiency, and increase background noise decreasing the sensitivity of detection [53]. The group of works collected in this review has been organized taking into account the different separation modes because the strategies employed depend on the working mode. Thus, works in which the chiral selector was used as a pseudophase in the separation media (EKC, MEKC, and NACE modes) will be described first followed by the use of chiral selectors immobilized in a stationary phase (CEC mode), which does not affect MS sensitivity.

In all EKC-ESI-MS methods described in Table 3, the introduction of the chiral selector in the MS detector was avoided. The different strategies used to avoid the entrance of the chiral selector into the mass analyzer, preventing a deterioration of the sensitivity, were the partial filling of the capillary with the separation media containing the chiral selector in combination with anionic CDs migrating in countercurrent [21, 22] or the use of anionic CDs migrating in countercurrent with suppressed EOF [43]. Table 3 reveals that LODs reached in works using EKC-ESI-MS were comprised from 3×10^{-5} to 4×10^{-9} M.

Rudaz *et al.* used the countercurrent migration of the negatively charged highly sulfated-γ-CD (HS-γ-CD) and the partial-filling technique to achieve the enantioseparation of amphetamine derivatives [21, 22]. The countercurrent migration technique is based on the control of the apparent mobility of the chiral selector to avoid that it reaches the MS detector, whereas in the partial-filling technique, a zone of the capillary (where the enantiomeric separation takes place) is filled with BGE containing the chiral selector while the zone close to the MS detector only contain buffer without chiral selector. The use of this strategy with a low concentration of HS-γ-CD in normal polarity mode allowed the rapid stereoselective separation of seven amphetamine derivatives in spiked plasma samples in analysis times less than 6 min (see Fig. 6) [21]. In a later work [22], an electrokinetic injection was tested to enhance the detection sensitivity. However, the mobility of the chiral selector opposite to the analyte

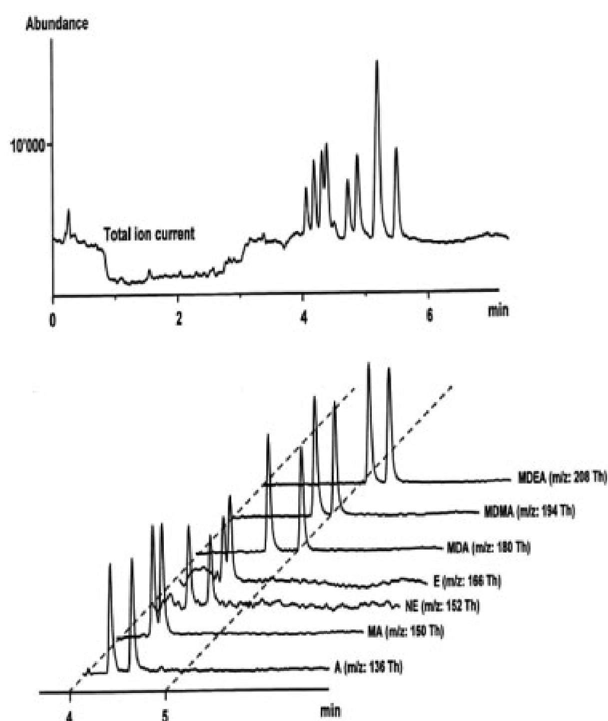

Figure 6. Chiral analysis of seven amphetamine derivatives with low concentration of HS-γ-CD and MS detection in spiked plasma sample after LLE. Electrophoretic conditions: fused-silica capillary, 64.5 cm total length (56 cm detector length) and 50 μm id; separation buffer, 20 mM ammonium formate (pH 2.5) containing 0.15% HS-γ-CD; separation temperature, 20°C; applied voltage, 25 kV; hydrodynamic injection, 250 mbar × s; UV detection at 200 nm. MS conditions: sheath liquid, isopropanol/water (50:50 v/v) with 0.1% formic acid; syringe flow rate, 3 μL/min. ESI capillary at 4.5 kV. Nebulizing pressure and drying gas flow rate, 4 psi and 4 L/min, respectively. Gas temperature, 200°C; fragmentor, 70 V (reprinted from ref. [21] with permission).

© 2008 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

could lead to numerous drawbacks during the electric field application. Thus, the voltage used to introduce cationic analytes into the capillary started the displacement of negatively charged CD within the capillary to the injection vial. Besides a sample contamination, the chiral selector quantity in the capillary decreased in an uncontrolled manner and the analysis time variability increased. To avoid this drawback, the introduction of an appropriate buffer plug length between the zone containing the chiral selector and the analyte injection was necessary. These conditions were applied to plasma samples after LLE and a concentration of 4×10^{-9} M of each enantiomer was detected (see Fig. 1). To the best of our knowledge, this sensitivity was the highest obtained for a chiral determination of drugs in plasma by CE-MS with a single quadrupole MS as detection device.

The use of another anionic CD, the heptakis(2,6-diacethyl-6-sulfato)-β-CD (DAS-β-CD), which moves to the inlet of the capillary and does not flow into the MS detector was used in a selective EKC-ESI-MS method for the simultaneous chiral separation of six drugs [43]. In this work, the EOF was also suppressed by adjusting the pH value of the electrolyte to less than 3. This method was applied to the analysis of human urine samples obtaining sufficient sensitivity (LODs from 10^{-7} to 6×10^{-8} M) to detect the concentrations of methamphetamine (MA), 3,4-methylenedioxyamphetamine (MDA), and amphetamine (AT) in addicts.

On the other hand, polymeric surfactants (also called molecular micelles or micelle polymers) are very attractive as alternative pseudostationary phases to conventional micelles for the coupling of the MEKC separation mode with ESI-MS [44–46]. They provide several advantages over conventional micelles for this hyphenation [44, 45]: (i) the covalent bonds between surfactant monomers are difficult to ionize in the electrospray resulting in less-background noise from surfactant monomers of low molecular weights in MEKC-MS applications, (ii) micellar solutions can be used at any polymer concentration due to zero CMC, consequently higher S/N are observed in MEKC-ESI-MS, and (iii) polymeric surfactants have lower surface activity and lower volatility, and are stable in the presence of a high content of organic modifier in the BGE, which also tends to enhance the ESI-MS intensity.

A new polymeric surfactant, poly(sodium *N*-undecenoxy carbonyl-L-leucinate) (poly-L-SUCL) was synthesized by Shamsi *et al.* and employed in a MEKC system [46]. In this work, poly-L-SUCL was used as chiral selector with tandem UV and MS detections and applied for the simultaneous enantioseparation of eight structurally similar β-blockers, weakly basic compounds having similar p*K*a values but different hydrophobic character and *m/z* values (see Fig. 7). MEKC-ESI-MS (LODs from 7×10^{-6} to 9×10^{-7} M) provided ~16 times better S/N ratio than MEKC-UV (LODs from 1.3×10^{-5} to 2×10^{-6} M) and high resolution. However, as it can be seen in Fig. 7, longer separation times were obtained by MS detection due to MS detection was performed at the end of the capillary (120 cm), whereas UV detection was

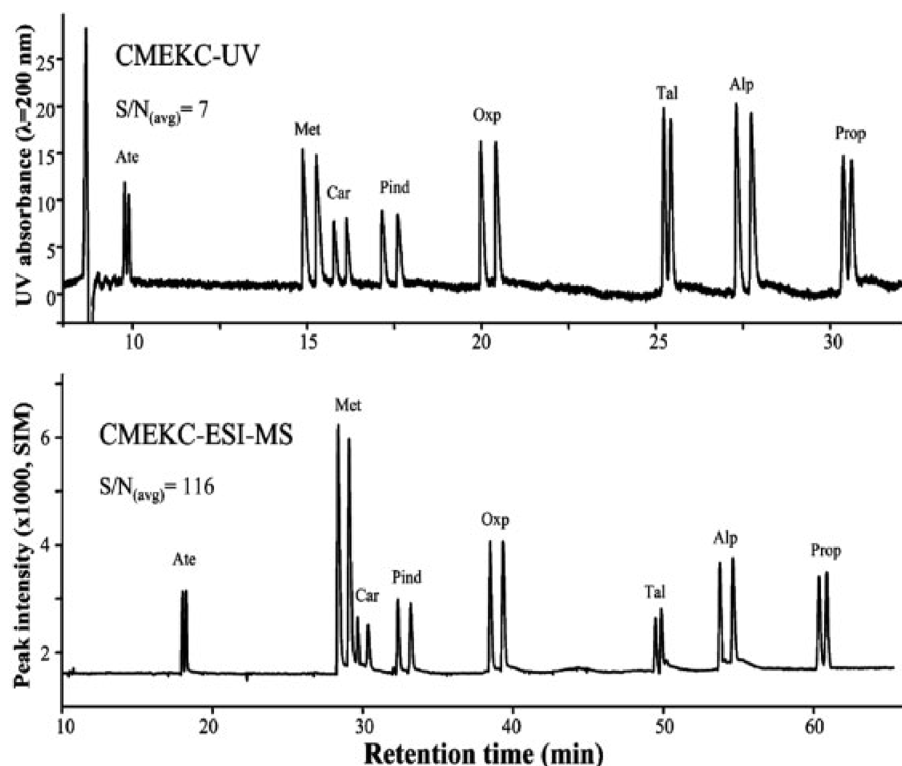

Figure 7. Electropherograms illustrating simultaneous UV (top) and MS (bottom) detection of β-blockers. Electrophoretic conditions: fused-silica capillary, 120 cm total length and 50 μm id; separation buffer, 20 mM ammonium acetate and 20 mM TEA (pH 8.5) containing 25 mM poly-L,L-SUCLV; separation temperature, 20°C; applied voltage, 30 kV; hydrodynamic injection, 50 mbar for 1 s. MS conditions: sheath liquid, 40 mM ammonium acetate in 80% v/v methanol (pH 8.0); syringe flow rate, 5 mL/min. ESI capillary at 2.5 kV. Nebulizing pressure and drying gas flow rate, 4 psi and 5 L/min, respectively. Gas temperature, 200°C; fragmentor, 85 V. Identification of peaks: (±)-atenolol (Ate), (±)-metoprolol (Met), (±)-pindolol (Pin), (±)-oxprenolol (Oxp), (±)-alprenolol (Alp), (±)-propranolol (Pro), (±)-carteolol (Car), and (±)-talinolol (Tal) (reprinted from ref. [44] with permission).

© 2008 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

achieved at half capillary length (60 cm), where the on-column detection window was placed. The same research group evaluated six amino acid-based polymeric surfactants for enantioseparation and ESI-MS detection of two benzodiazepines (oxacepam and lorazepam) and one benzoxazocine (nefopam) [45] in order to have further information on the applicability of polymeric surfactants. This study showed that polymeric surfactants are a promising class of chiral selectors for MS, which combined powerful chiral recognition in MEKC and high sensitivity of MS. Simultaneous enantioseparation of all three chiral analytes was achieved using poly(-sodium *N*-undecenoyl-L-leucinate) (poly-L-SUL) with 15% ACN in the BGE. Only partial chiral resolution and relatively low S/N for benzodiazepines were obtained. However, the use of a dipeptide polymeric surfactant, poly(sodium *N*-undecenoxy carbonyl-LL-leucyl-valinate) (poly-LL-SUCLV), provided the best MEKC conditions for chiral recognition of the two benzodiazepines.

Recently, Rizvi *et al.* [46] synthesized and compared three polymeric sulfated chiral surfactants for the enantioseparation of a broad range of structurally diverse racemic compounds (phenylethylamines, β-blockers, 2-(2-chlorophenoxy)propionic acid, benzoin derivatives, PTH–amino acids, and benzodiazepines) at very acidic, neutral, and basic pH conditions in MEKC using UV detection. In addition, a sensitive MEKC-MS method using poly(sodium *N*-undecenoxy carbonyl-L-leucine) sulfate (poly-L-SUCLS) was developed for one of the phenylethylamines (pseudoephedrine) in human urine. The LOD obtained at pH 2.0 was ~16 times lower than that obtained at pH 8.0. This work demonstrated for the first time the superiority of chiral separation and sensitive MS detection at low pH over conventional high pH, when anionic polymeric surfactants in MEKC-MS are employed. This fact can be understood taking into account that the sulfated polymeric surfactants are also negatively charged at low pH and the ionization efficiency of the MS, in the positive ionization mode, is higher when acidic solutions are used.

The elimination of the aqueous media in NACE can provide additional selectivity with respect to that obtained in aqueous CE, and favors the analysis of solutes with poor water solubility. In addition, due to the high volatility and low surface tension of organic solvents like methanol or ACN, the use of these media in ESI-MS may enhance ionization, resulting in improved detection limits compared to separation in aqueous buffer systems. The on-line coupling of NACE and ESI-MS was achieved to determine low levels of the enantiomers of a basic chiral drug (salbutamol) in biological samples [15]. The selected BGE (methanol acidified with formic acid containing heptakis(2,3-di-*O*-acetyl-6-*O*-sulfo)-β-CD (HDAS-β-CD)) offered good possibilities to be directly applied for MS coupling since the BGE contained volatile solvents and the nonvolatile CD migrated toward the capillary inlet (countercurrent technique) away from the MS detector. LODs were 3×10^{-8} M for the first enantiomer and 6×10^{-8} M for the second one.

CEC was also hyphenated to ESI-MS for the chiral analysis of drugs [47, 48]. In CEC, the separation was carried out mainly in a packed capillary column (PCC). Thus, Zheng and Shamsi [47] showed the feasibility of using a vancomycin CSP for the simultaneous enantioseparation of eight β-blockers by CEC-ESI-MS. Internally tapered capillaries were employed to pack CEC-MS columns (see Fig. 8A), which were fabricated by a novel procedure described by the same research group [48]. A comparison between external and internal tapered capillaries for on-line CEC-ESI-MS showed that the latter provided enhanced electrospray stability, sensitivity and robustness resulting in significantly lower noise [48]. Other problems associated with external tapering were successfully overcome with the internal taper, such as, the poor durability and tip breakage, as well as the possibility of using a harsh polar organic mobile phase, which was not previously successful using an external taper due to higher operating current and electrospray arcing. Next, data on the

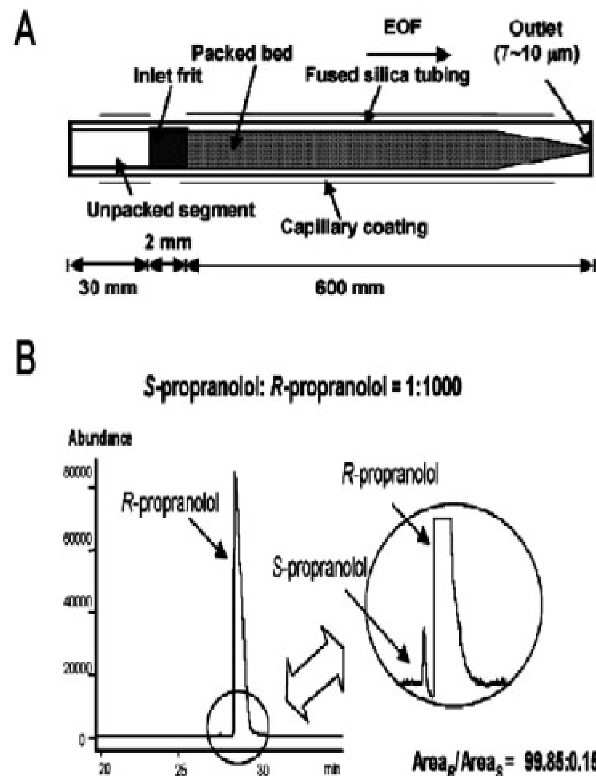

Figure 8. (A) Schematic of an internally tapered CEC/MS column showing dimension of packed and unpacked segments. (B) Electropherogram showing the analysis of the nonracemic mixture of propranolol. Electrophoretic conditions: fused-silica capillary, 63 cm total length and 75 µm id; mobile phase, MeOH/ACN/HOAc/TEA (70:30:1.6:0.2 by volume); stationary phase, 3 µm vancomycin CSP; applied voltage, 25 kV; electrokinetic injection, 5 kV for 60 s. MS conditions: sheath liquid, MeOH/H_2O (90:10 v/v) containing 50 mM NH_4OAc; syringe flow rate, 5.0 µL/min. ESI capillary at 3.0 kV. Nebulizing pressure and drying gas flow rate, 4 psi and 5 L/min, respectively. Gas temperature, 130°C; fragmentor, 80 V (reprinted from ref. [48] with permission).

© 2008 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

reproducibility of this CEC-MS system using warfarin and β-blockers as model analytes were presented. Last, the applicability of performing quantitative CEC-MS with these PPCs was demonstrated for the simultaneous enantioseparation of β-blockers achieving LODs $\sim 3 \times 10^{-8}$ M [47, 48]. Figure 8B shows the sensitive detection of one enantiomer (the pharmacological active *S*-propranolol) at 0.1% in *R*-propranolol, which is the limit above which impurities have to be identified according to ICH guidelines [1].

ECL was also used as an attractive detection method for chiral CE [23]. This detection technique has comparative sensitivity to LIF and MS detection. Moreover, it has other predominant qualities, including low equipments costs, ease to operate, high selectivity and no need for derivatization. In this end-column CE-ECL detection, the buffers of capillary inlet (separation buffer) and capillary outlet (detection solution) differed from each other in terms of pH value, the nature of the electrolyte, the ionic strength and the buffer composition. To achieve better detection sensitivity and good enantioresolution at the same time, the conditions of both buffers were systematically optimized. The baseline chiral separation and highly sensitive detection of disopyramide (a racemic antiarrhythmic drug) was achieved with LODs about 10^{-7} M for the enantiomers. This chiral method was applied to the enantioseparation of the racemic drug in spiked plasma samples after LLE (see Fig. 9). It can be observed that the enantiomers were successfully separated and no interferences appeared with ECL detection.

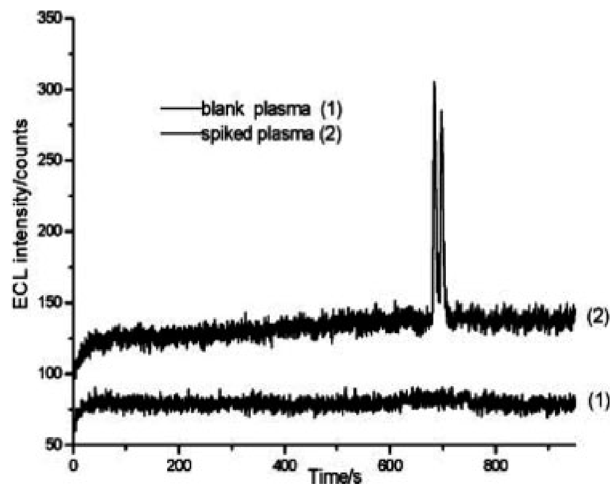

Figure 9. Electropherograms of extracts of blank plasma (1) and plasma spiked with a concentration of 4 mM racemic drug (2). Electrophoretic conditions: fused-silica capillary, 60 cm total length and 50 μm id; separation buffer, 40 mM acetate buffer (pH 4.5) containing 3 mg/mL HS-β-CD; applied voltage, 22 kV; hydrodynamic injection, 35 mbar for 5 s (reprinted from ref. [23] with permission).

3 Concluding remarks

Due to the low concentration sensitivity obtained in CE with UV–Vis absorption detection, limited by the path length of the capillary (usually 50–75 μm), different strategies were proposed to increase this detection sensitivity. In addition to the use of off-line extractive sample treatment techniques (SPE, SPME, LLE), different in-capillary preconcentration techniques based on electrophoretic phenomena can be employed in CE. They enabled important concentration sensitivity enhancements keeping good enantiomeric resolution. Other strategies employed the use of alternative detection systems to the UV–Vis detector. On-column and end-column detection systems were employed. On-column LIF detection in combination with in-capillary preconcentration techniques (LVSS and sweeping) provided the best detection sensitivity reported in this review (LODs up to 10^{-10} M). However, a derivatization step was needed for most applications reported in this article on the use of LIF as detection system. End-column MS detection is being increasingly used in chiral analysis of complex matrices by CE, mainly for biological samples, since the hyphenation CE-MS allows selective discrimination of enantiomers which have the same *m/z* ratio. However, MS detection not always enabled to improve the detection sensitivity with respect to UV–Vis detectors, results depending on analyte properties. Attempts to use other detection systems included ECL detection which showed good sensitivity when combined with LLE and no derivatization was needed.

A combination of the variety of strategies described in this article is considered the most promising way to perform sensitive chiral analysis by CE.

The authors thank the Ministerio de Educación y Ciencia (Spain) for the research project CTQ2006-03849/BQU and the Comunidad Autónoma de Madrid (Spain) for project S-0505/AGR/0312. Carmen García-Ruiz thanks the Ministerio de Ciencia y Tecnología (Spain) for the Ramón y Cajal program (RYC-2003-001). Laura Sánchez-Hernández thanks the Comunidad Autónoma de Madrid for her research contract.

4 References

[1] ICH Q3A, International Conference of Harmonisation, Impurities in New Drug substances. Step 4 version, dated 25 October 2006. http://www.ich.org/LOB/media/MEDIA422.pdf.

[2] Chankvetadze, B., *J. Chromatogr. A* 1997, *792*, 269–295.

[3] Chankvetadze, B., *Trends Anal. Chem.* 1999, *18*, 485–498.

[4] Chankvetadze, B., Blaschke, G., *J. Chromatogr. A* 2001, *906*, 309–363.

[5] Gübitz, G., Schmid, M. G., *Electrophoresis* 2007, *28*, 114–126.

[6] Geiser, L., Veuthey, J. L., *Electrophoresis* 2007, *28*, 45–57.

[7] García-Ruiz, C., Marina, M. L., *Electrophoresis* 2006, *27*, 195–212.

[8] Ha, P. T. T., Hoogmartens, J., Van Schepdael, A., *J. Pharm. Biomed. Anal.* 2006, *41*, 1–11.

© 2008 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

[9] Erny, G. L., Cifuentes, A., *J. Pharm. Biomed. Anal.* 2006, *40*, 509–515.

[10] Natishan, T. K., *J. Liq. Chromatogr. Relat. Technol.* 2005, *28*, 1115–1160.

[11] Hernández-Borges, J., Rodriguez-Delgado, M. A., Garcia-Montelongo, F. J., Cifuentes, A., *Electrophoresis* 2005, *26*, 3799–3813.

[12] Baldacci, A., Thormann, W., *J. Sep. Sci.* 2006, *29*, 153–163.

[13] Berzas-Nevado, J. J., Villasenor-Llerena, M. J., Guiberteau-Cabanillas, C., Rodriguez-Robledo, V., *Electrophoresis* 2006, *27*, 905–917.

[14] García-Ruiz, C., Alvarez-Llamas, G., Puerta, A., Blanco, E. *et al.*, *Anal. Chim. Acta* 2005, *543*, 77–83.

[15] Servais, A. C., Fillet, M., Mol, R., Somsen, G. W. *et al.*, *J. Pharm. Biomed. Anal.* 2006, *40*, 752–757.

[16] Asami, T., Imura, H., *Anal. Sci.* 2006, *22*, 1489–1493.

[17] Glowka, F. K., Karazniewicz, M., *Anal. Chim. Acta* 2005, *540*, 95–102.

[18] Cardoso, C. D., Jabor, V. A. P., Bonato, P. S., *Electrophoresis* 2006, *27*, 1248–1254.

[19] Afshar, M., Thormann, W., *Electrophoresis* 2006, *27*, 1517–1525.

[20] Theurillat, R., Knobloch, M., Levionnois, O., Larenza, P. *et al.*, *Electrophoresis* 2005, *26*, 3942–3951.

[21] Rudaz, S., Geiser, L., Souverain, S., Prat, J. *et al.*, *Electrophoresis* 2005, *26*, 3910–3920.

[22] Schappler, J., Guillarme, D., Prat, J., Veuthey, J. L. *et al.*, *Electrophoresis* 2006, *27*, 1537–1546.

[23] Fang, L. Y., Kang, J. Z., Yin, X. B., Xang, X. R. *et al.*, *Electrophoresis* 2006, *27*, 4516–4522.

[24] Fang, H. F., Liu, M. M., Zeng, Z. R., *Talanta* 2006, *68*, 979–986.

[25] Mikus, P., Kubacak, P., Valaskova, I., Havranek, E., *Talanta* 2006, *70*, 840–846.

[26] Almeida, V. K., Larive, C. K., *Magn. Res. Chem.* 2005, *43*, 755–761.

[27] Hedeland, Y., Lehtinen, J., Pettersson, C., *J. Chromatogr. A* 2007, *1141*, 287–294.

[28] Kirschner, D. L., Jaramillo, M., Green, T. K., *Anal. Chem.* 2007, *79*, 736–743.

[29] Ptolemy, A. S., Tran, L., Britz-McKibbin, P., *Anal. Biochem.* 2006, *354*, 192–204.

[30] Ptolemy, A. S., Le Bilhan, M., Britz-McKibbin, P., *Electrophoresis* 2005, *26*, 4206–4214.

[31] Chen, H., Terabe, S., in: Marina, M. L., Ríos, Á., Valcárcel, M. (Eds.), *Analysis and Detection by Capillary Electrophoresis*, Elsevier (CAC series), Amsterdam, The Netherlands 2005, pp. 135–172.

[32] Peng, X. J., Sternberg, E., Dolphin, D., *Electrophoresis* 2005, *26*, 3861–3868.

[33] Kavran-Belin, G., Rudaz, S., Veuthey, J. L., *J. Sep. Sci.* 2005, *28*, 2187–2192.

[34] Goossens, J. F., Mahieu, C., Dias, N., Bailly, C. *et al.*, *Electrophoresis* 2006, *27*, 4717–4729.

[35] Erny, G. L., Elvira, C., San Roman, J., Cifuentes, A., *Electrophoresis* 2006, *27*, 1041–1049.

[36] Carlavilla, D., Moreno-Arribas, M. V., Fanali, S., Cifuentes, A., *Electrophoresis* 2006, *27*, 2551–2557.

[37] Zhao, S. L., Yuan, H. Y., Xiao, D., *J. Chromatogr. B* 2005, *822*, 334–338.

[38] Quan, Z., Song, Y., Feng, Y. Z., LeBlanc, M. H. *et al.*, *Anal. Chim. Acta* 2005, *528*, 101–106.

[39] Zhao, S. L., Song, Y. R., Liu, Y. M., *Talanta* 2005, *67*, 212–216.

[40] Miao, H., Rubakhin, S. S., Sweedler, J. V., *Anal. Chem.* 2005, *77*, 7190–7194.

[41] Miao, H., Rubakhin, S. S., Sweedler, J. V., *J. Chromatogr. A* 2006, *1106*, 56–60.

[42] Sheeley, S. A., Miao, H., Ewing, M. A., Rubakhin, S. S. *et al.*, *Analyst* 2005, *130*, 1198–1203.

[43] Iio, R., Chinaka, S., Takayama, N., Hayakawa, K., *Anal. Sci.* 2005, *21*, 15–19.

[44] Akbay, C., Rizvi, S. A. A., Shamsi, S. A., *Anal. Chem.* 2005, *77*, 1672–1683.

[45] Hou, J. G., Rizvi, S. A. A., Zheng, J., Shamsi, S. A., *Electrophoresis* 2006, *27*, 1263–1275.

[46] Rizvi, S. A. A., Zheng, J., Apkarian, R. P., Dublin, S. N. *et al.*, *Anal. Chem.* 2007, *79*, 879–898.

[47] Zheng, J., Shamsi, S. A., *Electrophoresis* 2006, *27*, 2139–2151.

[48] Zheng, J., Norton, D., Shamsi, S. A., *Anal. Chem.* 2006, *78*, 1323–1330.

[49] Friedman, M., *J. Agric. Food Chem.* 1999, *47*, 3457–3479.

[50] Erbe, T., Brückner, H., *Eur. Food Res. Technol.* 2000, *211*, 6–12.

[51] Cheng, Y. F., Dovichi, N. J., *Science* 1988, *242*, 562–564.

[52] Merino, F., Rubio, S., Perez-Bendito, D., *J. Sep. Sci.* 2005, *28*, 1613–1627.

[53] Shamsi, A. A., *Electrophoresis* 2002, *23*, 4036–4051.

© 2008 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim