Focusing Review

High-speed Enantioseparation by Microchip Electrophoresis

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Abstract

Microchip electrophoresis (MCE) can achieve faster analysis of small amount of samples with higher separation efficiency. These characteristics are suitable for the enantioseparation and this analytical method enables chiral separations to be performed in seconds. Since a prerequisite for achieving sufficient resolution and detectability in the chiral analyses is the application of appropriate detection methods for target enantiomers with no dead volume between a separation channel and a detection site, various detection schemes have been introduced in the MCE chiral analysis. In the early stage of the investigation of the MCE chiral analysis, laser-induced fluorescence (LIF) spectrometry was mainly employed as a detection scheme. However, it is a drawback of LIF detection that most of analytes should be derivatized prior to analysis, which is not only labor-intensive and sometimes troublesome but strongly affects the chiral selectivity. Therefore, other detection schemes, e.g., UV absorption, electrochemical detection, conductometry, and so forth, have been also introduced in the MCE analysis of chiral compounds. This article gives an overview of original works done in the MCE chiral analysis with regard to approaches to improve resolution and sensitivity for enantiomers.

Keywords: Microchip electrophoresis; Chiral separation; Detection method; Cyclodextrin electrokinetic chromatography

1. Introduction

Microchip electrophoresis (MCE) has been recognized as an important separation technique on the research field of micro total analytical systems (μ-TAS) since the first publication on MCE was reported by Manz et al. in 1992 [1]. In MCE, there are several advantages, e.g., high speed analysis in seconds, small amount of sample, high separation efficiency, and high-throughput analysis by using microchips with highly integrated multi-separation channels, which provides superior performance especially for the analysis of biogenic compounds. Since these characteristics in MCE are very suitable for the analysis of enantiomers which often requires higher separation efficiency, the MCE method is expected to be applied to the high-throughput chiral analysis systems.

In capillary electrophoresis (CE) analysis of enantiomers, both electrokinetic chromatography (EKC) using cyclodextrins (CDs) as a chiral selector and capillary electrochromatography analysis with stationary phase packed or coated capillaries are generally employed. In the MCE analysis of enantiomers, on the other hand, cyclodextrin EKC (CDEKC) has been mainly applied, which attained the enantioseparation of amino acids, biogenic amines, amphetamines, arylpropionic acids, and so on [2].

In the MCE chiral analysis, the selection of the detection scheme is considerably important. When laser-induced fluorescence (LIF) spectrometry is employed as a detection scheme, a fluorescent derivatization of analytes is sometimes troublesome to reduce chiral recognition ability between selectors and analytes. In the case of off-channel detector configuration, which is primarily employed in electrochemical (EC), electroconductivity (ECD), and chemiluminescence (CL) detection, on the other hand, the separation efficiency is reduced by a dead volume formed between the separation channel and detector. Thus, the detection scheme strongly affects the chiral resolution in MCE. In this article, the chiral analysis in MCE is reviewed, mainly microchip CDEKC (MC−CDEKC), with categorizing by the detection methods.

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2. Chiral Analysis by MCE−LIF

Since the early stage of the development of MCE, LIF has been mainly employed due to its high sensitivity [1]. In the first report of the MCE chiral analysis, LIF was also used as the detection scheme [3]. However, this MCE chiral separation study was reported in 1999, i.e., after 7 years delay from the first MCE report in 1992. As mentioned above, this would be due to the need for the fluorescent derivatization of analytes in MCE−LIF, which reduces the selectivity of chiral selectors such as CDs.

Hutt et al. first reported the MC−CDEKC−LIF analysis of amino acids derivatized with fluorescein isothiocyanate (FITC) on glass microchips with cross-channel geometry, whose dimensions were 150 μm in width and 20 μm in depth [3]. Although racemic FITC-labeled Val, Ala, Glu, and Asp were successfully separated within 4 min, long separation channel length of 19 cm was needed, which is comparable with that in a conventional CE. In addition, resolution was insufficient, i.e., a baseline separation of these amino acids was attained at 10 ℃, while at room temperature these peaks were considerably overlapped. This lower separation efficiency would be caused by the relatively larger channel width. Rodriguez et al. reported fast and efficient MC−CDEKC separations of FITC-labeled amino acid enantiomers by employing shorter separation channel of 7 cm and γ-CD as a chiral selector [4]. Analysis times were ranging from 75 to 160 s with efficiencies up to 28000 (395000 m^{-1}). As well as FITC, 4-fluoro-7-nitrobenzofurazane (NBD-F) has been employed to the derivatization of amphetamines for MC−CDEKC [5]. NBD-F, which exhibits absorption around 488 nm and strong fluorescence in the visible region, is suitable for the derivatization of amines. In the MC−CDEKC separation of amphetamines labeling with NBD-F, though the separation time of 10 min was required due to the longer separation channel length of 16 cm, the use of highly sulfated γ-CD, which is expected to improve the column efficiency, brought a simultaneous chiral separation of seven drug components such as amphetamine and ephedrine.

To improve the analytical reproducibility, on the other hand, poly(vinyl alcohol) (PVA) coated microchannel, which gave suppressed electroosmotic flow, was employed to the chiral separation of FITC-labeled amines [6]. The PVA coating can also enhance the separation efficiency compared to the bare glass microchip. As for the MC−EKC−LIF chiral analysis without CDs, it was reported that native racemic gemifloxacin separated by using chiral crown ether was detected by laser induced native fluorescence with He-Cd laser (325 nm) [7]. Nakajima et al. also reported a ligand-exchange MCE separation of NBD-F labeled amino acids with the separation channel length of 90 mm [8].

As a high-throughput chiral analysis system, Gao et al. reported that multi-channel chip was applied to the screening of the experimental condition, e.g., the concentration and the type of CDs [9]. To achieve the simultaneous detection of FITC labeled three drug components, i.e., baclophen, norfenefrine and tocainide, a line-shaped laser and CCD camera were employed for excitation and detection, respectively. A four-separation channel chip provided a parallel analysis of four samples less than 100 s.

3. Chiral Analysis by MCE-UV

In the application of UV absorption detection to the MCE analysis, there are several drawbacks, e.g., low concentration sensitivity due to a short optical length, and limited wavelength region according to strong UV absorption of substrates such as polymer and Pyrex glass microchips. However, this detection scheme can analyze most of sample compounds without any derivatization, which should not require complicated and labor-intensive experimental procedures, so that UV absorption has been recognized as the most common detection scheme in CE. Though MCE−UV study has not been reported in 1990 s, an MCE system equipped with a linear imaging UV detector has been introduced by Shimadzu in 2000 [10]. This linear UV imaging detector can monitor the concentration profile throughout the separation channel. To reduce the background noise, furthermore, a quartz microchip with an optical slit along separation channel was employed.

In our research group, the chiral separation by MC−CDEKC was investigated with the linear imaging UV detector [11]. As a model system for the MC−CDEKC−UV, racemic 1-aminoindan (AI), which is a pharmaceutically important chiral amine as a key structural element in therapeutic agents under clinical investigations, e.g., Parkinson's and Alzheimer's disease, was selected as a test analyte. In the MCE analysis employing the linear imaging UV detector, the data obtained is different from that of the conventional single-point detection scheme, so that the linear imaging detector enables the observation of the real-time separation process of the racemic analytes. As shown in Fig. 1, a partial peak separation was already observed within 5 s after the injection of AI into the separation channel filled with a running buffer containing sulfated β-CD (S-β-CD). A baseline separation was achieved after 15 s utilizing a separation length of 8.1 mm. Finally, after 50 s resolution (*Rs*) of 3.4 was obtained using the full separation length of 25 mm. These results clearly demonstrated that a highly efficient and faster chiral separation of AI was achieved on the microchip in less than 1 min using S-β-CD as a chiral selector.

In the CDEKC chiral separation, the most important factor is the concentration of CDs. Fig. 2 shows the chiral separation of AI in 10 s employing the running buffer containing various concentrations of S-β-CD. The degree of separation was clearly dependent on the S-β-CD concentration. At S-β-CD concentrations of 1.3 and 2.5 mM, insufficient chiral separations were observed in 10 s,

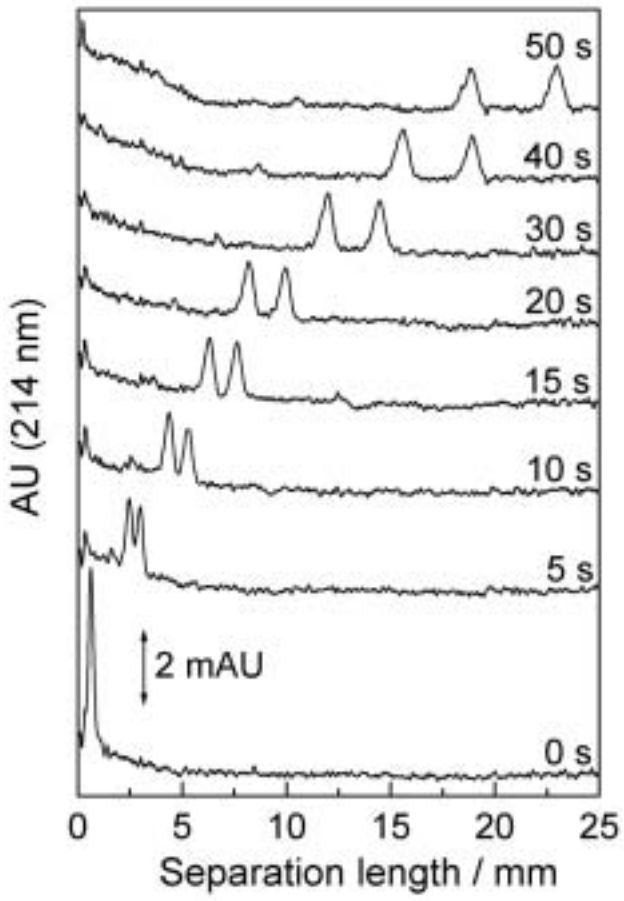

Figure 1. Separation length-based electropherograms obtained by entire separation channel detection in the analysis of an AI racemic mixture. Running buffer, 10 mM phosphate buffer (pH 7.0) containing 10 mM S-β-CD; sample concentration, 1.0 mg/mL; detection wavelength, 214 nm; temperature, 25 ℃. [Reprinted with permission from *Anal. Sci.* **2005**, *21*, 61-65. Copyright 2005 The Japan Society for Analytical Chemistry.]

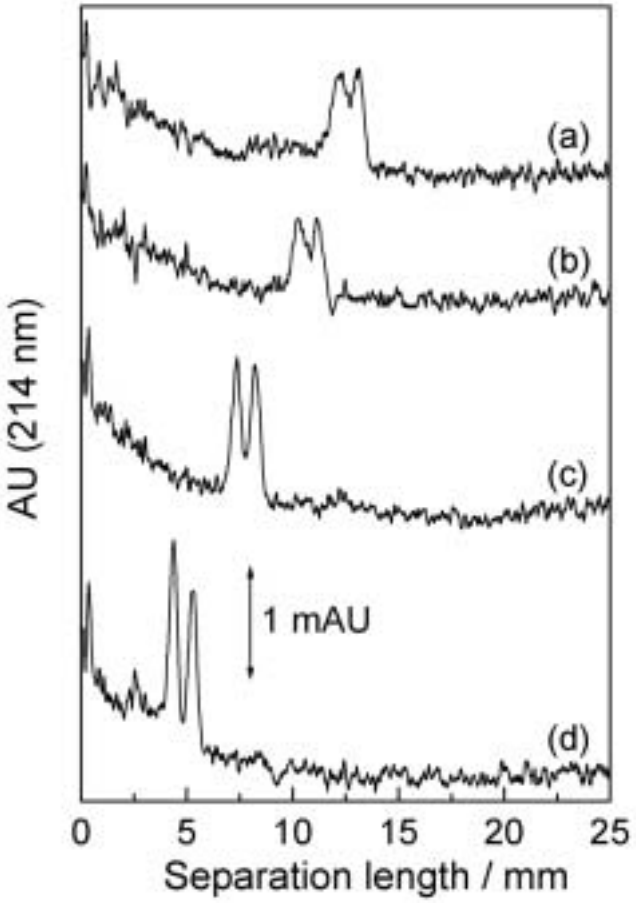

Figure 2. Separation length-based electropherograms of the chiral separation of AI in 10 s. Running buffer consisted of (a) 1.3, (b) 2.5, (c) 5.0, and (d) 10 mM S-β-CD in 10 mM phosphate buffer (pH 7.0). Other conditions are shown in Fig. 1. [Reprinted with permission from *Anal. Sci*. **2005**, *21*, 61-65. Copyright 2005 The Japan Society for Analytical Chemistry.]

whereas in 5.0 mM S-β-CD further resolved peaks were obtained. At 10 mM, the separation of racemic AI was already achieved in only 10, as also shown in the figure. As can be seen, 10 mM S-β-CD provided the best chiral separation, but resulted in a longer analysis time. This is because a higher chiral selector concentration would increase the interaction between AI and slowly migrating S-β-CD. Thus, the concentration of the chiral selector should be kept as low as possible for faster electrophoretic analysis employing the conventional single-point detection scheme. In the MCE apparatus with the linear imaging UV detector, however, the analysis can be immediately terminated when the desired separation of sample is achieved. When the running buffer containing 10 mM S-β-CD was used, the analysis time for the baseline separation (14 s) could be significantly shortened in comparison with that obtained in the conventional single-point detecting scheme (52 s). This approach is extremely effective for high-speed chiral separation in MCE. It should be noted that, however, too high CDs concentration generally gives smaller difference in the electrophoretic mobilities of the AI enantiomers, which brings a poor resolution [4, 11]. In the MCE−UV analysis, furthermore, a sufficient time resolution might not be obtained since the accumulation time is relatively longer in the detection due to its poor signal-to-noise ratio. The accumulation time was set at 0.5 s in Figs. 1 and 2, which caused a peak broadening according to smaller data points on the appeared peaks. Since the MCE chiral analysis requires high-speed and high-resolution, this poor time resolution is considerably problematic and the improvement of the MCE−UV instrument should be desired. Actually, the detection limit (S/N = 3) of AI was estimated to be 0.51 mg/mL, so that the detection sensitivity was apparently insufficient. The low detectability in the present chiral MCE analysis employing the linear imaging UV detection will be improved in combination with on-line sample preconcentration techniques, e.g.,

field amplification stacking and sweeping. As another report on MCE−UV, Ludwig et al. also reported the CDEKC chiral separation of 19 acidic and basic drug components by using highly sulfated CDs [12].

4. Chiral Analysis by MCE−CL

CL is a radiative deactivation process induced by chemical reactions, e.g. oxidation/reduction reaction, between analytes and CL reagents, so that an excitation light source should not be required. In CL, therefore, only the use of a photodetector such as photomultiplier tube, CCD camera, and so forth, enables sensitive detection, which is advantageous for the miniaturization of the MCE apparatus. In the case of MCE−CL, however, it is known that a band broadening accompanying the mixing of the CL reagents would reduce the separation efficiency and the detectability.

As an application of MCE−CL, chiral separation of racemic dansyl amino acids on poly(dimethyl siloxane) (PDMS) chips with three channel patterns (cross, cross combining with Y, and cross combining with V) was reported [13]. Liu et al. investigated the effect of the on-line mixing performance on the detection sensitivity, reproducibility, and peak symmetry in detail. As a result, the PDMS chip with the V-shape mixing channel provided the highest peak and the limit of detection for dansyl glycine was evaluated to be 0.39 μM with the reproducibility of less than 5%. When hydroxypropyl-β-CD was added into the running buffer, the chiral separation of dansyl phenylalanine was achieved with R_S of only ~ 0.8 in spite of utilizing the separation length of 30 mm. This might be due to a band broadening caused by the mixing process at the V-form channel.

5. Chiral Analysis by MCE−EC

In EC detection, sample derivatization is not required for electroactive analytes and relatively higher sensitivity can be obtained. Although suitable target analytes are limited to electroactive components, EC detection has been mainly applied to the MCE analysis of amines. In the MCE−EC analysis, amperometric detection is generally employed. It is advantageous that mole number of detected analytes can be directly calculated in amperometric EC detection since the peak area is proportional to the quantity of electricity of the analyte.

Schwarz and Hauser reported that successful MC−CDEKC−amperometric EC analysis of racemic adrenalins and ephedrines by employing carboxymethyl-β-CD without any derivatization [14]. The chiral separation was improved by adding crown ether, which resulted in the efficiency of 20000, R_S of 2.5, and the limit of detection of 0.1 μM. In addition, they also reported the application of EC detection to the chiral analysis of complex mixtures and succeeded the simultaneous separation of 9 neurotransmitter components within 160 s [15].

6. Chiral Analysis by MCE−ECD

Recently, the number of publications concerning MCE−ECD analysis has increased since the fabrication of electrodes on microchips has been well established. In the case of UV/LIF and EC detection, target analytes are limited to those having chromophoric and electroactive groups, respectively, while in ECD a universal detection can be performed since the detection principle is based on the difference in the conductivity between running buffer and sample zones.

Ölvecká et al. reported isotachophoresis (ITP) separation of Trp enantiomers on poly (methyl methacrylate) (PMMA) microchips with on-column conductivity detector coupled with separation channel [16]. A 94-mm separation length in ITP brought a complete separation of Trp by adding α-CD into a leading solution. Though the limit of detection was evaluated to be 8×10^{-5} M at the present stage, which was not sufficient concentration sensitivity, the application of on-line sample preconcentration by transient ITP is expected to achieve highly sensitive detection in microchip ITP−ECD.

7. Chiral Analysis by Microchip Electrochromatography

Above discussions, the chiral separations are mainly based on CDEKC. However, CDs are generally incompatible with a mass spectrometric (MS) detection due to its low volatility. Especially in electrospray ionization MS, low volatile CDs should cause a lower ionization efficiency and detectability. Thus, the low volatility of CDs would interfere the application of MS detection to the MCE analysis of enantiomers. For overcoming this drawback, microchip electrochromatography (MCEC) is considered to be an effective separation mode without any interference of chiral selectors in the detection process.

Bi et al. reported a fabrication of protein encapsulated alumina gel network on the inner surface of PMMA microchannel to form a bovine serum albumin (BSA) stationary phase for the MCEC analysis of racemic Trp [17]. On the basis of the chemical modification of a synthesized copolymer containing silane-functionalized scaffold, sol-gel reaction can provide the encapsulation of BSA on the PMMA surface. In the MCEC analysis with EC detection, Trp was efficiently separated with R_S of 1.57 within 60 s. The same authors also reported that BSA conjugated shortened carboxylic single-walled carbon nanotubes (SWNTs) were immobilized onto the surface of PMMA microchannel to attain the MCEC−EC chiral analysis of Trp [18]. A gradual solvent evaporation of BSA conjugated SWNTs suspension filled in the channel resulted in a stable stationary phase. In the prepared chips, successful separation of Trp enantiomers was achieved with R_S of 1.35 less than 70 s. Al-

though the MCEC−MS analysis of chiral compounds has not been reported, the application of MS detection should be promising a high performance MCE analysis system.

8. Conclusion

In this review, chiral analyses in MC−CDEKC and MCEC were reviewed with categorizing by the detection methods. Combination of MCE with sensitive (LIF and EC) or universal (UV and ECD) detection techniques provides high-speed chiral analysis with good separation ability. Especially, in linear imaging UV detection, the analysis time for the baseline separation could be significantly shortened under a higher S-β-CD concentration compared with that obtained by the conventional single-point detection scheme. This approach is extremely effective for high-speed chiral separation in MCE. Moreover, further high-throughput and high-performance chiral analysis systems are expected to be attained by the application of several detection schemes, e.g., MS, thermal lens microscopy and Raman scattering, and the development of microchips integrating on-line sample pretreatment and MCE separation/detection sites.

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