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Highly sensitive chiral analysis in capillary electrophoresis with large-volume sample stacking with an electroosmotic flow pump

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ABSTRACT

To improve the sensitivity in chiral analysis by capillary electrophoresis without loss of optical resolution, application of large-volume sample stacking with an electroosmotic flow pump (LVSEP) was investigated. Effects of the addition of cyclodextrin (CD) into a running solution on the LVSEP preconcentration was theoretically studied, where the preconcentration efficiency and effective separation length would be slightly increased if the effective electrophoretic velocity ($\nu_{ep,eff,BGS}$) of the analytes was decreased by interacting with CD. In LVSEP-CD-modified capillary zone electrophoresis (CDCZE) and LVSEP-CD electrokinetic chromatography with reduced $\nu_{ep,eff,BGS}$, up to 1000-fold sensitivity increases were achieved with almost no loss of resolution. In LVSEP-CD-modified micellar electrokinetic chromatography of amino acids with increased $\nu_{ep,eff,BGS}$, a 1300-fold sensitivity increase was achieved without much loss of resolution, indicating the versatile applicability of LVSEP to many separation modes. An enantio-excess (EE) assay was also carried out in LVSEP-CDCZE, resulting in successful analyses of up to 99.6% EE. Finally, we analyzed ibuprofen in urine by desalting with a C_{18} solid-phase extraction column. As a typical result, 250 ppb ibuprofen was well concentrated and optically resolved with 84.0–86.6% recovery in LVSEP-CDCZE, indicating the applicability of LVSEP to real samples containing a large amount of unnecessary background salts.

1. Introduction

Chiral compounds are recognized to play important roles in chemistry, biology, medicine, and pharmacology [1–3], so that the analytical methods for the chiral compounds require the high sensitivity, high optical resolution, and short analysis time. Among several chiral separation methods, such as high-performance liquid chromatography (HPLC), gas chromatography, and capillary electrophoresis (CE), CE exhibits high resolution with little sample consumption in a short analysis time. Several separation modes have been developed for chiral separation in CE, including micellar electrokinetic chromatography (MEKC), cyclodextrin (CD)-modified capillary zone electrophoresis (CDCZE), CD electrokinetic chromatography (CDEKC), CD-modified MEKC (CDMEKC), affinity capillary electrophoresis (ACE), and nonaqueous CE (NACE) [4–6]. However, the concentration sensitivity is quite poor because of the short optical path length and the small injection volume of sample solution.

To overcome such a drawback of CE, several online sample preconcentration techniques have been developed [7–21]. Although up to 1000-fold sensitivity increases have been achieved in chiral analysis [7–16], optimization of the preconcentration conditions is usually required because the resolution was reduced due to the decrease in the effective separation length accompanying the increase in the sample injection volume [17–19]. Since the enantioseparation is not so easy without the optimal electrolyte composition, additional optimization of the preconcentration condition is one of the most serious disadvantages. Moreover, highly efficient preconcentration techniques often require multi-step procedures [16,20], which are quite bothersome and often cause the reduction in the analytical reproducibility. Hence, we focused on an online sample preconcentration technique using field amplified sample stacking, large-volume sample stacking with an electroosmotic flow (EOF) pump (LVSEP) [21–23], which provides the high sensitivity with almost no loss of resolution in a simple experimental procedure. In our previous work [22], up to 780-fold sensitivity increases were achieved with good separation performance in the CE analysis of oligosaccharides. Moreover, we did not need to optimize the sample injection volume, because the sample filled in the whole capillary could be concentrated. Thus, the application of LVSEP to the chiral analysis in CE is expected to improve the sensitivity with high enantioseparation efficiency and to minimize the optimization procedure of the

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experimental conditions and the multi-step preconcentration procedure.

In spite of the high preconcentration and separation ability of LVSEP, there has been no report on the separation performance in combining LVSEP with any separation modes except for the most basic separation mode, capillary zone electrophoresis (CZE). In LVSEP, the separation performance is determined by the inversion position of the sample migration where the EOF velocity and electrophoretic velocity of the analyte in a background solution (BGS) is balanced [23]. Hence, the change in the effective electrophoretic mobility in the different separation mode can cause the increase or decrease in the resolution. It is important to consider the effect of the separation mode on the resolution both theoretically and experimentally.

Our aims in this study are to clarify the effects of the separation mode on the resolution in LVSEP and to achieve the efficient improvement of the concentration sensitivity without loss of optical resolution and without complicated experimental procedures including the optimization steps. Theoretical investigation on the resolution in the LVSEP-applied chiral analysis using CDs as chiral selectors was performed by estimating the inversion position, which is expected to directly affect the effective separation length. Three enantioseparation modes, CDCZE, CDEKC, and CDMEKC, were carried out to evaluate the performance of the sensitivity improvement and the enantioseparation. An enantio-excess (EE) assay was also carried out in LVSEP-CDCZE. Finally, we performed the analysis of a drug component dissolved in a urine matrix to show how to analyze real samples containing a large amount of unnecessary background salts. The purification using a C_{18} solid-phase extraction (SPE) column was applied for the LVSEP analysis of the urine sample.

2. Experimental

2.1. Materials and chemicals

A fused silica capillary was purchased from Polymicro Technologies (Phoenix, AZ, USA), poly(vinyl alcohol) (PVA, M_w = 88,000, 99% hydrolyzed) was obtained from Japan Vam and Poval (Osaka, Japan), warfarin was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany), thiourea, (±)-abscisic acid, racemic ibuprofen, (S)-(+)-2-(4-isobutylphenyl)propionic acid ((S)-ibuprofen), 2,6-di-*O*-methyl-β-cyclodextrin (DM-β-CD), and 2,3,6-tri-*O*-methyl-β-cyclodextrin (TM-β-CD) were purchased from Wako (Osaka, Japan), quaternary β-cyclodextrin (QA-β-CD) and DL-leucine were purchased from Sigma–Aldrich (St. Louis, MO, USA), and all other reagents were purchased from Nacalai Tesque (Kyoto, Japan). All solutions were prepared with deionized water purified by using a Direct-Q System (Nihon Millipore, Japan), and filtered through a 0.45 μm pore membrane filter (Nacalai Tesque) prior to use.

2.2. Derivatization of amino acids

Amino acids were derivatized with fluorescein isothiocyanate (FITC) for laser-induced fluorescence (LIF) detection as in the previous report [24]. Briefly, 5 μL of 50 mM amino acids and 5 μL of 50 mM FITC dissolved in 50 mM borate buffer (pH 9.5) were mixed and left for 24 h at room temperature. The solution was diluted with deionized water or a BGS for the appropriate concentrations.

2.3. SPE purification of urine sample

Urine samples spiked with ibuprofen were purified with a C_{18} SPE column (Inert Sep C_{18}, GL Science, Kyoto, Japan). Urine was sampled from a healthy male volunteer and filtered with a 0.45 μm pore membrane filter. Ibuprofen dissolved in methanol (1%, w/v) was spiked in the urine for certain concentration, followed by adjusting pH to around 3 by adding 6 M hydrochloric acid. After conditioning the SPE column with 1 mL methanol and 1 mL water, 500 μL of the urine sample was passed through the cartridge with a gentle gravity pressure at a flow rate of about 0.3 mL/min. The column was washed with 1.5 mL of water, 0.5 mL of 25 mM formic acid in ACN/water (20/80, v/v), and 1.5 mL of water again. Ibuprofen was then eluted with 0.5 mL of ACN. The eluent was lyophilized and the residue was diluted with 500 μL of water for the LVSEP analysis.

2.4. Capillary coating

A fused silica capillary was coated with PVA in the same way as the previous papers [22,25,26]. Briefly, the capillary was activated and washed with 1 M NaOH and water, followed by the injection of a 5% PVA solution into the whole capillary. Both the capillary ends were immersed in the same PVA solution and left at room temperature for 15 min. The PVA solution was then removed out of the capillary and the capillary was heated at 140 °C for 18 h under a nitrogen gas flow. The capillary was filled with deionized water and stored at room temperature. Prior to use, the capillary was flushed with a BGS for 15 min.

2.5. Apparatus

All CE experiments were performed on a P/ACE MDQ system (Beckman Coulter, Fullerton, CA, USA) equipped with a diode-array UV detector or a LIF detector. The LIF detector used in the LVSEP-CDMEKC analysis consisted of a 488 nm argon ion laser module and photomultiplier detector with a 520 nm band pass filter. UV detection was performed at 200 nm in LVSEP-CDCZE or 250 nm in LVSEP-CDEKC.

2.6. Analytical procedure

The capillary with total/effective lengths of 40/30 cm was employed in the CDCZE analysis and that of 60/50 cm in the CDEKC and CDMEKC analyses. They were conditioned with deionized water in applying LVSEP or with the BGS in the conventional CDCZE/CDEKC/CDMEKC analyses at 20 psi for 3 min prior to each run. Sample injections were performed with a pressure of 20 psi for 90 s (whole capillary injection) in the LVSEP-applied analyses or 0.3 psi for 3 s in the other conventional analyses. The applied voltage and the temperature were set at −30 kV and 25 °C, respectively, except in the CDCZE analysis of ibuprofen with voltage application of −25 kV.

3. Results and discussion

3.1. Theoretical consideration

In LVSEP-CDCZE/CDEKC/CDMEKC, the EOF-suppressed capillary is filled with a low ionic strength solution containing anionic analytes, whereas the inlet and outlet vials are filled with the high ionic strength BGS containing CD (Fig. 1a). After applying the voltage, anionic analytes are concentrated at the sample matrix (SM)/BGS boundary by the difference in the electric field strength between the two zones. The focused analytes move toward the cathode and the BGS is introduced into the capillary by the enhanced EOF in the low ionic strength SM (Fig. 1b). As the analytes migrate toward the cathode, the EOF velocity becomes slower and the electric field strength in the BGS becomes higher (Fig. 1c). When almost all the SM in the capillary is removed out from the cathodic capillary end, the electrophoretic velocity of the analytes exceeds the EOF rate, resulting in the inversion of the sample migration direction (Fig. 1d). After the complete removal of the SM, the analytes are