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Review

Recent applications of on-line sample preconcentration techniques in capillary electrophoresis

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ABSTRACT

This review highlights recent developments and applications of on-line sample preconcentration techniques in capillary electrophoresis (CE) from 2010 to April 2013. Various preconcentration techniques based on the analyte velocity change in two or three discontinuous solutions system including field-amplified stacking, transient isotachophoresis, pH-mediated stacking, sweeping, and their modified and combined techniques have been employed to enrich and separate biological, environmental, food, toxicological, forensic and nanoparticle samples in CE. More than 170 published research articles collected from Scopus databases from the year 2010 described the on-line sample preconcentration techniques. This review provides comprehensive tables listing the applications of the on-line sample preconcentration techniques with categorizing by the fundamental preconcentration mechanism and application area.

Contents

1. Introduction

Capillary electrophoresis (CE) is one of the candidates for the separation of a small amount of analytes in complex samples since it has much merits relative to conventional HPLC in terms of high separation efficiency, short analysis time, simple operation, small consumption of reagents, buffers and samples. Additionally, several separation modes and detection schemes are introduced in CE, so that the application area is extended to various analytes including ionic, neutral, small and large molecules. In spite of such versatilities, poor concentration sensitivity is still problematic due to a low detection volume in CE. To detect a low amount of

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analytes, three approaches are mainly selected, *i.e.*, highly sensitive detector, on-line and off-line sample preconcentration techniques. Among various detectors, laser-induced fluorescence (LIF), mass spectrometry (MS) and electrochemical (EC) detection are often employed for sensitive CE analysis. However, recent progresses of proteomics, metabolomics and glycomics need further high detection sensitivity to analyze a trace amount of biomolecules. Hence, a coupling of these sensitive detectors and on-line and off-line sample preconcentration techniques is desired. Especially for biosamples, off-line sample preconcentration techniques with sample pretreatment are necessary to extract, isolate and enrich the analytes of interest from complex sample matrices. In recent years, several off-line extraction techniques including solid phase microextraction (SPME), liquid-phase microextraction (LPME), hollow-fiber liquid-phase microextraction (HF-LPME), liquid–liquid–liquid microextraction (LLLME), dispersive LLME (DLLME), single-drop microextraction (SDME), ultrasound-assisted emulsification microextraction (USAEME), solvent-bar microextraction (SBME), electrokinetic membrane extraction (EME), and so on, are combined with CE [1–4]. These methods are effective to enrich and purify target analytes from crude sample matrices prior to the CE separation. To achieve high-throughput and automated analysis, however, these off-line preconcentration and pretreatment techniques are not favorable since they are often tedious due to increased processing steps and time.

Compared with off-line pretreatments, on-line sample preconcentration techniques, where most of the enrichment processes are performed in the separation capillary, can easily improve the detection sensitivity. The on-line sample preconcentration techniques applied to CE are classified to three categories: preconcentration by analyte velocity change in two or three discontinuous solutions system, focusing, and solid phase extraction (SPE). Focusing techniques such as isoelectric focusing (IEF) are used for the preconcentration and pre-separation [5]. SPE techniques concentrate analytes at the solid-phase immobilized capillary. Although the SPE approaches can give high preconcentration efficiency and/or highly selective separation performance, they often requires labor-intensive preparation processes and complicated experimental procedures. On the other hand, the on-line sample preconcentration based on the changes in the analyte migration velocity at the boundary of discontinuous solution systems can be carried out with easy experimental procedure and relatively short enrichment time. For the stacking at discontinuous solutions boundary, target analytes are dissolved in a sample matrix whose components differ from those in a background solution (BGS). At the boundary between the sample and BGS zones, the changes in the migration velocity of the analytes should occur according to the difference in the electric field, pH and the retention factor, resulting in the preconcentration of the analytes in the long sample zone. These techniques can be combined with several separation modes such as capillary zone electrophoresis (CZE), cyclodextrin modified-CZE (CDCZE), electrokinetic chromatography (EKC), micellar EKC (MEKC), cyclodextrin EKC (CDEKC), cyclodextrin modified-MEKC (CDMEKC), microemulsion EKC (MEEKC), capillary gel electrophoresis (CGE), non-aqueous CE (NACE) and isotachophoresis (ITP).

In this review, recent developments and applications of the on-line sample preconcentration techniques mainly based on the migration velocity change in "capillary"-based electrophoresis from the year 2010 are briefly reviewed because various review papers on the on-line sample preconcentration have been published [6–22]. For detailed mechanism and discussion, these papers and references therein should be referred. Principal on-line sample preconcentration techniques include field-amplified stacking, transient isotachophoresis, pH-mediated stacking, and sweeping techniques. Please refer to Tables 1–6 for an overview of applications of the on-line sample preconcentration techniques in CE.

2. Field-amplified stacking

2.1. Field-amplified sample stacking (FASS)

Among several on-line sample preconcentration techniques developed in CE, field-amplified sample stacking (FASS) is the simplest and most common technique. In FASS, a low-conductivity sample solution is hydrodynamically injected into the capillary filled with a high-conductivity BGS. Because the electric field strength is inversely proportional to the electrical conductivity, a higher field is applied to the sample zone relative to the BGS zone. Hence, the electrophoretic migration of ionic analytes in the sample zone is faster than that in the BGS, which causes the "stacking" of the analytes around the sample/BGS boundary. Although the conductivity of the sample is limited to be low in FASS, up to 100-fold increase in the detection sensitivity can be obtained by injecting a long sample plug (typically, <5 cm).

FASS and modified techniques were employed to many applications including biological, environmental, food, and pharmaceutical areas as summarized in Table 1 [23–44]. As a typical biological application, Liu et al. reported FASS-CZE-LIF analysis of fluorescein isothiocyanate (FITC)-labeled catecholamines in rat brain, which provided the enrichment factor of 118–132 and the limit of detection (LOD) of 22 pg/L [25]. For the determination of cationic and anionic neurochemicals, a PEO-based stacking technique was applied to CZE [32]. In the modified technique, in addition to the FASS effect, ionic neurochemicals can be stacked through the viscosity difference between a sample and BGS zones, giving 116–281-fold sensitivity improvements for biogenic amines and their metabolites. In recent years, FASS has been often combined with on-line or off-line extraction techniques, such as SPE and LLME, to purify crude samples and improve the detection sensitivity. For example, a coupling of FASS with dispersive LLME (DLLME) resulted in the 814-fold sensitivity improvement for an antidepressant drug, sertraline, which belongs to the class of selective serotonin reuptake inhibitors [38]. Khan and co-workers reported a novel quantification of microRNA based on off-line coupling of SPE using RNA binding protein-coated magnetic beads with FASS in a protein facilitated affinity capillary electrophoresis (ProFACE) assay for the detection of ultralow amounts of microRNA [41]. As a typical result, LOD was 0.5 fM or 30,000 microRNA molecules in 1 mL of serum. Such selective pre-extraction technique is quite effective for desalting of the bio-samples, which is requisite for the field-amplified stacking.

2.2. Field-amplified sample injection (FASI)

To obtain higher enrichment efficiency, a field-amplified sample injection (FASI) technique is available [45–70]. In FASI, ionic analytes are electrokinetically injected from the inlet vial filled with a sample solution to the capillary according to the FASS concentration mechanism, which can provide the introduction of a larger amount of the analytes compared to FASS. Jin et al. reported the sensitivity enhancement factors (SEFs) between 62,000 and 200,000-fold for the FASI-CDCZE analysis of β-blockers with the sample injection for 5 min at +10 kV after introducing a short plug of water for 10 s at 0.5 psi to improve the SEF and reproducibility [62]. In place of pure water plug, Lau et al. effectively used a solvent plug containing 45% acetonitrile. The solvent plug was introduced hydrodynamically into the capillary for 40 s before injecting four toxic metal ions electrokinetically at +10 kV for 60 s in the FASI step,