Online Coupling of Solid-Phase Microextraction and Capillary Electrophoresis

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Abstract

Solid-phase microextraction (SPME) and capillary electrophoresis (CE) are two of the main inventions that shaped 20th Century analytical chemistry. SPME is an effective microscale sampling and sample preparation technique, and CE is a high-efficiency microanalytical method. Online coupling of SPME with CE can be a powerful combination because of the significant advantages of the two techniques. The progress in the development of online SPME–CE coupling is surveyed in this review. Problems encountered and solutions reported are highlighted.

Introduction

Solid-phase microextraction (SPME) (1–3) and capillary electrophoresis (CE) (4–8) are the two main inventions in analytical chemistry in last two decades of the 20th Century. In 2001, the journal *Analytical Chemistry* honored six research themes as great ideas in the decade of 1989–1999 that shaped 20th Century analytical chemistry (9). SPME, applying CE, and DNA analysis by CE were three of the six themes, along with three others: electrospray and nanoelectrospray, matrix-assisted laser desorption/ionization, and micro-total analysis system (or lab on a chip).

SPME is a useful sampling and sample preparation technique. It simplifies chemical analysis by integrating sampling, sample preparation, and sample concentration into a single step of extraction on a microscale solid phase, with the convenient introduction of extracted analytes into analytical instruments. SPME offers several apparent advantages. First, it is a non-exhaustive approach. The analytes can be extracted from the sample matrix under pre-equilibrium conditions, which greatly reduces the extraction time. Second, SPME is a little-solvent-consuming—even solvent-free—technique, which is friendly to the environment and operators. Third, interference from the sample matrix can be effectively eliminated by choosing an appropriate extraction phase. For instance, in drug analysis of blood samples, interference of the proteins present in blood can be eliminated by using restricted-access, material-based SPME (10). Finally, this technique can be easily miniaturized to allow it to be used with both microscale analytical instruments and small living systems, such as single cells. Recently, SPME has been demonstrated as a tool for in vivo pharmacokinetic studies (11). So far, SPME has been widely applied to fundamental research initiatives and environmental, pharmaceutical, clinical, forensic, and food analyses (12).

CE is a revolutionary invention in analytical chemistry that offers significantly higher separation efficiency when compared with other liquid phase separation methods, such as high-performance liquid chromatography (HPLC). By using a microbore capillary as the separation column, Joule heat can be efficiently dissipated, allowing the use of a very strong electric field. The separations are thus efficient and rapid. The narrow diameter of the capillaries requires very small volumes of sample and reagents. CE systems are easy to automate. The throughput can be multiplied by using capillary arrays. Because of its multiple separation modes, CE is applicable for analysis of a broad range of analytes, including inorganic ion, small molecule, peptide, protein, DNA or RNA, virus, and cell. The separation power of CE for biological macromolecules, such as DNA and protein, is predominant among the separation methods available. It played an important role in the accomplished Human Genome Project (13). CE has been playing an increasingly important role in the ongoing proteomics studies (14,15).

Because of the significant advantages of SPME and CE, the combination of the two technologies can be a powerful analytical tool. There are two ways to realize the hyphenation: off-line and online coupling. Off-line coupling is quite straightforward. The analytes extracted by an SPME fiber or tube are desorbed into a certain volume of desorbing solvent/solution, and a portion of the obtained sample solution is then injected into a CE system for analysis. This type of coupling has been applied to analyses of a variety of compounds (16–25). Although off-line coupling can be easily reached, a disadvantage is very apparent. As the volume of the desorbing solvent/solution is usually much larger than the sample volume that can be injected into the CE system, the extracted analytes are diluted and the detection sensitivity is, therefore, relatively poor. A solution to this problem is to combine off-line SPME–CE coupling with online sample preconcentration

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techniques, which allow for injection of a large volume of sample into the capillary. The possibilities of such a combination have been demonstrated with several preconcentration modes, including normal stacking mode, field-enhanced sample injection, and stacking with matrix removal (25). Still, the complete transfer of all the extracted analytes into the CE system is still impossible with off-line coupling. With regard to instrumentation, off-line coupling is relatively lacking in challenge. Thus, it will not be discussed further in this review.

Online coupling is significantly challenging for several reasons. First, slow desorption kinetics in the liquid phase result in band broadening and analyte carryover. When SPME is coupled with gas chromatography (GC), analyte desorption can be completed in seconds by virtue of quick diffusion coefficient in gas phase, which is facilitated by the high temperature used in GC. As a comparison, when SPME is coupled with CE, the kinetics of desorption in the aqueous phase are much slower, which is worsened by the much lower temperature used (usually around room temperature). Thus, a much longer desorption time has to be used. However, there is a compromise between peak broadening and analyte carryover. To ensure complete desorption (no carryover), a longer desorption time is needed, which may result in peak broadening; to ensure high efficiency (less peak broadening), a shorter desorption time is required, which usually causes analyte carryover. As the sample volume injected in CE must be very small (usually several nanoliters) under plug injection mode, which is the main mode in CE, the problems of peak broadening and carryover are very apparent. Second, size match at microscale is another issue that must be taken into account. The bore size of the capillaries used in CE is usually 25–75-µm inner diameter (i.d.), whereas the SPME fibers commonly used are larger than 75-µm outer diameter (o.d.). Although the size of SPME fibers can be lowered to match the size of the capillary, it requires highly sensitive detection, such as by laser-induced fluorescence (LIF) or mass spectrometry (MS), because of the lower extraction capacity. When a less sensitive detector, such as a UV absorbance detector, is employed, a big SPME fiber is needed. To couple a big SPME fiber with a regular size capillary, an appropriate adapter is needed. A good adapter must be able to confine all the analyte molecules desorbed from the SPME fiber within a sample band of acceptable size and must approach zero extra-column effect.

Although one book chapter (26) introducing off-line and online SPME–CE coupling has been published in 1999, there is no review paper exclusively dealing with online SPME–CE coupling thus far. We survey the progress in the online coupling of SPME with CE in this review.

Several groups (27–30) reported coupling SPME with CE; however, the "SPME" used therein are essentially not SPME. Indeed, these "SPME" devices should be called micro-solid-phase extraction (micro-SPE). Micro-SPE and SPME are two alike terms, but they are distinct according to their functions and the concepts of these techniques. Micro-SPE is mainly used for sample enrichment and sample clean-up, whereas SPME is mainly for sampling, though it can perform sample enrichment and sample clean-up, too. Though micro-SPE is an exhaustive extraction method, SPME is a nonexhaustive one, which is usually operated under equilibrium or pre-equilibrium conditions. In this review, discussion of micro-SPE–CE coupling is not covered.

Discussion

Fiber SPME

Fiber SPME is the main format for the coupling, which can be directly exposed to the sample. The first online coupling of SPME and CE was reported by Nguyen and Luong (31). The CE instrument used was a Beckman P/ACE 5000 system (Beckman, Fullerton, CA). An SPME adapter was built at the inlet end of the capillary. The structure of the adapter is illustrated in Figure 1. The separation capillary (50-µm i.d. × 350-µm o.d.) was connected with a short capillary segment (1.5 cm × 180-µm i.d. × 380-µm o.d.) at the inlet end through a piece of heat-shrinkable tubing (2-cm length). The SPME fiber used was 150 µm in diameter, so it could be easily inserted into the 180-µm-i.d. capillary. If the contacting surfaces of the capillary segment and the separation capillary were well polished, zero dead volume could be approached. Sixteen polycyclic aromatic hydrocarbons (PAHs) were used as test compounds. The extraction phase was poly(dimethylsiloxane), coated over the glass core of the fiber. The separation mode, called cyclodextrin (CD)-modified CE by the authors, was essentially an electrokinetic chromatography mechanism, in which the analytes are separated according to differential partitioning coefficients between a pseudo-stationary phase and a mobile phase. Negatively charged sulfobutyloxy-β-CD (SBβCD) was used as the pseudo-stationary phase and neutral methyl-β-CD (MβCD) and α-CD were used as mobile phase modifiers. Methanol was used as desorbing solvent: it was injected for 3 s (injection condition not reported) and then remained for 5 min before separation. Figure 2 compares the electropherogram of 15 PAHs obtained by direct sample injection and that with SPME extraction. The sample for extraction was 100-fold more diluted than that for direct injection, but the peak heights for all species were reduced approximately 40–60% only. The results obtained with SPME clearly exhibited effective concentration effect. In addition, the SPME adapter nearly attained zero-volume connection, as manifested by the preservation of the migration order as well as the resolution power. Reproducibility of the SPME–CE coupling was investigated. With the same capillary

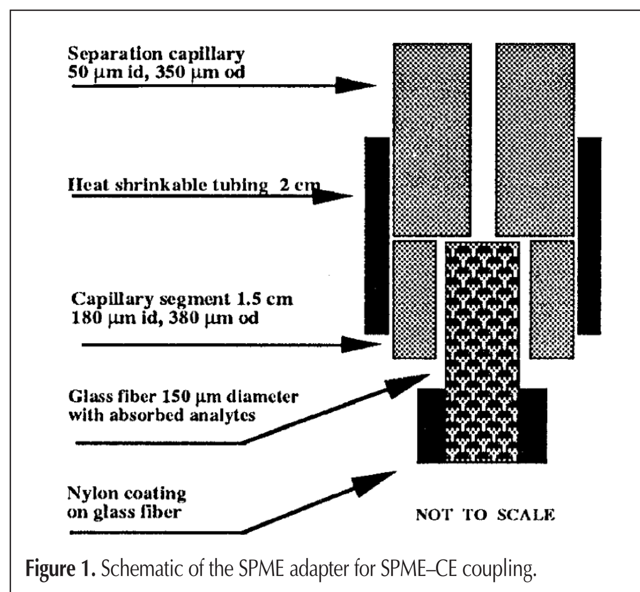

Figure 1. Schematic of the SPME adapter for SPME–CE coupling.

cassette, satisfactory fiber-to-fiber reproducibility was obtained, with a variation of < 5% and < 7% (n = 4) for migration time and peak area, respectively. However, significantly different migration times and peak areas (for some PAHs) were noticed from cassette to cassette. The unexpected behavior was explained by the nonzero dead volume between the extraction fiber and separation capillary. And the authors pointed out that zero-volume connection is a key feature for the SPME–CE coupling. With UV detection, pyrene as low as 8 parts per billion (ppb) was detected, and the highest limit of detection (LOD) was 75 ppb for acenaphthene. As a comparison, the LODs obtained without SPME were at ppm level.

In the previously mentioned study, online SPME–CE coupling was implemented using an adapter, which accommodated an SPME fiber of a size larger than the bore size of the separation capillary. Online SPME–CE hyphenation has been attained by using an interface that allowed direct insertion of an SPME fiber of small size into a separation capillary of large bore size (32). The SPME–CE system and the structure of the interface are illustrated in Figure 3. The CE instrument used was a Bio-Rad HPE 100 system, equipped with a UV detector. The separation capillary was 75-µm i.d., and the diameter of the SPME fiber was approximately 40 µm. The interface was made of a Teflon block, on which two conical tubes were perfectly aligned and fixed with epoxy glue. The gap between the two conical tubes was approximately 1 mm, which allowed electric contact and solution junction. The inlet end of the separation capillary was inserted into one of the conical tubes, and the other conical tube was used to guide the SPME fiber into the separation capillary. The inlet end of the CE capillary was etched to a conical shape by hydrofluoric acid, to facilitate fiber insertion. By virtue of this interface, the SPME fiber could be easily inserted into the separation capillary, generally taking less than 10 s. Before insertion of the SPME fiber, a 0.2M NaOH solution was injected into the inlet end (10 kV for 5 s), which functioned as desorbing solution. With poly(acrylate) (PA) phase fiber, 10 phenolic compounds were extracted, separated, and detected. Little efficiency loss was found. No carryover of analytes was observed in subsequent solvent desorption from the same fiber. The LOD for pentachlorophenol obtained by SPME–CE with UV detection was determined to be 2 ppb [signal to noise ration (s/n) = 3], two orders of magnitude lower than that obtained using conventional CE–UV detection without sample preconcentration.

When inserting an SPME fiber into a CE capillary, an interesting and useful phenomenon was found (33). As the commonly used fiber material (fused-silica) has high electric resistance, the presence of such a fiber within the separation capillary changes the distribution of the electric field. The electric field strength across the section where the SPME fiber presents is higher than that across the remaining section of the separation capillary, which follows the following equation:

$$\frac{E_1}{E_2} = \frac{D_2}{(D_2 - d^2)} \qquad \text{Eq. 1}$$

where E_1 and E_2 are the electric fields across the section where the SPME fiber presents and the remaining section, respectively; and D and d are the inner diameter of the separation capillary and the diameter of the SPME fiber, respectively. So under an electric field, the analytes desorbed from the SPME fiber will migrate faster in the section where the fiber presents and slow down when they reach the remaining section where there is no fiber. Such an effect results in a narrowed analyte band as compared

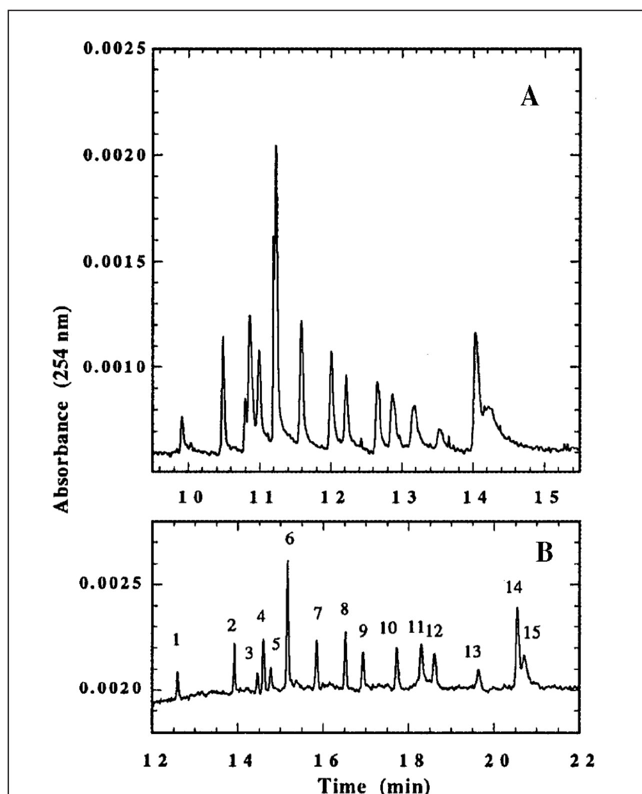

Figure 2. Electropherograms of 15 PAHs without microextraction, 40-fold diluted sample, 57-cm capillary (A) and with microextraction, 4000-fold diluted sample, 67-cm capillary (B). Run buffer: 35mM SBβCD, 10mM MβCD, 4mM α-CD in 50mM borate buffer, pH 9.2.

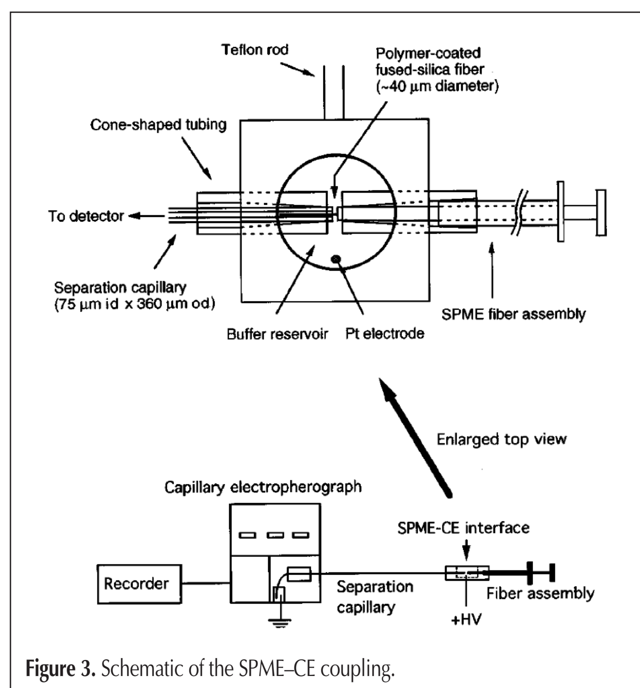

Figure 3. Schematic of the SPME–CE coupling.

with the original length (as long as the length of the extraction phase) and the compression factor is determined by the ratio of E_1/E_2. For example, when inserting a 65-µm core fiber into a 100-µm i.d. capillary, the experimental compression factor was 1.8, which was in good agreement with the theoretical values, 1.7 (33). Such a zone-narrowing effect is helpful to gain high separation efficiency. However, it is not a good idea to count on maximizing the compression factor alone to reduce the band to an acceptable size. If the ratio of the diameter of the SPME fiber over the i.d. of the capillary is 0.9, which seems to be the maximum value in practice, the theoretical compression factor is calculated to be 5.3. Such a compression factor may be high enough to compress the analytes desorbed from a short SPME fiber (i.e., 1 cm long) into a narrow band (1.9 mm long); however, the surrounding solution of the SPME fiber is subjected to a serious Joule heating, which can induce the temperature 5.3 times higher than that in the remaining section of the capillary if the heat dissipation rate is the same along the whole capillary. Therefore, it is a practical strategy to reduce the fiber size to an intermediate value and combine the zone-narrowing effect with other band sharpening mechanisms such as using solvent or desorbing solution.

Because of the excellent separation power of CE for biological macromolecules such as protein and DNA, SPME–CE coupling suitable for analysis of biological macromolecules is promising. Recently, online coupling of SPME and capillary isoelectric focusing (CIEF) has been accomplished and applied to protein analysis (34). The coupling strategy took full advantage of CIEF with whole column imaging detection (WCID). CIEF is a high-resolution CE mode, in which amphoteric analytes can be separated according to the differences in isoelectric points (pI). When coupling CIEF with WCID, amphoteric analytes can be focused into narrow stationary bands within the separation column. This convergent focusing mechanism offers a unique solution to the problems associated with SPME–CE coupling. On one hand, as the analyte molecules can be focused into a narrow band, the initial size of the analyte band does not influence the peak efficiency; therefore, the desorption process will contribute no band broadening. On the other hand, analyte carryover can be eliminated by simply extending the separation time. Moreover, this coupling offers two additional advantages. First, catholyte and anolyte, which are necessary electrolytes for CIEF, can be used as desorbing reagents, and no additional desorbing reagent or solution is needed. Under the electric field, proton or hydroxide ions migrate into the adapter and displace the protein molecules extracted. Such a desorption mechanism is favorable because the desorbing reagent does not contribute additional volume and thus no dilution effect is associated. Second, the desorption process is activated while the focusing is initiated, saving the total analysis time. This is different from static desorption, in which desorption must be completed prior to separation.

The experimental set-up for the SPME–CIEF–LIF–WCID is illustrated in Figure 4, which relied on a home-made CIEF–LIF–WCID system reported previously (35,36). As small SPME fibers (340-µm core × 8 mm) were used, LIF detection was employed to detect the limited amount extracted. The cartridge for the SPME–CIEF–WCID coupling had an adapter built within the catholyte reservoir of the capillary cartridge. Before experiments, the capillary was filled with CIEF separation medium, and anolyte and catholyte were added to the anolyte and catholyte reservoirs, respectively. An SPME fiber with extracted analytes was inserted into the adapter. Under an electric field, hydroxide ions migrated into the adapter so the local pH inside the adapter increased. Consequently, the extracted analytes on the SPME fiber were desorbed into surrounding solution and focused into certain positions inside the separation capillary where the pH

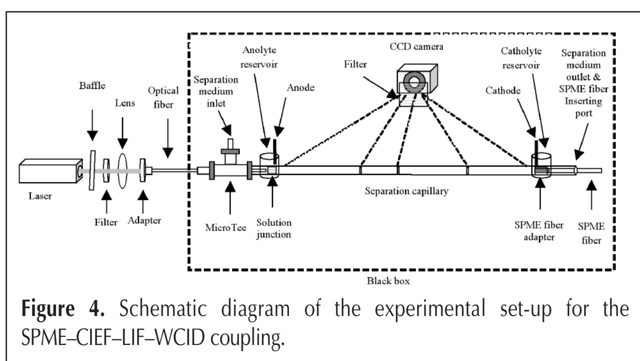

Figure 4. Schematic diagram of the experimental set-up for the SPME–CIEF–LIF–WCID coupling.

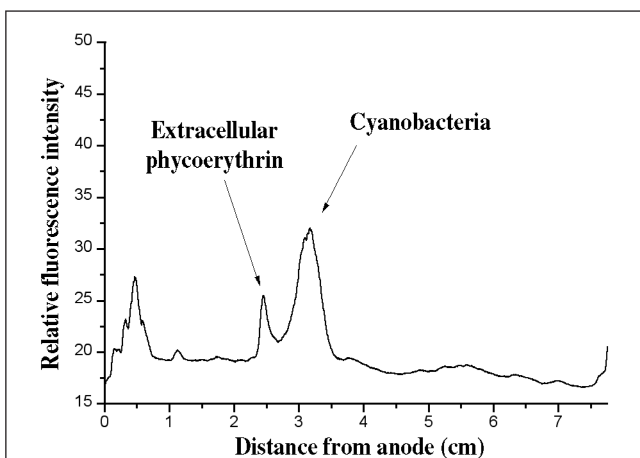

Figure 5. CIEF–LIF–WCID of the CCMP833 cyanobacteria with conventional sample injection. Sample mixture: 100-fold diluted CCMP833 containing 2% pharmalytes (pH 3–10) and 0.5% poly(vinylpyrrolidone).

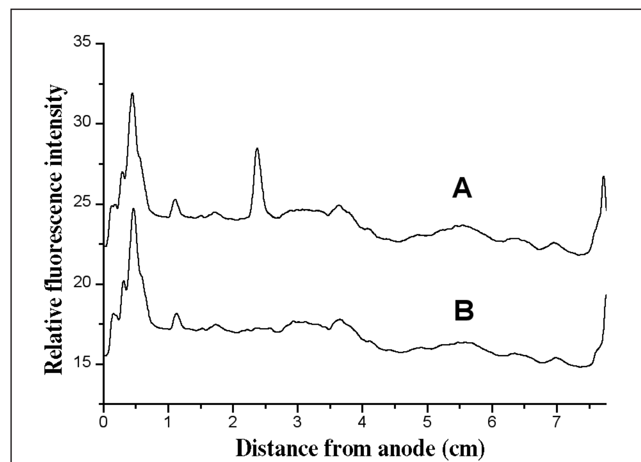

Figure 6. CIEF profiles for SPME extracted extracellular phycoerythrins from the cultured cyanobacteria sample (A) and a blank (B). Sample: (A) CCMP833, (B) none.

equaled to the pI of the proteins. A laser beam of 488 nm was axially illuminated from another end of the separation capillary (167-µm i.d. × 7.8 cm Teflon AF 2400) by liquid-core waveguide, and the fluorescence emitted by the analytes was perpendicularly detected with a charged coupled device camera. Two extraction phases, including (3-aminopropyl)triethoxysilane (APTES) and polypyrrole, were tested, and both exhibited selectivity to the test analytes, R-phycoerythrin and B-phycoerythrin (both are naturally fluorescent protein). By using this coupling method, R-phycoerythrin in water was extracted in 10 min and subsequently analyzed within 20 min, providing a LOD of 3.5×10^{-12} M (s/n = 3). The run-to-run and fiber-to-fiber reproducibility for peak position was excellent, with a relative standard deviation (RSD) value of 2% (n = 6). However, because the SPME preparation procedure was not optimized, the reproducibility for peak area was poor, with a RSD of 13% and 47% for run-to-run (n = 6) and fiber-to-fiber (n = 4), respectively. The method was applied to analysis of extracellular phycoerythrins in cultured cyanobacteria samples. Analysis of extracellular proteins is important because extracelluar proteins have been suggested as signaling markers for the assessment of cell viability (36). However, accurate analysis of extracellular phycoerythrins is problematic because of the presence of bacteria cells in the sample, which can release extra proteins during sample transportation and storage. The electropherogram for the bacteria sample obtained with conventional CIEF–LIF–WCID is shown in Figure 5, which exhibited a peak for the extracellular phycoerythrins and a peak for the cells. As a contrast, when the sample was extracted with SPME, only the peak for the extracellular phycoerythrins was observed (as shown in Figure 6). Thus, the interference from the bacteria cells was avoided by using SPME. With this method, the extracellular phycoerythrins in the cyanobacteria sample at the experiment time were estimated to correspond to 2nM R-phycoerythrin.

To couple SPME with CE through a microdialysis hollow fiber is a new strategy developed more recently (37). Although the performance of the coupling was demonstrated with a CIEF–WCID instrument, the strategy presented is valid for coupling with traditional single-point detection CE system. The principle of the coupling is illustrated in Figure 7. An SPME adapter was connected with the inlet end of the separation capillary through a microdialysis hollow fiber of known molecular weight cutting-off (MWCO) value. Before the experiment, the separation capillary and the adapter were filled with the separation buffer, and the separation buffer and desorbing solution were added to the buffer vial at the inlet end and the buffer vial on the adapter, respectively. After the SPME fiber was inserted into the adapter, a high voltage of appropriate polarity was applied across the two vials. Under the electric field, the desorbing ions migrated into the adapter and, consequently, the analytes extracted on the fiber were desorbed. The desorbed analytes migrated further into the microdialysis

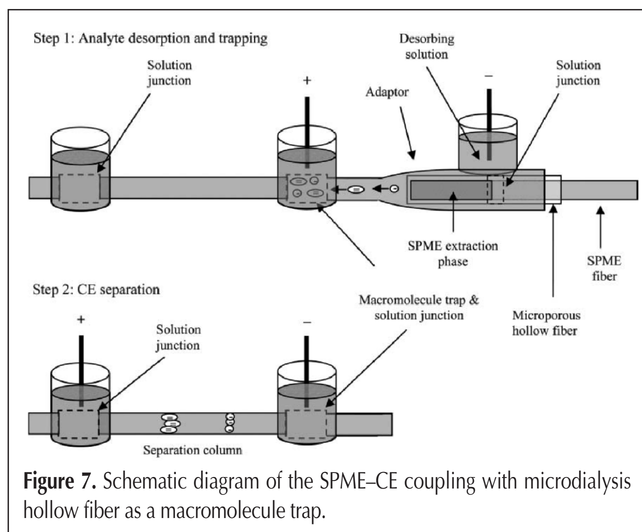

Figure 7. Schematic diagram of the SPME–CE coupling with microdialysis hollow fiber as a macromolecule trap.

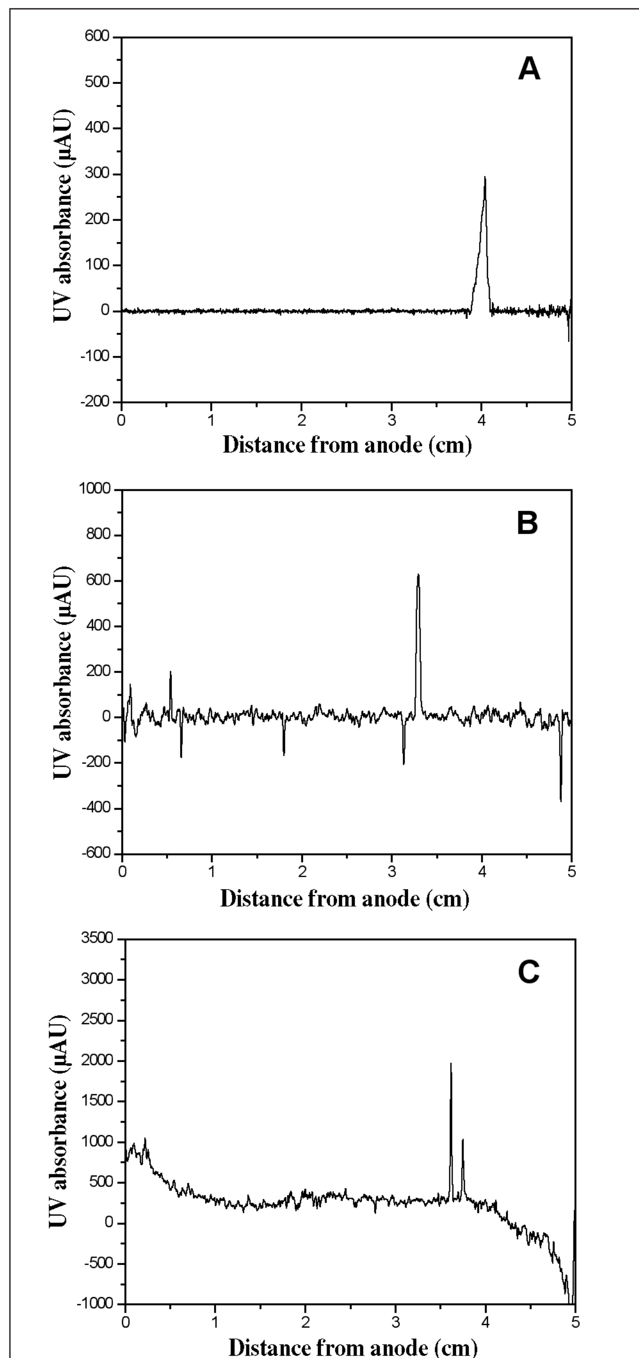

Figure 8. SPME–CZE–WCID of BSA (A), β-lactoglobulin A (B), and β-lactoglobulin A plus B (C). Sample: 38µM bovine serum albumin in 10mM phosphate buffer (pH 7.2) (A), 3µM β-lactoglobulin A in 10mM phosphate buffer (pH 7.2) (B), and 6.8µM β-lactoglobulin A plus B aqueous solution (C).

hollow fiber, where analytes with molecular weight greater than the MWCO were trapped because of the dialysis effect. CE separation was initiated by applying another electric field with different electric polarity across the separation capillary. In this coupling configuration, the microdialysis hollow fiber functioned as a macromolecule trap and a sample preconcentrator as well. Because the initial sample volume in the microdialysis hollow fiber was very small (at nL level), band broadening caused by coupling with SPME was eliminated. Meanwhile, like in SPME–CIEF–LIF–WCID coupling, the desorption time could be as long as needed, and analyte carryover was thereby eliminated. With confining the desorbed analytes in the very small volume of the trap, zone electrophoresis mode became possible on the Convergent Bioscience iCE280 system (Toronto, Canada), which is designed mainly for CIEF mode. Online coupling of SPME with capillary zone electrophoresis (CZE) and CIEF has been successfully achieved. The separation column was a 100-μm i.d. × 5 cm fluorocarbon coated fused-silica capillary. As UV–WCID was used, big SPME fibers (340-μm core size × 2.5 cm) were used. SPME fibers with APTES phase were used, which exhibited selectivity to several acidic proteins. Representative results of the SPME–CZE–WCID coupling are shown in Figure 8. By using a higher pH solution to sweep the analytes into the separation capillary, the separation efficiency was further improved. A pair of proteins with very close charge/mass ratio, β-lactoglobulin A and B, were baseline separated. The electropherograms for β-lactoglobulin A and B obtained with SPME–CIEF–WCID and with conventional CIEF–WCID with sample injection were compared. The result obtained with SPME–CIEF–WCID exhibited nearly the same focusing profile and resolution as those obtained with conventional CIEF–WCID. With UV–WCID, the LODs were 3.7×10^{-7} and 3.0×10^{-8} M (s/n = 3) for β-lactoglobulin A and ovalbumin, respectively. Coupling with capillary non-gel sieving electrophoresis (CNGSE) (37) was also attempted. However, it was found that the macromolecule trap made of microdialysis hollow fiber is not suitable for this purpose because multiple peaks were observed for a single protein tested. The reason was explained by insufficient replenishment of SDS anion because of the physical presence of the hollow fiber.

In-tube SPME

In-tube SPME is another format that has been coupled with CE, in which extraction material, including organic fiber, liquid chromatographic packing material, or chemically modified glass bead is packed in a short piece of tube. It looks like a micro-SPE cartridge. However, when it is built in a cross-shaped device or connected with a cross connector, it can be used for sampling. Jinno et al. (38) reported online coupling of fiber-in-tube SPME with CE. The fiber-in-tube SPME–CE system is shown in Figure 9. The SPME was a short piece of fused-silica capillary or GC column (250-μm i.d. × 10 mm) packed with zylon fiber (10-mm length). It was connected with a home-made CE system through a cross connector. The separation capillary was a 75-μm i.d. fused-silica capillary. A Microfeeder (Azuma Denki Kogyo, Tokyo, Japan) was used to deliver sample for extraction and to perform analyte desorption and injection. To minimize the band broadening effect, the gap between the separation capillaries inside the connector was strictly decreased (less than 10 μm). In the extraction process, the sample solution delivered from the Microfeeder pump passed continuously through the extraction medium for a certain time at a known flow rate. The analytes were adsorbed in the fiber-packed capillary. Next, another syringe containing the desorbing solvent, acetonitrile, was attached to the Microfeeder and the solvent was pumped through the extraction medium. Meanwhile, the desorbed analytes were directly transferred to the space in the cross connector by pumping an appropriate amount of the solvent. After the concentrated sample zone was injected with the cross connector, a high voltage was applied to the separation capillary, and the analytes were separated. Extraction and separation of four tricyclic antidepressant drugs, amitriptyline, imipramine, nortriptyline, and desipramine, were performed with the hyphenated system. CZE separations of the four drugs obtained by fiber-in-tube SPME–CE and direct CE analysis were compared. The

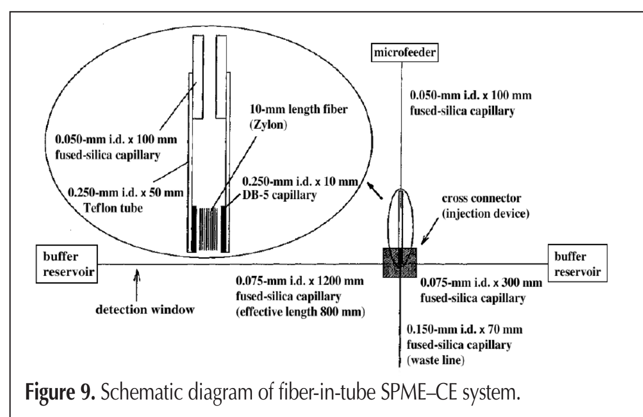

Figure 9. Schematic diagram of fiber-in-tube SPME–CE system.

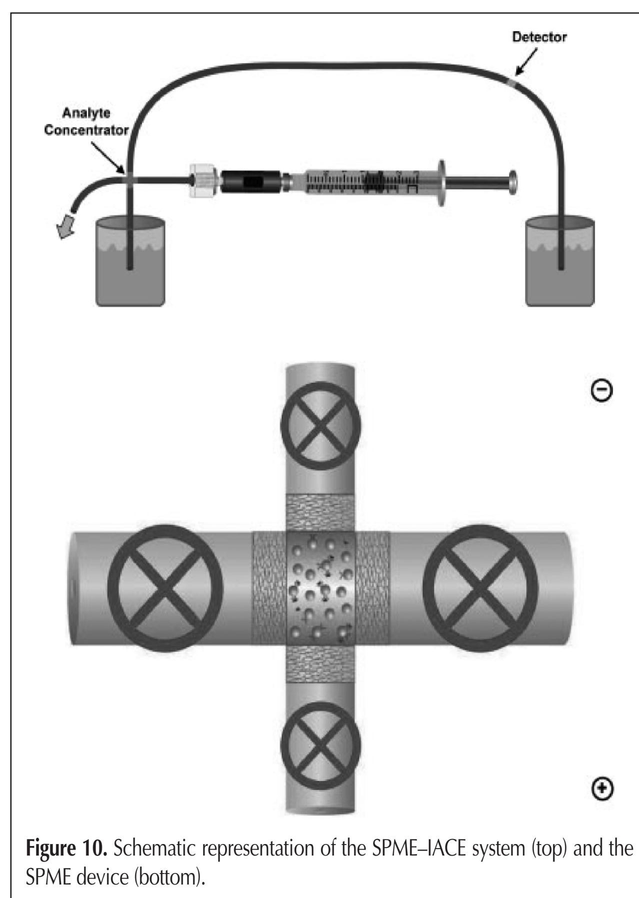

Figure 10. Schematic representation of the SPME–IACE system (top) and the SPME device (bottom).

detection sensitivity obtained with the fiber-in-tube SPME–CE was more than 100 times better than that with direct CE analysis without SPME. LODs ranged from 44 ng/mL for nortriptyline to 153 ng/mL for imipramine (s/n = 3). This SPME–CE method was applied to the analysis of amitriptyline in human urine. Amitriptyline of 200 ng/mL in the patient's urine was extracted and determined.

Recently, Guzman (39) reported online coupling of in-tube SPME with immunoaffinity CE (IACE). The instrumental set-up of the in-tube SPME–IACE system and the SPME device are illustrated in Figure 10. The system was composed of a cross-shaped SPME device and a home-made CE system. A 100-µm i.d. fused-silica capillary was used for the separation. The SPME device, which was of cruciform configuration, had four entrance–exit ports. In the horizontal position, there was a large-bore transport tube for sample introduction and buffer washes. In the vertical position, there was a small-bore fused-silica capillary for CE separation of the analytes. Four microfabricated values were built on the four ports to control the path of the fluid in the desired direction. High specificity polyclonal antibodies were employed as extraction phase, including Fab's fragment derived from antibodies raised against the acidic nonsteriodal anti-inflammatory drugs ibuprofen and naproxen, and Fab's fragments derived from antibodies raised against the neuropeptides angiotensin II and neurotensin. These immunoaffinity ligands were covalently immobilized to the surface of controlled-pore glass beads (3000-Å pore size, 200–400 mesh, irregularly shaped). The prepared beads were packed into the SPME device. With the valves for the separation capillary closed, sample solution of approximately 1 mL were introduced into the SPME device using a syringe by positive pressure or by employing a low vacuum aspiration system directly from the sample reservoir. After a few washes of the transport tube with 50mM sodium tetraborate buffer (pH 9.0), the valves were switched to the separation position. The analytes were finally eluted with a plug of approximately 100 nL pf 300 glycine buffer (pH 3.4). Then a high voltage was applied to the separation capillary to start the CE separation. Urine specimens spiked with the drugs or peptides were examined. Sharp peaks were observed for the drugs and peptides extracted, and the background was very clean, indicating the effectiveness of the coupling. With UV detection, the LODs were approximately 1 ng/mL for ibuprofen and naproxen and 0.5 nL/mL for angiotensin II and neurotensin. As similar devices can be used as a microreactor when immobilized enzymes are packed, the SPME device is also referred as to analyte concentrator–microreactor by the inventor.

Conclusion

Online SPME–CE coupling has been developed in the past several years. The problems encountered (i.e., band broadening, analyte carryover, and size match) have been solved with several strategies. For classification, these strategies include three types: (*i*) direct insertion with small fiber SPME, (*ii*) coupling through an adapter for big fiber SPME, and (*iii*) directly coupling with in-tube SPME. These strategies have their own advantages and disadvantages. For the direct-insertion type of coupling, the main advantage is its zero connection volume, but the main disadvantage is the difficulty in insertion when a very narrow bore-size capillary is used. For the through-adapter type of coupling, the main advantages are its compatibility with big SPME fibers and ease in operation. For the in-tube SPME type of coupling, the main advantage is its extraction capacity. But it has two main disadvantages. First, usually only a portion of desorbed analytes can be transferred into the separation capillary. Second, the in-tube SPME is prone to be blocked by dirty matrix or particles in the sample if the sample is not filtered before extraction. The two in-tube SPME devices reported (38,39) look very similar to micro-SPE, as they were operated under exhaustive conditions. However, in-tube SPME can also work under pre-equilibrium or equilibrium conditions, if all operational conditions are precisely controlled.

The use of electric fields in CE is a favorite factor for the coupling. Desorbing solvent or ion can be introduced with electroosmotic or electrophoretic forces. When ions such proton, hydroxide ion, or inorganic ion are used as desorbing reagent, their volume can be negligible. Therefore, there is no dilution effect associated with the desorption process. Besides, the desorbed analyte bands can be further sharpened by focusing mechanisms and online sample concentration techniques in CE. In addition to the focusing mechanism of CIEF, which has been employed (34), other concentration techniques such as field-enhanced sample stacking (40–42), sweeping (43,44), velocity-difference induced focusing (45,46), and chromatographic zone-sharpening (47) can be helpful means to reduce the analyte band. As the principles in CE have been widely employed in microfluidic systems, the SPME–CE coupling strategies developed should be useful for coupling SPME with microfluidic devices.

Future Trends

Several aspects might be the main trends in the development of online SPME–CE coupling in future. First, novel strategies will be developed to solve the problems associated with the coupling, though they have been partially or completely solved by the reported strategies. More CE modes such as capillary electrochromatography, capillary gel electrophoresis, or CNGSE will be used to separate analytes with different properties such as neutral compounds or nucleic acids. The focusing mechanisms and sample concentration techniques will be assets for the new strategies. Second, coupling of nano-SPME and CE or chip CE with highly sensitive detection will be a promising tool for in vivo sampling and analysis of single cells. Although the extraction capacity of nanoscale SPME is limited, ultrasensitive detection such as LIF (48,49) or MS (50,51) has developed to be able to detect zeptomole analytes and single molecules. Therefore, online coupling of nano-SPME–CE–LIF or nano SPME–CE–MS can be expected to be applicable for single-cell analysis and in vivo analysis. As there have been successful applications of microelectrodes in single-cell analyses (52,53), such a coupling method should be feasible. Third, SPME can be coupled with microfluidic systems, in which electrophoresis in the microchannel is the core for the separa-

tions. As microfluidic systems can perform multiple functions, functions that favor the coupling (i.e., fluorescent labeling) can be integrated into the system. Finally, SPME with highly specific selectivity will be used to extract a single or several analytes from very complicated samples (i.e., to extract a specific protein from a proteomic sample). For this purpose, aptamers (54), which are small segments of nucleic acids and can bind specifically with a variety of molecules, will be the affinity ligands of choice.

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Manuscript received September 14, 2005;
revision received December 30, 2005.