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On-Channel Micro-Solid Phase Extraction Bed Based on 1-Dodecanethiol Self-Assembly on Gold-Deposited Colloidal Silica Packing on a Capillary Electrochromatographic Microchip

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A fully packed capillary electrochromatographic (CEC) microchip with an on-column micro-solid phase extraction (SPE) bed for the preconcentration and separation of organic analytes was prepared. A linear microchannel with monodisperse colloidal silica packing was formed on a cyclic olefinic copolymer microchip with two reservoirs on both ends. Silver-cemented silica packing frit structure was formed at the entrance of the microchannel by electroless plating treatment as a base layer. A gold coating was formed on it by reducing Au^{3+} to gold with hydroxylamine. Finally micro-SPE bed was formed by self-assembly adsorption of 1-dodecanethiol on it. Micro-SPE beds were about 100-150 μm long. Approximately 10^3 fold sensitivity enhancements for Sulforhodamine B, and Fluorescein in nM concentration levels were possible with 80 s preconcentration. Basic extraction characteristics were studied.

Key Words : Solid phase extraction, Capillary electrochromatography, Microchip colloidal silica packing, Dodecanethiol self-assembly

Introduction

Analyses using capillary electrophoretic (CE) or CEC microchips have been popular research topics in the area of microfluidics because of their efficiency and versatility in various analytical separations. Practical analyses are possible with minimal amounts of samples and solutions on small microfluidic platforms. Numerous reports on microchip-based CE or CEC separations have been published in various areas, including environmental, medicinal and physiological sciences.[1-8]

Most of the capillary microchannels employed in the CE or CEC microchips were about 50 mm long. They were either open tubular or monolithic porous polymer-packed microchannels. The surface of the microchannels or monolithic porous channels could be modified to get specific interactions between the surface and analytes for effective separations. Recently we have reported a new CEC microchip platform having crystalline sub-micron silica bead packing through the whole microchannel, which shows competitive separation characteristics with only about 5 mm long separation microchannel. Because of the built-in nanofilter structure resulting from the colloidal silica packing the handling of solution and chip became much easier. Also the electroosmotic flow (EOF) control became more reproducible due to the packing structure with extremely large surface area holding solution in the microchannel.[9] The surface of the colloidal silica beads can be modified for desirable surface interaction with analytes.[10]

Various efforts have been reported to increase the analytical sensitivity by means of detection signal enhancement, sample stacking or preconcentration by employing SPE beds.[11-21] Among those preconcentration of the analytes utilizing the SPE technique would be a good choice for sensitivity enhancement. Desirable analytes can be extracted to the proper extraction bed from complex sample matrix, and then released for the separation by eluting with a solvent with different polarity. Recently, many works on the microchips with on-chip SPE capability have been reported.[14-21] Commercial C_{18}-silica beads were slurry-packed in a SPE chamber for the extraction of Rhodamine B with 70-220 fold concentration enhancements.[18] Relatively large SPE chamber about ~7 mm long and four times wider than the microchannel was formed for the packing of large C_{18}-silica beads (3 μm) to get proper extraction capacity. So extended sample releasing time causing band broadening was required. A disk-type polymeric monolith SPE chamber having anionic functionality was also formed at microchannel-cross for the extraction of catecholamine.[15] Similarly elution peaks were broad and overlapped due to the large diameter of the SPE chamber (~1.8 mm) and large pore size of the monolithic structure. Various on-channel monolithic SPE beds on the microchips were also prepared and examined for sample extraction or extraction followed by CE or CEC separation.[14-16] In most cases the length of the SPE bed ranged from 2 to 7 mm although the width was similar to the microchannel. Enough surface area for proper sample adsorption could not be afforded with short bed length because of large pore size (~10 to 20 μm) of polymeric monolith. Several hundred fold enrichments were reported with millimeter scale SPE chambers in most works.

Recently a perm-selective micro-preconcentrator based on

carboxylate-type polyacrylamide gel was reported.[21] Anionic gel micro-plug (~500 μm) was formed near the channel cross by photo-polymerization. Electrostatic repulsion of the anionic gel limited the passage of anionic fluorescein isothiocyanate (FITC)-labeled amino acids through the gel plug effectively resulting 10^5 fold enrichments in 3 min. It is an excellent example of real micro-scale on-chip preconcentrator showing extremely high efficiency. This work showed effective interaction between the preconcentration bed and analytes is essential for high efficiency in micro-scale SPE. Nevertheless effective micro-SPE beds on the CE or CEC microchips are still desired for sensitive microchip analysis of neutral or less ionic analytes.

In these circumstances, here we report a C_{12}-modified colloidal silica-based on-channel micro-SPE formed at the entrance of the microchannel of the colloidal silica packed CEC microchip mentioned earlier. The surface area for the sorbent was enlarged by employing colloidal silica bead packing. The surface concentration of the sorbent, 1-dodecanethiol, was further increased by self-assembly adsorption on gold coating on the surface of the colloidal silica beads. Therefore the effect of the size reduction to micrometer scale was compensated while the sensitivity was enhanced further. Detailed preparation procedures and the extraction characteristics of the micro-SPE bed are described.

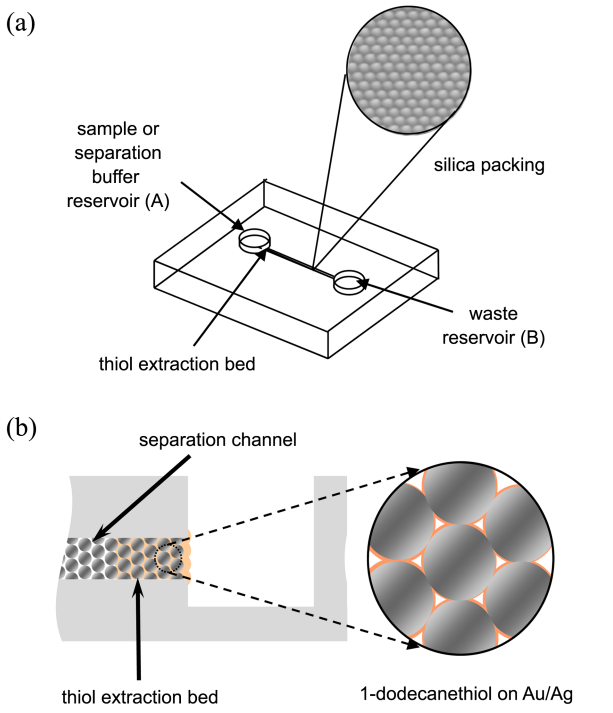

Figure 1. Schematic diagrams of the microchip with a micro SPE bed. (a) Overall feature of the microchip. (b) Details of the SPE bed at the entrance of the separation channel.

Materials and Methods

Reagents and Materials. Unmodified monodisperse colloidal silica beads (800 nm) were purchased from Bang's Laboratories, Inc. (Fisher, IN, USA). Rhodamine 110, Sulforhodamine B and Fluorescein were obtained from Sigma-Aldrich and used without further purification. Solutions for fluorescence detection were prepared one day before CEC experiments and kept in a refrigerator. The solutions were used without filtration because the microchip had built-in nano-filter structure at the entrance of the microchannel. Cyclic olefinic copolymer (COC) resins (Topas 8007, 5013) were obtained from Ticona (Summit, NJ, USA).

Microchip Fabrication. Spontaneous three dimensional crystalline packing of the silica beads in the linear microchannel was performed similarly to the previous work.[9,10] A 35 mm long straight microchannel with 80 μm width and 50 μm depth was patterned on a COC plastic microchip substrate by hot press molding technique using an electroformed nickel mold. After hydrophilic treatment with air plasma an end of the microchannel was carefully dipped into an aqueous solution of monodisperse colloidal silica beads (0.05 wt %, 40 °C) in a polyethylene beaker. The colloidal silica solution moved upward to the other end of the microchannel by capillary action. Spontaneous three-dimensional crystalline packing of the silica beads started from the top end of the microchannel, and continued downward slowly by the slow evaporation of water. After reaching the desirable packing length, excess silica at dipped area was washed with water. The packing was dried at room temperature.

The packed microchip substrate was bonded with a cover plate as follows: the glass transition temperature (T_g) of the packed COC substrate and the cover plate was 134 °C. A plain COC plate with low T_g (78 °C) was placed on the packed substrate, and then a cover COC plate was placed on top of it. The sandwiched COC plates were heated at 125 °C in a convection oven for 3 h so to soften the middle COC plate. Finally, a weight preheated at the same time was placed on the stack of the COC substrates for 4 h to bond both outer COC plates together by the softened middle layer, and cooled down to room temperature. After bonding it was fabricated to a microchip as shown in Figure 1. Two reservoirs with 12 mm distance were drilled on the microchannel with a milling bit (2.5 mm in diameter). The microchip was 20 mm (w) × 25 mm (l) × 2.5 mm (h) in size with a 12 mm long colloidal silica-packed microchannel at the center. The micro-SPE bed was formed at one end of the packed microchannel.

Extraction Bed Formation. Gold-coated silica frit structure was prepared at one end of the microchannel for the formation of the micro-SPE bed. Gold plating was performed over the silver-cemented frit structure prepared by simple silver mirror reaction similarly to previous work.[22] The microchannel was wet with 3.7% formaldehyde solution from one of the reservoirs. After completion of wetting, the formaldehyde solution was added to the other reservoir, and then removed from both reservoirs. A drop of 5 mM $Ag(NH_3)_2^+$ in 0.1 M NH_3 was added to both reservoirs, and allowed to diffuse into the packing for 30 s. After removing the silver solution, the reservoirs were filled again with the formaldehyde solution promptly. The microchip was placed

on a preheated hot plate at 40 °C for 5 min for the completion of electroless silver plating on the silica packing at the ends of the microchannel. The reservoirs were washed thoroughly with deionized water. A drop of 8.5 mM $HAuCl_4$ solution was added to one of the reservoirs and allowed to diffuse in for 35 s at room temperature. After removing the gold solution, a drop of 20 mM hydroxylamine in 40 mM NaOH was added to reservoir for the deposition of gold on the silver coating in the microchannel. After 4 min, the reservoirs were washed thoroughly with deionzed water and then with 30% methanol. A scanning electron microscope (SEM) (JSM 5200 from Jeol) was used for the morphology observation.

For the adsorption of the 1-dodecanethiol, the microchip was immersed in 0.8 mM 1-dodecanethiol in ethanol for 3 h. The microchip was washed with ethanol and deionized water thoroughly. The microchannel was further washed electroosmotically with 30% methanol/20 mM borate buffer (pH 8.5) to remove extra 1-dodecanethiol and reducing agents until stable background obtained. After the completion of cleaning, the microchip was stored in 20 mM borate buffer (pH 8.5) until use.

LIF Detection. A charge coupled device (CCD) multichannel spectrometer (S2000 PCI, Ocean Optics) was connected to a camera port of a fluorescence microscope (Olympus BMX 51) using optical fiber for the detection of analytes. LIF detection was carried out by using a microchip stage block with a built-in blue diode laser source (405 nm) with a focusing lens underneath a slit unit. The position of the laser block can be controlled by a micrometer control knob to the slit position for desirable length for separation. The slit unit has 50 μm slits in 1 mm intervals in series along the microchannel from the channel-cross. Details on the structure and use of the microchip stage block were described in previous work.[23] A built-in filter block in the microscope with band pass of 400-440 nm and barrier filter of > 475 nm was used for the selection of the excitation and detection wavelength. A bandwidth of 200 nm for detection was set at 550 nm. The signal was integrated every 200 ms. Fluorescence light induced by the laser coming through the slit underneath of the microchip was collected through an objective lens (× 50), and then analyzed by the CCD detector.

Extraction and Electrochromatographic Measurements. For the EOF drive of the solutions, a multichannel high voltage power supply from Lab Smith (HVS 448-3000V) was used. Rhodamine 110 and Sulforhodamine B, Fluorescein were used as probe molecules. Before the extraction of the analytes the microchannel was cleaned with acetonirile (ACN)/20 mM borate separation buffer and then a blank borate buffer. An aliquot of aqueous sample buffer solution containing the probe molecules was added to the sample/running buffer reservoir where the micro-SPE bed formed. The sample buffer solution was eluted for a given time to accumulate the analytes at the 1-dodecanethiol extraction bed by applying 180 V typically. Then, the sample reservoir was washed with blank buffer solution again. After adding a drop of ACN containing separation buffer solution to the sample reservoir, the elution for the separation was initiated immediately by applying separation voltage, typically 300 V, across the microchannel. Extra elution of the ACN solution was allowed for 100 s after confirming the last analyte in order to flush out any organic analyte or contaminants left in the microchannel. Then the ACN solution was removed from the microchannel by eluting the blank buffer solution for 100 s for subsequent experiment.

Results and Discussion

Microchip Fabrication and Micro-SPE Bed Formation. Direct coating of the gold on the silica beads was tried for the self-assembly adsorption of dodecanethiol on it. However, it was not feasible because of gold nanoparticle formation which did not adhere to the silica bead surface. It is likely the gold nanoparticles would have aggregated together and precipitated in the voids of the silica packing rather than coating the silica surface. Indeed, the extraction efficiency decreased gradually during repeated use of the microchip, possibly because of the physical instability of the gold coating. So, a silver coating was applied as a base layer for the gold coating. The stability of the silver coating was demonstrated in our previous work on silver-cemented frit structure formation for packing structure stabilization in the microchannel.[22] Improved stability of the extraction bed was achieved with the gold coating on the silver base coating formed by silver mirror reaction. It is likely the silver coating served as a seed layer for gold deposition. The surface morphologies of the micro SPE bed at the entrance of the

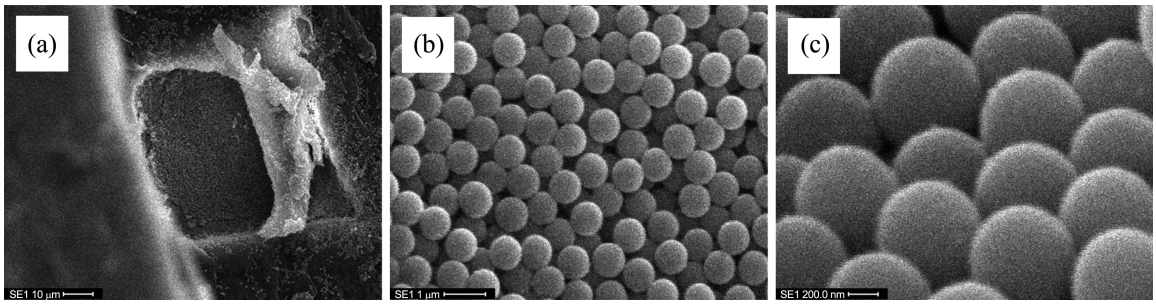

Figure 2. SEM images of the silica packing of the micro-SPE at the entrance of the microchannel. (a) × 1000, (b) × 15000, (c) × 50000, 25 kV.

microchannel are shown in Figure 2. The silica packing at the entrance seemed to be held tightly by the plated metals. The metal coated surface of the silica beads was round and smooth although some pimply deposits can be seen. The voids of the silica packing were not clogged by the metallic deposits. Some burr of the COC plastic formed during the drilling process of the reservoir was also observed. However the burr did not clog the microchannel with significant problem because of enough opening of the microchannel. The coating procedure was improved empirically as described in the experimental section for the high stability of the extraction bed. Three hours of self-assembly adsorption of 1-dodecanethiol was enough for the best results. Longer adsorption caused extra oily thiol to float in the microchannel, and extended the cleaning time of the thiol from the microchannel. The length of the extraction bed was controlled by changing the duration of the soaking time of $Ag(NH_3)_2^+$ and Au^{3+}. However precise control of the length was not feasible as desired. The length of the micro-SPE beds was estimated using the optical microscope and ranged about 100-150 μm.

Extraction Characteristics of Micro-SPE bed. Rhodamine 110 is highly hydrophobic and Fluorescein is moderate, but Sulforhodamine B is water soluble. Nevertheless, all the analytes were hydrophobic enough to be extracted by the 1-dodecanethiol extraction bed from aqueous sample solution. The ACN/buffer solution was also strong enough to wash all the analytes out from the extraction bed. All three probe molecules extracted were separated effectively with only 6 mm separation channel length. (not shown). Least polar Rhodamine 110 was eluted first, then, Sulforhodamine B followed by Fluorescein. However, the elution behavior of Rhodamine 110 was suspected because it was always eluted at the position of the background front of the ACN/buffer eluting solution. Extremely high hydrophobicity of Rhodamine 110 did not allow regular chromatographic or electrophoretic interaction. It was eluted without any retention. Furthermore, the peak height was continuously growing without steady state saturation upon the extension of preconcentration time while the others were saturated eventually to steady state values (will be shown Figure 5(a) below). It is thought that Rhodamine 110 was trapped on the dodecanethiol extraction bed, but the excess adsorbed irreversibly on the silica surface in the separation channel during preconcentration. All Rhodamine 110 trapped on the SPE bed and adsorbed on the silica beads could be washed out easily by the ACN/buffer solvent to show the oversized peak at the solvent front. Irreversible adsorption of extra Rhodamine 110 on the surface of the silica packing in the separation channel was confirmed using a microchip without extraction bed treatment by observing fluorescence from the entire packed microchannel. So Rhodamine 110 was excluded in the study of the extraction characteristics of the SPE bed.

In contrast, Sulforhodamine B and Fluorescein revealed better separation behaviors. It seems that highly negative Sulforhodamine B had less interaction with the negatively charged silica surface, and it was eluted electrophoretically along with EOF. Meanwhile, less polar Fluorescein showed adsorption chromatographic behavior resulting slower migration. The ACN content in the eluting solvent affected migration time extensively as it can be seen in Figure 3. As the ACN content decreased, the migration time and the peak width of Fluorescein increased gradually although the EOF increased by the polarity increase of the solvent. It would be due to the dominant adsorptive interaction of the Fluorescein to the silica surface. In contrast, Sulforhodamine B was so polar that its migration behavior was affected extensively by the EOF change resulting from the change of ACN content. In fact, the migration of Sulforhodamine B became faster because of increased EOF as the ACN content decreased. However, such chromatographic behavior is not conclusive because more systematic works have to be done. So we left them for further study at this moment, and our discussion is limited to extraction characteristics hereafter.

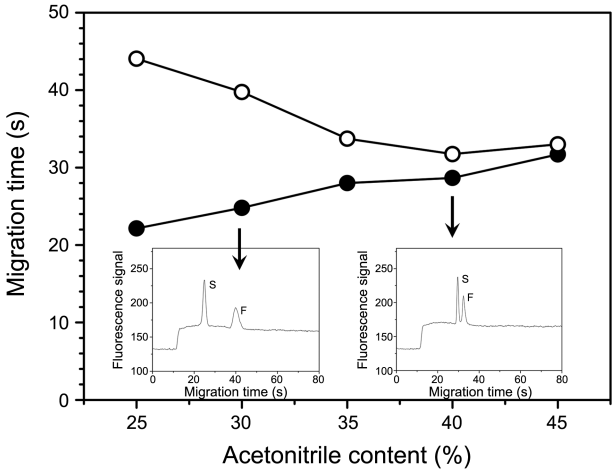

Figure 3. Effect of elution buffer on the migration time. Open circle: Fluorescein, solid circle: Sulforhodamine B, 50 nM each. 80 s extraction with samples in 30 mM $Na_2B_2O_7$ (pH 8.5) at 180 V. Eluted with 30% ACN/60 mM $Na_2B_2O_7$ (pH 8.5) at 300 V. Detected at 6 mm from the SPE bed.

The sensitivity was improved as much as 10^3 folds by the preconcentration capability of the extraction bed as shown in Figure 4. 50 nM of Sulforhodamine B and Fluorescein were collected to the extraction bed for 80 s in Figure 4(a). In comparison, the 50 μM analytes were separated using a fully packed microchip without extraction bed in Figure 4(b). Pinched sample loading and separation with back pushing technique were applied. The same LIF detection setting and separation channel length were selected. Although direct comparison between two separations was not possible due to the different elution conditions, briefly, the sensitivity enhancements of 780 fold for Sulforhodamine B and 1010 fold for Fluorescein were achieved.

In Table 1 the extraction characteristics are compared for typical microchips with SPE bed reported previously. They are mostly flow-through type SPE beds trapping by attractive surface interactions except the perm-selective type in the last. The enrichment factor of the micro-SPE in this work was far less than that of the perm-selective polyamide gel

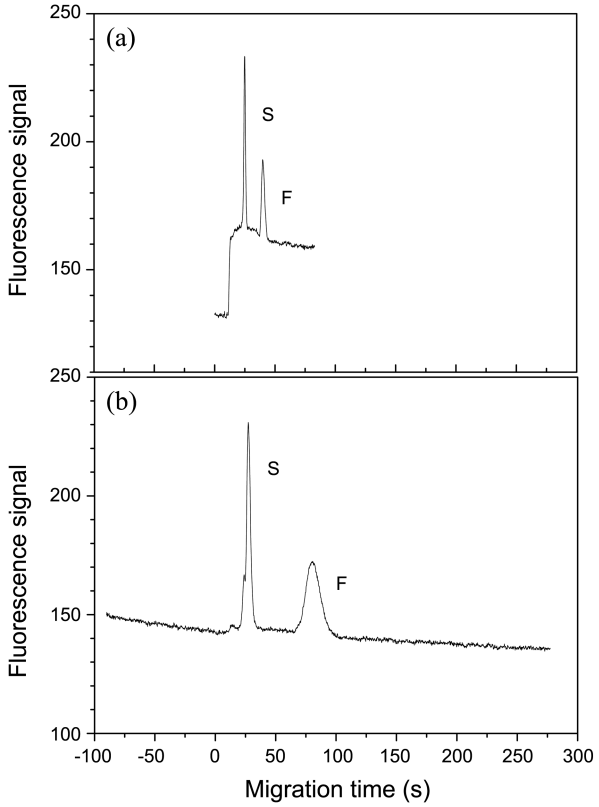

Figure 4. Sensitivity comparison between the microchips with and without micro-SPE bed. S: Sulforhodamine B, F: Fluorescein. (a) With micro-SPE bed. 50 nM each. 80 s extraction with samples in 30 mM $Na_2B_2O_7$ (pH 8.5) at 180 V. Eluted with 30% ACN/60 mM $Na_2B_2O_7$ (pH 8.5) at 300 V. Detected at 6 mm from the SPE bed. (b) Without micro-SPE bed, 50 μM each. The sample in 15% ACN/60 mM $Na_2B_2O_7$ (pH 8.5) was loaded at 180 V, and eluted at 300 V. Detected at 6 mm from the channel cross.

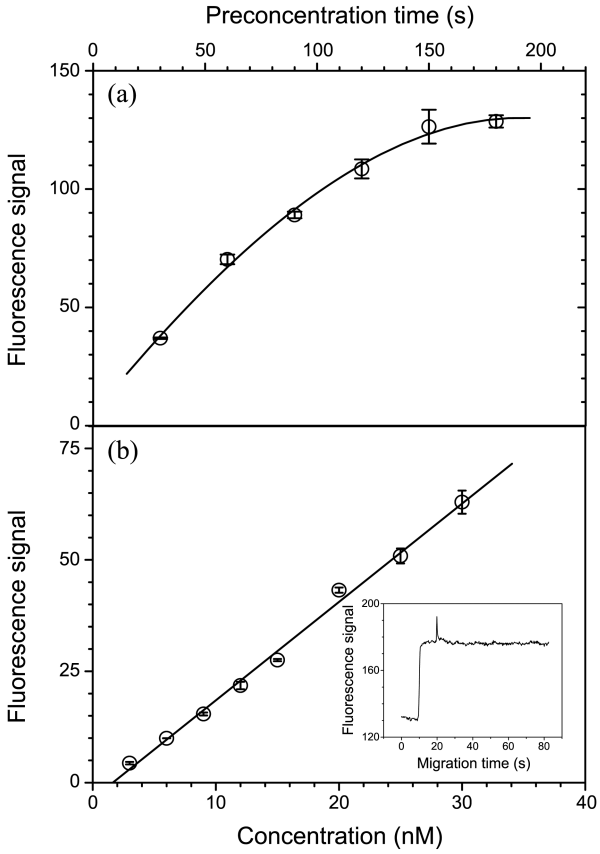

Figure 5. Saturation behavior of the micro-SPE bed and linear calibration curve for Sulforhodamine B. (a) Saturation behavior of the micro-SPE bed, 30 nM of sample in 25 mM $Na_2B_2O_7$ (pH 8.5) was extracted at 180 V, and eluted with 40% ACN/60 mM $Na_2B_2O_7$ (pH 8.5) at 300 V. Detected at 6 mm from the micro-SPE bed. (b) Linear calibration curve. 120 s extraction. Other conditions are same as Figure a. Inset: 0.1 nM of Sulforhodamine B. Extracted for 600 s, and then eluted with 50% ACN/buffer 60 mM $Na_2B_2O_7$ (pH 8.5) at 300 V. n=3 for the error bars.

trap. However it was three to ten times higher than other flow-through type SPE beds although the bed length was only several tenths to hundredths of theirs. Such high enrichment factor was owing to the high surface concentration of the dodecanethiol on the gold-coated colloidal silica packing.

Figure 5(a) shows the saturation behavior of the extraction bed for Sulforhodamine B. The fluorescence signal increased

Table 1. Extraction characteristics of typical microchips with SPE bed

SPE Type	Bed dimension (μ-channel[a])	Analytes	Enrichment factor (time)	Separation method	Ref.
C_{12}-SH SAM on colloidal silica	100-150 μm long (80 μm × 50 μm × 12 mm)	Sulforhodamine B Fluorescein	760-1000 (80 s)	CEC	This work
Ion exchange or hydrophobic polymer monolith	7 mm long (100 μm × 40 μm × 60 mm)	Coumarin 519	337 (66 s) 1650 (1700 s)	Frontal analysis	14
Boronic acid polymer monolith	~1.8 mm in diameter[b] (150 μm × 50 μm × 54 mm)	Catecholamine	92-178 (15 min)	N/A	15
PolyE-323 coated monolith	1 mm long, 140 μm wide (56 μm × 20 μm × 35 mm)	BODIPY	105 (30 min)	ES/SMS	16
3 μm C_{18}-silica	~4 mm long[b], 210 μm wide (55 μm ×15 μm × 50 mm)	Rhodamine B Coumarin 314 & 334	220 (90 s)	MEKC	18
Perm-selective anionic polyamide gel	100~500 μm long 100 μm × 30 μm × ?[b]	Fluorescein 8-aminonaphthalene-1,3,6-trisulfonic acid	10^5 (3 min)	CE	21

[a]Denotes the dimension of the microchannel (width × depth × length). [b]Estimated from the figure therein.

gradually as the preconcentration time increased until the extraction bed saturated at near 150 s. For the extension of the extraction capacity, larger surface area of the gold coating is desirable, on which more 1-dodecanethiol can be adsorbed. The amount of the gold coating could be increased by increasing either the soaking time or the concentration of the gold plating solution. However, severe band broadening by the extended length of the extraction bed or clogging of the microchannel was experienced as well.

The concentration dependence of the peak area for Sulforhodamine B was also examined from 3 nM up to 30 nM at fixed extraction time of 120 s and shown in Figure 5(b). A linear calibration curve with a regression coefficient of 0.997 was obtained for the calibration range. The relative error for each point was less than 6% (n=3). The migration time was reproducible with less error than 1.7%. Further increase in extraction sensitivity down to 0.1 nM was possible by increasing the preconcentration time to 600 s (Inset of Figure 5(b)). However, quantitative analysis was not possible because the experimental conditions for trace level analysis are not optimized yet. The evaporation of the sample solution, adsorption of the analytes on the plastic reservoir t and trace level impurities in the solvent would disturb quantitative analysis. Careful extraction conditions with system upgrades should be established to increase analytical sensitivity further.

Conclusions

In this paper, a highly effective on-column micro-SPE bed was formed successfully at the entrance of a microchannel packed with monodisperse colloidal silica beads on a CEC microchip. The gold coating on the silver seed layer on the silica packing was effective for the formation of the self-assembled 1-dodecanethiol layer for the extraction bed. The on-column preconcentration capability of the fully packed microchip will be beneficial for the sensitivity improvement in CEC microchip analysis. Further studies on the separation behaviors after enrichment and improvement in micro-channel designs for practical applications are in progress.

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