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Trapping of Bead-Based Reagents within Microfluidic Systems: On-Chip Solid-Phase Extraction and Electrochromatography

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A 330-pL chromatographic bed was fabricated on a glass substrate as part of an electroosmotically pumped microfluidic system. Two weirs within a sample channel formed a cavity in which octadecylsilane (ODS) coated silica beads (1.5−4 μm diameter) were trapped. ODS beads were mobilized into and out of the cavity using electroosmotic flow through a bead-introduction channel which accessed the cavity. This procedure allowed the beads in the cavity to be repeatedly exchanged. A 1 nM solution of a nonpolar analyte (BODIPY 493/503) in buffer was loaded onto the beads for different lengths of time using an electroosmotic flow of 1.2 nL/s. The material retained on the ODS phase was then eluted by electroosmotic flow of acetonitrile with a concentration enhancement of up to 500 times. Capillary electrochromatography was conducted using a similar device. BODIPY and fluorescein were loaded onto a 200-μm-long chromatographic bed and then separated in an isocratic CEC run with an acetonitrile/buffer mobile phase. Complete separation was achieved in less than 20 s with a 2-μm plate height.

Recent developments in the area of Micro Total Analysis Systems (μ-TAS) have included systems that perform chemical reactions, separation, and detection on a single microchip.[1−3] Most microfluidic devices have been based on open tubular flow designs and solution-phase reagents. Very few have employed packed reactor beds incorporating beads with immobilized reagents or stationary phases, despite how ubiquitous bead-based materials have become in immunoassays, reactor beds, and chromatography. It appears that the difficulty of packing portions of a complex microfluidic manifold with beads has hindered the utilization of these ideal reagent delivery vehicles within microfluidic devices. A convenient method of trapping beads on-chip and of packing the trapping zones, should significantly expand the range of the microfluidic toolbox and extend the number of applications of such devices. A packed-bed chromatographic device with a bead-trapping frit was fabricated previously in a silicon substrate,[4] but the packing material could not be readily exchanged, nor could electrokinetic pumping be used to pack the bed, owing to the conductivity of silicon. Here we report on a design that allows exchange of packing materials which has led to on-chip solid-phase extraction (SPE) and capillary electrochromatography (CEC), and can be further extended to include integrated packed-bed immuno- or enzyme reactors.

Sample preconcentration by SPE is often required to overcome detection-limit problems or to eliminate a potential interferent. To date, preconcentration within microchips has been performed by sample stacking[5] using "isoelectric focusing". Unlike sample stacking, SPE can be made selective for a particular analyte and does not require precise control of buffer concentrations. For SPE the amount of preconcentration is limited by the preconcentration time, which makes it more flexible than sample stacking. The SPE of an analyte can be beneficial not only for analyte preconcentration but also for removing other impurities or changing solvent conditions. The coupling of SPE with chips has been accomplished,[6] but the SPE component was made in a capillary or similar cartridge external to the chip. An integrated, on-chip SPE component is ultimately easier to manufacture, does not require low dead volume coupling to the chip, and eliminates sample handling losses or contamination problems arising from the off-chip sample manipulation. Routine incorporation of SPE onto a chip will reduce problems with on-chip detection limits and will improve the range of sample preparation steps which can be integrated.

Capillary electrochromatography has recently received significant attention due to the fact that it combines the separation power of both liquid chromatography and capillary electrophoresis. To date the difficulty associated with packing chromatographic material within devices has focused most previous chromatographic efforts upon open channel methods.[7−10] Devices with

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(1) Harrison, D. J.; Fluri, K.; Seiler, K.; Fan, Z.; Effenhauser, C. S.; Manz, A. *Science (Washington, D.C.)* **1993**, *261*, 895−897.

(2) *Micro Total Analysis Systems'98, Proceedings of the μTAS '98 Workshop*; Harrison, D. J., van den Berg, E., Eds.; Kluwer: Dordrecht, 1998.

(3) Coyler, C. L.; Tang, T.; Chiem, N.; Harrison, D. J. *Electrophoresis* **1997**, *18*, 1733−1741.

(4) Ocvirk, G.; Verpoorte, E.; Manz, A.; Grasserbauer, M.; Widmer, H. M. *Analytical Methods and Instrumentation* **1995**, *2*, 74−82.

(5) Jacobson, S. C.; Ramsey, M. *Electrophoresis* **1995**, *16*, 481−486.

(6) Figeys, D.; Aebersold, R. *Anal. Chem.* **1998**, *70*, 3721−3727.

(7) Manz, A.; Miyahara, Y.; Miura, J.; Watanabe, Y.; Miyagi, H.; Sato, K. *Sens. Actuators, B* **1990**, *B1*, 249−255.

(8) Jacobson, S. C.; Hergenröder, R.; Koutny, L. B.; Ramsey, J. M. *Anal. Chem.* **1994**, *66*, 2369−2373.

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channel widths of 2 μm or less are required to improve mobile-phase transfer in open columns leading to other practical considerations such as clogging and a short path length for detection. Problems with the reproducibility and the cost of stationary-phase coating in such structures could also prove formidable.

Packed-bed chromatography has the benefit of providing low mobile-phase mass transfer resistance, and a wide variety of stationary phases are available. We present the use of an off-chip prepared stationary phase, which offers the advantage that it eliminates the need for coating the chip and allows optimization of the stationary-phase preparation.

EXPERIMENTAL SECTION

Solutions and Reagents. Acetonitrile (BDH, Toronto, ON) was filtered through a 0.45-μm Nylon-66 filter (Altech, Deerfield, IL) prior to use. Otherwise, the acetonitrile was used as received, with no added electrolyte. The 50 mM potassium phosphate (pH 7.0) and ammonium acetate (pH 8.5) buffers were prepared in ultrapure water (Millipore Canada, Mississauga, ON). A 1:1 (v/v) mixture of acetonitrile and buffer was also prepared. A stock solution of 0.10 mM, 4,4-difluoro-1, 3, 5, 7, 8-penta methyl-4-bora-3a,4a-diaza-(*S*)-indacene, BODIPY 493/503 (Molecular Probes, Eugene, OR) was prepared in HPLC grade methanol (Fisher, Fair Lawn, NJ). A 1 mM stock solution of fluorescein di-sodium salt (Sigma) was prepared in phosphate buffer. Both stock solutions were then diluted in the 50 mM phosphate and 50 mM ammonium acetate buffers to give 1.0 μM solutions, which were then diluted to 1.0 nM. This 1.0 nM solution served as the sample for preconcentration and electrochromatography. All aqueous (buffer and sample) solutions were filtered through a cellulose acetate syringe filter (0.2-μm pore size) (Nalgene, Rochester, NY) prior to use.

Reverse-phase chromatographic beads were used to formulate the SPE bed on-chip. The beads were Spherisorb ODS1 (Phase Separations, Flintshire, UK), porous C-18 beads whose particles ranged from 1.5 to 4.0 μm in diameter, as determined by scanning electron microscopy. A slurry of approximately 0.003 g/mL of ODS-1 was prepared in acetonitrile. This slurry was used to pack the SPE bed on-chip, as described in the subsection titled Chip Design and Operation.

Instrumentation. The power supply and relay system used to control the electrophoretic voltages necessary for bead packing and all liquid handling on-chip has been described previously.[11] LabVIEW programs (National Instruments, Austin, TX), written in-house, were used for computer control of the voltage system and for data acquisition.

The laser-induced fluorescence detection system consisted of a 488-nm argon ion laser (Uniphase, San Jose, CA), operated at 4.0 mW, and the associated focusing optics[7] (Melles Griot, Irvine, CA). Fluorescence emitted from the BODIPY sample was collected by a 25X, 0.35 NA microscope objective (Leitz Wetzlar, Germany). The images were observed with a SONY CCD-IRIS camera. Alternatively, a 530-nm emission filter and a photo multiplier tube (PMT) (R1477, Hamamatsu, Bridgewater, NJ) were used as a detector, positioned so that the channel between the chamber and reservoir 2 could be monitored. Data were collected from the section of wide channel just next to the chamber. The weir was just out of the field of view. The PMT was biased at 530 V while the PMT signal was amplified, filtered (25 Hz Butterworth), and sampled at a frequency of 50 Hz.

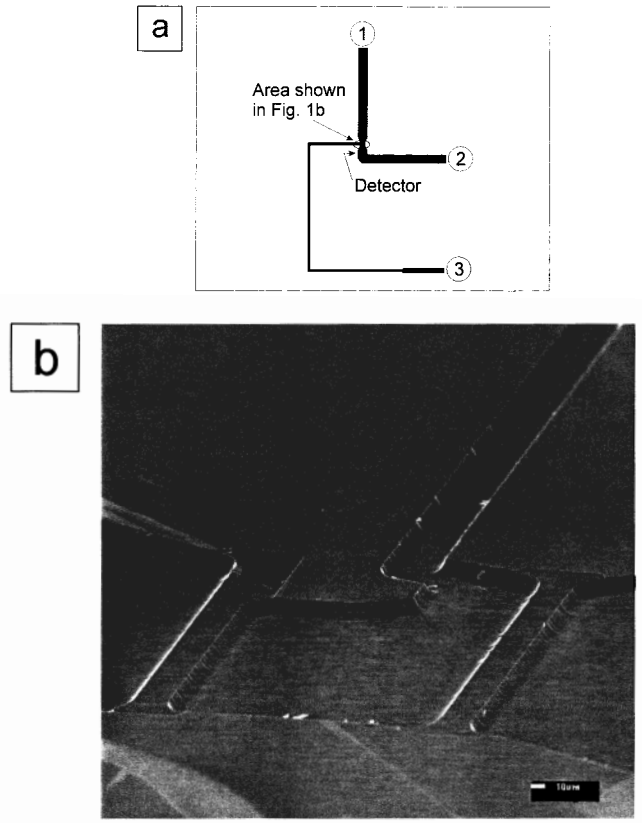

Figure 1. (a) Drawing of microchip device used for packing and solid-phase extraction. Reservoirs are numbered for reference in text: 1, outlet buffer channel; 2, inlet sample/buffer channel; 3, bead-introduction channel. (b) Image of cavity taken with scanning electron microscope, showing weir placement and hooked bead channel entrance. Etched channel widths are 580 μm for the wide channels and 30 μm for the bead introduction channel. Approximate chamber volume is 330 pL. Channels are 10 μm deep; the weirs are 9 μm high.

The fluorescence of the buffer, acetonitrile, and 1.0 nM BODIPY in both buffer and acetonitrile was measured using a Shimadzu RF-5301PC Spectrofluorophotometer.

Chip Design and Operation. Microchip devices were prepared in Corning 0211 glass by the Alberta Microelectronic Corp. (Edmonton, AB, Canada), using previously published chemical etching procedures.[12] Figure 1 shows the device used for these experiments and an image of the chamber obtained with a scanning electron microscope (JEOL X-Vision JSM6301FXV, Peabody, MA). The reservoirs are numbered for reference. Two photomasks were required to create the device: the first was used to to etch the tops of the weirs to a depth of 1 μm and the second was utilized to etch the channels (10 μm deep). The smaller channel leading into the chamber from reservoir 3 was used to direct the stationary-phase packing material into the chamber using electrokinetic pumping.[13,14]

(9) Kutter, J. P.; Jacobson, S. C.; Matsubara, N.; Ramsey, J. M. *Anal. Chem.* **1998**, *70*, 3291−3297.
(10) He, B.; Tait, N.; Regnier, F. *Anal. Chem.* **1998**, *70*, 3790−3797.
(11) Fluri, K.; Fitzpatrick, G.; Chiem, N.; Harrison, D. J. *Anal. Chem.* **1996**, *68*, 4285−4290.
(12) Fan, Z. H.; Harrison, D. J. *Anal. Chem.* **1994**, *66*, 177−184.
(13) Yan, C. U. S. Patent 5,453,163, 1995.

The chips were not conditioned with any aqueous solutions prior to use. All channels were first filled with acetonitrile. The chamber was packed with the ODS beads by replacing the acetonitrile in reservoir 3 with the ODS/acetonitrile slurry and then applying positive high voltage at reservoir 3 with reservoirs 1 and 2 grounded. The voltage was ramped from 200 to 800 V over approximately 5 min to effect packing.

Once the chamber was packed, a step gradient was performed to introduce aqueous solution to the channels and beads. A 1:1 (v/v) mixture of acetonitrile and buffer was placed in reservoirs 1 and 2. Acetonitrile replaced the slurry in reservoir 3. The voltage on reservoir 1 was ramped from 200 to 800 V, with reservoir 3 biased at 400 V and reservoir 2 grounded. After 2−5 min at 800 V, the acetonitrile/buffer mixture in reservoirs 1 and 2 was replaced with buffer, and the same voltage program repeated. The chamber was monitored visually to ensure that the acetonitrile was completely replaced by buffer and that the packing material did not shift or unpack during this procedure. (The beads could be seen to agglomerate as the acetonitrile was expelled, and the change in the index of refraction at the water/acetonitrile interface was clearly visible.)

Solid-Phase Extraction. BODIPY was loaded with 200 V from reservoir 2 to 1 with reservoir 3 floating. BODIPY was eluted with acetonitrile in the reservoirs and potentials of 500 V and ground applied to reservoirs 1 and 2, respectively, with reservoir 3 floating. When the acetonitrile came in contact with the beads, the BODIPY was eluted from the beads in a relatively narrow band.

Capillary Electrochromatography. BODIPY and fluorescein samples were loaded onto the column with 500 V applied from reservoir 2 to 1, with reservoir 3 floating. After a specified loading time the sample was replaced with buffer and voltage reapplied for 30 s to wash the remaining sample within the channel onto the column. The buffer was then replaced with the CEC mobile phase, which ranged in composition from 10−30% acetonitrile in the run buffer. Seven hundred and fifty volts was applied to effect the CEC separation.

RESULTS AND DISCUSSION

Packing and Unpacking of Chromatographic Material in the Bead Chamber. An integrated solid-phase extraction bed was prepared using a double weir design to construct a cavity in which beads coated with a stationary phase could be trapped. This layout was originally designed to trap cells and so did not include an injector[1] or a solvent gradient generator.[9] The 30-μm-wide side channel forming part of a three-way junction was designed to feed beads into or out of the chamber created by the two weirs on either side of the side channel, as illustrated in Figures 1 and 2. Sample could then be delivered from reservoir 2, along the inlet channel, across the chamber, and on toward the outlet (reservoir 1). The volume of the chamber was 330 pL, while the volume of the outlet and inlet channels was 1.5×10^{-7} and 4.1×10^{-8} L, respectively. The inlet and outlet channels had much lower flow resistance than the side channel, despite the weirs, given their wide dimensions (580 μm, tapering to 300 μm at the weirs). The flow resistance was manipulated by the selection of these channel dimensions in order to encourage flow between reservoirs 1 and 2, rather than into the bead-introduction channel during sample loading and elution.

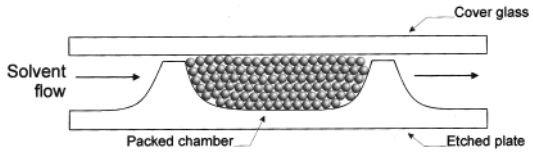

Figure 2. Drawing of cross-section of packed chamber, showing weir heights in relation to channel depth and particle size. Electroosmotic flow is driven by walls and by free silanol groups on particles. Solvent flow direction is indicated for preconcentration step.

Reverse-phase beads were used in the SPE device because of their extensive use for the chromatography of proteins, peptides, and tryptic digests,[15,16] as well as for other applications of SPE and CEC.[17,18] Electrokinetic packing of conventional capillaries has been demonstrated, and we have adapted the method for the microchip.[13,14] The packing procedure involved applying a positive voltage (200−800 V) to bead reservoir 3, while grounding reservoirs 1 and 2. The applied voltage induced EOF to flow down the bead channel, carrying the beads into the cavity. An organic solvent was required to suspend the chromatographic beads and prevent them from aggregating and plugging the relatively narrow bead channel. Studies have shown that capillaries filled with acetonitrile exhibit substantial electroosmostic flow.[19−22]

At the early stages of packing, the beads entering the cavity contacted the weir structures on either side of the cavity shown in Figure 3a. The beads were unable to traverse the weir because the distance from the top of the weir to the bottom of the cover plate (1.0 μm) was less than the diameter of the individual particles of the packing material (1.5−4.0 μm). The cavity continued to pack until it was entirely filled with chromatographic material, as shown schematically in Figure 2. Several authors have described the difficulties associated with reproducibly fabricating frits for retaining packing material in conventional capillaries.[23−26] The two-weir design of the microchip used in the present work circumvented this problem, and the electrokinetic packing of the beads provided an even distribution of beads throughout the chamber with no observable voids. It was also possible to pack the cavity by applying vacuum at reservoirs 1 and 2, although this was less convenient when electrokinetic flow was used for sample loading and elution.

Figure 3a shows an image of the bead cavity midway through the packing procedure, while Figure 3b shows the cavity 10 s later.

(14) Yan, C.; Dadoo, R.; Zhao, H.; Zare, R. N.; Rakestraw, D. J. *Anal. Chem.* **1995**, *67*, 2026−2029.
(15) Hancock, W. S.; Chloupek, R. C.; Kirkland, J. J.; Snyder, L. R. *J. Chromatogr., A* **1994**, *686*, 31−43.
(16) Nielsen, R. G.; Riggin, R. M.; Rickard, E. C. *J. Chromatogr.* **1989**, *480*, 393−401.
(17) Seifar, R. M.; Kok, W. Th.; Kraak, J. C.; Poppe, H. *Chromatographia* **1997**, *46*, 131−136.
(18) Knox, J. H.; Grant, I. H. *Chromatographia* **1991**, *32*, 317−328.
(19) Wright, P. B.; Lister, A. S.; Dorsey, J. G. *Anal. Chem.* **1997**, *69*, 3251−3259.
(20) Lister, A. S.; Dorsey, J. G.; Burton, D. E. *J. High Resolut. Chromatogr.* **1997**, *20*, 523−528.
(21) Schwer, C.; Kenndler, E. *Anal. Chem.* **1991**, *63*, 1801−1807.
(22) Salimi-Moosavi, H.; Cassidy, R. M. *Anal. Chem.* **1995**, *67*, 1067−1073.
(23) Boughtflower, R. J.; Underwood, T.; Paterson, C. J. *Chromatographia* **1995**, *40*, 329−335.
(24) Van den Bosch, S. E.; Heemstra, S.; Kraak, J. C.; Poppe, H. *J. Chromatogr., A* **1996**, *755*, 165−177.
(25) Colon, L. A.; Reynolds, K. J.; Alicea-Maldonado, R.; Fermier, A. M. *Electrophoresis* **1997**, *18*, 2162−2174.
(26) Majors, R. E. *LC-GC* **1998**, *16*, 96−110.

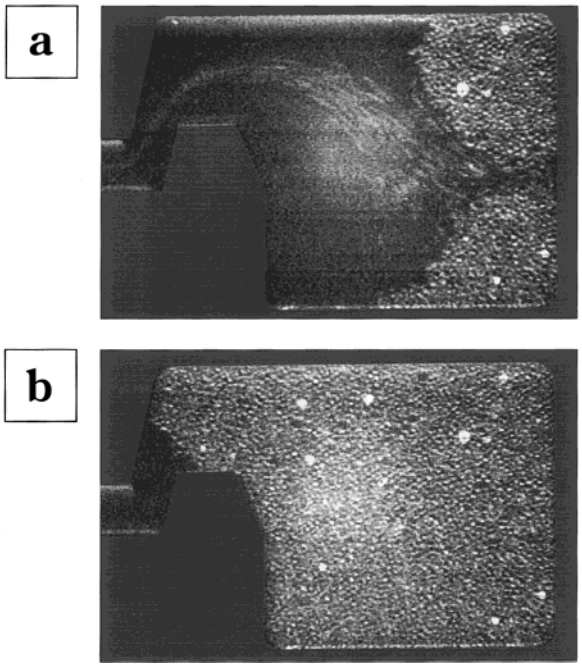

Figure 3. Images of the chamber (a) at an initial stage of electrokinetic packing and (b) after it is completely filled with beads. Image background has been colored to enhance contrast.

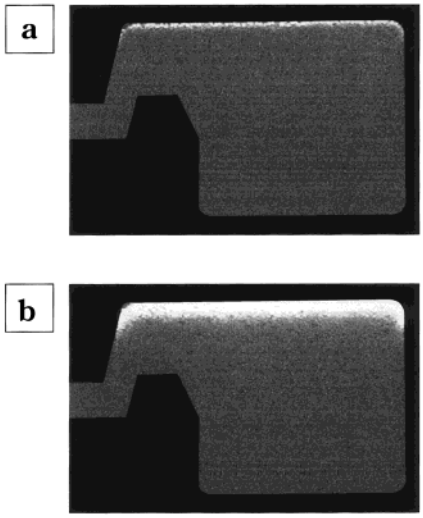

Figure 4. Preconcentration of a 1.0 nM BODIPY solution on a packed bed on-chip. (a) BODIPY concentrating on the first layer of beads at the weir/bed interface at the right edge of Figure 1b after 1 min of preconcentration. (b) Later stage of preconcentration, showing BODIPY adsorbing further into the bed as bead capacities are reached at the weir/bed interface after 2 min of preconcentration. Image background has been colored to enhance contrast.

If the beads did not pack tightly they were removed from the chamber by simply reversing the voltages and then repacking the bed. Once aqueous solution was introduced to the chamber, the reversed-phase beads tended to aggregate and were more difficult to remove. However, subsequent removal was accomplished by flushing the aqueous solution out with acetonitrile, using either EOF or vacuum or a combination of the two. The ability to remove beads that have been packed enables one to either refresh used chromatographic beads, or substitute a more applicable material.

A design utilizing a hook at the chamber entrance (Figure 1b) yielded the most favorable results, enabling the chamber to be packed and remain so after removal or alteration of voltages. During the packing step, bead reservoir 3 had a positive bias applied, with reservoirs 1 and 2 grounded. The hooked structure caused the electric-field lines to follow a curved pathway into the cavity. Consequently, as the chromatographic beads followed the electric-field lines into the cavity they appeared to be "sprayed", as if from a snow blower (Figure 3a), to become uniformly packed into the chamber.

During the packing procedure the cavity filled only to the beginning of the hook structure (see Figure 3b). Once filled, the beads were observed to flow down the sides and up the middle of the bead channel (toward reservoir 3) mimicking the solvent back flow generated in a closed electrophoretic system.[27] In such a system EOF is directed along the walls until it reaches the end of the chamber, where pressure causes the solution to reverse direction and flow back up the center of the bead-introduction channel.

Cavities constructed without the hooked entrance were not easy to pack well. Because of its symmetric design, this type of chamber may exhibit solvent back flow, after it has filled to a certain extent. That is, the partially filled chamber may resemble a closed or restricted system. Such an occurrence would preclude the filling of the symmetric chamber with beads and is consistent with the behavior of the side channel discussed above. In contrast, the asymmetric chamber (hooked entrance) is less likely to experience back flow directly into the bead-introduction channel. All subsequent experiments were performed using chambers with the asymmetric design.

Solid-Phase Extraction (SPE) On-Chip. Preconcentration is a valuable tool that can be used to enhance the sensitivity of microfluidic devices. To determine the ability of a packed SPE bed constructed on a microchip to preconcentrate an analyte, we concentrated a 1.0 nM solution of BODIPY reagent from 50 mM phosphate buffer. Solution conditions utilized were similar to those used for protein and peptide analysis in HPLC-CE systems.[28,29] The BODIPY reagent, when diluted in aqueous buffer, exhibits a high affinity for ODS material and is an excellent fluorophore. The preconcentration and elution of the BODIPY reagent was carried out in four steps: equilibration of the SPE bed with buffer; sample introduction; buffer flush; and elution of analyte.

Following rinsing of the packed bed with phosphate buffer, a solution of 1.0 nM BODIPY was placed in reservoir 2, and +200 V was applied for 2 min, with reservoir 1 grounded. The EOF (0.2 mm/s, 1.2×10^{-9} L/s) flowed toward reservoir 1, carrying the BODIPY onto the SPE bed during the loading step. Fluorescence of the adsorbed BODIPY occurred initially at the first few layers of beads only, as shown in Figure 4a. Figure 4b shows the SPE bed after 1.5 min, with a total of 1.4×10^{-16} moles of BODIPY reagent loaded on the bed, assuming complete capture of the dye. No sample breakthrough was observed with BODIPY, due to its high affinity for the ODS material. In fact, visual observation

(27) Shaw, D. J. *Introduction to Colloid and Surface Chemistry*, 3rd ed.; Butterworth: London, 1980.

(28) Bushey, M. M.; Jorgenson, J. W. *Anal. Chem.* **1990**, *62*, 978−984.

(29) Castagnola, M.; Cassiano, L.; Rabino, R.; Rossetti, D. V. *J. Chromatogr.* **1991**, *572*, 51−58.

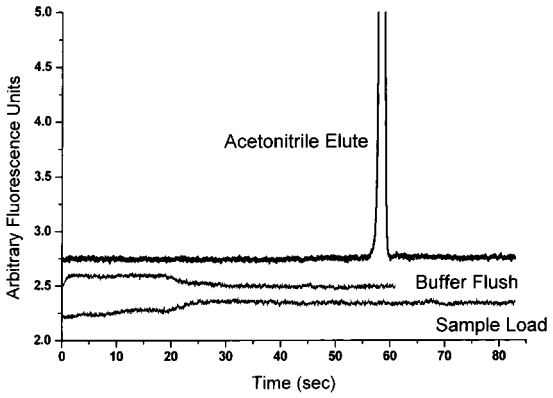

Figure 5. Plot of fluorescence intensity vs time, showing fluorescence of 1.0 nM BODIPY sample during loading, followed by a buffer flush and then preconcentrated BODIPY during elution with acetonitrile (ACN). The time origin represents the point at which a given solvent/solution composition was introduced to the reservoirs and the potentials were turned on. Traces have been offset upward slightly to allow easier viewing.

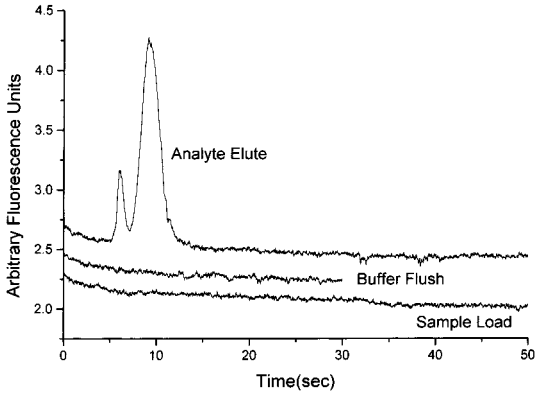

Figure 6. Electrochromatogram of BODIPY and fluorescein, showing different steps of the separation: first a 100-s load; second, 30-s buffer flush; third, an isocratic elution from the 200-μm-long column with a 30% acetonitrile/70% 50 mM ammonium acetate mobile phase. Fluorescein was the first to elute. Traces have been offset slightly to allow easier viewing.

indicated that, after concentrating 1.0 nM BODIPY solution for two minutes, only 5% of the physical volume of the SPE bed was utilized, suggesting the capacity of the 330 pL bed was $\sim 2.8 \times 10^{-15}$ moles of analyte.

A buffer wash step was used after loading to wash sample remaining within the channel onto the bed. The solutions in reservoirs 1 and 2 were then replaced with acetonitrile. Furthermore, the dye was eluted with solvent moving in the same direction as the initial loading step or, by reversal of the potential gradient during the elution step, it could be directed back toward the original sample reservoir. Both procedures work well, but the latter was more convenient for our testing.

Figure 5 shows the 3-step preconcentration experiment for a 1.0 nM BODIPY sample following bed equilibration. The 90-s loading step showed an increase in signal as the fluorescent sample passed by the detector positioned as shown in Figure 1a. This was followed by a 60-s rinse step. Acetonitrile was then used to elute the BODIPY reagent off the bed in the opposite direction to which it was loaded, eliminating the need for detector repositioning. The BODIPY reagent eluted in a relatively narrow 3-s band following a 90-s preconcentration step, exhibiting a many-fold concentration increase compared with that of the original sample. The fluorescence of the BODIPY (1.0 nM) reagent was tested in both buffer and acetonitrile and did not show a significant difference in intensity for either of the solvents. The preconcentration factor (P. F.) can be estimated using eq 1

$$\mathrm{P.F} = \frac{V_\mathrm{i}}{V_\mathrm{f}} = \frac{t_\mathrm{pre}\, f_\mathrm{buff}}{t_\mathrm{elute}\, f_\mathrm{elute}} \qquad (1)$$

where V_i is the volume of buffer containing analyte and V_f is the volume of acetonitrile containing analyte. The volume V_i is the product of the preconcentration time (t_pre, s) and the electroosmotic flow of the sample being concentrated (f_buff, L/s) while V_f is the product of the width of the eluted analyte peak (t_elute, s) and the flow rate of the eluting solvent (f_elute L/s). For this case, the analyte was preconcentrated by a factor of at least 100 times. After sufficient concentration, the BODIPY is easily observed visually on the SPE bed.

Different sample-loading times were utilized to increase the amount of preconcentration. Preconcentration times ranging from 120 to 532 s were studied yielding preconcentration factors of 80−500. Peak area (rsd 3−11%) plotted versus preconcentration time yielded a linear relationship ($r^2 = 0.9993$) over the studied conditions.

Capillary Electrochromatography (CEC) On-Chip. The ability to trap chromatographic material has enabled packed-channel CEC to be achieved on-chip. Reversed-phase-mode CEC was performed on a chamber packed with octadecyl silane-coated beads equilibrated with buffer. Because of the lack of an injector within the chip design, the samples were loaded onto the front of the chromatographic bed in 50 mM ammonium acetate buffer, pH 8.5 (see Experimental Section). Both compounds were totally retained under these conditions, as indicated by a lack of analyte signal in the loading and flush steps. The loading step functioned to both introduce the sample and preconcentrate the retained analytes at the front of the bed.[30] Figure 6 shows the three steps involved in the CEC separation of BODIPY and fluorescein with a mobile-phase composition of 30% acetonitrile/ 70% aqueous 50 mM ammonium acetate. Once the mixed mobile phase reaches the bed, both compounds begin to undergo chromatography and are eluted from the bed. The compounds are completely eluted and separated in less than 20 s on less than 200 μm of chromatographic bed, yielding a plate height of 2 μm ($N = 100$ plates or 500 000 plates/m) for the fluorescein peak. Under these conditions, the fluorescein is eluted prior to the BODIPY reagent. Peaks were identified by comparing retention times of the standards with those of the mixture. At pH 8.5, fluorescein possesses a net (−2) charge while BODIPY is neutral. In a normal CZE separation, the electrophoretic mobility of fluorescein would oppose the EOF, causing the BODIPY to elute prior to fluoroscein. In this case, the elution order of the two components is reversed, indicating an interaction between the analytes and the stationary phase. The BODIPY, being more hydrophobic, has a higher

(30) Swartz, M. E.; Merion, M. *J. Chromatogr.* **1993**, *632*, 209−213.

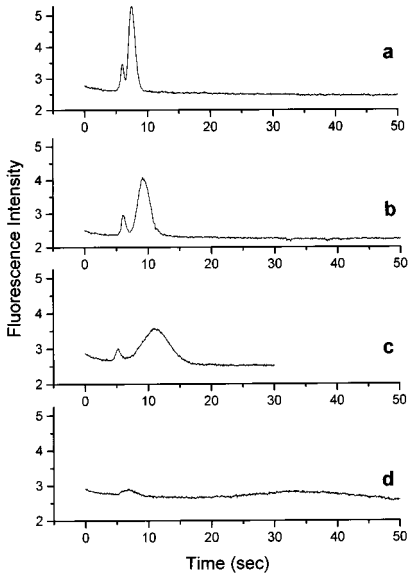

Figure 7. Electrochromatograms of BODIPY and fluorescein with different concentrations of acetonitrile in the mobile phase: a (30%), b (22%), c (15%), and d (10%). Sample was injected for 100 s with a 30-s buffer flush. Fluorescein was the first to elute.

affinity for the chromatographic material than does fluoroscein, causing the BODIPY to be eluted later.

Figure 7 shows the CEC separation of BODIPY and fluorescein utilizing mobile phases with different concentrations of acetonitrile. The increased acetonitrile concentration lowers the polarity of the mobile phase, decreasing the amount of time required for the BODIPY to elute. The elution time for fluorescein does not change, indicating little to no chromatographic retention except at low percent of acetonitrile. Decreasing the acetonitrile concentration provides baseline resolution, but leads to more extensive band broadening.

As was the case in the infancy of HPLC and GC, the most efficient stationary-phase configuration for microfluidics is yet to be determined. Our present results with a nonoptimized design lie about midway between the range of 200 000−1 700 000 plates/m reported for open tubular CEC on a chip.[8−10] Now, with the advent of an efficient method for packing and unpacking channels on-chip, the relative advantages and disadvantages of open versus packed columns can be examined.

CONCLUSIONS

The preparation of a solid-phase-extraction bed on-chip offers distinct advantages over current on-line preconcentration methods. The method is inherently simple, requiring no complex manufacturing or drilling, and it eliminates the dead volume associated with connectors.

It is apparent that the overall microchip design presented here is not optimized as a SPE or CEC device. However, it does demonstrate the ability of a two-weir device to be used as a cavity to trap chromatographic material. Improvements in the design could include better placement of the hooked channel to make the chamber more symmetrical, lowering the possibility for band broadening during elution. The volume of the chamber could be increased or decreased, depending upon the application. Clearly, if the application is CEC, the column length will need to be extended to increase the absolute number of theoretical plates. In addition, an injector and solvent mixer could be included in the design to reliably introduce samples and supply solvent gradients to allow gradient elution.

The ability to pack channels on microchip devices will allow several techniques to be integrated in on-chip processes in the future, as suggested by this study. Using different types of packing materials makes possible methods such as SPE, enzyme-linked assays, and CEC. The integration of a variety of techniques based on packed beds that are complementary to CE will expand the capability of microfluidics to analyze compounds that were previously beyond the scope of microchip-based CE. These techniques may soon be added to the microfluidics arsenal, moving us ever closer to the true micro TAS concept.

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