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Toward sol-gel electrochromatographic separations on a chip

A porous photopolymerized sol-gel (PSG) monolith was synthesized in the separation channel of a borosilicate glass chip via UV irradiation (5 min) of a mixture of 3-methacryloxypropyltrimethoxysilane, an acid catalyst, a porogen, and a photoinitiator. The PSG monolith adhered strongly to the chemically untreated channel walls. The chip was fabricated using standard lithography procedures to give channels that are 35-μm deep and 90-μm wide. Masking the other channels defined the 4.7-cm PSG section in the separation channel. Two dyes Coumarin 314 and 510 were successfully separated within baseline resolution in 225 s when fluorescent detection occurred immediately after the PSG section. The separation time was reduced to 80 s with little loss in resolution by detecting the dyes 1.2 cm from the front of the PSG monolith.

Key Words: Photopolymerized sol-gel; Capillary electrochromatography; Chip

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1 Introduction

Microfabricated devices, such as glass chips, for chemical and biological analyses have received increasing attention in recent years [1]. Free-solution electrophoresis has been easily implemented in the narrow channels of chips [2–5]. Electrochromatography, which combines the features of capillary electrophoresis and liquid chromatography, however, has been more difficult to implement in chips. Several routes have been proposed, including packing [6], open-tubular (coating) [7], in situ polymerization of monoliths [8], and in situ micromachining of monolithic support structures [9]. The packing route is time-consuming and requires the use of frits or restriction points to prevent leakage of the chromatographic particles. Open-tubular formats are easier to prepare but give poor chromatographic performance. Monoliths are readily prepared in situ and are tunable in charge and hydrophobicity. In situ polymerization can be thermally or photochemically initiated, with the latter producing monoliths having higher efficiencies [10]. A majority of the monoliths used in capillary electrochromatography (CEC) separations are purely organic and are prepared from methacrylate monomers [11–16]. The usefulness and versatility of such monolithic structures in CEC and chip electrochromatography (ChEC) have been demonstrated for a wide range of neutral and charged analytes [11, 17–19]. Alternatively, we reported that photopolymerized sol-gel (PSG) monoliths may be used for the preparation of organic-inorganic hybrid porous monoliths for use in CEC separations [20, 21] and preconcentration [22, 23]. Sol-gel based materials have also been used as wall coatings in open-tubular electrochromatography and as stationary phases in CEC [24–28].

Following our recent success with the preparation of porous photopolymerized sol-gel (PSG) monoliths in capillary columns [20], we anticipated that this approach would be well-suited for microfabricated chip devices. This paper describes the fast and simple preparation of a PSG monolith in the separation channel of a glass chip. The usefulness of PSG for CEC is demonstrated in the separation of two test analytes, Coumarin 314 and 510. A comparison is made of separation times at two different detection points. One is immediately after the PSG section; the other is on the monolith 1.2 cm from the front of the PSG section.

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2 Experimental

2.1 Materials and reagents

3-Trimethoxysilylpropylmethacrylate, toluene, Coumarin 314, and Coumarin 510 were purchased from Sigma-Aldrich (Milwaukee, WI) and used as received. The photoinitiator Irgacure 1800 was obtained from Ciba (Tarrytown, NY).

2.2 Instruments

For the synthesis of the PSG monolith we use a Spectronics XL-1500 UV crosslinker (Westbury, NY) equipped

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with six blacklight tubes (15 W) of predominantly 365-nm wavelength as the irradiation source with an intensity of 90 mJ/cm^2.

We monitor separations with a Zeiss Axiovert 135 inverted fluorescence microscope (Thornwood, NY). The light beam from a Zeiss XBO 75 W/2 arc lamp passes through a 420-nm excitation filter and into a 40 × microscopic objective lens. The objective lens focuses the beam onto the chip. Fluorescence signal is collected with a 490-nm emission filter and detected by a Hamamatsu R4632 photomultiplier tube (Bridgewater, NJ). Data collection and control of electronics are accomplished using codes written with LabView (version 5.0). A home-built power supply is used to apply voltages to all four channels of the chip.

2.3 Chip fabrication

Glass chips used in this study were made in the Stanford Nanofabrication Facility at Stanford University. The channels in the fluidics layer of the chip were etched into a 500-μm thick × 100-mm wide Corning 0211 borosilicate glass plate (Precision Glass & Optics, Santa Ana, CA) using standard lithography procedures [29, 30]. The fluidics layer of the chip consists of four linear channels, 90-μm wide and 35-μm deep, that are connected to a 180-μm long, double-T injection section (**Figure 1**). The channels terminated into 1.2-mm diameter holes that were drilled into the fluidics layer using a diamond bit. A cover plate made from the same thickness of borosilicate glass was thermally bonded at 550°C for 5 h to the fluidics layer. We glued borosilicate glass tubes (1.2-cm long × 6-mm diameter) over each of the four wells to create four large-volume (100–150 μL) reservoirs.

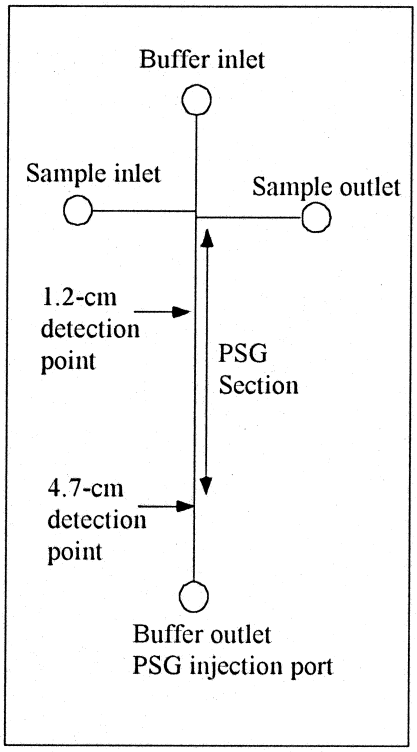

Figure 1. Schematic diagram of the fluidics layer of a glass chip.

Prior to filling the channel with the sol-gel reaction solution, we cleaned the channel with a series of solvents in the order: (1) methanol (5 min); (2) distilled (DI) water (5 min); (3) 1 M NaOH (10 min); (4) DI water (10 min); and (5) 1 M HCl (10 min). Each of the liquids was pumped through the channel at pressures up to 90 psi. After cleaning with concentrated nitric acid, we dried the chip in an oven at 100°C for approximately 1 h.

2.4 PSG reaction solution

We fabricate the PSG monolith using a published procedure [20] with slight modifications. A monomer stock solution was prepared with 575 μL of 3-trimethoxysilylpropoyl-methacrylate and 100 μL of 0.12 M HCl, which was stirred for 15 min in the dark at room temperature. 20 mg of Irgacure 1800 was dissolved in 312 μL of toluene followed by the addition of 88 μL of the monomer stock solution to create the final reaction solution, which was stirred for 3 min in the dark at room temperature.

2.5 Preparation of the PSG monolith in a channel

We used black electrical tape as a mask to prevent PSG formation except for a 4.7-cm long section, which serves as the separation channel. Approximately 100 μL of the final sol-gel reaction solution was placed into the buffer waste reservoir. This solution entered by capillary action and filled the entire separation channel. To avoid the introduction of air bubbles inside the separation channel, we filled the three remaining reservoirs with the sol-gel solution.

The chip was placed in a black box that contained an irradiation window. The black box ensured that light did not enter the masked areas. The chip was irradiated for 5 min at 365 nm in the UV crosslinker.

After irradiation, the channels were washed with ethanol for approximately 5 min using a vacuum pump (at approximately 90 psi) attached to the buffer outlet reservoir. All four of the reservoirs on the chip were filled with the separation solution and sealed with silicone elastomer caps to prevent evaporation of acetonitrile. We electrokinetically conditioned the PSG monolith at 2.5 kV for approximately 10 min after which time a stable current was achieved.

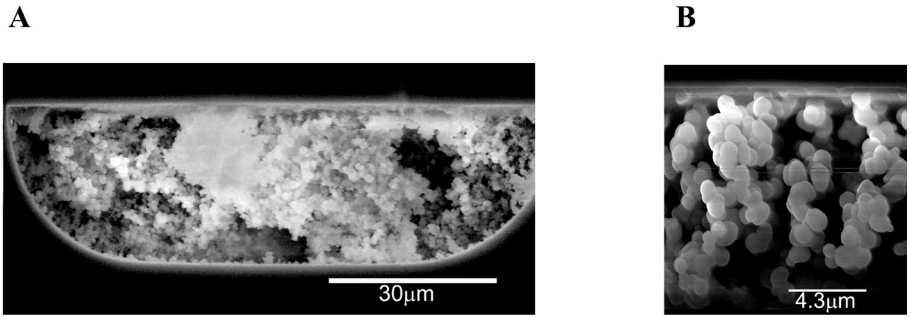

Figure 2. Scanning electron microscope images of a PSG-filled channel in a glass chip: (A) cross-sectional view, and (B) 7 × magnification of (A).

2.6 Sample loading and separation

The buffer inlet, sample outlet, and buffer outlet reservoirs were filled with 100 μL of the separation solution. We placed the same volume of sample solution in the sample inlet reservoir. Loading and separation voltages were applied via a home-built power supply; voltages and signal were controlled and collected using Labview 5.0. We electrokinetically loaded the sample into the double-T section of the chip by applying 1.5 V to the sample inlet reservoir, 1.2 V to the buffer inlet reservoir, ground to the sample outlet reservoir, and 2.1 V to the buffer outlet reservoir. The sample plug was moved into the channel by applying the following voltages: 1.2 V, 2.1 V, 1.1 V, and ground, to the sample inlet, buffer inlet, sample outlet, and buffer outlet reservoirs, respectively. We used the same voltages for separation. The epifluorescent signal was optically (420 nm filter) and spatially (40 × objective lens) filtered and imaged onto a photomultiplier tube. All experiments were performed at 20°C.

2.7 Solutions

Each stock solution of the coumarin consists of 1 mg/mL in acetonitrile. We prepared sample solutions with a ratio of 1/3/6 (*v*/*v*/*v*) for buffer/water/acetonitrile, and they were sonicated prior to its use. The separation solution consisted of 1/3/6 (*v*/*v*/*v*) 50 mM ammonium acetate (pH 6.5)/water/acetonitrile. We degassed the separation solution by sonication prior to use.

3 Results and discussion

3.1 PSG monolith

A porous PSG monolith that supports electroosmotic flow is rapidly prepared in the separation channel of a glass chip without the need for pretreatment of the glass surface. **Figure 2** shows scanning electron microscope (SEM) micrographs of the cross-section of a PSG-filled channel in a glass chip. Figure 2.A shows that the PSG monolith fills the channel and is bonded to the channel wall. In Figure 2.B, a magnified image (7 ×) of this cross section reveals a porous network of interconnecting nearly spherical structures that is similar to that observed in capillary columns [20, 21].

3.2 Chromatographic performance

We evaluated the PSG-filled separation channel using a test sample of two neutral Coumarin dyes. **Figure 3** illustrates the reversed-phase separation of Coumarin 314 (peak 1) and Coumarin 510 (peak 2) using a mobile phase of 50 mM ammonium acetate (pH 6.5)/water/acetonitrile (1/3/6, *v*/*v*/*v*) and fluorescent detection. Efficiency, as measured by the theoretical plate number *N*, was calculated from the expression, $N = 5.54\left(\frac{t_R}{fwhm}\right)$, where *fwhm* is the full-width-half-maximum of the analyte peak and t_R is the elution time of the analyte. Resolution was determined by, $R_s = \frac{(t_2 - t_1)}{\frac{1}{2}(\omega_1 + \omega_2)}$, where t_2 and t_1 are the retention times and ω_2 and ω_1 are the widths at the base of peaks 2 and 1.

In Figure 3.A, where the detection occurs immediately after the 4.7-cm PSG section, the peaks elute in less than 250 s. The peak shapes are symmetrical. Single chip run-to-run reproducibility ($n = 5$) was better than 1% RSD. The resolution is 1.26 ($n = 5$, 3.05% RSD), where an R_s value of 1 or greater indicates successful separation. We achieved efficiencies of up to $N = 18{,}500$ ($n = 5$, 1.56% RSD) and $N = 5{,}700$ ($n = 5$, 2.34% RSD) plates/meter for peaks 1 and 2, respectively.

By moving the detection point closer to the front of the PSG section, the elution times of the two dyes were decreased, and detection occurred on the PSG monolith. Figure 3.B illustrates a fast separation of the coumarin dyes with little loss in resolution, $R_s = 1.01$ ($n = 5$, 5.12% RSD). Both peaks are eluted within 80 s. The analytes are detected at 1.2 cm from the front of the PSG monolith. Run-to-run reproducibilities ($n = 5$) are 3.13% RSD for peak 1 and 5.18% RSD for peak 2. Peak tailing in both peaks 1 and 2 may be contributing to the higher RSD

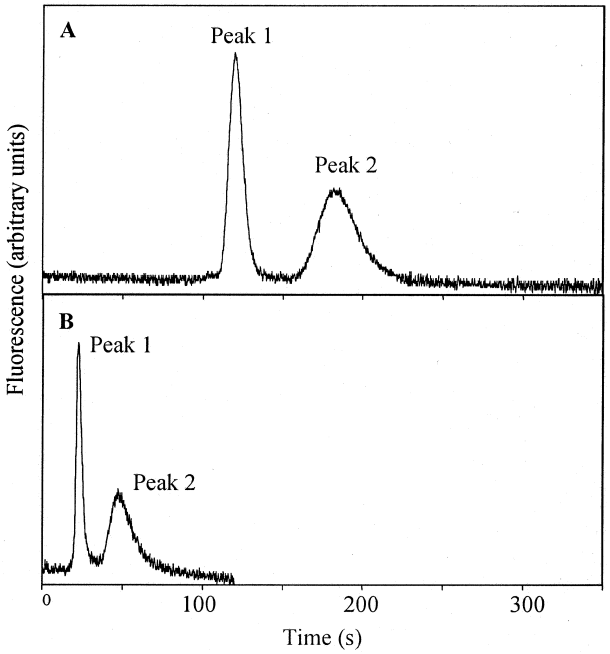

Figure 3. Electrochromatograms for the separation of a mixture of Coumarin 314 (peak 1) and Coumarin 510 (peak 2) (A) detected immediately after the PSG section whose length is 4.7 cm, and (B) detected on-column 1.2 cm from the start of the PSG section. The separation solution is 1/3/6 (*v*/*v*/*v*) 50 mM ammonium acetate (pH 6.5)/water/acetonitrile. The separation voltage is 2.0 kV for (A) and 2.5 kV for (B). Detection is by fluorescence, which is excited at 420 nm. The concentration of each of the coumarin dyes in the separation solution is 0.6 mg/mL.

values with peak 2 having significantly more tailing than peak 1. The efficiencies are comparable to those obtained for the 4.7-cm detection point. Efficiencies of peaks 1 and 2 are $N = 21{,}300$ ($n = 5$, 9.93% RSD) and $N = 6{,}100$ ($n = 5$, 5.80% RSD) plates/meter, respectively.

By moving the detection window, the length of the PSG monolith can be varied in a single chip. Analysis times can be shortened while maintaining resolution simply by repositioning the detection point. The need to create microfabricated chip devices with separation channels containing different lengths of PSG sections may become unnecessary for some purposes.

4 Concluding remarks

A photopolymerized sol-gel (PSG) monolith is easily prepared in a channel of a glass chip. We have demonstrated its use for the efficient and reproducible reversed-phase chromatographic separation of a mixture of neutral Coumarin dyes. By moving the detection point closer to the start of the PSG-filled separation channel, we were able to achieve a fast separation with little loss in resolution and efficiency.

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