ON-CHIP POROUS POLYMER MONOLITHS FOR SOLID PHASE EXTRACTION USING DIGITAL MICROFLUIDICS

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ABSTRACT

We present the first use of digital microfluidic techniques to perform on-chip solid phase extraction (SPE). The material used for SPE was a porous polymer monolith with 12-carbon functionality (C12 PPM) which was photopolymerized on-chip and used to desalt a peptide sample prior to nano-electrospray ionization mass spectrometric analysis (nano-ESI-MS). We anticipate this technique may be useful for preparative-scale sample cleanup and concentration.

KEYWORDS: Digital microfluidics, porous polymer monoliths, solid phase extraction, preparative-scale

INTRODUCTION

The high surface-to-volume ratios and short diffusion distances in microchannels makes microfluidics an attractive platform for sample extraction and preconcentration. These techniques take many different forms, involving functionalized channel walls [1], porous membranes [2], packed microparticles [3], or PPMs [4]. While these methods are promising for analytical applications, they are not well suited for preparative-scale applications, as the small samples handled in microchannels are difficult to recover. On the contrary, digital microfluidics (DMF) is a relatively new technique characterized by the manipulation of discrete droplets on an open array of electrodes [5]. While DMF shares many characteristics with microchannels (automation, integration, etc.), it is particularly well-suited for preparative processes. Here, we introduce the first method using DMF for on-chip SPE. The new method involves on-chip formation and activation of a C12 PPM for sample clean-up and desalting of peptide mixtures prior to sample recovery and analysis by nano-ESI-MS.

EXPERIMENTAL

Devices were fabricated in the University of Toronto Emerging Communications Technology Institute (ECTI) fabrication facility using photolithography and wet etching. The device design, shown in Figure 1a, featured an array of eighty-eight actuation electrodes (2.2 × 2.2 mm ea.) connected to ten reservoir electrodes (5 × 5 mm ea.), with inter-electrode gaps of 40 μm. Devices were assembled with an unpatterned ITO–glass top plate and a patterned bottom plate separated by a spacer formed from three pieces of double-sided tape (total spacer thickness 270 μm). Unit droplets (covering a single driving electrode) were ~1 μL. To actuate droplets, driving potentials (220-300 V_{pp}) were generated by amplifying the output of a function generator operating at 18 kHz.

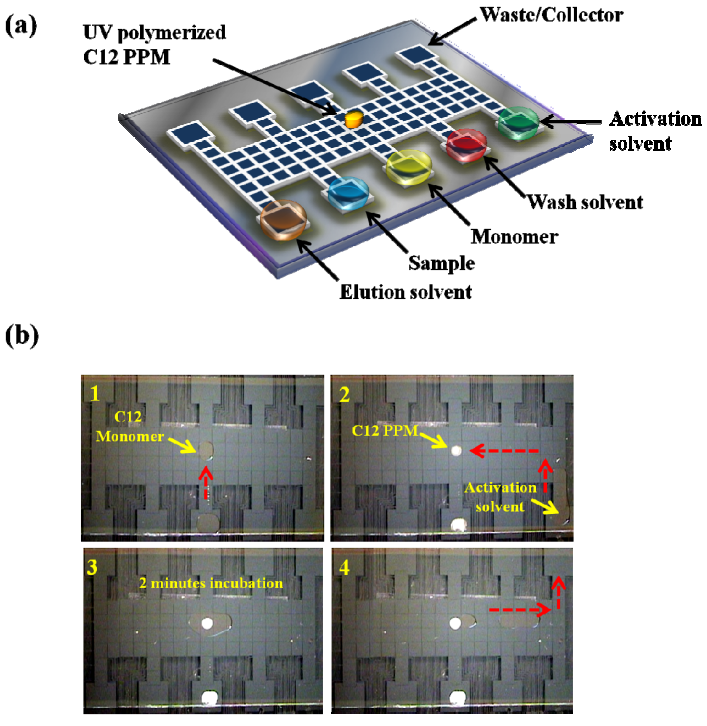

Figure 1: Digital Microfluidic (DMF) Device Design and Operation. (a) Schematic. (b) Frames (1-4) from a movie illustrating the formation and activation of C12 PPM on chip for SPE.

Porous polymer monoliths (PPMs) were prepared via on-chip photopolymerization of a casting solution droplet manipulated by DMF. The casting solution was prepared by mixing 279 μL of butyl acrylate, 150 μL of 1,3-butanediol diacrylate, 69 μL of lauryl acrylate, 2.5 mg of 2,2-dimethoxy-2-phenyl-acetophenone, and 1 mL of porogen which comprised a 4:1:1 ratio of acetonitrile, 95% ethanol, and 5 mM phosphate buffer at pH 6.8. As illustrated in Figure 1b, the PPM was formed by pipetting 5 μL casting solution onto the middle reservoir electrode and then dispensing a 1 μL droplet and translating it to a central electrode (frame 1) where it was polymerized by exposure to UV radiation (100 W, 365 nm, 5 min). Next, the PPM was activated by dispensing and passing a 5 μL droplet of acetonitrile followed by a 5 μL droplet of formic acid (0.5% v/v) over the monolith (frame 2). After incubating for 2 minutes (frame 3), the activation solvent droplet was moved away from the C12 PPM to the waste reservoir (frame 4). Once activated, one or more 1 μL sample droplets were dispensed and actuated to the PPM. After incubation, unbound samples were moved away and the PPM

was washed with wash solvent (2 x 5 μL, 0.5% formic acid in water), followed by eluting the bound sample with elution solvent (2 x 5 μL, 0.1% formic acid in acetonitrile).

RESULTS AND DISCUSSION

To evaluate the PPMs formed on chip, a model substrate, fluorescein, was used to optimize parameters such as sample loading time and number of elution steps. For sample loading time optimization, fluorescein (5 μM in 1% v/v formic acid) droplets were driven to PPMs and were allowed to incubate for 2, 4 or 8 minutes. After incubation, sample droplets were moved away from the PPM and their fluorescent intensities were measured. As shown in Figure 2a, over 80% of the sample was loaded onto the PPM after only 2 minutes of incubation time. For elution optimization, a fixed amount of fluorescein was loaded onto the PPM, and the fluorescein was eluted with 1, 2, or 3 droplets of acetonitrile. As shown in Figure 2b, 2 droplets of acetonitrile were sufficient to elute over 90% of the fluorescein. To validate the technique for preparative-scale applications, an on-chip desalting of angiotensin II (AngII, 1 μM, MW 1046 Da) in a solution containing sodium chloride

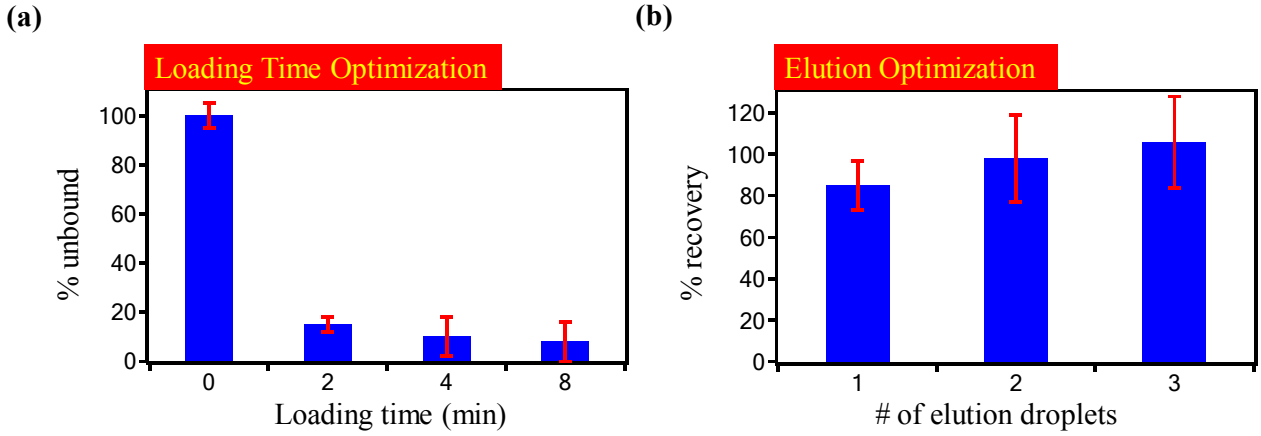

Figure 2: On-Chip SPE Parameter Optimization. Loading time (a) and elution (b) optimization using fluorescein as model system.

(100 mM) was performed, after which the sample was collected and analyzed by nano-ESI-MS. As shown in Figure 3a, the spectrum of the non-desalted sample yielded no peak for AngII at m/z 1047 because of ionization suppression by sodium chloride. In contrast, a high-intensity peak at m/z 1047 (Figure 3b) was observed after on-chip desalting. In on-going work, we are developing PPMs with other functionalities (SCX, immunochemistry) on chip for preparative sample purification.

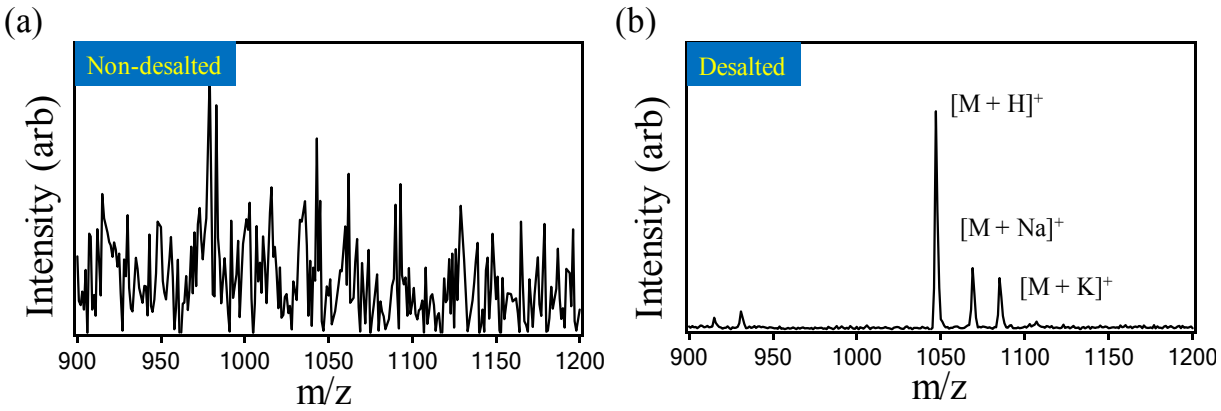

Figure 3: NanoESI Mass Spectra of non-desalted (a) and desalted (b) Angiotensin II.

CONCLUSIONS

We introduced the first combination of porous polymer monoliths and digital microfluidics, and the first implementation of solid phase extraction by digital microfluidics for preparative-scale applications. We anticipate that this technique may be useful for a wide range of applications requiring preparative sample cleanup and concentration.

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REFERENCES

[1] Kutter, J.P., Jacobson, S. C., Ramsey, J. M., "Solid phase extraction on microfluidic devices", *Journal of Microcolumn Separations **12***, 93-97 (2000)

[2] Foote, R. S.; Khandurina, J.; Jacobson, S. C.; Ramsey, J. M. "Preconcentration of proteins on microfluidic devices using porous silica membranes", *Analytical Chemistry **77***, 57-63 (2005)

[3] Oleschuk, R. D.; Shultz-Lockyear, L. L.; Ning, Y.; Harrison, D. J. "Trapping of bead-based reagents within microfluidic systems: On-chip solid-phase extraction and electrochromatography", *Analytical Chemistry **72***, 585-590 (2000)

[4] Yu, C.; Davey, M. H.; Svec, F.; Frechet, J. M. J. "Monolithic porous polymer for on-chip solid-phase extraction and preconcentration prepared by photoinitiated in situ polymerization within a microfluidic device", *Analytical Chemistry **73***, 5088-5096 (2001)

[5] Abdelgawad, M., Wheeler, A. R., "The digital revolution: A new paradigm for microfluidics", *Advanced Materials* **21**, 920-925 (2009)

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