

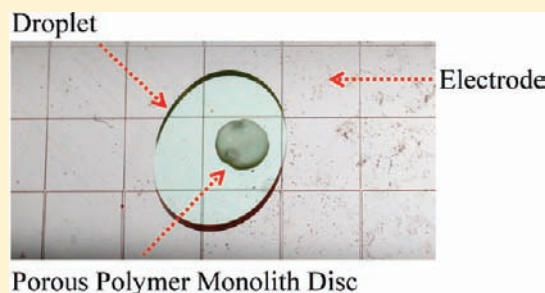
A Digital Microfluidic Method for in Situ Formation of Porous Polymer Monoliths with Application to Solid-Phase Extraction

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ABSTRACT: We introduce the marriage of two technologies: digital microfluidics (DMF), a technique in which droplets are manipulated by application of electrostatic forces on an array of electrodes coated by an insulator, and porous polymer monoliths (PPMs), a class of materials that is popular for use for solid-phase extraction and chromatography. In this work, circular PPM discs were formed in situ by dispensing and manipulating droplets of monomer solutions to designated spots on a DMF device followed by UV-initiated polymerization. We used PPM discs formed in this manner to develop a digital microfluidic solid-phase extraction (DMF-SPE) method, in which PPM discs are activated and equilibrated, samples are loaded, PPM discs are washed, and the samples are eluted, all using microliter droplets of samples and reagents. The new method has extraction efficiency (93%) comparable to that of pipet-based ZipTips and is compatible with preparative sample extraction and recovery for on-chip desalting, removal of surfactants, and preconcentration. We anticipate that DMF-SPE may be useful for a wide range of applications requiring preparative sample cleanup and concentration.



In the past two decades, there has been great interest in the development of micro total analysis systems (μ TAS) that integrate sample delivery, separation, and detection on miniaturized devices.¹ However, a major challenge for such methods is sample complexity; many samples require processing prior to analysis to purify and extract the desirable analytes. Among the various sample processing techniques, the most prominent method is solid-phase extraction (SPE), which exploits interactions between a liquid sample and a solid stationary phase material. In a typical SPE, the sample is retained by a stationary phase and is subsequently eluted in a more concentrated, purified form.

Microfluidic SPE techniques reported previously have taken many different forms, using stationary-phase materials formed on channel walls,^{2,3} packed beds of beads,^{4–11} porous membranes,^{12–15} and porous polymer monoliths (PPMs).^{16–18} The latter technique, relying on PPMs, is particularly attractive because of the capacity to easily form the stationary phase in microchannels (i.e., by filling channels with a solution of monomers followed by UV- or heat-curing to form polymer monoliths). Despite these promising developments, the microfluidic SPE methods reported previously are not a perfect match for preparative applications, as samples handled in microchannels are often difficult to recover.¹⁹ Ideally, a method could be developed combining the advantages of μ TAS (i.e., integration of many automated steps on a single platform) with the capacity to perform preparative sample cleanup, such that samples could be recovered and used for various applications.

An alternative miniaturized fluid handling format to microchannels is digital microfluidics (DMF), a technique in which

discrete fluidic droplets are manipulated by electrostatic forces on an array of electrodes coated with an insulating dielectric.^{20–22} While DMF shares many characteristics with microchannels, DMF is a distinct paradigm from microchannels. In DMF, droplets can be dispensed, merged, mixed, and split independently from each other, making this technique appropriate for carrying out multistep reactions. Moreover, DMF is particularly well-suited for preparative processes, as samples with volumes as large as milliliters²³ can be manipulated. We have used DMF previously for many preparative applications in the past, including processing of samples containing proteins^{24,25} and hormones²⁶ followed by analysis off-line by mass spectrometry. Here, we report a new digital microfluidic method to form PPM discs in situ, with application to on-chip SPE. This is the first example of combining PPMs with digital microfluidics, and we anticipate that this combination may be useful for a wide range of applications requiring preparative sample cleanup and concentration.

METHODS

Reagents and Materials. Unless otherwise stated, all chemicals were obtained from Sigma-Aldrich (Oakville, ON) and were used without further modification. All buffers were formed using

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deionized water that had a resistivity of 18 M Ω cm at 25 °C, filtered with nylon syringe filters from Millipore (Billerica, MA, 0.2 μ m pore diameter), and sonicated (5 min) prior to use. Ethanol (95%) and NaOH were purchased from ACP Chemicals (Montreal, QC). NaCl was purchased from Mallinckrodt Baker (Phillipsburg, NJ). Fluorescein was purchased from Life Technologies (Burlington, ON). Cleanroom reagents and supplies included Parylene C dimer from Specialty Coating Systems (Indianapolis, IN) and Teflon-AF from DuPont (Wilmington, DE). C18 ZipTips were purchased from Millipore.

All solutions used on DMF devices were used within 3 days of preparation. A C12 casting solution was prepared by mixing 279 μ L of butyl acrylate, 150 μ L of 1,3-butanediol diacrylate, 69 μ L of lauryl acrylate, 2.5 mg of 2,2-dimethoxy-2-phenylacetophenone, and 1 mL of porogen comprising a 4:1:1 ratio of acetonitrile, 95% ethanol, and 5 mM phosphate buffer at pH 6.8. For digital microfluidic solid-phase extraction experiments, activation solvent was 0.1% (v/v) formic acid in acetonitrile, equilibration and wash solvents were 0.5% (v/v) formic acid in deionized water, and elution solvent was 500 mM sodium borate in deionized water at pH 9.0 (for elution of fluorescein) or 0.1% (v/v) formic acid in acetonitrile (for elution of peptides). A fluorescamine-labeled peptide standard was prepared using methods similar to those described previously.²⁷ Briefly, a stock solution was formed by mixing 100 μ L of fluorescamine (3 mg/mL in acetone), 10 μ L of angiotensin IV (10 mM in 10 mM borate buffer at pH 9), and 890 μ L of acetone. The reaction mixture was allowed to incubate for at least 2 h before being diluted 10 \times with 0.1% formic acid for analysis.

Digital Microfluidic Device Fabrication and Operation. Digital microfluidic devices were fabricated in the University of Toronto Emerging Communications Technology Institute (ECTI) cleanroom facility, using a transparent photomask printed at Pacific Arts and Design (Markham, ON). Glass devices bearing patterned chromium electrodes were formed by photolithography and etching as described previously²⁸ and were coated with 7 μ m of Parylene-C and 50 nm of Teflon-AF. Parylene-C was applied using a vapor deposition instrument (Specialty Coating Systems), and Teflon-AF was spin-coated (1 wt %/wt in Fluorinert FC-40, 1000 rpm, 30 s) followed by postbaking on a hot-plate (160 °C, 10 min). The polymer coatings were removed from contact pads by gentle scraping with a scalpel to facilitate electrical contact for droplet actuation. In addition to patterned devices, unpatterned indium tin oxide (ITO) coated glass substrates (Delta Technologies Ltd., Stillwater, MN) were coated with Teflon-AF (50 nm, as above).

Two device designs were used, shown in Figures 1a and 5a. The former featured an array of 88 actuation electrodes (2.2 \times 2.2 mm each) connected to 10 reservoir electrodes (5 \times 5 mm each), with interelectrode gaps of 40 μ m. The latter design featured 12 large actuation electrodes (7.5 mm \times 7.5 mm each) for moving sample and solvent droplets, and 19 small actuation electrodes (2.2 mm \times 2.2 mm each) for moving monolith casting solution and elution solvent droplets. 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 μ m), such that “unit” droplets (i.e., the droplet size covering a single electrode) were 1 μ L for 2.2 \times 2.2 mm electrodes and 12 μ L for 7.5 mm \times 7.5 mm electrodes.

To actuate droplets, driving potentials (220–300 V_{pp}) were generated by amplifying the output of a function generator

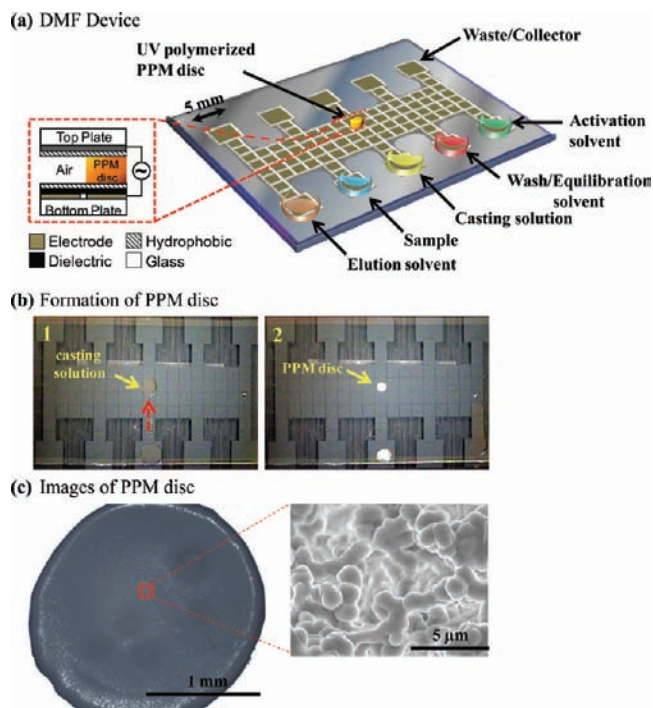


Figure 1. Digital microfluidic (DMF) device design and porous polymer monolith (PPM) disc. (a) Schematic of the device, which features reservoirs dedicated to five different reagents, including elution solvent, sample, monomer, wash solvent, and activation solvent. The reservoirs are connected to an array of 88 actuation electrodes. (b) Frames from a movie illustrating the formation of a PPM disc. In frame 1, a 1 μ L droplet of casting solution was dispensed and driven to the middle of the device. The PPM disc was formed (frame 2) as a result of exposure to UV irradiation (100 W, 365 nm, 5 min). (c) A bright-field microscope image of a PPM disc formed on chip (left) and a high-resolution SEM image of the interior of a PPM disc (right). Scale bars are 1 mm and 5 μ m, respectively.

(Agilent Technologies, Santa Clara, CA) operating at 18 kHz. As described elsewhere,^{24,28} droplets were sandwiched between a patterned bottom plate and an unpatterned (Teflon–ITO–glass) top plate and actuated by applying driving potentials between the top electrode (ground) and sequential electrodes on the bottom plate via the exposed contact pads. Droplet actuation was monitored and recorded by a CCD camera mounted on a lens. For the device shown in Figure 1a, 1 μ L unit droplets were dispensed from larger volumes in reservoirs by actuating three adjacent electrodes in series as described previously.²⁹ On the same device design, larger 5 μ L droplets were dispensed from reservoirs and actuated by applying potentials to multiple electrodes simultaneously.

Porous Polymer Monolith Formation and Characterization. Porous polymer monoliths (PPMs) were prepared via on-chip photopolymerization of a C12 casting solution droplet manipulated by DMF. As illustrated in Figure 1b, each PPM was formed by dispensing a 1 μ L droplet of casting solution onto the array of actuation electrodes and translating it to a central position where it was polymerized by exposure to UV radiation (100 W, 365 nm, 5 min). As shown, the resulting PPM took the shape of a circular disc with radius \sim 1 mm and height of 270 μ m. Two additional types of PPMs were formed, (a) in a glass pipet, and (b) in a “dummy substrate”. The former (in pipet) was used to evaluate the quality of the casting solution. These PPMs were formed by aspirating \sim 50 μ L of casting solution into the glass

pipet and exposing to UV radiation as described above. The latter (in dummy substrates) was required for N₂ adsorption experiments because the method used (see below) was not sensitive enough to evaluate the tiny PPM discs formed in digital microfluidic devices. These discs were formed by polymerizing 15 μ L droplets sandwiched between pieces of unpatterned glass coated with 50 nm Teflon-AF (formed as above) with 450 μ m spacing between them. PPMs formed in this manner had approximate diameters of 5 mm and masses of 3.1 mg.

The surface morphologies and surface areas of PPMs were characterized by scanning electron microscopy (SEM) and N₂ adsorption, respectively. Surface morphologies of PPMs formed on-chip and in glass pipettes were observed using a Hitachi S-5200 SEM (Hitachi, Mississauga, ON); prior to analysis, specimens were carbon-coated to prevent charging. Surface areas of PPMs formed in dummy substrates were determined using an Autosorb-1 instrument from Quantachrome Corp. (Boynton Beach, FL). The samples were outgassed at 50 °C for 4 h under vacuum, and the specific surface area (SSA) was obtained from N₂ (purity 99.999%) adsorption at 77 K using Brunauer, Emmett, and Teller (BET) theory.³⁰ For the calculation of the BET SSA, the relative pressure range was assumed to be 0.05–0.1 mbar. At least three different PPMs were evaluated for all characterization experiments.

Standard Digital Microfluidic Solid-Phase Extraction Process. All digital microfluidic solid-phase extraction (DMF-SPE) experiments were carried out using a variation on the following four-step procedure. Step 1, activation/equilibration: A PPM disc was activated by dispensing and driving a 5 μ L droplet of activation solvent onto the monolith. The droplet was actuated back and forth across the PPM disc five times and then was allowed to incubate on the disc for 1 min before it was driven away and collected in the waste reservoir. An identical process (dispense, actuate, incubate, move to waste) was then implemented for a 5 μ L droplet of equilibration solvent. Step 2, sample loading: A 1 μ L sample droplet was dispensed and actuated to the PPM and was actuated back and forth across the PPM disc five times before being incubated for 2, 4, or 8 min, and then the droplet was moved to waste. Step 3, monolith washing: Droplets of wash solvent (2 \times 5 μ L) were dispensed, actuated onto the monolith (and back and forth across the PPM disc five times), and then moved to waste. Step 4, elution: One, two, or three 5 μ L droplets of elution solvent were dispensed, actuated onto the monolith (and back and forth across the PPM disc five times), and then moved to a sample collection reservoir.

Digital Microfluidic Solid-Phase Extraction Process Optimization. Fluorescein (5 μ M in 0.5% v/v aqueous formic acid) was used as a model analyte to optimize the DMF-SPE process on the device shown in Figure 1a for (i) sample loading time and (ii) elution volume. For the former (optimization of sample loading time), a truncated DMF-SPE method was performed incorporating only steps 1 and 2 from above. After the sample droplet was incubated on the PPM for 2, 4, or 8 min, the sample droplet was moved to a fresh spot on the device and dried by heating on a hot plate (50 °C, \sim 2 min). The precipitate was manually resolubilized in 50 μ L of borate buffer (500 mM, pH 9) and transferred to a 384-well plate for fluorescence measurement (λ_{exc} 480 nm; λ_{em} 520 nm) using a PHERAstar plate reader (BMG Labtech, Durham, NC). For the latter (optimization of elution volume), the full four-step DMF-SPE process was used (with a 2 min incubation in step 2). In step 4, the samples were eluted in 1, 2, or 3 droplets of elution solvent, which were pooled

and moved to a fresh spot on the device, and dried, resolubilized, and interrogated for fluorescence intensity as described above. For both optimizations (i and ii), the fluorescence intensities of the experimental samples were compared to the fluorescence intensities of control experiments in which 1 μ L fluorescein droplets were driven across the device without any exposure to a PPM and then dried and resolubilized as above. All experimental and control experiments were conducted in triplicate using three different PPMs/devices.

Solid-Phase Extraction Efficiency. A fluorescamine-labeled peptide (10 μ M angiotensin IV in 0.1% formic acid) was used as a model analyte to evaluate the extraction efficiency of DMF-SPE on the device shown in Figure 1a and by commercially available ZipTips. For the former (DMF-SPE), the labeled peptide sample was extracted using the four-step procedure described above with a 2 min incubation in step 2 and with two droplets of elution solvent in step 4. The eluted droplets were pooled and moved to a fresh spot on the device, and dried, resolubilized in 50 μ L of elution solvent, and interrogated for fluorescence intensity using the plate reader (with λ_{exc} 390 nm; λ_{em} 460 nm) as described for process optimization, above. For the latter (conventional SPE), 5 μ L aliquots of labeled peptide sample were extracted on C18 ZipTips as per the manufacturer's instructions. The extracted samples were dried and dissolved in 50 μ L of elution solvent for analysis using the plate reader. For both cases (DMF-SPE and ZipTip), the fluorescence intensities of the experimental samples were compared to the fluorescence intensities of control samples (1 μ L for DMF-SPE, 5 μ L for ZipTip), which were not extracted, but dried and resolubilized as above. All experimental and control experiments were conducted in triplicate using three different PPMs/devices or ZipTips.

Digital Microfluidic Solid-Phase Extraction for Preparative Sample Cleanup. DMF-SPE was validated for use for preparative sample cleanup by extraction of two solutions of angiotensin II (Ang II, 1 μ M) on the device shown in Figure 1a: (1) in a solution containing 100 mM sodium chloride, and (2) in a solution containing 0.05% w/v Pluronic F68. For both experiments, 1 μ L sample droplets were extracted using the four-step procedure described above with a 2 min incubation in step 2 and with two droplets of elution solvent in step 4. The eluted droplets were pooled and moved to a fresh spot on the device and were dried at room temperature. The eluate was manually resolubilized in 50 μ L of 50% acetonitrile containing 0.1% formic acid and analyzed via nanoESI-MS (LTQ-Finnigan, Thermo Electron Corp., FL). The applied spray voltage was varied between 1.7 and 2.0 kV, and the flow rate of the syringe pump and capillary temperature were kept constant at 0.5 μ L/min and 200 °C, respectively. As a control, 1 μ L aliquots of the same samples (without desalting or removal of surfactant) were dried, resolubilized, and analyzed by nanoESI-MS for comparison. Each experiment and control was performed in triplicate using separate devices/PPMs.

Digital Microfluidic Solid-Phase Extraction for Sample Concentration. The device shown in Figure 5a was used to evaluate the potential of DMF-SPE for sample concentration. A variation of the four-step procedure described above was implemented with fluorescein as the sample (5 μ M in 0.5% v/v aqueous formic acid). In steps 1–3, the volumes of activation, equilibration, sample, and wash droplets were 12 μ L (instead of 1 or 5 μ L). Step 4 was similar to the procedure outlined above but used a single 1 μ L elution droplet. After the extraction was complete, the eluted droplet was pipetted off the device and diluted

in 49 μL of elution solvent. The fluorescence intensity was measured using a plate reader as above and compared to that of a control sample comprising 1 μL of fluorescein sample, not extracted, diluted with 49 μL of borate buffer (500 mM, pH 9).

RESULTS AND DISCUSSION

Porous Polymer Monolith Formation and Characterization. We report here the first combination of digital microfluidics and porous polymer monoliths (PPMs). PPMs have been used previously in enclosed microchannels^{16,17,31–34} and capillaries^{35–37} by polymerizing a casting solution containing monomers that is surrounded on all sides by the walls of the channel or capillary. Monoliths formed in this manner take the shape of the channel or capillary containing the casting solution. Here, we introduce a new format for PPMs, in which droplets of casting solution are dispensed onto an array of electrodes where they are manipulated into position and then polymerized. This format confers some advantages: the PPMs can be formed in any desired location on the device, multiple PPMs may be formed in parallel, and the PPMs are open on all sides (in the plane of the device), which facilitates a wide range of solid–liquid interactions.

A schematic of a device is shown in Figure 1a, and pictures depicting the formation of a monolith are shown in Figure 1b. As shown, PPMs formed in this manner take the shape of a circular disc, which is slightly smaller than the droplet from which it was formed (e.g., a 1 μL droplet with ~ 2.2 mm diameter forms a disc with ~ 2 mm dia.). The size of the discs is fairly reproducible; for example, when five discs were formed on five different devices, the coefficient of variation in diameter was 10%. Magnified images of PPM discs formed on DMF devices are shown in Figure 1c. The discs have a skin-like outer layer and an interior with the characteristic popcorn-like structure to PPMs formed in conventional formats. The average surface area of PPM discs formed on substrates approximating DMF devices was found to be 3.7 ± 0.2 m²/g by N₂ adsorption, a value that is similar to what has been reported for PPMs formed in capillaries.³⁸

To our knowledge, this article describes the first combination of digital microfluidics with three-dimensional structures positioned on an array of DMF electrodes. As we began this work, we hypothesized that there might be two potential pitfalls. First, because droplets manipulated by DMF are known to pin (i.e., get stuck) on heterogeneous regions on device surfaces,³⁹ we hypothesized that droplets might experience similar problems when they encountered PPM discs. In fact, this was observed for cases in which the droplets were smaller than PPM discs; in such cases, droplets were observed to become stuck on the PPMs, resulting in no further movement. To circumvent this problem, we used sample or solvent droplets that were larger than the PPMs, such that a small fraction of the droplet could stick to the monolith, while a large fraction of the droplet could move away. We have observed similar phenomena, dubbed “passive dispensing”, for the process of translating a droplet across a patterned hydrophilic region for cell culture.⁴⁰ For the 2.2 mm diameter/1 μL droplets and ~ 2 mm diameter PPM discs used here, we estimate that when a droplet is driven onto and off of a monolith disc, the volume remaining on the monolith is less than 10% of the original droplet volume. Second, there was concern that PPMs (which, in this case, are not covalently bound to the device surfaces and can be removed/replaced using tweezers when the device is disassembled) might become mobile when they

(a) Activation/Equilibration of PPM disc



(b) Sample Loading



(c) Washing



(d) Elution



Figure 2. Frames from a movie (left-to-right) depicting the four-step digital microfluidic solid-phase extraction procedure. In (a), a PPM disc is activated by dispensing a 5 μL droplet (frame 1) of activation solvent and driving it onto the monolith. After incubation (frame 2), the droplet is moved away to the waste reservoir (frame 3). Similar steps are repeated for PPM equilibration (not shown), followed by sample loading (b), PPM washing (c), and sample elution (d).

encounter moving droplets. Interestingly, this phenomenon was not observed during the course of hundreds of experiments. We hypothesize that the friction forces between the PPM discs and the device surfaces are larger than the forces associated with moving droplets.

Digital Microfluidic Solid-Phase Extraction. After developing means to form PPM discs in situ on digital microfluidic devices, a four-step procedure was developed to implement digital microfluidic solid-phase extraction (DMF-SPE), which is depicted in Figure 2. First, the PPM was activated in a nonpolar solvent and then equilibrated in a polar solvent (Figure 2a). Second, a small sample droplet was loaded (Figure 2b), and, third, any unbound sample was washed away by a large droplet of wash solvent (Figure 2c). Fourth, a droplet of elution solvent was used to extract the target analyte from the PPM disc (Figure 2d).

A fluorescence method was developed to optimize the DMF-SPE procedure for loading time and elution volume, using fluorescein as a model substrate. For the former (loading time), fluorescein droplets were allowed to incubate on PPM discs for 2, 4, or 8 min before collecting the sample droplet and measuring the remaining analyte as a function of the fluorescence intensity. As shown in Figure 3a, after incubation for 2 min, less than 20% of the fluorescence (relative to the control) remained in the sample droplet. A *t* test revealed no significant differences between 2 or 4 min of incubation ($p = 0.30$), so a 2 min incubation period was used

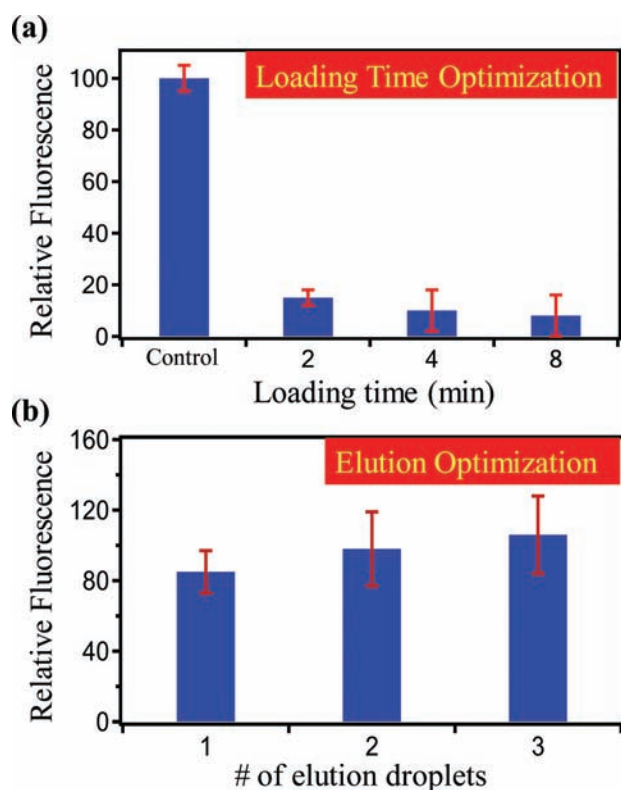


Figure 3. DMF-SPE optimization. Loading time (a) was optimized by evaluating the fluorescence of droplets that had incubated 2, 4, or 8 min on a PPM disc relative to a control sample. Elution volume (b) was optimized by evaluating the number of elution droplets required to extract a fluorescent analyte from a PPM disc.

for all subsequent experiments. For the latter (elution volume), fluorescein was loaded onto PPM discs, rinsed, and then eluted in 1, 2, or 3 5 μ L droplets of elution buffer. As shown in Figure 3b, 2 elution droplets extracted over 90% of bound sample from the PPMs. A *t* test revealed no significant difference ($p = 0.25$) between 2 droplets and 3 droplets, so 2 elution droplets were used for all subsequent experiments.

The optimized DMF-SPE method was validated by comparing its extraction efficiency to that of the commercially available ZipTip method. Fluorescamine-labeled angiotensin IV was used as a model substrate, and after comparison with controls, the DMF-SPE method had an extraction efficiency of $93 \pm 14\%$, and the ZipTip had an extraction efficiency of $92 \pm 5\%$. This is a remarkable similarity given that the DMF-SPE monolith was formed with C12 monomers, and the ZipTip possessed C18 functionality. We propose that in the future, methods for forming PPMs with C18¹⁷ or other functional groups^{16,35,36} might be adapted for use in DMF. In addition, future methods will be combined with automated feedback-controlled dispensing⁴¹ to control and reduce the variability of dispensed droplet size, which we speculate will improve the precision of the DMF-SPE technique.

Digital Microfluidic Solid-Phase Extraction for Preparative Analysis. To validate DMF-SPE for preparative processes, two applications were implemented on-chip followed by analysis by mass spectrometry: sample desalting and removal of surfactant. For the former (desalting), a solution containing 1 μ M angiotensin II and 100 mM NaCl was evaluated. As shown in Figure 4a,

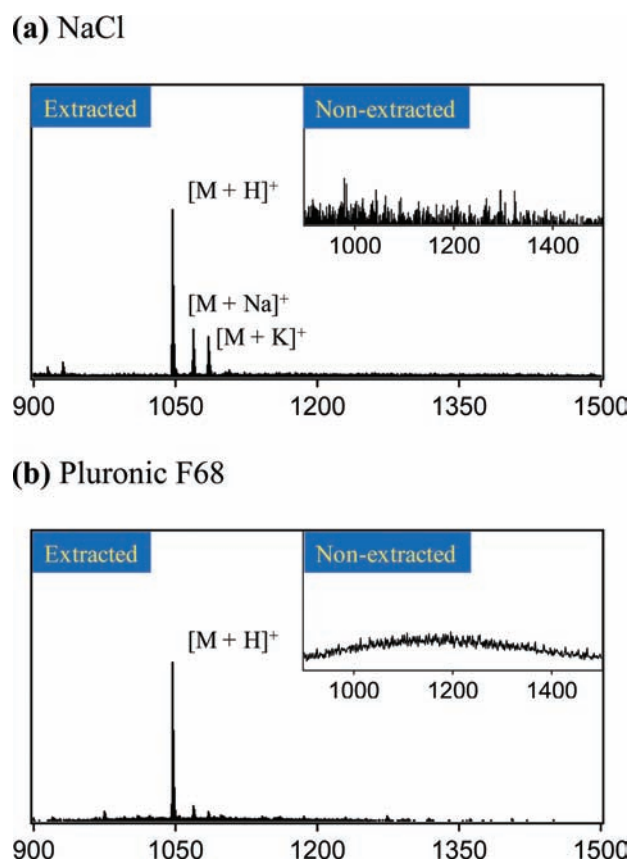


Figure 4. Preparative DMF-SPE. NanoESI mass spectra generated from solutions of angiotensin II (MW 1046) containing (a) 100 mM NaCl or (b) Pluronic F68 (0.05% w/v). The spectra in the main panels were generated from solutions that had been extracted, and the spectra in the insets were generated from solutions that had not been extracted.

the nonextracted sample had no peak at $m/z = 1047$ (the $[M + H]^+$ peak for angiotensin II) because of ion suppression by the salt matrix, but after desalting, a strong signal at $m/z = 1047$ was observed. For the latter (removal of a surfactant), a solution containing 1 μ M angiotensin II and 0.05% Pluronic F68 was evaluated. This case is particularly important for digital microfluidics, as we^{24,25,28,40,42–44} and others^{45,46} have used various Pluronics as solution additives to prevent adsorption of peptides and proteins onto DMF device surfaces. Unfortunately, Pluronics, like other surfactants, are known to be strong ion suppressors for MS,^{47,48} making the ability to remove the additives prior to analysis critical for obtaining useful information. As shown in Figure 4b, the nonextracted sample had no peak at $m/z = 1047$, whereas the extracted sample (Figure 4b) had a strong signal.

The data shown in Figure 4 are superficially similar to data obtained with methods demonstrated previously for sample preparation for MALDI-MS.^{49,50} In the previous work, sample droplets were allowed to dry on flat DMF device surfaces, after which droplets of water were driven over the spots to rinse them. The dried samples were found to be substantially purified. This previous method^{49,50} represented an important milestone for DMF, but the new method reported here is likely superior for several reasons. First, the three-dimensional PPM discs reported here have a larger surface area than a flat surface of equal size, which suggests that the analyte loading capacity for the new method is higher. Second, in the new method, analytes are eluted

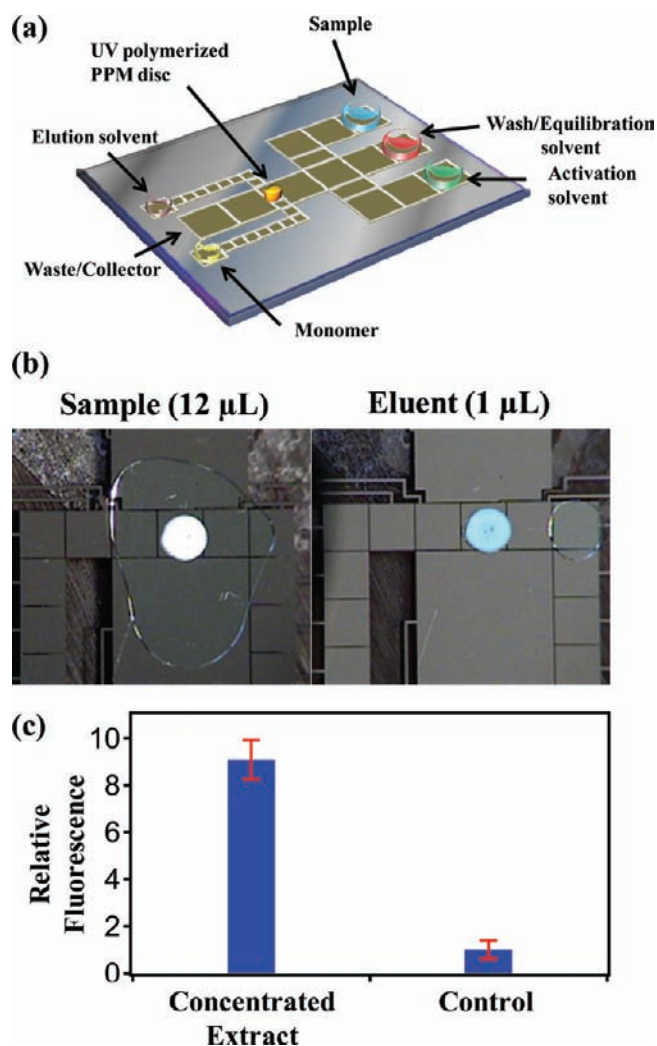


Figure 5. DMF-SPE for concentration. (a) Schematic of device featuring large electrodes (7.5 mm × 7.5 mm) for sample, activation, and washing solvents and small electrodes (2.2 mm × 2.2 mm) for elution solvent and monomer casting solution. (b) Frames from a movie illustrating the process of concentrating a sample on-chip using a PPM disc. In frame 1, a 12 μL sample droplet was actuated onto an activated PPM disc. In frame 2, the disc was washed, and a 1 μL droplet of elution buffer was driven onto it to extract the sample. (c) Bar graph demonstrating a concentration factor of ~ 9 in extracted samples versus controls.

from the PPM disc and can be delivered to any desired location, whereas in the previous method, the sample is permanently bound to a particular spot on the device surface. Third, PPMs can be formed with a wide variety of chemical functional groups,^{16,17,35,36} while the previous method is limited to the device surface material (Teflon-AF).

Digital Microfluidic Solid-Phase Extraction for Sample Concentration. A critical application for SPE is sample concentration, and a new device, shown in Figure 5a, was designed to probe this application for DMF-SPE. This device featured a combination of large electrodes used to deliver 12 μL sample droplets, and small electrodes that were used to deliver 1 μL elution solvent droplets. The process is illustrated in Figure 5b, in which a large sample droplet is loaded onto a PPM disc followed by elution in a much smaller droplet. Using this design,

a concentration factor of ~ 9 for sample droplets of fluorescein was achieved as shown in Figure 5c.

The data and method depicted in Figure 5 are preliminary. The concentration factor of ~ 9 was smaller than the expected value of 12. We speculate that this will be improved in future work, when PPM disc size and shape and electrode layout are optimized. Regardless, these proof-of-principle data fit comfortably in the range of concentration factors for ZipTips (i.e., 2.5–10) and suggest that DMF-SPE may be a useful new tool for applications that are commonly executed using the more conventional technique.

CONCLUSION

We have successfully combined two technologies, digital microfluidics (DMF) and porous polymer monoliths (PPMs). In this work, PPM discs were formed in situ on DMF devices by dispensing droplets of monomer solutions onto an array of electrodes followed by UV-initiated polymerization. The PPM discs were used for preparative solid-phase extraction (SPE) with all fluidic handling steps carried out by DMF. The new method had extraction efficiencies comparable to those of commercially available ZipTips and was compatible with straightforward sample extraction and recovery. We anticipate that this technique may be useful for a wide range of applications requiring preparative sample cleanup and concentration.

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