

# Strong and small: strong cation-exchange solid-phase extractions using porous polymer monoliths on a digital microfluidic platform

Jared M. Mudrik, Michael D.M. Dryden, Nelson M. Lafrenière, and Aaron R. Wheeler

**Abstract:** We present the first method for digital microfluidics-based strong cation-exchange solid-phase extractions. Digital microfluidics is a microscale fluid handling technique in which liquid droplets are actuated over an array of electrodes by electrodynamic forces. Strong cation exchange has gained considerable importance in the field of proteomics as a separation mode for protein and peptide extractions. The marriage of these two techniques is achieved by incorporating sulphonate-functionalised porous polymer monolith discs onto digital microfluidic chips. By manipulating sample and solvent droplets onto and off of these porous polymer monoliths, proteins and peptides are extracted by controlling solution pH and ionic strength. This novel microscale extraction method has efficiency comparable to commercially available strong cation-exchange ZipTips and is highly effective for sample cleanup. We anticipate that this digital microfluidic strong cation-exchange extraction technique will prove useful for microscale proteomic analyses and other applications requiring separation of cationic compounds.

**Key words:** microfluidics, digital microfluidics, solid-phase extraction, strong cation exchange, ion exchange, porous polymer monoliths.

**Résumé :** Nous présentons ici la toute première méthode d'extraction sur phase solide à échange cationique fort basée sur la microfluidique digitale. La microfluidique digitale est une technique de la manipulation des volumes de fluides micro-échelles, dans laquelle des gouttelettes liquides sont déplacées sur une matrice d'électrodes par des forces électrodynamiques. Le phénomène d'échange cationique fort, utilisé comme mode de séparation dans l'extraction des protéines et des peptides, a pris une importance considérable dans le domaine de la protéomique. La combinaison de ces deux techniques (extraction sur phase solide et microfluidique digitale) est atteinte par l'intégration des disques de monolithes polymériques poreux possédant des groupements fonctionnels sulfonate sur les puces microfluidiques. En manipulant des gouttelettes d'échantillon et de solvant sur et hors de ces monolithes polymériques poreux, des protéines et des peptides sont extraits en contrôlant le pH et la force ionique de la solution. Cette méthode novatrice de microextraction, aussi efficace que la technique commercialisée à échange cationique fort ZipTips, est très efficace pour nettoyer les échantillons. Nous prévoyons que cette technique d'extraction basée sur l'échange cationique fort et utilisant la microfluidique digitale s'avèrera utile pour les analyses protéomiques micro-échelles et pour d'autres applications nécessitant la séparation de composés cationiques.

**Mots-clés :** microfluidique, microfluidique digitale, extraction sur phase solide, échange cationique fort, échange d'ions, monolithes polymériques poreux.

## Introduction

Solid-phase extraction (SPE) is an invaluable technique in the analyst's toolbox. Through interactions between components of a liquid sample and a solid stationary phase, SPE allows analytes of interest to be wholly separated from their liquid matrix. In a typical SPE procedure, analytes are loaded onto a solid phase, unretained matrix components are washed away, and analytes are finally eluted in an appropriate solvent. SPE techniques have been developed for a variety of sample preparation purposes, including cleanup, preconcentration, and enrichment.<sup>1-3</sup>

Solid phases bearing negatively charged surface moieties can be used to bind cationic analytes in a process known as strong cation exchange (SCX). SCX is particularly useful for processing peptides and proteins, having been adopted for various proteomic work-

flows in both SPE and chromatographic formats; applications include protein isolation,<sup>4,5</sup> phosphopeptide enrichment,<sup>6-8</sup> peptide fractionation,<sup>9</sup> and proteomic reactors.<sup>10,11</sup> To facilitate SPE of proteomic samples on small, microlitre-scale samples, various commercial micro-SPE products have been adopted, such as micropipette tips with integrated solid-phase materials,<sup>12-14</sup> centrifugal spin columns,<sup>15-17</sup> and SPE microwell plates.<sup>18,19</sup> While these products are certainly useful, they can be tedious to use, requiring multiple pipetting and handling steps, and are typically implemented offline.

In recent decades, there has been an effort to develop microfluidic technologies incorporating sample preparation, separation, and detection onto miniature devices known as micro total analysis systems.<sup>20,21</sup> One platform that has become popular for use in

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these systems, digital microfluidics (DMF), is defined by the actuation of discrete liquid droplets in two dimensions over an array of dielectric-coated electrodes via electrodynamic forces.<sup>22–24</sup> Droplets can be individually actuated in two dimensions, dispensed, merged, mixed, and split. These processes can be easily automated using simple electronics<sup>25</sup> without the need for costly robotic fluid handling systems. DMF has lent itself to a wide variety of analytical applications,<sup>26</sup> including DNA processing,<sup>27,28</sup> protein digestion,<sup>29,30</sup> chemical synthesis,<sup>31,32</sup> tissue analysis,<sup>33</sup> and blood analysis.<sup>34,35</sup> We recently reported the first technique for performing SPE on DMF,<sup>36</sup> which used reverse-phase porous polymer monoliths (PPMs) with C12 moieties as a solid phase; droplets are driven to and from the (stationary) PPM structure to enable the various steps required for an extraction (equilibration, loading, rinsing, eluting, etc.). This DMF–PPM–SPE format allows extractions to be performed in-line with analytical processes on DMF chips without the need for manual intervention and paves the way for the development of automatic DMF micro total analysis systems that integrate SPE for sample preparation. Building on our previous work,<sup>36</sup> we report herein a novel technique for performing SCX–SPE on DMF using sulphonate-functionalised PPMs. This represents the first instance of SCX–SPE in DMF and will be integral for DMF-based protein and peptide analyses, allowing users to avoid offline micro-SPE techniques.

## Methods

### Reagents and materials

Unless otherwise specified, all chemicals were purchased from Sigma-Aldrich (Oakville, Ontario) and were used without further modification. All buffers were prepared in deionised water ( $\rho = 18 \text{ M}\Omega \text{ cm}$ ,  $25 \text{ }^\circ\text{C}$ ). NaOH and KCl were purchased from ACP Chemicals (Montreal, Quebec). NaCl was purchased from Mallinckrodt Baker (Phillipsburg, New Jersey). Cleanroom supplies included Parylene C dimer from Specialty Coating Systems (Indianapolis, Indiana) and Teflon AF from Dupont (Wilmington, Delaware). SCX ZipTips were purchased from EMD Millipore (Billerica, Massachusetts). Teflon AF-coated glass slides were prepared by spin coating (30 s, 1000 rpm) a solution of Teflon AF (DuPont) in Fluorinert FC-40 (1% w/w) on Corning glass slides (75 mm  $\times$  50 mm  $\times$  1 mm) (Corning, New York).

### DMF device fabrication and operation

DMF devices were fabricated in a two-plate format with a 19-by-4 array of actuation electrodes (2 mm  $\times$  2 mm) and 10 reservoir electrodes (5 mm  $\times$  5 mm). To form electrode-bearing DMF bottom plates, glass slides (75 mm  $\times$  50 mm  $\times$  1 mm) were obtained precoated with chromium (200 nm) and photoresist (AZ1500) (530 nm) from Telic Co. (Santa Clarita, California) and were patterned via photolithography and wet etching with a transparent photomask (Pacific Arts and Design, Markham, Ontario) in the Toronto Nanofabrication Centre cleanroom at the University of Toronto as described previously.<sup>37</sup> The finished DMF bottom plates were coated with a dielectric layer of Parylene C (7  $\mu\text{m}$ ) via vapour deposition (Specialty Coating Systems) and spin coated with a layer of Teflon AF (50 nm, 30 s, 1000 rpm) followed by postbaking on a hot plate (160  $^\circ\text{C}$ , 10 min).

DMF top plates were formed from indium tin oxide coated glass (Delta Technologies Ltd., Sillwater, Minnesota) spin coated with Teflon AF (50 nm, as above). DMF devices were assembled by sandwiching the top and bottom plates together with three layers (270  $\mu\text{m}$ ) of double-sided tape (3M, London, Ontario) as spacers. Droplets were actuated by applying sine wave potentials ( $\sim 400 \text{ V}_{\text{p-p}}$ , 18 kHz) between successive actuation electrodes and the top plate using a function generator (Agilent 33220A, Santa Clara, California) and amplifier (Trek PZD700, Lockport, New York).

### PPM formation and characterisation

SCX PPMs were formed by photoinitiated polymerisation of methacrylate monomers in a porogenic solvent following a

procedure adapted from Ueki et al.<sup>38</sup> A casting solution was prepared by dissolving the monomers glycidyl methacrylate (0.9 mL) and ethylene glycol dimethacrylate (0.3 mL) along with the photoinitiator 2,2-dimethoxy-2-phenylacetophenone (12 mg) in a solvent comprising water (0.15 mL), 1-propanol (1.05 mL), and 1,4-butanediol (0.6 mL). As illustrated in Fig. 1a, casting solution (100  $\mu\text{L}$ ) was sandwiched between two Teflon AF-coated glass slides separated by 270  $\mu\text{m}$  of double-sided tape and exposed to a UV lamp (Entela, Upland, California) until polymerisation was complete (5 min, 100 W, 365 nm). Small discs (2.5 mm diameter) were cut from the resulting PPM sheet with a biopsy punch (2.5 mm diameter) (Miltex Inc., York, Pennsylvania). The discs were then thrice soaked in methanol (5 min), incubated in sodium sulphite (1 mol/L, 80  $^\circ\text{C}$ , 48 h), thrice rinsed with nitric acid (10 mmol/L), and incubated in nitric acid (10 mmol/L, 10 min). Finally, the finished SCX PPM discs were thrice rinsed with water and placed on a DMF bottom plate, straddling the border between two electrodes as shown in Fig. 1b. The top plate was then positioned on top of the monolith, sandwiching it between the two plates.

Negative control PPMs were prepared following the procedure described above, omitting sodium sulphite incubation. Negative control and functionalised PPMs were analysed via X-ray photoelectron spectroscopy (K-Alpha, Thermo Scientific, Waltham, Massachusetts) at the Surface Interface Ontario facility at the University of Toronto. PPM morphology was measured via scanning electron microscopy (S-5200) (Hitachi, Mississauga, Ontario) at the Centre for Nanostructure Imaging Facility at the University of Toronto (2.0 kV, secondary electron mode).

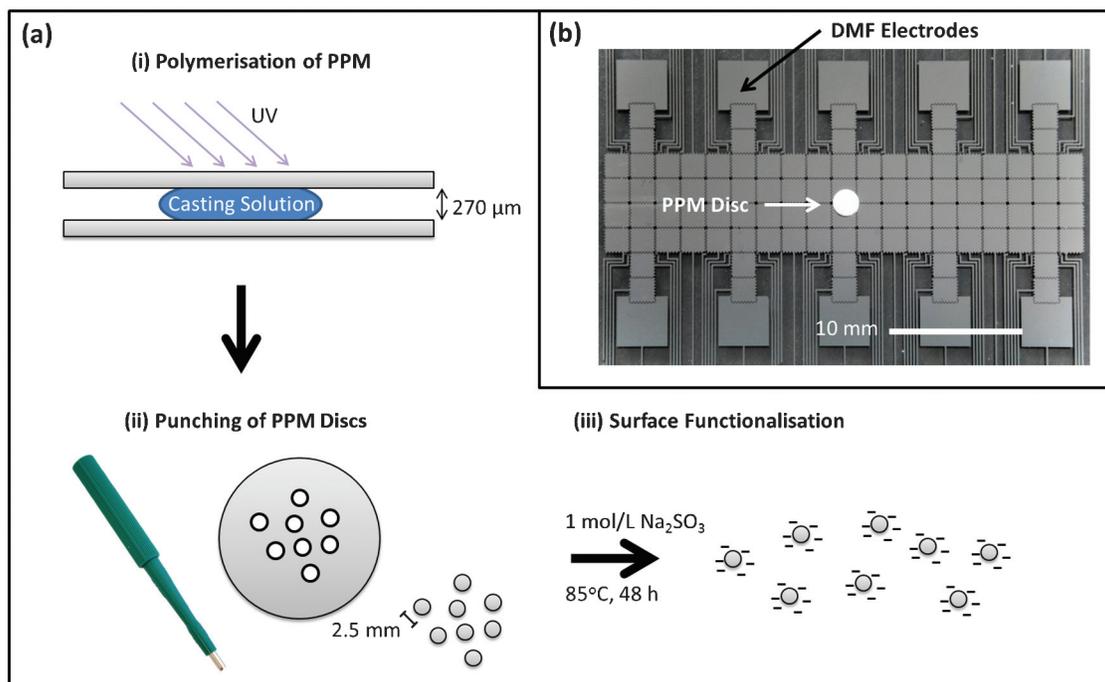
### DMF extraction procedure

DMF-based extractions were performed in five steps over the course of 28 min. First, aliquots of sample, sample solvent, and elution solvent (as defined for particular experiments below) were loaded into their designated reservoirs. Each reservoir held a single premeasured volume of liquid for use in a particular step of the extraction. Second, two sample solvent droplets (10  $\mu\text{L}$  each) were dispensed from separate reservoir electrodes and successively brought to the PPM, where they were incubated (2 min) and carried back to reservoir electrodes as waste. Third, a sample droplet (3  $\mu\text{L}$ ) was dispensed from a reservoir and brought to the PPM for a loading and incubation period (usually 10 min; other times as indicated below). After incubation, the sample droplet was actuated off the PPM and carried to a reservoir electrode as waste. Fourth, two sample solvent droplets (10  $\mu\text{L}$  each) were dispensed from separate reservoirs and successively carried onto the PPM where they were incubated (2 min) and actuated away as waste. Fifth, two droplets of elution solvent (10  $\mu\text{L}$  each) were successively dispensed and moved onto the PPM, incubated (5 min), and carried away to reservoir electrodes for collection by pipette (20  $\mu\text{L}$ ) for analysis. During all loading and incubation steps, mixing was achieved by actuating the droplet engulfing the PPM back and forth linearly.

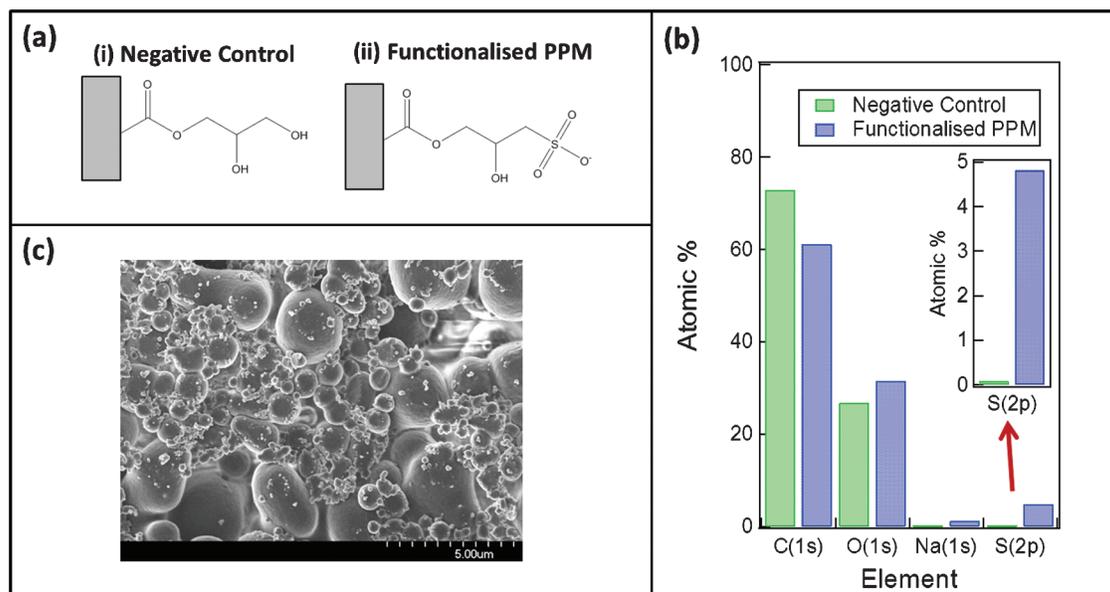
### Extraction efficiency determination

DMF-based SCX extractions were performed as described above using Cy3-streptavidin (10  $\mu\text{g/mL}$  in sample solvent) as the sample and sodium citrate buffer (10 mmol/L, pH 3, 0.1% Pluronic F-68) as the sample solvent. For ionic strength-controlled elution, 1 mol/L KCl in sodium citrate buffer (10 mmol/L, pH 3, 0.1% Pluronic F-68) was used as the elution solvent. For pH-controlled elution, sodium hydroxide solution (100 mmol/L, pH 13, 0.1% Pluronic F-68) was used as the elution solvent. For comparison, the same extraction protocols were performed using SCX ZipTips following manufacturer's directions. Briefly, two sample solvent volumes (10  $\mu\text{L}$  each) were aspirated and dispensed (three cycles each) with the ZipTip and then expired to waste. Second, a volume of sample (3  $\mu\text{L}$ ) was loaded onto the ZipTip with repeated aspiration and dispensing (10 cycles) and expired to waste. Third, the ZipTip

**Fig. 1.** Formation and integration of porous polymer monolith discs for DMF-PPM-SPE. (a) Outline of the fabrication process: casting solution (100  $\mu\text{L}$ ) is sandwiched between Teflon AF-coated glass slides and exposed to UV light for 5 min to carry out polymerisation (i), a biopsy punch is then used to cut 2.5 mm diameter discs from the resulting PPM (ii), and finally, the PPM discs are reacted with sodium sulphite (1 mol/L) for 48 h at 85  $^{\circ}\text{C}$  to carry out surface functionalisation (iii). (b) Picture of a PPM disc on a DMF device, held in place between the top and bottom plates (the top plate is transparent and is not visible).



**Fig. 2.** PPM characterisation. (a) Structures of surface moieties in the negative control (i) and functionalised (ii) PPMs for elemental analysis. (b) X-ray photoelectron spectroscopy elemental analysis results for functionalised PPMs (blue) and negative controls (green). The presence of sulphur in functionalised PPMs versus the control indicates successful surface sulphonation. (c) Scanning electron micrograph of the side-edge of a functionalised PPM disc showing its porous popcorn-like morphology. Color in online version only.

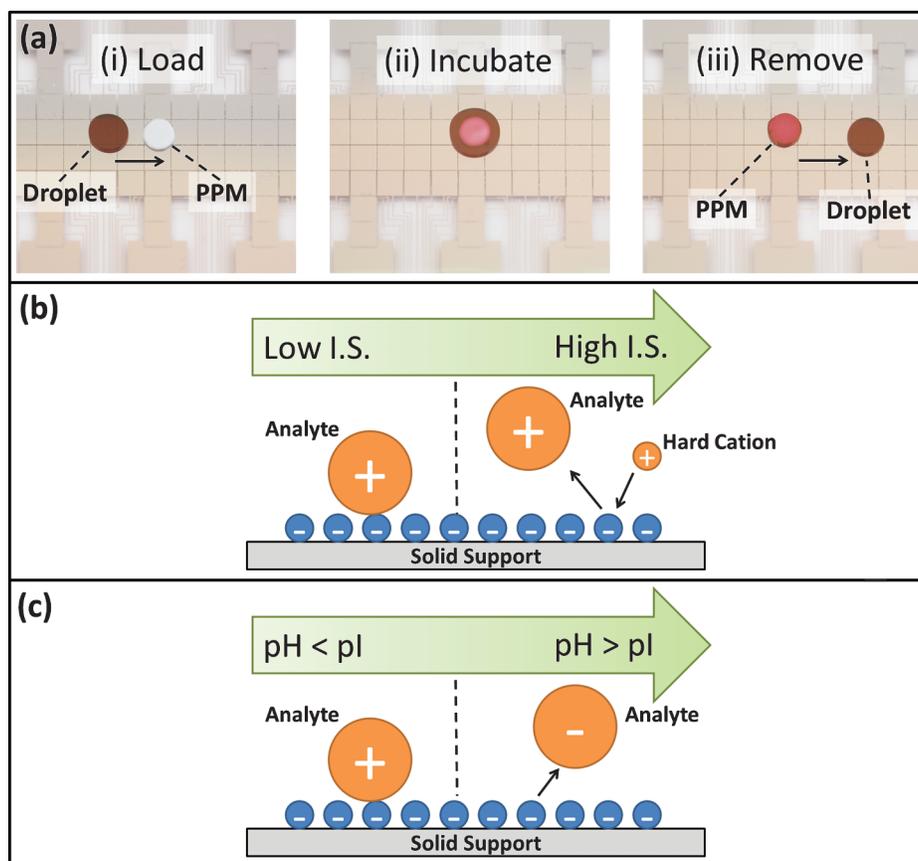


was washed with two volumes of sample solvent (10  $\mu\text{L}$  each) with repeated aspiration and expiration (three cycles each). Finally, the bound sample was eluted with two volumes of elution solvent (10  $\mu\text{L}$  each), which were aspirated and expired (five cycles) and then collected. Recovered Cy3-streptavidin was quantified via fluorescence using a Typhoon Trio fluorescence scanner (GE Life

Sciences, Piscataway, New Jersey) ( $\lambda_{\text{ex}}$ , 532 nm,  $\lambda_{\text{em}}$ , 570 nm, 450–475 V gain). Ionic strength- and pH-based experiments were each performed in triplicate.

DMF-based SCX extractions of angiotensin IV (200  $\mu\text{mol/L}$  in sample solvent) were carried out with the same sample solvent as above. Elutions were performed in triplicate with both a

**Fig. 3.** SCX solid phase extraction. (a) Image series illustrating the loading (i), incubation (ii), and removal (iii) of a droplet of red food dye onto and off of a PPM on a DMF device. In DMF-SPE, all extraction steps are carried out by loading appropriate droplets of sample solvent, sample, sample solvent again, and elution solvent, respectively, onto and off of a PPM following these steps. (b) Illustration of the ionic-strength-controlled SCX elution process. A soft cationic analyte is loaded onto the negatively charged SCX surface in a low-ionic-strength (I.S.) solution. To elute, the ionic strength of the surrounding solution is raised, causing the abundant hard metal cations to displace the analyte cations on the SCX surface. (c) Illustration of the pH-controlled SCX elution process. Analytes are loaded at a pH in which they are cationic and bind to the negatively charged SCX surface. To elute, the solution pH is adjusted such that the analyte becomes anionic and is no longer retained on the SCX surface.



negative control elution solvent (1 mmol/L sodium citrate buffer, pH 3) and a standard elution solvent (1 mol/L NaCl in 10 mmol/L sodium citrate buffer, pH 3). Collected eluates were labeled off-chip with fluorescamine using a procedure adapted from a method described previously.<sup>39</sup> Briefly, sodium bicarbonate buffer (20  $\mu$ L, 100 mmol/L, pH 9) and fluorescamine (10  $\mu$ L, 500  $\mu$ mol/L in acetonitrile) were added to eluates in a 384-well plate. The labeled samples were immediately analysed using a PHERAStar fluorescence plate reader (BMG Labtech, Durham, North Carolina) ( $\lambda_{\text{ex}}$ , 480 nm,  $\lambda_{\text{em}}$ , 520 nm, gain optimised to the most fluorescent sample for each run).

#### Kinetics analysis

DMF-based extractions of Cy3-streptavidin (10  $\mu$ g/mL) were carried out in triplicate as described above with loading times varied at 15, 30, 45, 60, 120, and 300 s. After loading, a portion of the sample droplet (1  $\mu$ L) was collected from the PPM with a micropipettor. The resulting samples were diluted to 20  $\mu$ L with deionised water and quantified via fluorescence using a Typhoon Trio fluorescence scanner ( $\lambda_{\text{ex}}$ , 532 nm,  $\lambda_{\text{em}}$ , 570 nm, 500–550 V gain). Curve fitting was performed using nonlinear least squares, with fit suitability verified by observation of residuals.

#### Sample cleanup

A peptide sample mixture of angiotensin I and bradykinin (100  $\mu$ g/mL each) was prepared in sodium citrate buffer (10 mmol/L,

pH 3) with a poly(ethylene glycol) (PEG) matrix (1 g/mL). To extract the peptides from the PEG, a pH-controlled DMF SCX extraction was performed as described above. The sample solvent used was sodium citrate buffer (10 mmol/L, pH 3) and the elution solvent was a NaOH solution (10 mmol/L, pH 12). The extracted and nonextracted samples were analysed via MALDI-MS (MALDI Micro MX, Waters, Milford, Massachusetts). To prepare MALDI spots, a sample droplet (1  $\mu$ L) was mixed with a droplet of sinapic acid solution (1  $\mu$ L, 20 mg/mL in 1:1 acetonitrile – 0.1% trifluoroacetic acid) on a MALDI target plate (Waters) and allowed to dry at room temperature. Laser power was adjusted and the laser spot moved for each sample to achieve optimal signal-to-noise ratio. Data were baseline subtracted and the centroid spectrum plotted.

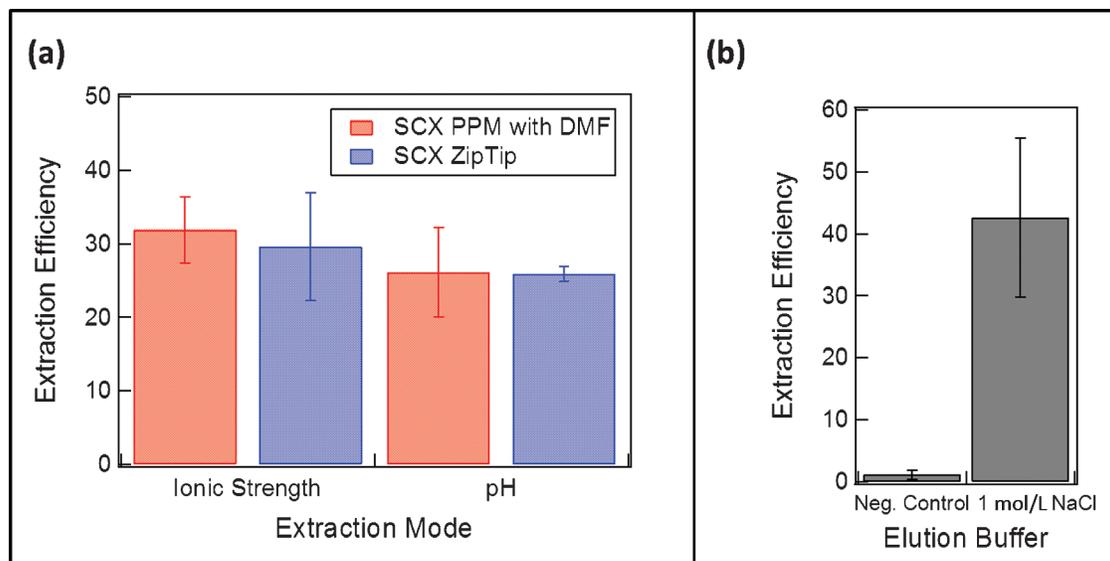
## Results and discussion

#### PPM formation and characterisation

PPMs have been developed and used extensively for microchannel and capillary-based separation and extraction applications.<sup>38,40–48</sup> Recently, we presented the first application for PPMs on DMF<sup>36</sup> using PPM discs with C12 moieties as a reverse phase for SPE. With the goal of expanding this technique to include SCX extractions, we have optimised the method to make use of PPMs bearing sulphonate surface moieties.

PPMs were formed by UV-photopolymerisation of a casting solution of methacrylate monomers (including glycidyl methacry-

**Fig. 4.** SCX–SPE efficiency. (a) Comparison of extraction efficiencies for Cy3-streptavidin (10  $\mu\text{g}/\text{mL}$ ) purified on SCX PPMs on DMF (red) and the commercial SCX ZipTip product (blue). Extractions were performed in both ionic strength and pH elution modes. Efficiencies in both modes are similar for the DMF-based method and the commercial ZipTip product. (b) Extraction efficiency of angiotensin IV (200  $\mu\text{mol}/\text{L}$ ) using SCX PPMs on DMF when eluted with a low-ionic-strength negative control buffer (1  $\text{mmol}/\text{L}$  sodium citrate, pH 3) and a standard high-ionic-strength elution buffer (1  $\text{mol}/\text{L}$  sodium chloride, 10  $\text{mmol}/\text{L}$  sodium citrate, pH 3). The significantly higher extraction efficiency achieved with the high-ionic-strength buffer versus the negative control indicates that recovery is a result of elution of bound analytes rather than sample carryover. Color in online version only.



late) in a porogenic solvent between two Teflon AF-coated glass slides (Fig. 1a). Following polymerisation, a biopsy punch was used to cut the resulting sheet of PPM material into small discs for use on DMF devices. These discs were incubated in sodium sulphite to allow reaction between epoxide moieties from incorporated glycidyl methacrylate and sulphite anions, generating sulphonate functionality on the PPM surface. These negatively charged sulphonate moieties served as the source of SCX functionality in the PPMs. Functionalised PPM discs were used by sandwiching them between the top and bottom substrates of a DMF device (Fig. 1b). The friction on the PPM disc in this configuration sufficed to hold it in place during droplet movement onto and off of the PPM.

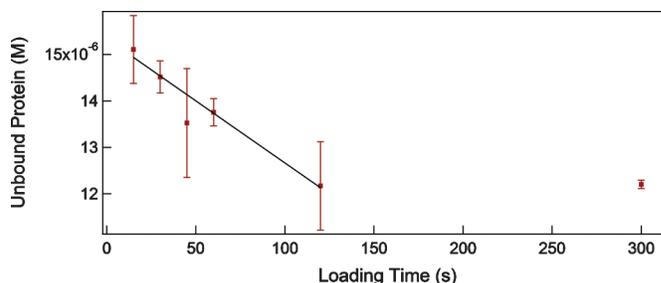
To verify surface sulphonation of PPMs, X-ray photoelectron spectroscopy was used to perform elemental analysis on functionalised and nonfunctionalised PPMs (Fig. 2a). While sulphur (2p) was absent in the nonfunctionalised negative control PPMs, it was present (5 atomic %) in the functionalised sample (Fig. 2b). In addition, the lack of a stoichiometric quantity of sodium to sulphur indicates that the sulphur present was not adsorbed by sodium sulphite. These results indicate that sulphonation was successful.

Qualitative characterisation of PPM morphology was performed via scanning electron microscopy. A view of the side edge of a PPM sample (Fig. 2c) reveals that a highly porous popcorn-like structure is available to droplets being introduced onto the monolith. This structure provides a large surface area on which cationic analytes may bind.

#### DMF extraction procedure

DMF SCX extractions were performed in four steps by loading, incubating, and removing appropriate droplets onto and off of PPMs (Fig. 3a). In the first step, the PPM is conditioned with sample solvent to prepare it for sample loading. This step ensures that loading will occur at the correct pH and ionic strength. In the second step, the sample solution is loaded onto the PPM and incubated to allow analyte binding to reach equilibrium. The pH of the sample solvent is chosen to ensure that analytes of interest are cationic. Repeated actuation of the sample droplet in contact with

**Fig. 5.** Analysis of the binding kinetics of Cy3-streptavidin (10  $\mu\text{g}/\text{mL}$ ) on SCX PPMs. The protein loads with (apparent) linear zeroth-order kinetics over 2 min after which equilibrium is reached.

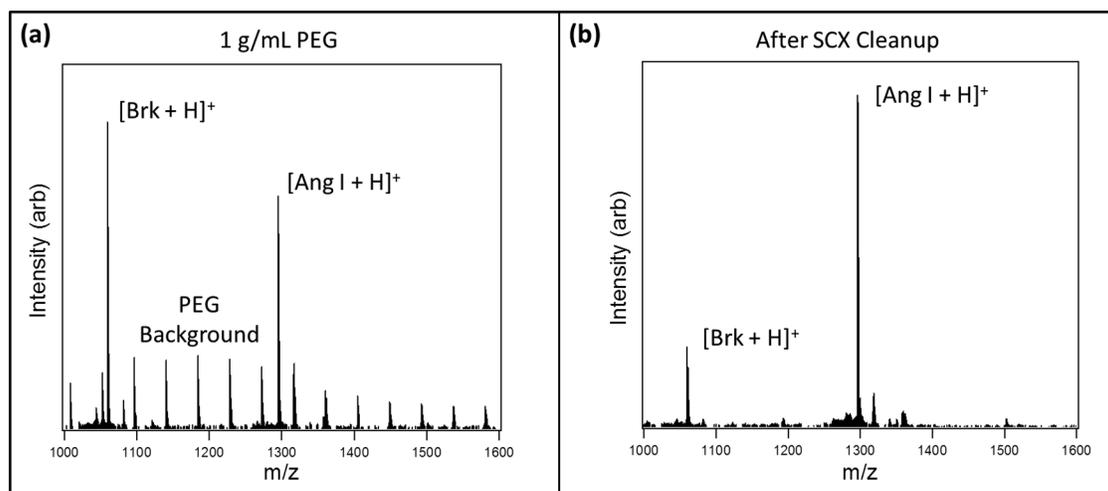


the PPM serves to mix the sample droplet to prevent the formation of a concentration gradient as analyte binding occurs. In the third step, the PPM is again rinsed with sample solvent to remove any unbound sample components, preventing contamination of the eluate. In the fourth step, an appropriate elution solvent is used to release bound analyte from the PPM. This can occur in one of two ways. In the first elution technique, illustrated in Fig. 3b, ionic strength is used to control analyte binding; the cationic sample is loaded in a buffer with low ionic strength and eluted in a buffer with high ionic strength. The hard cations in the high-ionic-strength buffer displace the soft analyte cations from the SCX binding sites, causing them to elute. In the second elution technique, illustrated in Fig. 3c, pH is used to control analyte binding; the sample is loaded at a pH in which it is cationic and eluted at a pH in which it is neutral or anionic. In our studies, DMF-based SCX extractions were evaluated in both ionic strength and pH elution modes.

#### Extraction efficiency determination

The efficiency of DMF-based extractions with SCX PPMs for protein samples was evaluated and compared with the commercial ZipTip microvolume SPE product. SCX extractions of Cy3-streptavidin were performed in both ionic strength and pH

**Fig. 6.** Mass spectra of 100  $\mu\text{g/mL}$  bradykinin (Brk) and angiotensin I (Ang I) with 1 g/mL PEG (a) before and (b) after removal of PEG via DMF-based SCX extraction. After cleanup, the peptide peaks are clear and the PEG background signal is significantly reduced.



elution modes on DMF chips with SCX PPMs and with SCX ZipTips. The extraction efficiencies, compared in Fig. 4a, were similar between both SPE techniques in each mode, with ionic strength and pH elutions yielding efficiencies of approximately 30% and 25%, respectively.

To ensure analyte retention occurred because of SCX binding and not carryover, extractions of angiotensin IV peptide were performed in ionic strength elution mode using a standard high-ionic-strength elution solvent and a negative control low-ionic-strength elution solvent. As seen in Fig. 4b, the negative control elution solvent yielded near-zero extraction efficiency, while the standard elution solvent yielded an efficiency of  $43\% \pm 13\%$ . This result is indicative of retention caused by the desired SCX binding.

It is noteworthy that the extraction efficiencies of Cy3-streptavidin are somewhat low for both the DMF method and for ZipTips, in both ionic strength and pH elution modes. To improve extraction efficiencies in the future, multiple extractions from one sample droplet could be performed in series using multiple PPM discs on a single DMF device.

### Kinetics analysis

The binding kinetics of Cy3-streptavidin on SCX PPMs was evaluated by varying sample loading time and quantifying unbound protein. As shown in Fig. 5, the sample saturates the monolith during the first 2 min of loading, after which equilibrium is reached and no further binding is observed. Because of the high error in the slope, likely caused by imperfect temporal resolution by the experimenter, the apparent zero-order kinetic rate constant could not be reasonably determined. The  $\sim 120$  s timescale is comparable to the loading procedure for SCX ZipTips, which involves repeated aspiration and expiration steps.

### Sample cleanup

To demonstrate the utility of DMF-based SPE using SCX PPMs for sample purification, extractions were carried out for a mixture of the peptides angiotensin I and bradykinin from a concentrated (1 g/mL) PEG matrix. Before extraction, MALDI-MS analysis yielded a spectrum of the peptides obfuscated by a series of intense PEG peaks (Fig. 6a). Following purification of the peptides via DMF-based SCX extraction with pH-controlled elution, the peptide sample was sufficiently purified such that the PEG matrix was no longer detected via MALDI-MS, yielding a clear spectrum of angiotensin I and bradykinin (Fig. 6b). From these results, it can be concluded that SCX PPMs present an effective means to purify cationic samples with noncationic matrices using DMF. We note that MALDI-MS is typically nonquantitative, and the changes in

peak heights for bradykinin and angiotensin do not necessarily indicate differences in extraction efficiency for the two analytes.

The demonstrated utility of SCX PPMs for digital microfluidic SPE makes them a promising tool for DMF-based proteomic workflows. In past work, DMF has been used as a platform for integrated proteomic sample processing, combining various protein digestion steps into a microscale workflow prior to offline analysis via macroscale SPE fractionation.<sup>29</sup> The novel SCX-PPM-SPE technique presented herein represents a promising addition to DMF-based proteomic workflows, paving the way for the integration of protein processing and SCX separation in a single DMF device.

### Conclusion

We have developed a novel technique for performing strong cation-exchange solid-phase extractions on a digital microfluidic platform using functionalised porous polymer monoliths. This method is highly effective for the extraction of proteins and peptides, with extraction efficiencies comparable to commercial ZipTips in both ionic-strength- and pH-controlled elution modes. Having demonstrated its utility for peptide sample cleanup, we anticipate that this new method will find great use in digital microfluidic proteomic workflows, enabling the integration of strong cation-exchange separation steps.

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