Journal of Chromatography A, 1218 (2011) 4039–4044

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

On-chip solid phase extraction and enzyme digestion using cationic PolyE-323 coatings and porous polymer monoliths coupled to electrospray mass spectrometry

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ARTICLE INFO

Article history:
Received 10 February 2011
Received in revised form 10 April 2011
Accepted 11 April 2011
Available online 16 April 2011

Keywords:
Microfluidics
Monolithic column
Mass spectrometry
Trypsin digestion
Solid phase extraction

ABSTRACT

We evaluate the compatibility and performance of polymer monolith solid phase extraction beds that incorporate cationic charge, with a polycationic surface coating, PolyE-323, fabricated within microfluidic glass chips. The PolyE-323 is used to reduce protein and peptide adsorption on capillary walls during electrophoresis, and to create anodal flow for electrokinetically driven nano-electrospray ionization mass spectrometry. A hydrophobic butyl methacrylate-based monolithic porous polymer was copolymerized with an ionizable monomer, [2-(methacryloyloxy)ethyl] trimethylammonium chloride to form a polymer monolith for solid phase extraction that also sustains anodal electroosmotic flow. Exposure of the PolyE-323 coating to the monolith forming mixture affected the performance of the chip by a minor amount; electrokinetic migration times increased by ~5%, and plate numbers were reduced by an average of 5% for proteins and peptides. 1-mm long on-chip monolithic solid phase extraction columns showed reproducible, linear calibration curves (R^2 = 0.9978) between 0.1 and 5 nM BODIPY at fixed preconcentration times, with a capacity of 2.4 pmol or 0.92 mmol/L of monolithic column for cytochrome *c*. Solution phase on-bed trypsin digestion was conducted by capturing model protein samples onto the monolithic polymer bed. Complete digestion of the proteins was recorded for a 30 min stop flow digestion, with high sequence coverage (88% for cytochrome *c* and 56% for BSA) and minimal trypsin autodigestion product. The polycationic coating and the polymer monolith materials proved to be compatible with each other, providing a high quality solid phase extraction bed and a robust coating to reduce protein adsorption and generate anodal flow, which is advantageous for electrospray.

1. Introduction

Microfluidic systems in which multiple sample preparation processes are integrated into the same device may provide a significant advantage in time, speed and automation for proteomics applications [1–3]. Electroosmotic flow (EOF) provides a powerful means of controlling and delivering fluids in an integrated system. We envision a system with electrokinetically delivered protein collection by a solid phase extraction (SPE) column, before or after upstream separation, followed by protein digestion and elution to downstream separation and electrospray mass spectrometry (ES-MS). Such a system requires a number of materials and surface properties be in place within the microfluidic devices, including a cationic surface to generate anodal flow for electrospray when detecting positive ions, a surface that has good properties for electrophoretic separation of proteins and peptides, and an SPE bed that is compatible with the cationic surface in terms of function and fabrication. Here, we evaluate the compatibility and performance of polymer monolith SPE beds that incorporate cationic charge, with a polycationic surface coating, PolyE-323, fabricated within fused silica capillaries and microfluidic glass chips.

Porous hydrophobic polymer monolithic columns for on-chip SPE are receiving increasing attention as an alternative to bead packed beds [4–7], which require retaining frits, and have been reported for both offline and online coupling to ES-MS [4,7–13]. Co-polymerization of ionic functionalities, such as [2-(methacryloyloxy)ethyl] trimethylammonium chloride (META) [14–16] and 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) [8,17], with hydrophobic polymerization mixtures have been reported. Proteins adsorbed to commercially available micro-beads in vials or packed in columns [18–21] can be effectively digested while adsorbed, via the addition of trypsin solution. Here we report on META-monolith fabrication on microchip for

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doi:10.1016/j.chroma.2011.04.027

SPE, on-bed trypsin digestion of captured protein, and elution for ES-MS, all driven electrokinetically.

The polymeric amine, PolyE-323, provides a coating that generates sufficient anodal EOF for nano-ES-MS and effectively eliminates protein and peptide adsorption in fused silica capillaries [22,23]. We explore the compatibility of PolyE-323 coatings with META-monolith formation, and the impact of this combination on capillary electrophoresis separation and SPE performance. The integration of electrokinetically driven SPE, on-bed tryptic digestion, elution and ES-MS of several proteins was demonstrated, illustrating that this pairing of organic coatings and beds are suitable for coupling these steps with separation techniques such as CE and CEC.

2. Materials and methods

2.1. Chemicals, materials and microchips

With the exceptions below, all reagents were from Sigma–Aldrich (Milwaukee, WI, USA), and solvents were HPLC or LC-MS grade. Benzoin was from General Intermediates of Canada (Edmonton, AB, Canada). BODIPY 493/503 (4,4-difluro-1,3,5,7,8-penta methyl-4-bora-3a,4-diaza-*s*-indacene) was from Molecular Probes (Eugene, OR, USA). Butyl methacrylate (BMA), ethylene glycol dimethacrylate (EDMA), [2-(methacryloyloxy) ethyl] trimethylammonium chloride (META) and water were purified as described previously [24]. Protein and peptide stock solutions (1–2 mg/mL) were prepared in Milli-Q water and 0.1 mM BODIPY in methanol, stored at −20 °C, then adjusted to room temperature and diluted as required prior to use. All aqueous solutions were prepared with Milli-Q water and filtered through 0.2 μm Nylon syringe filters (Chromatographic Specialties Inc., Brockville, ON, Canada). Fused silica capillary (50 μm i.d., 365 μm o.d.) was from Polymicro Technologies Inc. (Phoenix, AZ, USA).

Corning 0211 glass microfluidic devices were fabricated as previously described [24,25], at the University of Alberta NanoFab (Edmonton, AB, Canada); 3.5 cm long channels were etched 56 μm wide and 20 μm deep, with a 140 μm wide, 1 mm long segment etched at the center of the device, Fig. 1(a), and 1 mm wide access ports on the cover plate. At the end of the channel, a 4 cm long 50 μm i.d. gold coated nano-electrospray, pulled, fused silica capillary tip was inserted, as described previously [25,26]. For CE separation of proteins and peptides as well as measuring the EOF flow velocity of the coated chip using the neutral marker BODIPY, a P/ACE 5010 Beckman Instrument (Fullerton, CA, USA) and a microfluidic device with "double T injector" were used [27], the latter with 10 μm deep and 30 μm wide separation channels.

2.2. Channel coating and photo-polymerized monolith formation

PolyE-323 was synthesized from 1,2-bis(3-aminopropylamino) ethane and epichlorohydrin and channels or capillaries were coated with it according to published procedure [22]. The device was first conditioned with 1 M NaOH for 30 min and rinsed with Milli-Q water for 15 min with suction from house vacuum, then flushed with 7.5% (w/w) PolyE-323 solution (adjusted to pH 7 with acetic acid) for 30 min, then rinsed with 10 mM NH_4Ac (pH 8.0).

The monolith beds were prepared using the polymerization mixture described in Table 1, after sparging with nitrogen for 5 min to remove dissolved oxygen prior to mixing with initiator. The PolyE-323 coated channels were then filled with the polymerization mixture and the access ports were sealed. A transparency mask was used to selectively expose the 140 μm wide, 1 mm long channel segment to the UV source described previously [24].

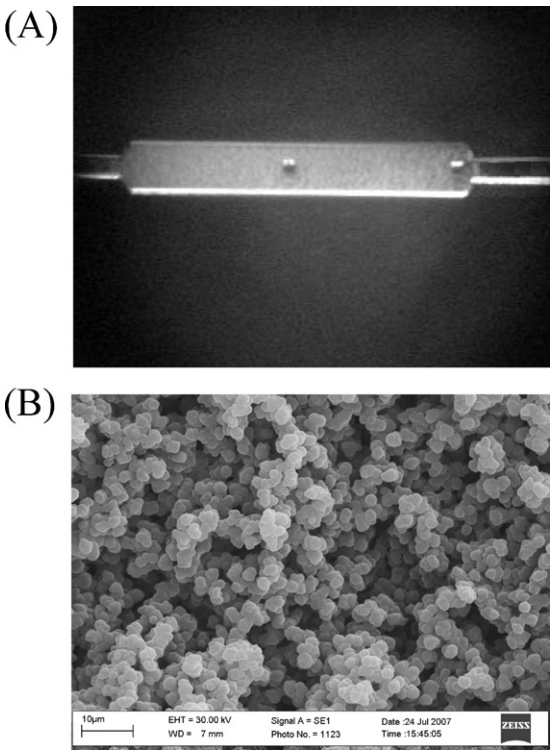

Fig. 1. (A) Optical micrograph of a 1 mm long monolith polymer bed, with 30 μm^2 posts, prepared in 20 μm deep, 140 μm wide microchannel; (B) SEM image of the monolith showing details of nodules and pores. The monolith has sharp edges and uniform structure.

Table 1
Polymerization mixtures and reaction conditions used for the preparation of monoliths.

Polymer	A[a]	B[a]
EDMA, g	0.24	0.24
BMA, g	0.36	0.36
META/H_2O[b], g		0.09
Benzoin, g	0.006	0.006
1-Octanol, g	0.9	
1-Propanol, g		0.54
1,4-butandiol, g		0.27
Exposure time, min	8	4

[a] (A) neutral hydrophobic monolithic polymer and (B) positively-charged monolithic polymer.
[b] META/H_2O: (0.2 g of 75% aqueous META solution/1.8 g H_2O).

Monoliths were then flushed with methanol/water mixture (1:1, v/v) at 0.05 μL/min flow rate for 5 min to remove un-reacted reagents. When not in use, the device was stored in buffer. Scanning electron microscopy (SEM) of the bed cross sections on-chip and mercury porosimetry of bulk monolith materials were done as described previously [24].

2.3. Instrumentation

The computer controlled power supply and relay arrangement has been described elsewhere [28], as has the laser induced fluorescence system [29], which used a 530 nm emission filter. A PE/Sciex API 150 EX single quadrupole mass spectrometer (PerkinElmer/Sciex, Concord, ON, Canada) was operated in positive ion mode. The gold coated nanoelectrospray tip operation was optimized by electrokinetically infusing bradykinin fragment (1-5), positioning it 5–10 mm from the orifice at tip voltages from 3.2 to 3.5 kV. Masses were scanned from *m/z* 500–1200 at 0.5 amu per step with a dwell time of 1 ms, unless otherwise stated.