Supporting Information for Publication

Utilizing Microchip Capillary Electrophoresis Electrospray Ionization for Hydrogen Exchange Mass Spectrometry

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Table of Contents

Microchip Design and Fabrication

A schematic of the CE-ESI microchip used in this work is shown in Figure S1. As described previously for CE-ESI, [1,2] the microchip contains an injection cross, a separation channel, an electroosmotic pump, and an ESI emitter. The reservoir labels correspond to sample (S), background electrolyte (B), sample waste (W), and electroosmotic pump (EO). The length of the separation, EO pump, sample, background electrolyte, and sample waste channels were: 10 cm, 2.5 cm, 1 cm, 0.8 cm, and 0.8 cm, respectively. For the complex mixture separation, the design was similar except the separation channel was 23 cm long while the EO pump channel was 3 cm long. Microchips were fabricated using 0.5 mm thick B270 glass obtained from Perkin Elmer (Waltham, MA). Microchips were fabricated in house using previously described photolithography, wet chemical etching, and thermal bonding techniques.[2] All channels were etched to 10 μm deep and 70 μm wide. Glass cylinders, 8 mm in diameter, were attached to the devices with a chemically resistant epoxy to serve as solvent reservoirs (Loctite E-120HP, Henkel Corporation, Germany).

Microchip Surface Coating

Microchips were coated with APDIPES in the gas phase using a commercially available Labkote chemical vapor deposition (CVD) system (Yield Engineering Systems, Livermore, CA) as described previously.[3] Briefly, microchips were placed inside the vacuum chamber of the CVD system. The coating protocol involved dehydration of the vacuum chamber, injection and vaporization of the silane reagent, a soak period where microchips were exposed to vaporized silane, and evacuation purges to remove reagent vapors. This protocol was repeated three times for a total coating time of 1 hour before removing the device from the CVD system. Following CVD coating, microchips were cooled to room temperature before further surface modification.

After coating the microchips with APDIPES, the sample, background electrolyte, sample waste, and separation channels were modified with a PEG reagent in the liquid phase as described previously.[4] An NHS-PEG solution was prepared in 100 mM phosphate buffer (pH 7.5) at a concentration of 5 mg/mL and flowed through the specified channels for a period of 1 hour. During PEGylation, the S, B, and W reservoirs were filled with PEG reagent and a head pressure of 25 psi was applied to each reservoir. The EO pump reservoir was filled with background electrolyte (BGE) with vacuum applied at the ESI tip (Figure 1). PEGylated channels were subsequently rinsed with DI water for 10 minutes followed by BGE for 10 minutes. After rinsing the device, the external surface of the ESI emitter was coated with trichloro-(1H,1H,2H,2H-perfluorooctyl)-silane to increase the surface hydrophobicity and facilitate ESI.

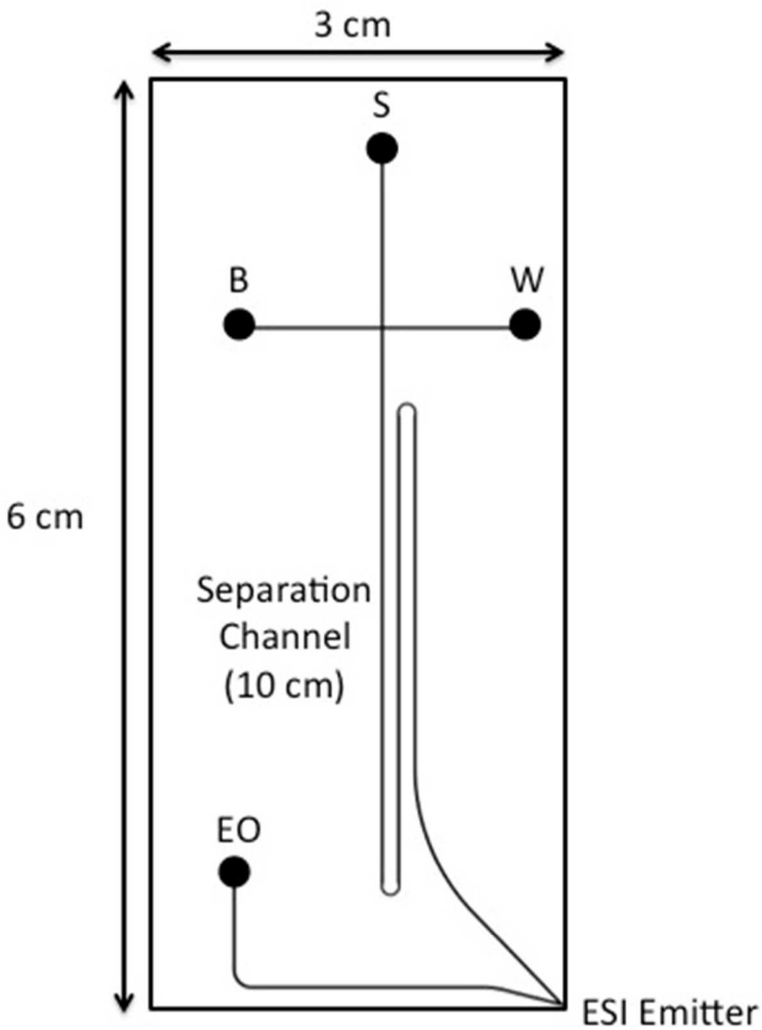

Figure S1. Schematic of CE-ESI Microchip. The reservoir labels correspond to sample (S), background electrolyte (B), sample waste (W), and electroosmotic pump (EO). The separation channel measures 10 cm long. The dimensions of the chip are approximately 3 cm wide by 6 cm long.

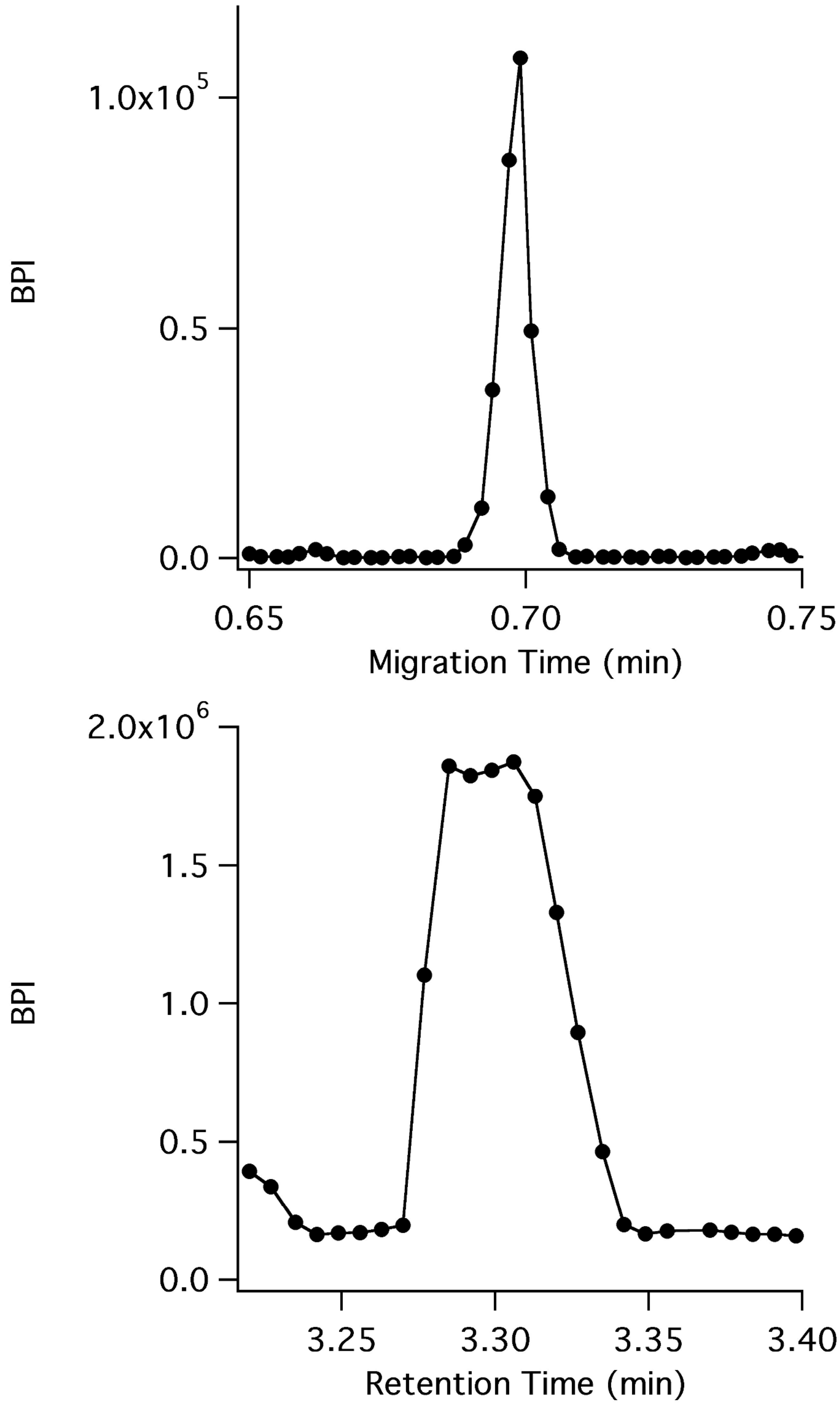

Figure S2. Representative peak profiles from bovine hemoglobin pepsin digest showing data points across peak for CE (top) and LC (bottom).

(1) Mellors, J. S.; Jorabchi, K.; Smith, L. M.; Ramsey, J. M. *Anal Chem* **2010**, *82*, 967-973.
(2) Mellors, J. S.; Gorbounov, V.; Ramsey, R. S.; Ramsey, J. M. *Anal Chem* **2008**, *80*, 6881-6887.
(3) Batz, N. G.; Mellors, J. S.; Alarie, J. P.; Ramsey, J. M. *Anal Chem* **2014**, *86*, 3493-3500.
(4) Redman, E. A.; Batz, N. G.; Mellors, J. S.; Ramsey, J. M. *Anal Chem* **2015**, *87*, 2264-2272.