GRADIENT ELUTION MICROCHIP ELECTROCHROMATOGRAPHY USING A MONOLITH STATIONARY PHASE

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ABSTRACT

This work describes the first integration of microchip electrochromatography using an organic monolith with gradient elution. In addition, we demonstrate the ability to localize the monolith within the separation channel without generating undesirable pressure driven flow at the open-channel/monolith interfaces. The chromatographic performance of this material was evaluated by a van Deemter analysis of three charge neutral peptides. Gradients in the mobile phase composition were readily generated on-chip and both isocratic and gradient separations of the selected peptides compared. To illustrate the separation power of this device, tryptic digests of bovine serum albumin were also analyzed.

KEYWORDS: electrochromatography, polymer monolith, gradient elution

INTRODUCTION

Organic porous polymer monoliths have been successfully employed as stationary phases in microchip electrochromatography [1, 2]. However, the application of this technique for the separation of complex samples has been previously limited by the use of isocratic mobile phase conditions. Gradient elution in electrochromatography provides many of the same advantages as in liquid chromatography, specifically reduced analysis times while increasing the overall resolution and sensitivity.

EXPERIMENTAL

Negatively charged porous polymer monoliths were localized in the separation channel by the photopolymerization of acrylate monomers following established procedures [1]. A photograph of the glass microchip device is shown in Figure 1.

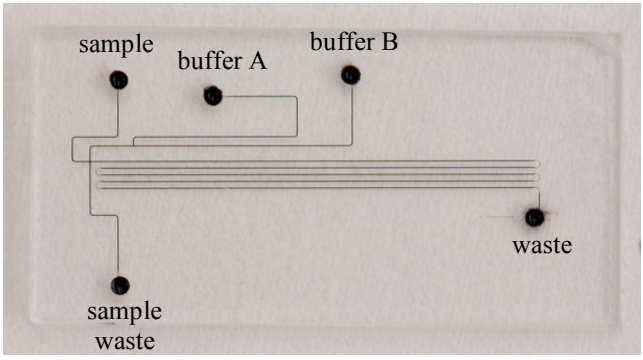

Figure 1. Photograph of the microchip device

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The separation channel is 13 x 95 microns with a length of ≈ 18 cm from the injection cross to the detection point. All experiments were preformed with a 10 mM sodium phosphate buffer at pH 7. Confinement of the stationary phase to the separation channel decreases the time required to load samples under aqueous conditions and reduces the injection bias based on the hydrophobicity of the analyte.

The difference in electroosmotic (EO) mobility between the open-channel and monolith filled regions leads to the generation of pressure driven flow at their interface. It is advantageous to eliminate this pressure gradient as it complicates the fluid control within the device and introduces additional band broadening, lowering separtive performance. To correct this mismatch in EO mobility, the amount of negatively charged monomer was systematically adjusted to equalize the EO flow in the open-channel and monolith filled regions.

RESUTS AND DISCUSSION

To test the chromatographic performance of the device, three short peptides were chosen such that their net charge after labeling with rhodamine (TRITC) was approximately zero. This was verified as the analytes coelute at the dead time when the mobile phase is comprised of over 50% acetonitrile (ACN). A van Deemter plot of these analytes separated at 30% ACN is show in Figure 2.

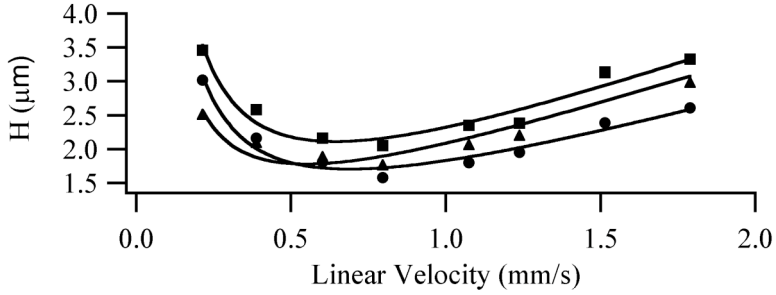

Figure 2. A van Deemter plot for three peptides. G-P-R (squares), G-F-R (triangles), Y-G-G-F-M-R (circles). 30% ACN.

The ability to create linear mobile phase gradients was incorporated onto the device by mixing of two buffers with different organic composition as previously reported for open-channel microchip electrochromatography [3, 4]. Figure 3a shows the separation of rhodamine labeled, charge neutral peptides with an organic composition of 25% ACN. As expected for a reversed-phase chromatographic separation, increasing the solvent strength resulted in a decreased interaction with the stationary phase until ultimately all four peptides coelute at 50% ACN shown in Figure 3b. Figure 3c shows the separation of the same analytes employing a linear gradient form 20% to 50% ACN. It can clearly be seen that the resolution between the first two peptides was increased while focusing the later eluting components. Figure 4 shows the separation of peptides resulting from a tryptic digest of bovine serum albumin with a calculated peak capacity of 145.

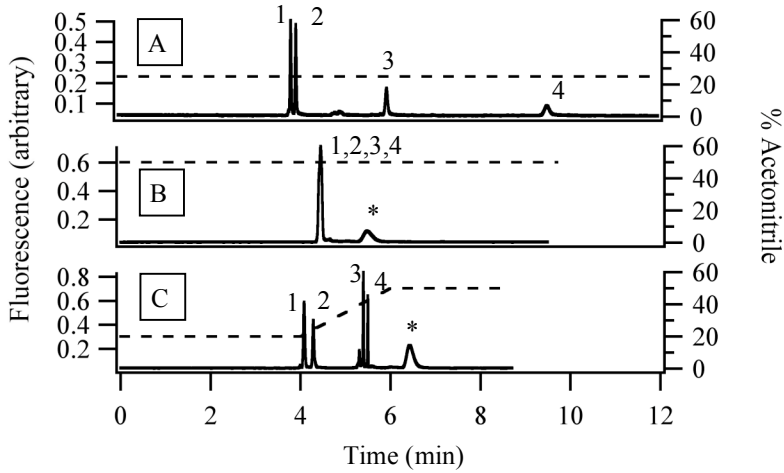

Figure 3. Isocratic and gradient separations of peptides. (1) G-P-R, (2) G-F-R, (3) Y-G-G-F-M-R, (4) Y-G-G-F-M-R-G-L, (*) free dye. E = 0.4 kV/cm

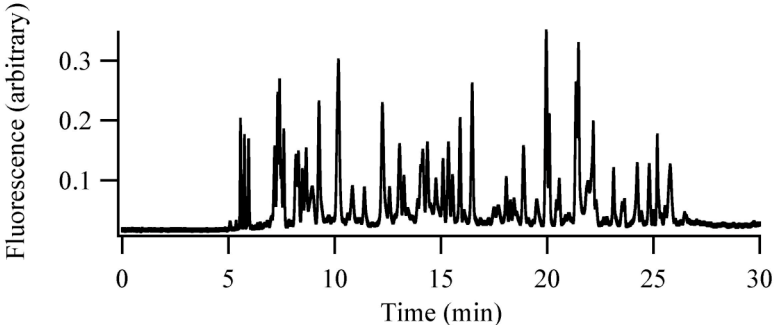

Figure 4. Separation of a BSA digest; 0-50% ACN in 33 min; E = 0.3 kV/cm

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