ANALYSIS OF PEPTIDES USING AN INTEGRATED MICROCHIP HPLC-MS/MS SYSTEM

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Abstract

An on-chip HPLC was fabricated using *in situ* polymerization of both fixed and mobile polymer monoliths. Integration of the chip with a nanospray MS emitter enables identification of peptides by the use of tandem MS. The chip is capable of analyzing of very small sample volumes (<200 pl) in short times (<3 min).

Keywords: HPLC, mass spectrometry, LC, MS/MS, flow control , microvalve

1. Introduction

Hyphenated LC-MS techniques are quickly becoming the standard tool for proteomic analyses. For large homogeneous samples, bulk processing methods and capillary injection and separation techniques are suitable. However, for analysis of small or heterogeneous samples, techniques that can manipulate picoliter samples without dilution are required or samples will be lost or corrupted; further, static nanospray-type flowrates are required to maximize SNR. Microchip-level integration of sample injection with separation and mass spectrometry allow small-volume analytes to be processed on chip and immediately injected without dilution for analysis.

2. Materials and methods

Fused silica wafers are constructed using traditional wet-etch techniques. Separation media are introduced via stearyl acrylate-based photopatterned porous polymer monoliths or via slurry packing of functionalized beads. High pressure fluidic connections are opened and closed on-chip via photopatterned mobile polymer elements that move in reponse to pressure differentials[1,2]. The functionality of a high-pressure Teflon-Teflon seal is emulated by forming compression seals between the fluorinated polyacrylate polymer and the fluoroalkyl-modified silica substrate (micrograph in Fig 1). The fluorinated monoliths are resistant to common HPLC solvents such as acetonitrile and methanol, and function at pressures up to 5000 psi. HPLC separations are performed on-chip and detected using both laser-induced fluorescence and mass spectrometry. Microchips were connected online to an ion-trap mass spectrometer via a commercial nanospray emitter at ~75 nl/min flowrate.

3. Results and discussion

Zero-dead-volume microchip injectors (e.g., Fig 1) are used to connect buffer and sample lines in microchip HPLC systems. Repeated injections (Figs 2 and 4) demonstrate reproducible injection volumes, with a retention time RSD of 3.6% and peak area RSD 3.9% over 104 injections; minimum injection volumes are 180 pl, limited by the external pressure switching [3]. The data (Fig 3) show a linear relation between the volume injected and the length of the injection time, allowing for predictable and precisely controlled injections. The injector did not show any detectable signs of leakage, and consistent injections were performed with as much as a one-week delay between injections.

We have demonstrated use of both polymer monolith (Figs 4,5) and packed-bed separations in a microchip format. Monolith-based materials allow for facile production of HPLC microdevices, while pressure-packed particle columns enable the use of a wide selection of chemical functionalities and porosity. Both types of separation material are compatible with our on-chip

injection valves, and we have demonstrated integrated HPLC devices using C18-functionalized beads and stearyl acrylate-based monoliths.
The on-chip injector and HPLC separation are integrated with tandem mass spectrometry analysis, enabling high-resolution detection and identification of peptides (Fig 6).

4. Conclusions

The ability to combine high-speed, low sample volume analysis with MS-based identification will significantly improve high throughput protemic studies. The device described in this work utilizes a reversed-phase separation. Currently we are investigating the use of other chip-based packed bed columns, such as cation exchange. Packed-bed columns not only broaden the range of separation modes, but will also enable multi-dimensional separations.

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References

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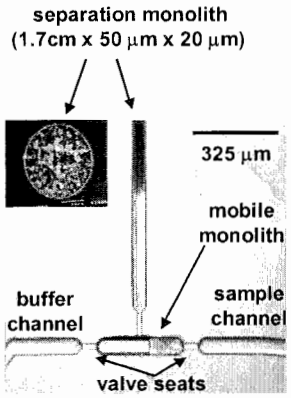

Figure 1. Micrograph of injector valve and beginning of separation media. Inset is electron micrograph of separation media polymerized inside 150 μm ID glass capillary.

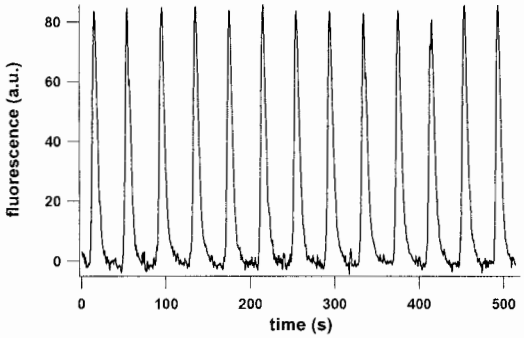

Figure 2. Rhodamine 560 injections, repeated at 0.025 Hz and measured using laser induced fluorescence. Injections consisted of 640 pl of 6.8μM Rhodamine 560 in 30% ACN, injected at 450 psi with 750 ms injection duration. Buffer used was 30% ACN with 0.2% HFBA, and separation was performed at 300 psi.

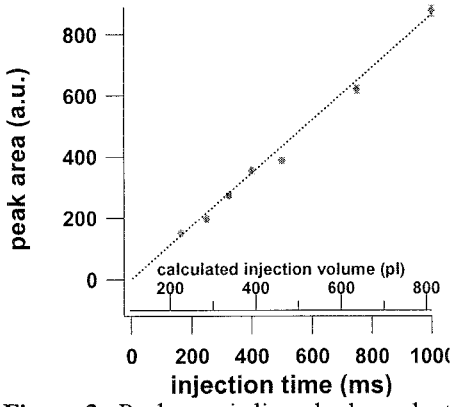

Figure 3. Peak area is linearly dependent on injection duration. Rhodamine 560 injections were performed at 450 psi, buffer flow was constant at 300 psi. Error bars are standard deviation, with at minimum 9 replicates at each injection duration.

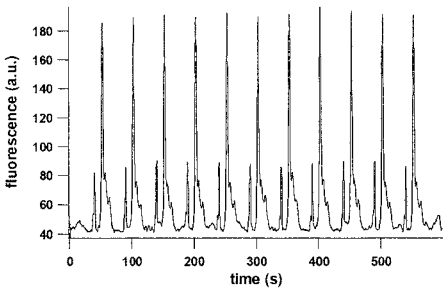

Figure 4. Repeated 470 pl injections of a peptide mixture. Isocratic separation using 30% ACN with 0.1% TFA in 5M phosphate buffer (pH 6.8) at 300 psi.

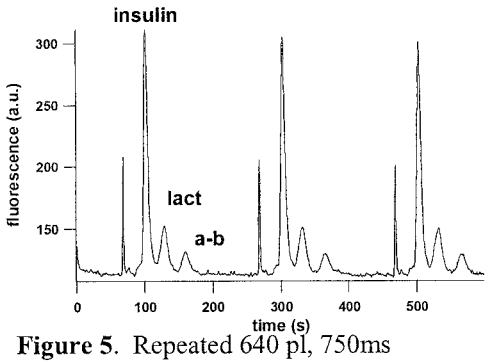

Figure 5. Repeated 640 pl, 750ms injections of a protein mixture. Isocratic separation was performed using 24% ACN + 0.16% HFBA in 5M phosphate buffer (pH 6.8) at 300psi. Peak identities: a. insulin b. anti-biotin c. lactalbumin

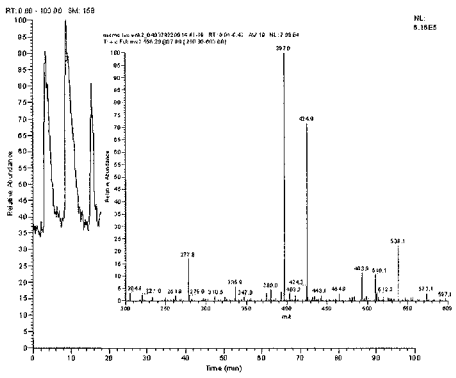

Figure 6. Repeated ~800 pl injections of 4 μM leucine enkephalin, detected using an ion-trap MS with a nanospray source. Inset spectrum shows successful identification of leucine enkephalin via tandem MS of the 556.2 m/z parent ion.