

Microchip Integrated Separation Systems for Proteomic Applications

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Keywords

Microfluidics, mass spectrometry, proteomics.

Introduction

Novel microfluidic devices that integrate all functional elements necessary to perform separations in a capillary electrochromatography (CEC) or liquid chromatography (LC) format have been developed. The research focused on optimizing designs that enable efficient handling of small sample amounts, complete integration that facilitates stand-alone operation and multiplexing, and easy interfacing to mass spectrometry (MS) detection. Mixtures of protein digests (0.1-1 microM) were utilized to evaluate the performance of these microfluidic platforms, and amounts as small as a few hundred amoles were detected with the present configurations.

Methods

Microfluidic devices were fabricated from glass using chrome/photoresist pre-coated substrates (Hoya, Shelton, CT). Sample and eluent microchannels (2-50 micrometers in diameter) were prepared by exposing the substrates through a photomask to UV irradiation, etching the exposed areas and thermally bonding a cover plate. Microfluidic manipulations were accomplished using either electrical fields or electroosmotically generated pressure, and were visualized with a Nikon Epi-fluorescence microscope. The microchip integrated electrospray interface consisted of a porous glass semipermeable gate and an ESI emitter. Mass spectrometric detection was accomplished with a Mariner time-of-flight instrument (Applied Biosystems, Framingham, MA) and an LCQ Deca XP ion trap (Thermo Finnigan, San Jose, CA).

Preliminary results

Mass spectrometry has become an indispensable tool in protein and peptide sample analysis. A complete characterization of all protein components of a cell requires the development of new techniques and instrumentation that can handle complex mixtures in a wide range of concentrations and in a timely fashion. Microfluidic devices present unique opportunities for integration, multiplexing and capability of handling small sample quantities, and represent an optimal platform for proteomic applications.

While in a capillary layout LC was proven to perform better than CEC for the separation of complex peptide mixtures, the integration of CEC on a microfluidic device is straightforward. We have designed some novel microchip platforms that incorporate all necessary components to operate both separations, CEC or LC, in a stand-alone format, i.e., micropumps, valves, injectors, separation elements, and MS interfaces (Figure 1). Pumping elements for the LC separations were capable of delivering up to

400 nL/min and generate pressures in excess of 100 psi. Valving components for pressurized LC and double-T injectors for CEC were designed to accommodate a simple injection step, or to incorporate a preconcentration step, as well. Separations in CEC were performed on polymeric monolithic stationary phases prepared by photopolymerization directly in the separation channel. The CEC injector was optimized to operate adequately under conditions of non-uniform electroosmotic flow. Separations in LC are under development. The microchip ESI interface, consisting of a porous glass disc and ESI emitter, allowed for hours of stable operation at the flow rates generated by the CEC monolithic column or the LC micropump. Multiplexed configurations incorporating up to 8 processing lines were designed and tested with a complex mixture of tryptic peptides produced by simultaneous digestion of a protein mix. Low and sub-femtomole amounts of peptides were analyzed with these microchip devices (Figure 2).

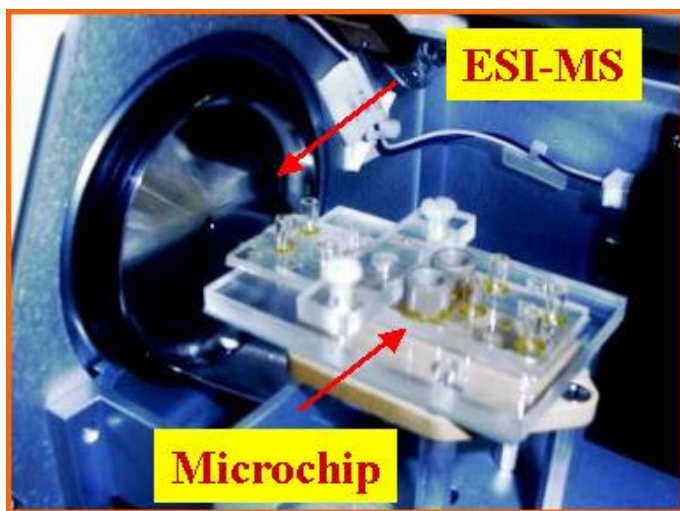


Figure 1. CEC microchip interfacing to ES/MS

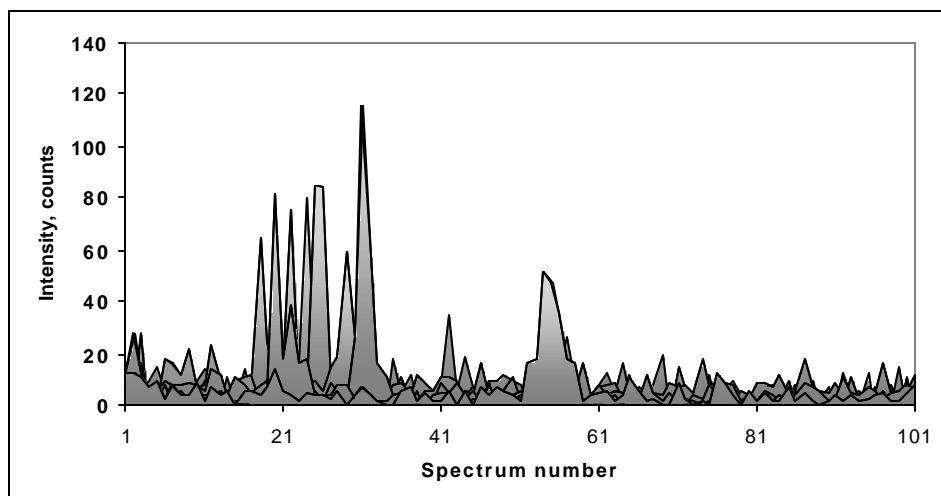


Figure 2. CEC separation of a protein digest on a microchip - TOFMS analysis platform (800 amol on-column injection).