CHIP-LC/MS: HPLC-MS USING POLYMER MICROFLUIDICS

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ABSTRACT

Microfluidic devices are shown to achieve the standards of high performance liquid chromatography (HPLC) by integrating the elements of sample enrichment, pressure driven liquid chromatography and nano-electrospray ionization tips in a common polymer structure, which we call Chip-LC/MS. These Chip-LC/MS devices have demonstrated high LC efficiency, resolution and state of the art mass spectrometry sensitivity using a low cost microfluidic device.

Keywords: liquid chromatography, electrospray, mass spectrometry, microfluidic

1. INTRODUCTION

Microfluidic technology is ideal for processing precious samples of limited volumes. Some of the most important classes of biological samples are both high in sample complexity and low in concentration. Combining the elements of sample pre-concentration, chemical separation and high sensitivity detection with chemical identification is essential for realizing a micro-total analysis system.

Previous reports have described the microfabrication and performance of single and multiple electrospray ionization (ESI) tips for infusion mass spectrometry using off-line purified samples.[1, 2] Recently, we described microfluidic devices that combining the elements of stationary-phase-based sample enrichment and pressure driven liquid chromatography columns with an integrated ESI tip to achieve Chip-based high performance liquid chromatography or Chip-LC/MS[3]. The efficiency, resolution and sensitivity of these Chip-LC/MS microfluidic devices are shown to be high by eliminating the use of bio-retentive materials, minimizing connecting volumes and by optimizing the ESI performance of polymeric spray tips.

2. EXPERIMENTAL

Direct write UV laser ablation was used to rapidly fabricate Chip-LC/MS devices from polyimide films: a biocompatible, solvent resistant and flexible polymer material. The laser used was a diode-pumped solid state (DPSS) laser, frequency tripled to 355 nm in the UV. Polyimide film with thickness from 50 to 125 microns was placed on a motorized X-Y stage and patterned by the laser using a computer driven CAD/CAM

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system. Layers were selectively metallized near the tip with evaporated gold to form an internal electrical contact to the fluid for electrospray bias. The films were cleaned before being laminated into multilayer device structures. The chemically retentive stationary phase for the sample enrichment and liquid chromatography column is packed into closed channels in the devices. LC columns were fabricated 20 to 45 mm in length. Electrospray tips with outer dimension of 50 μm and inner of 15 μm are formed by ablatively trimming away the polymer material concentrically around a finished channel structure.

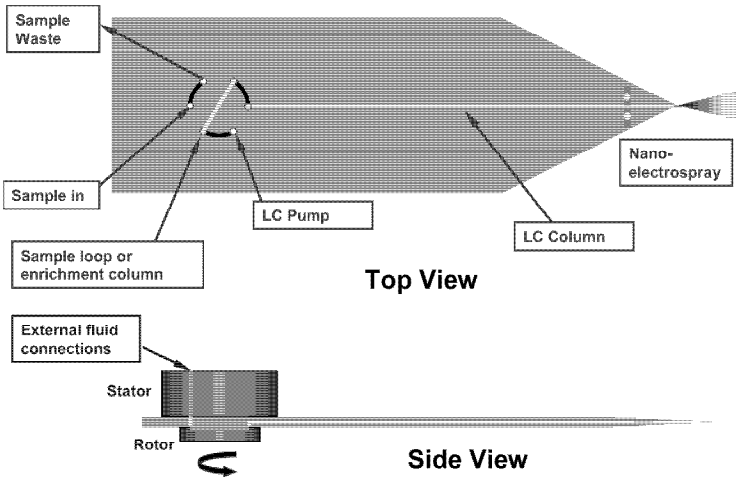

Figure 1: Schematic diagram of a laser ablated polyimide Chip-LC/MS device with the high-pressure, multiport rotary valve interface.

A novel microfluidic to rotary valve interface solves the problem of the macro to micro device fluid transition and enables, leak free, high-pressure fluid switching simultaneously between multiple ports of the microfluidic Chip-LC/MS device. The polyimide Chip-LC/MS device is placed between the stator and rotor of the valve and clamped in place. Figure 1. Fluid containing biological samples or the LC solvents enters the chip by standard capillaries connected at the valve stator. The LC solvents for reverse phase LC were delivered to the valve interface of the Chip-LC/MS device by an Agilent 1100 nanoflow pump running at 200nL/min to 300nL/min. An Agilent 1100 micro well plate auto sampler and capillary pump were used for fast, automated sample loading to the Chip-LC/MS enrichment column.

The valve and Chip-LC/MS assembly is positioned with the spray tip oriented orthogonal and a few millimetres away from the sample inlet of an Agilent 1100 ion Trap SL. With fluid flowing through the LC column, a voltage of -2.5 kV applied at the mass spectrometer inlet and the chip fluid contact grounded, a positive ion nano-electrospray is generated to allow LC-MS and tandem LC-MS/MS data collection.

3. RESULTS AND DISCUSSION

Biological samples of enzymatically-digested proteins were used to evaluate the performance of the Chip-LC/MS microfluidic devices. Liquid chromatography separations were performed using 40 mm channels packed with 3.5 μm, C18 functionalized stationary phase separation media. A LC mobile phase gradient from 2% to 85% acetonitrile/water with 0.1 % formic acid was delivered to the Chip-LC/MS interface at a flow rate of 300nL/min for ~ 1 hr. (Figure 2.) Sample injection volumes of 1to 5 μL of protein digest were preconcentrated on the 5 μm, C18 packed enrichment column at 2 - 4 μL/min prior to valve switching and sample transfer to the LC analytical column.

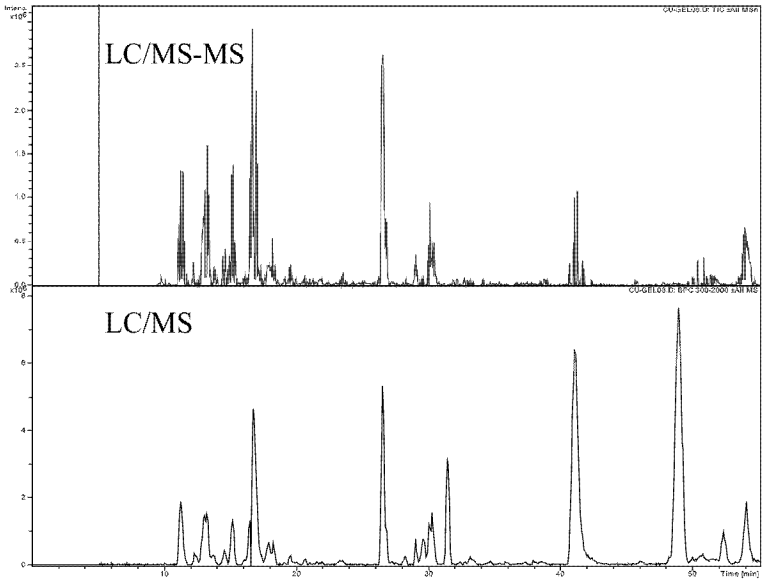

Figure 2: Chip-LC/MS and LC/MS-MS reverse-phase high performance liquid chromatogram from a tryptically digested mammalian protein. LC elution time is 55 min at 300 nl/min flow rate with 2-85% ACN/H_2O solvent gradient & 0.1% formic acid.

The liquid chromatograms recorded are sharp and well resolved, especially given the short 40 mm length of the LC column. There is no evidence of band tailing under normal operating conditions. Dispersion downstream of the LC column is effectively minimized by the seamless channel connecting the analytical column to the electrospray tip.

The mass spectrometry limit of detection for the conditions above is found to be the low fmol to high amol range. There is no evidence of background contamination from the polyimide material used to fabricate the device. The high sensitivity allows unknown protein samples to be routinely identified by searching the MS/MS spectral data against protein databases with high confidence peptide scoring. The sensitivity of the Chip-LC/MS devices has been tested using protein digest samples at the extremes of aqueous and high solvent conditions and the performance found to be at state of the art for nano-electrospray systems.

4. CONCLUSIONS

High performance liquid chromatography and sample enrichment has been integrated with nano-electrospray and combined with mass spectrometry detection to achieve Chip-LC/MS: a HPLC capable, low cost, polymer microfluidic device. We envision this as the first of a new class of microfluidic devices that will enable the next generation of nano-LC/MS with even greater sensitivity and functionality.

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