Nanoliter Solid Phase Extraction (SPE) Using Chromatographic Hollow Fibers for Sample Preparation for Mass Spectrometry

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INTRODUCTION
The sample preparation techniques may be modified to improve the signal sensitivity and selectivity for mass spectrometric analysis. The sample preparation in micro and nano liter range is of less sample in still a great challenge. Different methods for the sample preparation in Nano-HPLC or Nano-HSAMS are laborious and require few chromatographic chemicals and reagents. Here we describe the use of chromatographic hollow fibers, when the chromatographic material is immobilized on the inner wall of a capillary. These chromatographic fibers are well suited for sample preparation for mass spectrometry in such liter range. The samples are directly spotted at the atmosphere-pressure matrix-assisted laser desorption/ionization (AP-MALDI) as MALDI target plate or connected to mass spectrometer for electro spray ionization (ESI).

EXPERIMENTAL
Materials
Molecular Biology Grade Water from Bio Scientific (Walkersville, MD, USA) was used for the matrix and sample preparation solutions. Ovalbumin and fibrin were obtained from Sigma. The matrix material, α-cyano-4-hydroxycinnamic acid (4-HCCA) was purchased from Sigma (St. Louis, MO, USA). 4-HCCA was used for the matrix and dried at room temperature. Matrix composition was 1.15 mg/ml of 4-HCCA in 0.1% TFA in 5% ACN.

The digestion was carried out by using 10 µl of 1 mM µl of the purified peptide mixture with 1.0 µl of 2% ACN gold-plated target plate by mixing aliquots of 1.0 µl of the purified peptide mixture with 1.0 µl of 2% ACN gold-plated target plate.

RESULTS
In this paper, we have studied the purification and partial separation of peptides mixture after tryptic digestion and chromatographic hollow fibers. The chromatographic material is only on the inner wall of the fiber, therefore, there is hardly any or very little resistance or back pressure as compared to filled HPLC columns or nano-SPE cartridges. The fibers are made of inorganic material such as silica or polystyrene which are amenable to extraction under ambient conditions. These fibers are very resistant to any specific application and can be used as disposable SPE and this avoid cross contamination.

In Figure 2, we demonstrate the partial separation (A) after tryptic digestion of ovalbumin (B) and cellulase (A). The samples were eluted with step gradient and directly spotted on MALDI target plate.

Mass Spectrometry
Experiments were carried out in a Thermo Finnigan LTQ (San Jose, CA, USA) LCQ Deca XP mass spectrometer integrated with an AP MALDI ion source equipped with a 10 Hz nitrogen laser, and an integrated PerSeptive Biosystems) was obtained from Applied Biosystems (Foster City, CA, USA). Different affinity materials mini columns (TopTip) were obtained and modified (NanTip) in the micropipette tips by using Glygen (Columbia, MD, USA) Technology.

In Figure 1, we demonstrate the sample preparation (A) after tryptic digestion of ovalbumin and cellulase. The samples were eluted with step gradient and directly spotted on MALDI target plate.

LC/Fiber
Capillaries of different i.d. and length are made with the chromatographic particles directly attached to the inner orifice of the end of the capillary. Chromatographic particles of five to ten micron diameter are used. The chromatographic materials such as C-18, polymer based reverse phase and carbon particles are attached to the inner wall of the capillary. The samples of matrix are loaded through a fused silica capillary and are analyzed by the Thermo Electron Corp., a subsidiary of Thermo Fisher Scientific Inc, 1355 East Mathis Avenue, Waltham, MA 02451-5234, USA.

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DISCUSSION
The Chromatographic Hollow Fiber (LC-Fiber) has following advantages:

* No matrix contamination due to solid support
* Reproducible for nanometers
* Cost effective for several micro and nano-liters
* Minimal sample losses
* Still the loading of these fibers is a challenge due to small diameters and pressure

CONCLUSION
The LC-Fiber can be used as:

*NPSGC for volatile sample prep.
*Nano and micro SPE for sample cleanup before injecting the sample in a source of nano-HPLC.

*Capillary Electro-Chromatography.

Sample enrichment by using of different chromatographic materials such as C-18, immobilized polymer based EIC and immobilized enzyme. Process is a LCF. Baffles of different i.d. and length are made with the chromatographic particles directly attached to the inner wall of the capillary. Chromatographic materials such as C-18, polymer based reverse phase and carbon particles are attached to the inner wall of the capillary. The samples of matrix are loaded through a fused silica capillary and are analyzed by the Thermo Electron Corp., a subsidiary of Thermo Fisher Scientific Inc, 1355 East Mathis Avenue, Waltham, MA 02451-5234, USA.

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FIGURE 2

**Figure 1. AP-MALDI SPECTRA OF FETUIN AND OVALBUMIN**

**Figure 2.** LC/Fiber SPECTRA OF FETUIN AND OVALBUMIN (tryptic digest without purification and concentration)