

Chip-based electrospray interface for nanoLC-MS

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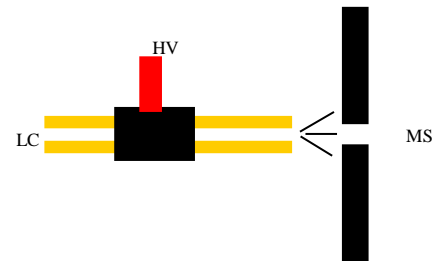


Overview

Electrospray is a popular atmospheric pressure ionization method for introducing HPLC eluent streams into a mass spectrometer for detection, analysis and identification. This API approach is utilized in a wide range of analytical fields and applications. When operating at very low flow rates typical of those employed in proteomics studies (100's of nL/min), ESI interfaces provide exceptional sensitivity. Accompanying this clear advantage is a level of difficulty in implementation and usage that requires training and patience and often leads to variability. The primary difficulties stem due to the requirement that dead volumes (typically located in connections) be minimized. We present here an approach to making and using nano-ESI sprayers that employ a high-performance chip-based microconnector and sprayer that give very good performance with greatly improved ease-of-use.

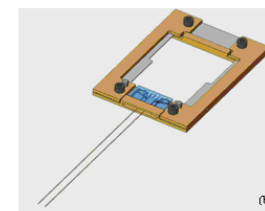
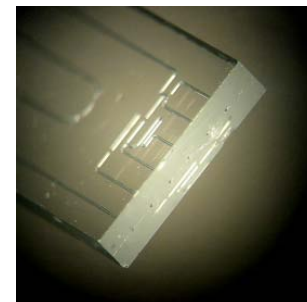
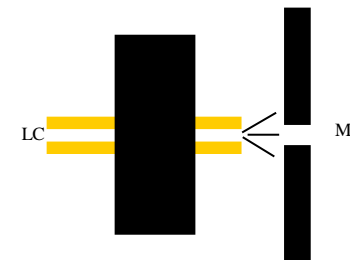
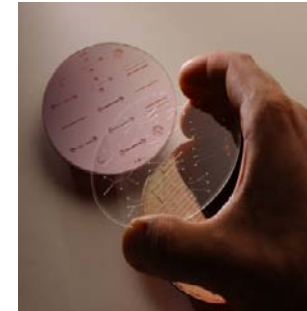
Introduction

- NanoESI is a popular nanoLC-to-MS interface.
- The general approach uses a pulled capillary tip; high voltage is most frequently applied at a liquid junction or by using a tip coated with electrical conducting material.
- There are several variants on this approach.
- While offering high performance, traditional approaches to using pulled tips in nanoESI interfaces present challenges in ease-of-use and robustness.
- Microfluidic devices have inherent appeal as capillary replacement for low flow rate applications because microchannels are defined lithographically and are highly reproducible.
- Microfluidic devices require a chip-to-world interface which does not sacrifice the performance gains from the microdevices themselves.
- In this poster, we will discuss the use of newly-developed, microfabrication-enabled devices for nanoESI to enhance the ease-of-use and reproducibility of proteomic experiments.



Methods / Devices

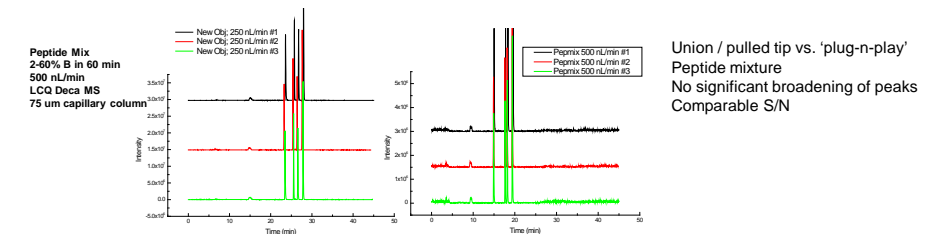
- Fused silica substrates were used to make a family of microfluidic chips used in this study.
- Microfluidic networks were formed in the chip using standard and proprietary microfabrication methods. Channels are formed in a variety of geometries, including cylindrical channels.
- Chips are chemically inert, mechanically suitable for high pressure. Similar approaches are used to make the microfluidic columns used in the cHiPLC-nanoflex module.
- For some chip designs, pulled silica sprayer tips (New Objectives), capillaries (Polymicro) and electrical leads are inserted into the cylindrical channels to form low dead volume liquid junctions. These are referred to as the "simple chip interface"
- In all cases, microfluidic channels are routed to edge of chips for implementation of the high-performance eksport™ microconnector design developed by Eksigent.
- The eksport™ connector allows formation of several microfluidic junctions with sub-nL dead volumes in a very easy-to-use format. The approach, akin to using a USB memory stick, allows a change of microfluidic chips in seconds with high performance at high pressures and low flow rates.



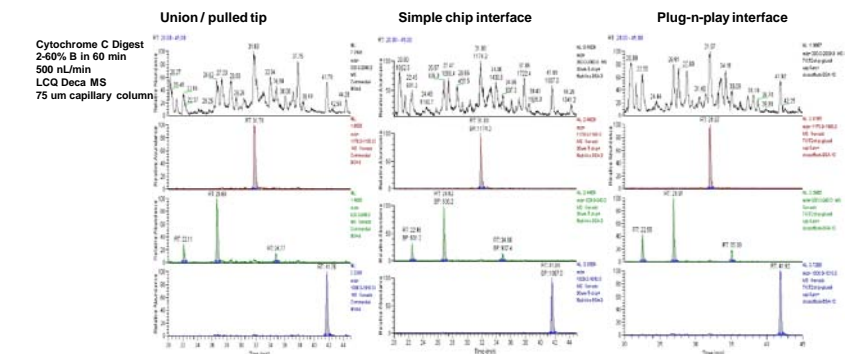
- The connector design used for the 'plug-n-play' interface is based upon the same architecture as the eksport™ microconnector system and allows through-connections of microfluidic channels as well as connections of electrical leads.
- Sprayer chips are mounted in easy-to-use carriers, similar to those used for the cHiPLC nanoflex columns and trap columns. Sprayers are connected to system plumbing voltages by sliding the carrier into place and securing it as seen in the figure.
- Sprayer chips can be replaced in seconds with confidence that very low dead-volume connections are made very repeatably and that high voltage connections are in place with no risk typically associated with exposed high voltage leads.
- Sprayer tip replacement is made independent of columns, traps, etc. to allow more effective replacement scheduling and provide consistency between experiments.

Results

- For preliminary testing, the chip-based sprayers were mounted onto the manipulator stages of a New Objectives platform for alignment. Performance was tested by carrying out nanoLC separations of peptide and protein digest mixtures; standard water / acetonitrile gradients were delivered by eksigent nanoLC systems into an LCQ Deca MS for detection. Direct comparison between 1) tips in traditional unions with liquid junctions, 2) 'simple chip interfaces' and 3) 'plug-n-play interfaces' were made.



For the comparison experiments, the chromatograms were collected sequentially on the same system (LC, MS column, sample, tip size, etc.). Particular care was given to the union-based interface to establish the best possible baseline performance for the comparison. The interface was changed between experiments and the position of the source was adjusted without making other system adjustments. We observed similar chromatographic performance with comparable S/N and no significant broadening of peaks by the chip-based sprayers relative to the union / pulled-tip interface. While we expect tip-clogging to remain an issue, the approach should reduce clogging associated with capillary fragments since the connection mechanism does not require cleaving or compressing any of the capillary-based components in the system. While the chip-based tips used in these experiments did not clog during usage, we do not want to make any conclusions on this aspect of the device based upon the limited data.



Conclusion

A plug-n-play nanoESI interface has been developed and demonstrated. The microfabrication-enabled ESI interface platform leverages many of the advantages that microfluidics has to offer. The device demonstrates high performance, robustness and ease-of-use. The interface allows changing sprayer tips in seconds. Moreover, the design requires little expertise to make a repeatable, high integrity connection. This aspect will be critical as proteomics moves from discovery through biomarker verification and validation. While this platform addresses many of the challenges associated with nanoESI, others issues remain such as tip clogging. Those are the subject of on-going research and development.