

Towards total ion utilization: Electrospray ionization in a sub-ambient pressure environment for high sensitivity mass spectrometry

Jason S. Page, Ioan Marginean, Ryan T. Kelly, Keqi Tang, and Richard D. Smith

Pacific Northwest National Laboratory, Richland, WA 99352

We aim to develop MS technologies which will enable proteomic analyses of limited amounts of sample (i.e. small tissue sections and individual cells); however, the large sample losses associated with the inefficient transmission of typical MS inlets [1,2] reduces the MS sensitivity limiting the effectiveness of detecting the cellular content of these samples. We demonstrate that these large ion losses from the inlet can be eliminated by operating the electrospray inside the first vacuum region of the mass spectrometer, which is maintained at pressures that facilitate efficient ion transmissions. The two main components of the low pressure ESI source, which we termed subambient pressure ionization with nanoelectrospray (SPIN), are 1) an ESI device which enables an electrospray at the entrance of an rf ion guide and 2) a tapered rf ion guide, called an ion funnel, with a large entrance i.d. which captures the entire electrospray plume and facilitates desolvation [3]. We demonstrate the performance the SPIN source compared to typical capillary inlets using both infusion experiments and LC/MS analyses of a protein digest solution.

The low pressure ESI device houses the counter electrode for electrosprays. The electrospray emitter protrudes ~ 2 mm through a small hole in the counter electrode, and a low flow sheath gas or voltage gradient ensures that the electrospray plume is effectively captured by the ion funnel, Fig. 1. Ion desolvation in the SPIN source was enhanced by increasing the pressure at which electrospray ions are created, all the while maintaining high transmission efficiencies. This improvement was accomplished by increasing the rf frequency and voltage of the ion funnel. These increases were enabled by greatly reducing the capacitance of the device, which required a total re-engineering of the construction process. In addition, we incorporated a low-pressure electrospray desolvation chamber that efficiently transmits the ions into the ion funnel, thereby increasing the pressure around the electrospray improving ionization efficiency and instrument sensitivity.

Characterization of the electrospray process at 760 to 25 Torr was carried out in a chamber, using a charge collector and in-house developed hardware and software to detect the electrospray current and analyze its characteristics. We discovered a possible new scaling law which related the electrospray current to the applied voltage as a function of the chamber pressure. In addition we verified that we can operate electrosprays from LC compatible solvents in a reproducible and controlled fashion down to 25 Torr. The SPIN source was installed on a single quadrupole mass spectrometer and evaluated by analyzing a protein digest solution using gradient elution HPLC MS. The SPIN source provided an ~ 8-fold average increase in sensitivity for detecting tryptic peptides compared to the standard ESI source with higher charge state peptides showing the largest increase in signal. Although MS

sensitivity improved, it was lower than predicted as the standard inlet used for the comparison had a ~1% transmission efficiency. To further improve performance, the pressure of the ESI source was increased beyond 30 Torr via an ultra-low capacitance ion funnel. This new interface design provided three main benefits; namely, 1) the electrical capacitance of the device was greatly lowered, which allowed higher rf frequencies and voltages to be used that increased the effective potential of the ion funnel and decreased space-charge limitations, 2) the higher pressures increased collisional energy transfers from the background gas, which improved desolvation/ionization efficiency, and 3) the breakdown voltage was increased allowing the use of electrospray emitter arrays. In addition, a desolvation chamber in the front of the ion funnel was designed and tested. The chamber allowed the electrospray to occur at higher pressures relative to the ion funnel and provided efficient ion transfer from the ESI source, which further improved ionization efficiency and instrument sensitivity.

- 1) Page, J. S. et al. *J. Am. Soc. Mass Spectrom.* **2007**, *18*, 1582.
- 2) Marginean, I. et al. *Anal. Chem.* **2008**, *80*, 6573.
- 3) Page, J. S. et al. *Anal. Chem.* **2008**, *80*, 1800.

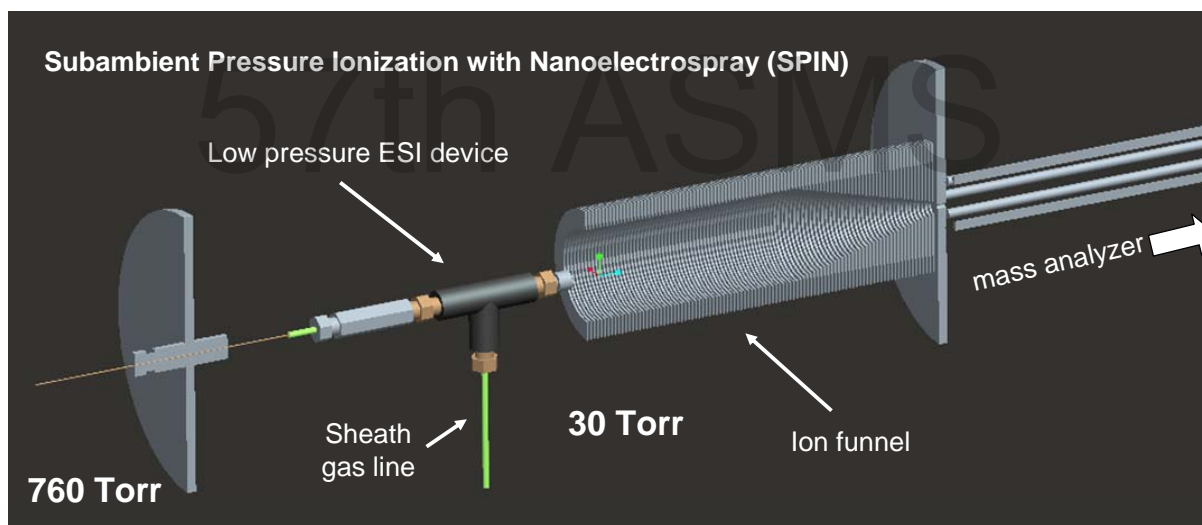


Figure 1. Drawing of the low pressure ESI source and MS interface which enables HPLC ESI MS without an atmospheric inlet to the instrument and the associated sample losses.