A miniaturized nanospray probe source for high sensitivity nanoLC-MS

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Introduction

NanoLC-MS for proteomics has been expanding in mass spectrometry core facilities. A recent popular trend in nanospray source design for nanoLC-MS is to have a spray emitter in a fixed position with respect to the mass spectrometer inlet (e.g., Michrom CaptiveSpray™ source and the Bruker Apollo NSI source). This design has the advantage of almost fool-proof operation, but imposes considerable restrictions, e.g. > 500 nL/min flowrates, factory-supplied flat-cut nanospray emitters and in the case of the Bruker NSI source, reduced sensitivity limit, which may be unacceptable to users who demand the best possible results for their samples.

We report a new micro/nanospray miniaturized "probe" source that eliminates these restrictions while maintaining user-friendliness in a core facility. The probe source can be directly inserted into the ESI housing of the Bruker Amazon ion trap mass spectrometer. The probe source allows 3-D fine adjustment of the position of the nanospray emitter which is essential for maximizing sensitivity. Sheath gas can be used to help vaporization if desired. A variety of nanospray emitters including those integrated onto a capillary nanoLC column as well as column heaters are accommodated. Moreover, the spray can be viewed with a camera, and the closed environment of the source is maintained.

This report presents the following:

1) The design of this flexible miniaturized nanospray probe source for a column with an integrated nanospray emitter
2) >20x improvement in sensitivity: 100 amol of BSA digest vs. 5 fmol of the Bruker NSI source.
3) Much higher coverage achieved by the new probe source : ~5x the number of peptides identified as the NSI source for the same amount of sample (500 ng of cell lysate).
4) Superior peak shapes and coverage by heating the sheath gas and the column system together with a novel "butterfly" heater.
5) The benefit of using sheath gas for nanospray <500 nL/min flowrates probably because

Probe source design

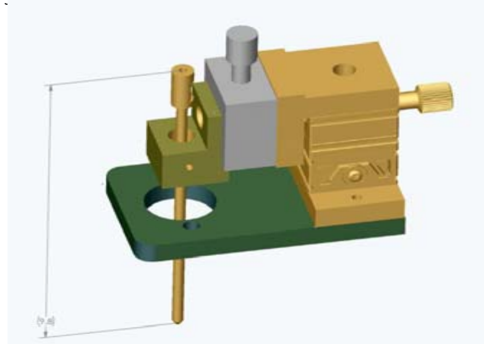


Figure 1: The probe source design. The nanospray probe, the part with the largest dimension in the source, is about 10 cm in length.

- The probe source is a slim tube (~3 mm) in diameter with a sheath gas nozzle at one end and at the other end, a sheath gas intake coupler which also holds the column/nanospray emitter in place.
- A miniature 3-D positioner is used to optimize the probe source position in front of the mass spectrometer inlet
- The whole probe source is mounted into one of the windows of the Bruker Apollo source
- A fused silica nanospray emitter or column is inserted into the probe through the sheath gas nozzle and secured at the sheath gas intake coupler.
- A camera and an LED light are installed at two of the windows of the Apollo source
- The OEM NSI or ESI source may be kept in the Apollo source if desired.

Experimental

Figure 2: the nanospray probe source is installed at one of the windows of the Apollo source. A camera is also mounted to facilitate optimizing the nanospray emitter position with respect to the MS inlet, and also the quality of the spray.

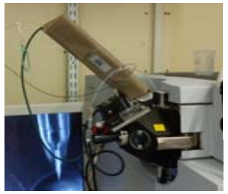


Figure 3: For the heated column and sheath gas experiments, the trap column, filter, analytical column and sheath gas tubing were enclosed in the "Butterfly" heater mounted close to the source.

Figure 4: the "Butterfly" heater was open to reveal an example of an LC system housed in the heater. For the heated sheath gas +column experiments reported here, the LC system comprised a 5 cm SCX column (but SCX was not performed), a 5cm RP trap column (Jupiter Proteo), filter, T for the vented trap and analytical column (eFrit™ , PicoFrit™ or flame-pulled capillaries packed with Halo RPA) which was partially in the probe source. The internal temperature of the Apollo source was at 48 °C and the column heater was maintained at 50 or 55 °C. The sheath gas tubing is made of viton and is not shown.

LC/MS

Bruker Amazon MS with the Apollo source and Easy NanoLC

Samples:

A. Sensitivity measurements: Bovine Serum Albumin (BSA) digest standard from 100 amol to 10 fmol;

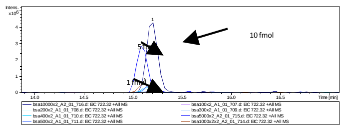
B. Peptide coverage measurements: E. coli lysate, 200 ng and 500 ng

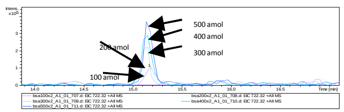
C. Heated column and sheath gas measurements: 20 fmol BSA digest standard

The LC/MS conditions for each set of experiments will be described with each set of results.

Results

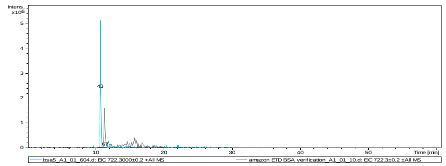
A. Sensitivity measurements for the probe source





- Trap column and an analytical column with flame-pulled tip packed primarily with Jupiter Proteo resin
- MeOH+0.1% formic acid as the organic phase
- Both trap and analytical columns were heated to 55 °C
- The experiments were started at 100 amol and increased stepwise to 10 fmol to avoid effects of carry-over.
- The probe source demonstrated a sensitivity limit of 100 amol (with 11 peptides identified) vs the 5 fmol of the Bruker NSI source.

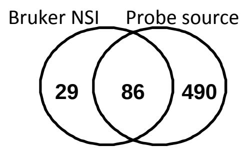
Results



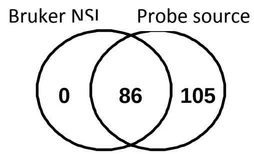
B. Peptide Coverage Measurements

Number of proteins identified from

i) **500** ng E. coli lysate

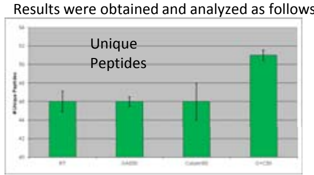


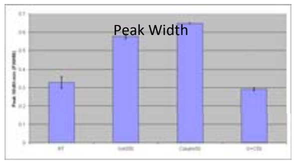
ii). **200** ng E. coli lysate

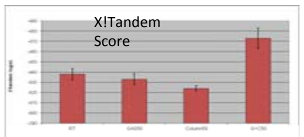


C. Heated column and sheath gas measurements

Mobile phase: A -10mM ammonium formate pH=2.1, B-MeOH, 2-hour gradient
Spray conditions: 1.8 KV-2.2 KV, 300 nL/min, 6 psi sheath gas
Temperature conditions: i) Room temperature for all (RT) , ii) 50 °C sheath gas only, (Gas50); iii) 50 °C column only (Column50); iv) 50 °C column + sheath gas (G+C50).
"Column" here means the entire column system as described in Figure 4.
Results were obtained and analyzed as follows:







Error bars represent 1 standard deviation from the mean value of data from triplicate runs. Good correlation between peak width and both X!Tandem score and #unique peptides was demonstrated. The substantial improvement due to heating the column system and sheath gas is expected to further improve with complex samples.

Summary and Conclusions

1) The Probe source with sheath gas and tapered emitter mounted on a 3-D positioner produced excellent MS sensitivity (100 amol with 11 identified peptides) even with the use of a trap column and an autosampler.
2) This sensitivity is >20x that of the OEM Bruker NSI source.
3) The peptide coverage by the Probe source was ~5x that of the Bruker OEM source with the same 500 ng of E. coli lysate sample loading.
4) Heating the whole column system and the sheath gas improved both the peak width and also the number of identified peptides substantially when compared to room temperature analysis, but heating the column system alone or the sheath gas alone produced poorer chromatography and coverage than those at room temperature.
5) All the results shown here were obtained with sheath gas even at flowrates of <500 nL/min. Performing nanospray-MS with sheath gas produced more stable spray, improved chromatography and more unique peptides identified. These improvements were likely due to better desolvation as indicated by the disappearance of droplets on the spray shield of the MS inlet as observed with the camera when sheath gas was used.