Broad Mass Range Ion Transmission and Improved Peptide Detection Using QTOF MS Equipped With New Atmospheric Pressure Interface

ASMS 2010

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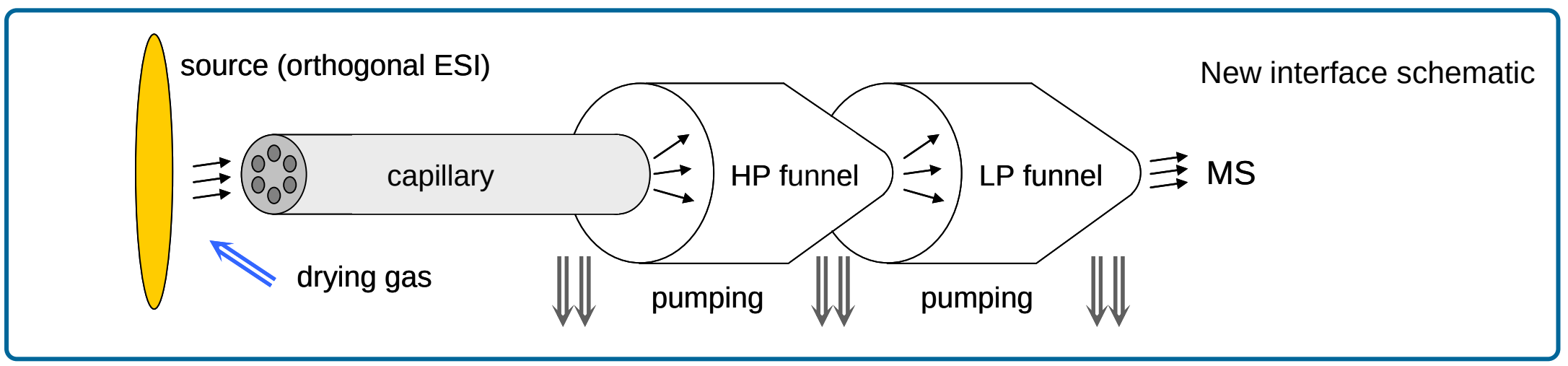
Introduction

One of the key advantages of Time-of-Flight (TOF) mass spectrometry consists in the extremely wide range of ion masses that can be analyzed simultaneously. A variety of applications, including high throughput proteomics and tissue imaging benefit from this unique feature of TOF technology. When atmospheric ionization techniques are used, ions are required to travel through a wide range of pressures, and are subjected to the action from a variety of optical elements. As a result, mass discrimination, rather than wide range transmission, is a norm in the world of mass spectrometry.
Handling ions at high pressure is particularly challenging due to the big variations in mass and ion mobility. We report the results of broad mass range transmission optimization of the new atmospheric pressure interface based on a novel dual electrodynamic ion funnel design in combination with multichannel capillary.

Experimental setup

Quadrupole TOF mass spectrometer was equipped with a dual differentially pumped ion funnel which replaced the standard skimmer interface. This allowed for increase of the gas sampling rate into the QTOF MS by up to 15^x using a multibore capillary.

ESI source with and without Agilent Jet Stream technology operating in infusion mode was used to evaluate broad mass range transmission. Nano-flow LC coupled to Agilent Chip-Cube was used for injecting E-Coli digest samples using typical proteomics analysis conditions (75 µ ID x 150 mm SB300 C18 column, 300 nL/min flow rate, 3-90% ACN gradient over 85 minutes).



Theoretical considerations

Key advantage of increased gas sampling: *Higher amount of analyte molecules delivered into the mass analyzer*

Potential drawbacks of high gas sampling

1) Increased amount of drying gas is required for providing efficient desolvation
2) Increased amount of heat in the system
3) Higher pumping requirements
4) Higher pressure in ion optics leading to ion losses
5) Unfavorable gas dynamics at the inlet

Goal: Optimize the gas flow parameters throughout the pressure interface for the highest gains in sensitivity, including:

1) Length, size and number of capillaries
2) Temperature and amount of the drying gas flow
3) Conductance of ion funnels
4) Inlet geometry

Transmission of dual funnel interface

The following factors limit the low and high mass transmission of ion funnels (see schematic on the right) [1]:

Low mass

1) Potential confinement instability in the narrow exit regions
3) Axial trapping in the exit regions

High mass

2) Insufficient effective potential at high pressure (P_1)
4) Losses due to gas flow into the pumping port (Θ_4)

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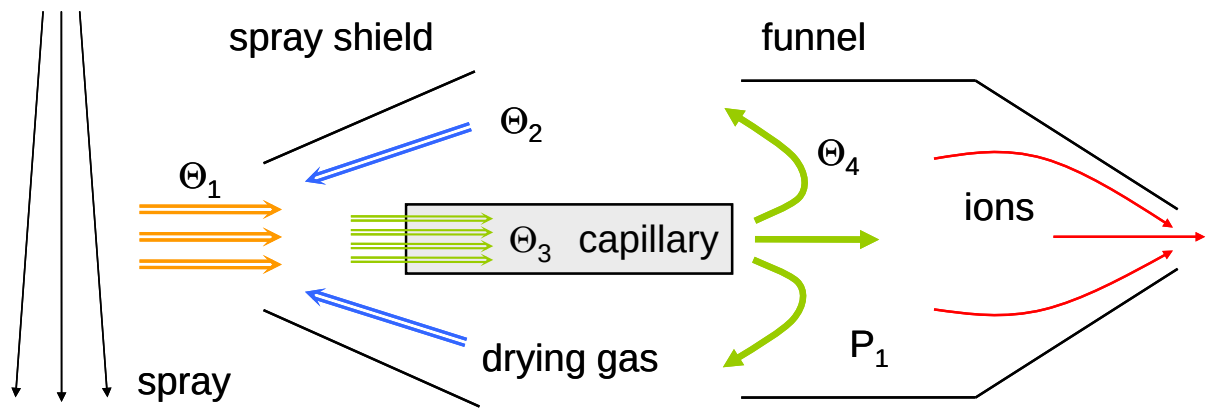
Theoretical considerations



Parameters defining gas sampling

Θ_1 – inlet gas flow
Θ_2 – heated drying gas flow
Θ_3 – gas flow through capillary
Θ_4 – gas flow into the pump
P_1 - pressure in the first funnel

Sampling and desolvation efficiency. Transmission through the capillary

High conductance of the capillary leads to high flow Θ_3 into the mass spectrometer. As a result, higher flow Θ_2 of drying gas is required for efficient desolvation of ions. If Θ_2 is too high then the counterflow $\Theta_1 = \Theta_3 - \Theta_2$ can become too large and result in poor collection of low mobility ions in the source region. The geometry of the spray shield must provide adequate compromise between the heat delivery and counterflow. Higher temperature of dying gas improves the desolvation, but could lead to higher diffusion-related losses in the capillary (particularly for high mobility ions).

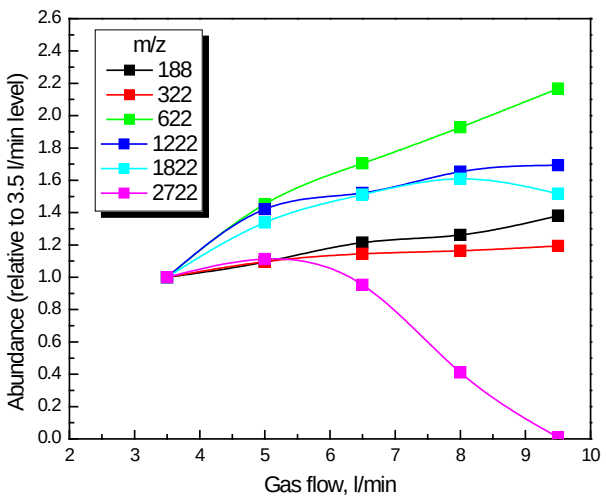
Using a source with high temperature assisted ionization (such as AJS) should decouple the desolvation region from the pressure interface. Reduced drying gas requirements the should achieve additional gains from the new interface.

Experimental results

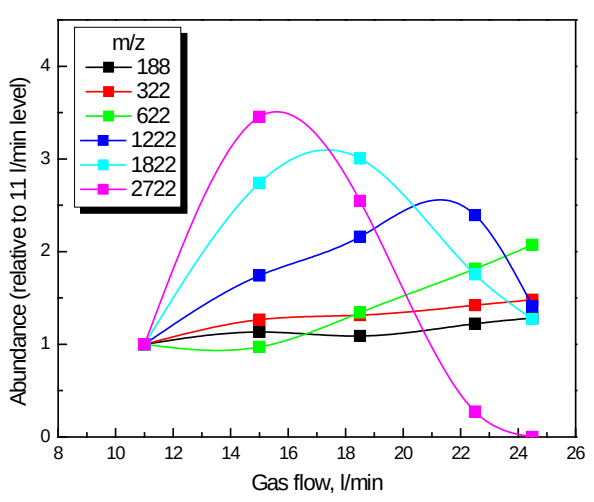
Based on considerations discussed in the previous section the number of capillary channels (6), the channel length (90 mm) and radius (0.3 mm) were selected to provide optimum conductance while maintaining laminar flow of gas.

The following plots illustrate the effects of drying gas flow on sensitivity when using the the standard and multibore inlets. The optimum is at about 3^x of the capillary conductance for the std. design and about 1.5^x for the 6 channel design. If a source with thermal gradient focusing technology (TGFT) is used, lower gas flow can be used while still providing adequate desolvation.

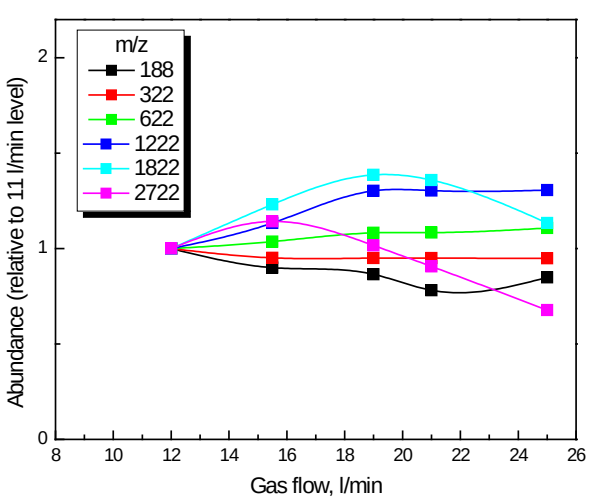
Standard interface



Multi-bore capillary interface



Multi-bore capillary with TGFT



Optimization of drying gas temperature (not shown) also reveals a compromise between the low mass and high mass sensitivity. This is mostly due to the balance between the desolvation efficiency and the diffusion losses inside the capillary.

As a result of the optimization of all the interface parameters, the broadest mass range gains (compared to the skimmer interface) were achieved without the need of mass-specific tuning of RF voltages on both funnels (see plots on the right panel).

Ion transmission improvements of up to 10 times were obtained using the new atmospheric pressure interface with ESI source. Highest gains were observed in the low-to-middle mass range, which can be attributed mostly to the improved sampling and lower diffusion losses in the capillary. However, significant improvements are confirmed even for the highest m/z ions.

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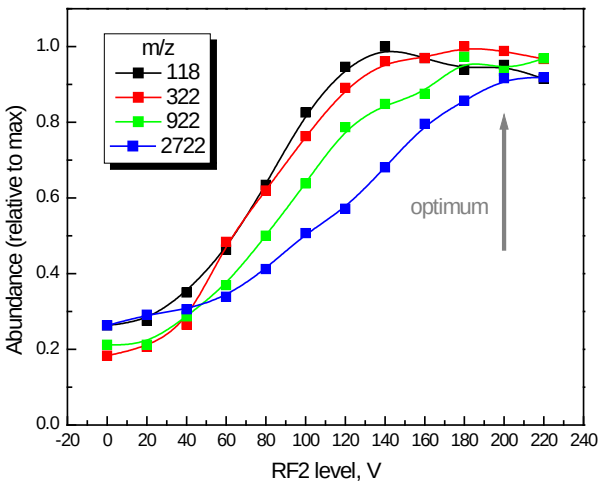
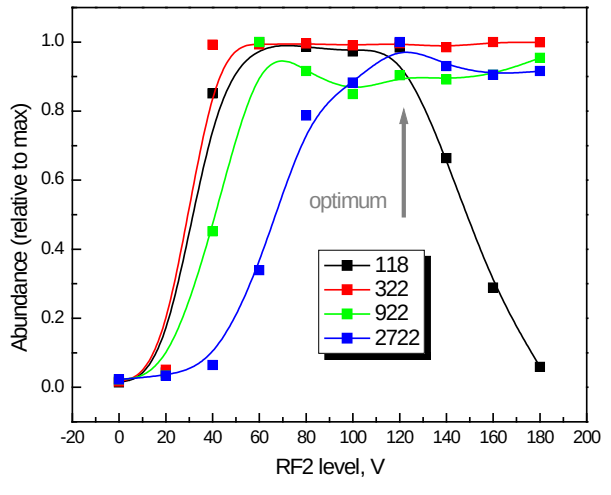
Experimental results

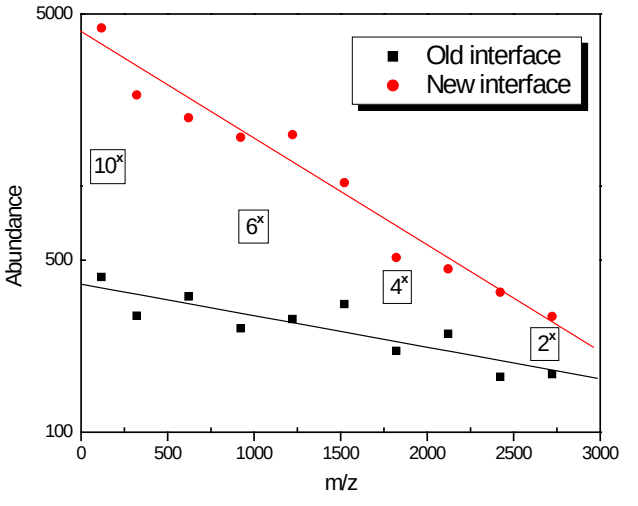
HP Funnel RF voltage optimization



LP Funnel RF voltage optimization



Optimized signal gains

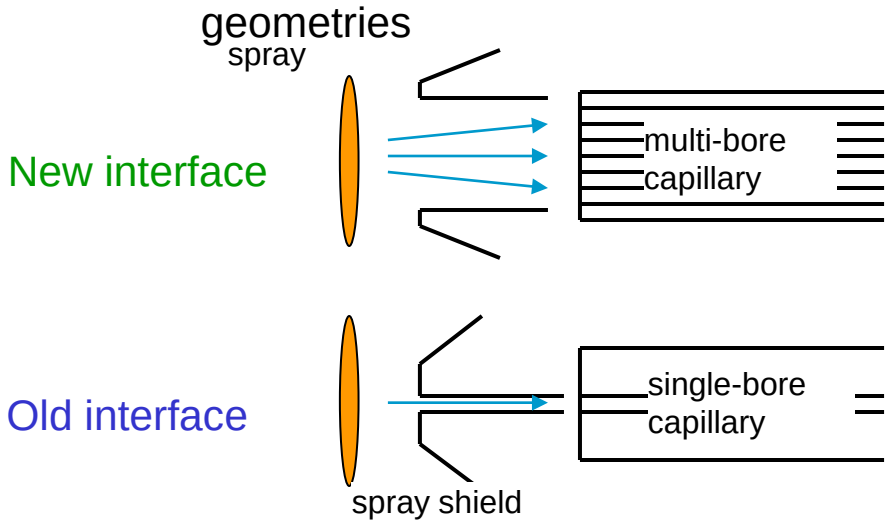


Application of the new interface in the nano-electrospray configuration

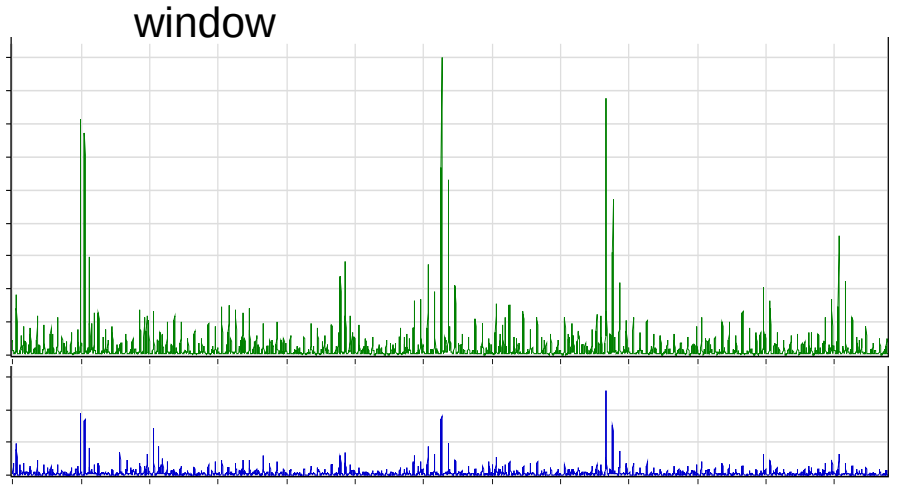
High throughput data-dependent "shotgun" proteomics analysis relies on efficient separation and detection of peptides as well as high in-scan dynamic range in MS/MS mode in order to achieve high peptide and protein identification scores. Any significant increase in ion sampling and transmission of an LC TOF MS/MS system is expected to lead a larger number of proteins identified.

Initial experiments with the HPLC coupled to a nano-electrospray source demonstrated very high ion sampling efficiency. Preliminary data showed increase in peptide abundances of five-fold or more compared to an instrument without the new interface. This should permit identifications at lower peptide levels on-column, or permit more precursors to be examined per unit time. Shown below are the schematics of nano-spray sampling geometries, as well as the plots illustrating relative abundances of a series of E-Coli digest peptides obtained by using the old and the new interface.

Nano-electrospray sampling geometries



Peptide mass spectra in a small mass window



Conclusions and References

Ion transmission improvements of 5 times or more were obtained using the new atmospheric pressure interface. Although the highest gains were observed in the low-to-middle mass range, the sensitivity improvements were noted across the entire range examined (118 to 2721 m/z) without additional adjustments to the interface operation conditions. The practical advantage of these gains was demonstrated in the experiment environment typical for high throughput proteomics analysis. Future goals include both further optimization of the atmospheric interface with the help gas dynamics modeling, as well as the demonstration of practical benefits from this technology (such as increased efficiency of protein identification).

[1] A. Tolmachev, T. Kim, H. Udseth, R. Smith, T. Bailey, J. Futrell, Int. J. Mass Spectr., 203, 31-47 (2000).