Sensitivity Increase Resulting from Design Improvements for a High Pressure MALDI Source on an FTMS

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Recently, we described a prototype high pressure MALDI (HP-MALDI) source, built in-house, coupled to an FTMS.[1] The reported sensitivity for this source was 10 fmol for a vasopressin sample. Desorption of ions occurs under high-pressure conditions (~ 3 mbar) using a nitrogen gas pulse in the MALDI plume to collisionally cool the ions and this feature was shown to greatly decrease metastable fragmentation.[1,2] The current source design incorporates an X-Y stage that accommodates a 384-well Bruker® sample plate and a dual UV/IR laser system (Bioptic Lasersysteme, Berlin Germany) to conduct experiments at both 355 nm and 2940 nm.

The new source design demonstrated some of the cooling capabilities of the HP-MALDI FTMS prototype, however cooling capacity was somewhat reduced. This was likely due to an aerodynamic problem resulting from the gas delivery into the MALDI plume. A new high pressure channel plate was designed and implemented into the HP-MALDI source, resulting in enhanced collisional cooling of the MALDI generated ions and in increased sensitivity.

In multishot accumulation mode,[3-5] 15 laser shots are acquired and the desorbed ions are accumulated in the hexapole before being transferred to the cell for analysis. This accumulation method increases the sensitivity substantially and optimization of this method is ongoing. One drawback of this method is that it not only accumulates analyte ions, but it accumulates matrix ions as well. This becomes problematic as it results in space charging in the cell. In order to overcome this problem, methods must be developed in order to maximize the accumulation of analyte while minimizing the accumulation of matrix.

The sensitivity of the HP-MALDI source was tested using standard peptides, such as angiotensin and the phosphopeptide, RRREEE(pS)EEEAA, and tryptic digest peptides from horseradish peroxidase and bovine serum albumin. The effects of different matrices (HCCA, DHB, SA) were evaluated, with HCCA being optimal for peptide analysis. The use of nonconductive, hydrophobic surfaces, such as Parafilm™ and Teflon™ were found to enhance sensitivity ~ 2-3 fold as compared with samples analyzed from a stainless steel plate. This is likely due to concentration of the sample by spotting on a hydrophobic surface. The smaller spot size meant that the entire sample spot was irradiated with the laser. In addition, the use of disposable sample surfaces insured that there was no carryover from previous analyses. Figure 1 shows the spectra of 30 attomoles of angiotensin spotted on Parafilm™ and 30 attomoles of RRREEETEEE spotted on Teflon™.

The current design has demonstrated sensitivity of 300 zmol (0.3 μL of a 10^{-12} M solution of RRREEE(pS)EEEAA applied to a Teflon target) using multishot accumulation (Figure 2). This represents an improvement of 5 orders of magnitude over our previous report. As in all MALDI methods, a major limiting factor in sensitivity is the interference of chemical noise.[6,7] The highest level of sensitivity achieved for this source was only possible because this particular analyte was located in a region free of chemical noise. The chemical noise can be shifted in this system by adjusting the time-of-flight of the ions trapped in the cell. However, as this is not a practical method for achieving high sensitivity by MALDI analysis, future work will focus on the identification and reduction of chemical noise.

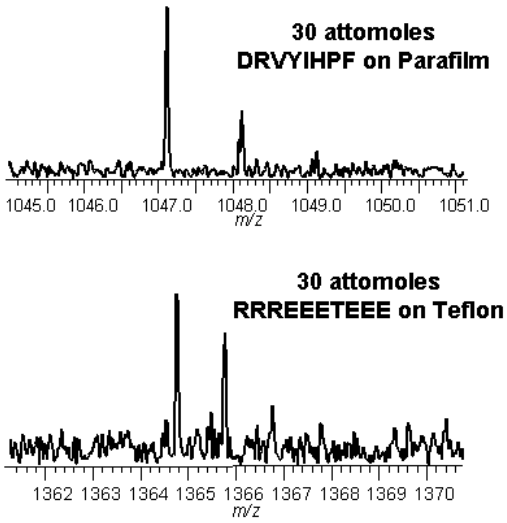

Figure 1: HP-MALDI of peptides from nonconductive surfaces.

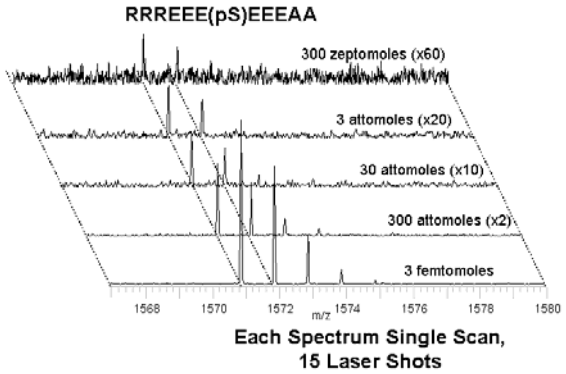

Figure 2: HP-MALDI of the phosphopeptide, RRREEE(pS)EEEAA.

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