Multi-functional *Vacuum* Ionization Source for MAI, LSI, and MALDI: Operational from AP for Comprehensive, Low-Cost Data-Mining in Mass Spectrometry

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Introduction

Unprecedented ionization processes developed into powerful methods have attributes highly desirable for MS and include high sensitivity, low cost, simplicity, ability to directly analyze biological and synthetic materials, potential for high throughput, automation, exceptional robustness, and wide applicability, especially in environments outside analytical laboratories. Initial matrix-assisted ionization (MAI) results showed different selectivity relative to ESI or MALDI providing information not readily obtained with current methodologies. Here, we demonstrate the first vacuum ionization source with multi-ionization capabilities on the same high-resolution API-mass spectrometer for a range of analytical problems with sensitivity in low fmol and detection limit in low amol ranges. The potential for achieving MS and MS/MS analysis speeds of *ca.* 4 seconds/sample in a simple low-cost fashion is demonstrated.

Methods

The prototype MS™ multi-functional vacuum ionization source with MAI and MALDI options was retrofitted with a Nd:YAG laser (266-1060 nm), and operated with MAI, LSI, and MALDI using respective matrices (3-nitrobenzonitrile, 1,2-dicyanobenzene, 2-nitrophloroglucinol, 2,5-dihydroxyacetophenone, 2,5-dihydroxybenzoic acid, and α-cyano-4-hydroxycinnamic acid). The source uses the optics and voltage available with the instrument and consists only of a flange, sample plate guide, and sample plate (glass, quartz, metal, Teflon etc.). Exposing samples rapidly in succession to the vacuum of the mass spectrometer (Waters SYNAPT-G2S) is implemented for a variety of standards and "real" samples, including animal tissue (drugs, lipids, proteins), urine (drugs), blood (proteins and digests, e.g. phosphohistidine-containing acid sensitive modifications), synthetic polymers, dendrimers, Langmuir-Blodgett monolayer films, and carbohydrates.

Preliminary Data

We report the first applications of a truly novel multi-functional vacuum ion source capable of rapid sequential acquisition of mass spectra utilizing vacuum MAI, LSI, or MALDI with operation directly from AP and, consequently, its ability to switch multi-sample substrate plates in <5 seconds. Mass spectra are acquired sequentially without carryover in <4 seconds/sample. The simplicity of the source design, operation, and utility is unprecedented in MS. A variety of small to large analytes (e.g., drugs, lipids, carbohydrates) were measured at high resolving power and at the mass accuracy

inherent with the mass spectrometer; for peptides, we demonstrate the ability to obtain nearly exclusively singly- or multiply-charged ions whereas proteins and synthetic polymers are highly charged. On the SYNAPT-G2S, switching between the MS^{TM} prototype multi-functional source *versus* the Waters (nano)ESI source, including pump down, requires about 1-hour, similar to ESI/vacuum MALDI switchover. Once the multi-functional source is installed, operational differences relate to only voltages, gases, matrices, and in case of a laser, fluence and wavelength. The substrate can be made of metal, certain polymers, or glass. Glass or quartz slides are used with transmission geometry laser alignment (LSI, MALDI). Finally, 'real sample' applications using this new source for a variety of analyses using MS, ion mobility-MS, and -MS/MS, of materials such as biological tissue, surface monolayers, dendrimers, phosphohistidine-containing proteins and digests, and biological blood biomarkers (intact proteins, digests), and drugs from urine are examples. Especially with complex mixtures of analytes, this multi-functional source provides the ability to observe a wider array of compounds than either vMAI or MALDI. Sensitivity and dynamic range are comparable to, if not better than the commercial ESI source on this instrument. Robustness, spatial resolution measurements with and without the use of a laser, and the potential for imaging, e.g. tissue sections, will also be discussed.