Investigating Gas Composition on Transport and Desolvation of High *m/z* Species in the First Vacuum Stages of a Mass Spectrometer

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Iain Campuzano[1], Kevin Giles[1], Manajit Hayer-Hartl[2]
[1]Waters Corporation MS Technologies Centre, Wythenshawe, Manchester, UK; Max Plank Institute of Biochemistry, Cellular Biochemistry, Martinsried, Germany

OVERVIEW

- **Using SF6 as a cone gas has a beneficial effect on the transport of high *m/z* ions in to the mass spectrometer.**
- **Investigating effects of using polyatomic gases within different regions of a mass spectrometer on high *m/z* ion transmission and ion mobility separation.**
- **Evaluation on a hybrid quadrupole/IMS/oa-ToF instrument (Synapt HDMS).**

INTRODUCTION

Nanoelectrospray is an ionization technique which can efficiently generate large biological gas-phase ions. Transfer of non-covalently associated protein-protein complexes from solution to the gas phase generally results in the formation of ions possessing relatively few charges, and consequently *m/z* values are often above 10,000, depending on the size of the protein complex under investigation.

Biological samples analysed under non-denaturing aqueous conditions are heavily solvated, resulting in non-gaussian mass spectral peak shape. An efficient desolvation process results in an accurately measured intact mass of the complex, due to the stripping of non-specific solution adducts.

Here we present a method providing efficient desolvation and effective transport of large biological ions from atmospheric pressure into the vacuum system of a mass spectrometer through modification of the atmospheric pressure gas composition.

METHODS

Instrumentation

The instrument used in these studies was a Synapt HDMS System (Waters Corporation), shown in **Figure 1,** which has a hybrid quadrupole/IMS/oa-ToF geometry. Briefly, samples were introduced by a borosilcate glass nanoelectrospray-spray tip and sampled into the vacuum system through a Z-Spray source. The ions pass through a quadrupole mass filter to the IMS section of the instrument. This section comprises three travelling wave (T-Wave) ion guides. The trap T-Wave accumulates ions whilst the previous mobility separation is occurring, then these ions are released in a packet into the IMS T-Wave in which the mobility separation is performed. The transfer T-Wave is used to deliver the mobility separated ions into the oa-ToF analyser.

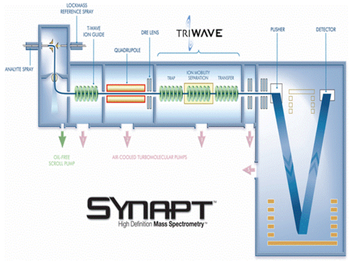

Figure 1 *Diagram of the Synapt HDMS System instrument.*

Samples and Gases

Alcohol dehydrogenase (147.5kDa), GroEL-SR (400kDa, single ring) Proteasome-S (700kDa) and GroEL (800kDa) were all buffer exchanged into an aqueous solution of 100mM ammonium acetate, to a final working protein concentration of 1.5uM. Sulphur Hexafluoride (SF6) obtained from BOC Gases LTD. Octafluoropropane obtained from F2-Chemicals, UK. CsI was at a concentration of 50ug/uL.

Experimental

SF6 and octafluoropropane were introduced as cone gas through the sheath cone assembly (Figure 2). The flow rate was controlled by means of a software controlled Bronkhorst gas flow controller (standard). The flow rate was varied form 0L/hour to 70L/hour to investigate optimal conditions for GroEL (800kDa) detection and transmission.

SF6 and octafluoropropane were compared to argon and xenon, as a trap/Transfer gas, for the transmission of high *m/z* CsI cluster ions.

Ion mobility separation is demonstrated using SF6 as an IMS gas. The central IMS cell was operated at a pressure of 0.3mbar of SF6.

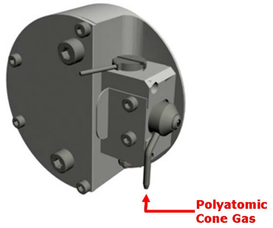

Figure 2. *Source Block, Sample Cone and method of cone gas delivery into the source of the Synapt HDMS system.*

RESULTS

In all previous published experiments on enhancing high mass ion transmission on an orthogonal time-of-flight mass spectrometer, additional gas has been introduced into the initial ion optics of the instrument, the efficiency of the pumping in the source region has been reduced, or replacing argon in the collision cell with a larger gas (1, 2, 3 & 4). Here we demonstrate that by introducing a polyatomic gas, such as SF6, as a cone gas, with no other source modifications, detection of high m/z ions, such as GroEL, can be enhanced (**Figure 2**).

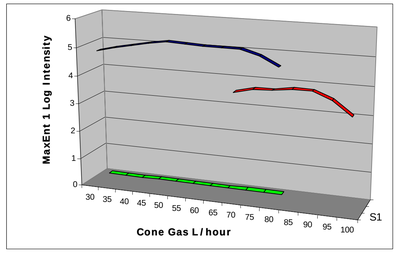

Figure 3. *Investigating the effect of cone gas **SF6**, **octafluoropropane** and **Argon** indicated flow rates (L/hour) on the transmission of intact GroEL (800kDa).*

To enable accurate comparisons of GroEL transmission when assessing SF6 and octafluoropropane, the deconvolution algorithm Maximum Entropy 1 was used to generate an intensity value for the entire GroEL multiply charged envelope. This figure was then plotted against the cone gas flow rate (**Figure 3**). SF6, when used as a cone gas "cools" the large ions more efficiently than octafluoropropane. The multiply charged GroEL ions are not even detectable when Nitrogen, Argon or Xenon (data not shown) used as a cone gas.

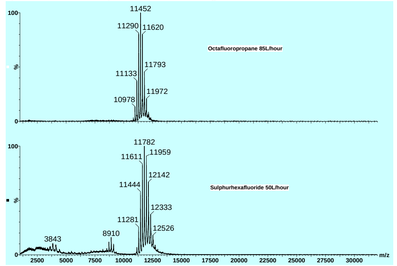

Figure 4. *Multiply charged GroEL spectra obtained at optimal flow rates of octafluorpropane (85L/hour) and SF6 (60L/hour)*

Large (>150kDa) multiply charged ions possess a large energy spread within the mass spectrometer. The kinetic energy spread needs to be reduced so the ions can be focused and ultimately detected by the mass spectrometer. The use of polyatomic gases, such as sulphur hexafluoride and octafluoropropane lend themselves particularly well to "cooling" or "thermalising" large ions (**Figure 4**). Polyatomic gases possess far more degrees of freedom than a monoatomic gas, such as argon or xenon. For example, SF6 (Mw 146) and octafluorpropane (Mr 188) possess 21 and 33 degrees of freedom (3N) respectively, as opposed to the 3 degrees of freedom of xenon (Mr 131).

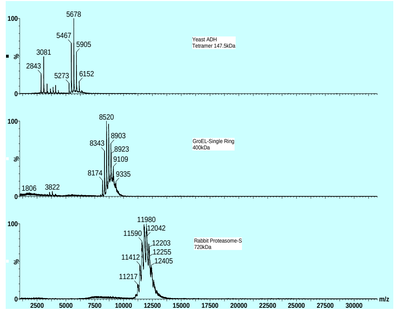

Figure 5. *TOF-MS data acquisition of Yeast ADH, GroEL-SR and Rabbit Proteasome-S, using SF6 as a cone gas.*

SF6 and Octafluoropropane as a Trap/Transfer Gas

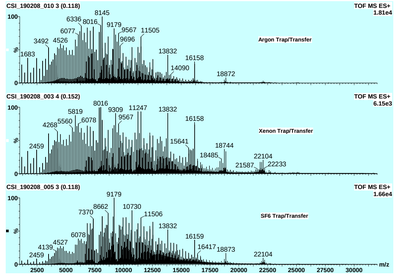

Figure 6a. *Comparison of Argon, xenon and SF6 as a Trap/Transfer gas on TOF-MS transmission of CsI clusters over the m/z range 1000-32,000.*

The properties of polyatomic gases were also investigated in the travelling ion guide regions of the instrument. Caesium iodide was used initially to assess the ability of SF6 to "cool"

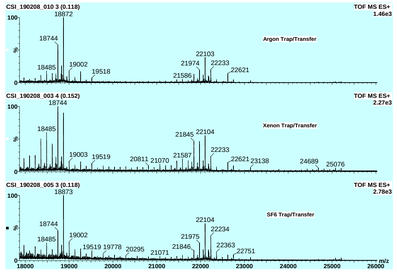

Figure 6b. *Zoomed-in region (m/z 18,000–26,000) of Figure 6a.*

ions of high *m/z* (Figure 6a). Utilising SF6 within the Trap/Transfer region of the Synapt, ions of high *m/z*, such as *m/z* 18,873 and *m/z* 22,104 show a 20% increase in signal intensity over that obtained with xenon, and a 50% increase over argon (**Figure 6b**).

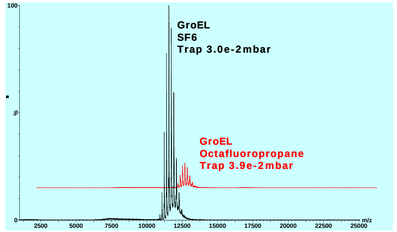

Figure 7. *TOF-MS of GroEL using different trap/transfer gases.* ***Red: Octafluoropropane; Black: SF6.***

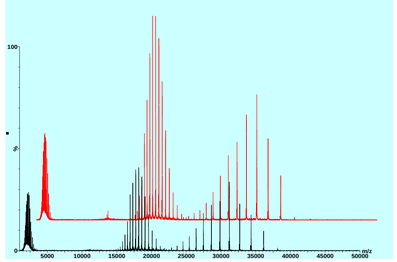

Figure 8. *TOF-MS/MS of GroEL precursor ion m/z 11,130 (+72). Collision energy was set to 160V for MS/MS.* ***Red: Octafluoropropane; Black: SF6.***

Operating in TOF-MS mode, SF6 provides improved desolvation and transmission over that obtained with octafluoropropane when operating at low activation energies (Trap 40V, **Figure 7**). In TOF-MS/MS mode with a trap voltage of 160V, the larger polyatomic gas, octafluoropropane, provides improved focussing and therefore, transmission of the high *m/z* GroEL product ions. For example (**Figure 8**) the 13mer is 3x as intensity when performing MS/MS in octafluoropropane. The 12mer is 2x as intense in octafluoropropane.

SF6 as an Ion Mobility Gas

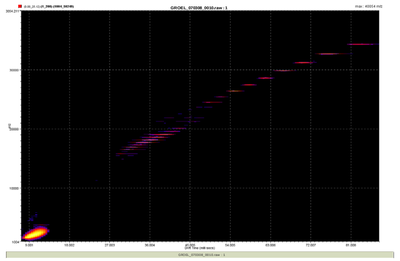

Figure 9. *Drift-time vs m/z plot for the MS/MS-IMS (precursor ion m/z 11,130). Trap collision energy was 160V. The trap/transfer region was filled with SF6.*

Nitrogen is a commonly used neutral gas for ion mobility experiments. **Figure 9** shows the ion mobility separation obtained in SF6 on the product ions generated from the MS/MS activation of the GroEL precursor ion *m/z* 11,130. All product ions produced over the *m/z* range 1000 to 50,000 can be well resolved and accurate Arrival Time Distributions (ATDs) calculated. This has important implications when measuring CCSs of stripped oligomeric species and modelling unfolding intermediates.

CONCLUSION

SF6 used as a cone gas provides excellent collisional cooling and focusing of large ions prior to their entry into the mass spectrometer, thus allowing for their detection.

SF6 and octafluoropropane used in the trapping and transfer region of the Synapt HDMS, especially when operated in MS/MS mode, provides improved collisional cooling over that obtained with argon and xenon.

SF6 can also be used as an IMS gas, providing very well defined ion mobility arrival time distributions for large ions.

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