Enhancing Secondary Ion Yields in Time of Flight-Secondary Ion Mass Spectrometry Using Water Cluster Primary Beams

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Abstract: Low secondary ion yields from organic and biological molecules are the principal limitation on the future exploitation of time of flight-secondary ion mass spectrometry (TOF-SIMS) as a surface and materials analysis technique. On the basis of the hypothesis that increasing the density of water related fragments in the ion impact zone would enhance proton mediated reactions, a prototype water cluster ion beam has been developed using supersonic jet expansion methodologies that enable ion yields using a 10 keV (H₂O)₁₀₀₀⁺ beam to be compared with those obtained using a 10 keV Ar₁₀₀₀⁺ beam. The ion yields from four standard compounds, arginine, haloperidol, DPPC, and angiotensin II, have been measured under static+ and high ion dose conditions. Ion yield enhancements relative to the argon beam on the order of 10 or more have been observed for all the compounds such that the molecular ion yield per a 1 μm pixel can be as high as 20, relative to 0.05 under an argon beam. The water beam has also been shown to partially lift the matrix effect in a 1:10 mixture of haloperidol and dipalmitoylphosphatidylcholine (DPPC) that suppresses the haloperidol signal. These results provide encouragement that further developments of the water cluster beam to higher energies and larger cluster sizes will provide the ion yield enhancements necessary for the future development of TOF-SIMS.
yields has been obtained but rarely above a factor of 5 increase. The successes of these approaches have been quite sample specific, and once the additive is sputtered away, its benefits are lost. Since the advent of polyatomic beams, it has been observed that water in the sample, present either adventitiously or as a consequence of freezing the analyte in an ice can, under bombardment by C\textsubscript{60}\textsuperscript{+}, gives rise to enhanced [M + H]\textsuperscript{+} yields.\textsuperscript{12–14} Enhancements in the range of 10 to 100 have been reported, but only very few molecules have been studied. These observations have been complemented by a study in which water was admitted to the analysis chamber just above the analyte\textsuperscript{15,16}. An enhancement of 10\times in the molecular ion of Irgafos 168 was observed with smaller increases for polymers. Using D\textsubscript{2}O, it has been suggested that these enhancements are associated with protonation activity in the presence of surface water, possibly as a consequence of the formation of H\textsubscript{3}O\textsuperscript{+} in the bombardment region.\textsuperscript{17,18} Such a suggestion accords with the phenomenon underlying proton transfer reaction mass spectrometry (PTRMS) and selected ion flow tube MS (SIFTMS), usually used to detect organics in the atmosphere.\textsuperscript{17,18} Because the proton affinity of most organic molecules is higher than that of water, 691 kJ/mol, H\textsubscript{3}O\textsuperscript{+} is highly reactive to many organic analytes such that the kinetics are determined by the collision rates and significant protonated ion yields are obtained. On the basis of this thinking, we are in the process of developing a water cluster primary ion beam by ionization of a neutral cluster formed by supersonic jet expansion (as distinct from an electrospray type method)\textsuperscript{19,20} using a similar methodology as for argon cluster beams.\textsuperscript{21–23} The hypothesis being that, in addition to benefits of enhanced low damage sputtering by a large cluster beam, sputtering with such a beam will generate a high density of water molecules and fragments in the emission zone that will enhance the formation of [M + H]\textsuperscript{+} ions and possibly [M − H]\textsuperscript{−} ions. If the density of proton donating species can be made high enough, ion yields might saturate to a relatively uniform level and this may have the benefit of ameliorating the other bugbear of SIMS and other desorption mass spectrometries, the matrix effect.

### COMPARING TOF-SIMS SPECTRA FROM 10 keV \textsubscript{Ar}\textsubscript{1000}\textsuperscript{+} AND \textsubscript{(H\textsubscript{2}O)\textsubscript{1000}}\textsuperscript{+} RESULTS

This Letter reports on initial data from our prototype water beam system based on a previous argon beam design\textsuperscript{23} that has been mounted on the J105 TOF-SIMS instrument.\textsuperscript{7} This very preliminary data shows that, relative to yields obtained from 10 keV \textsubscript{Ar}\textsubscript{1000}\textsuperscript{+}, [M + H]\textsuperscript{+} ion yields from arginine, [Asn\textsuperscript{1} Val\textsuperscript{5}] angiotensin II, dipalmitoylphosphatidylcholine (DPPC), and [Asn\textsuperscript{1} Val\textsuperscript{5}] angiotensin II are all enhanced by \textasciitilde 10\times or more. Further experimental details can be found in the online Supporting Information.

In Figure 1, the positive ion SIMS spectra from the lipid DPPC obtained using \textsubscript{Ar}\textsubscript{1000}\textsuperscript{+} and \textsubscript{(H\textsubscript{2}O)\textsubscript{1000}}\textsuperscript{+} primary beams under 1 \times 10\textsuperscript{11} ions cm\textsuperscript{−2} dose conditions are compared. These analysis conditions are somewhat above static, resulting in the removal of 5% to 10% of the surface volume sampled (termed static\textsuperscript{+}). Broadly, the spectra obtained are very similar. There are two obvious differences: first, the ion yields under water bombardment compared to argon are very significantly increased, m/z 184 by a factor 16 and [M + H]\textsuperscript{+} m/z 735 by a factor 17. Second, the ratio of the [M + H]\textsuperscript{+} to the [M + Na]\textsuperscript{+} peaks has reversed, from 0.7 under argon to 2.0 under water. It is clear that water bombardment has a profound effect on the
ion formation process and, while all ion yields are enhanced, protonation is favored. Figure 2 summarizes the data obtained from all the samples under the same dose conditions. In all cases, the enhancement relative to argon is around 10 or more. DPPC and haloperidol show the highest enhancements of close to 20; indeed, in the case of haloperidol, the yield of the [M + H]⁺, m/z 376 ion was so high the detector system was saturated. Hence, we have plotted the lower [M − OH]⁺ species at m/z 358.

The yield enhancement of [M + H]⁺ from angiotensin II is almost exactly 10. The enhancement of the fragment ions follows quite closely the enhancement of the molecular ion. For example, m/z 254, which is the b₂-NH₃ fragment, is also enhanced by a factor of 10. This seems to be true for most of the molecules we have studied. The fragment to molecular ion ratio is very similar under both beams. This behavior would be expected if the water beam predominantly enhances the yield of the [M + H]⁺ ion and most fragment formation results from fragmentation of this ion. Very early, 24,25 and recent MSMS studies from this laboratory would support this expectation. It is not true for all fragment ions, e.g., the m/z 95 from haloperidol where the ratio changes from ~0.4 under argon to ~0.2 under water. This may cast light on a changing mechanism of fragmentation with the involvement of water fragments in the near surface region.

It is interesting that the water cluster analysis of a second DPPC sample that had a higher yield of [M + Na]⁺ under the argon beam showed only a 10X enhancement of the [M + H]⁺ ion, and the [M + Na]⁺/[M + H]⁺ ratio which was about 1.0 under argon did not change. The interference of alkali metal ions in the formation of protonated secondary ions has been studied in this laboratory, and this work shows that salt can inhibit the formation of these [M + H]⁺ ions. 26,27 As in these earlier studies, the present results might suggest that the extent of salt inhibition is concentration related.

Although at this stage we have not carried out detailed depth profiling or sputter yield measurement, all of the samples were subjected to extended sputtering under water cluster bombardment up to a dose of about 1.7 × 10¹² ions cm⁻². For most of the samples, the signal profiles up to this dose were quite stable. Assuming a molecular volume of about 1 nm³ and a sputter yield of ~50 to 100 molecules per primary impact, 28 the 1.7 × 10¹² ions cm⁻² dose is well beyond the static limit and almost all the molecules in a 1 μm² × 10 nm surface volume will be removed. In light of our interest in subμm analysis, Table 1 compares the number of molecular secondary ions detected under our instrumental conditions from a 1 μm² surface using the argon and water beams under the static+ dose of 1 × 10¹² ions cm⁻² and when the whole surface layer is sputtered by 1.7 × 10¹² ions cm⁻² and most of the resulting secondary ions are collected.

It is clear that the yields/1 μm² under our static+ conditions are around 5 to 10 times the expectations for strict static conditions. Given ionization probabilities in the order of 10⁻³, they are in line with the higher ion dose. The present water beam improves the situation by a factor of 10, but the ability to accumulate all or most of the ions from a given area for analysis starts to lift the ion yield into a useful region. If the sputter yield is 50 to 100 per impact, the ionization probability under the water beam is ≥ 10⁻³.

The second major impetus for these studies was that the use of water primary beams might modify matrix effects in the case of [M ± H]⁺ ions. We have shown that the use of the hydrogen containing a polyatomic ion beam, coronene C₉₆H₄₇⁺, can help to lift the matrix effect. 29 Previous studies have shown that DPPC exerts a very significant suppression effect in the formation of the [M + H]⁺ ion of haloperidol. 30,31 As a preliminary study, a 1:10 mixture of haloperidol in DPPC was prepared by dissolving appropriate amounts of the two compounds in a methanol chloroform mixture and spin-casting the resulting solution onto a silicon wafer. Figure S.1, Supporting Information, summarizes the outcome of a comparative analysis of this mixture with the argon and water beams. The expected mixture ratio 0.09Y_Hal/(0.09Y_Hal + 0.091Y_DPPC) has been calculated using the observed m/z 358 ion yield from pure haloperidol, Y_Hal, and the m/z 735 ion yield from pure DPPC, Y_DPPC, under the argon and water beams. This is compared with the observed Y_Hal/(Y_Hal + Y_DPPCmix) from the mixture. Similar data for DPPC is also presented in Figure S.1, Supporting Information. It can be seen that under argon bombardment there is significant suppression of the haloperidol ion yield ratio by almost 5X from the expected 0.86 down to 0.19 and a corresponding enhancement of DPPC by more than 6X from 0.13 to 0.81. Under water bombardment however, the mixture ratio for haloperidol is close to 50% of that expected. In contrast, the DPPC ratio is still some 5 times higher than expected. After a dose of 1.7 × 10¹² ions cm⁻², the relative enhancement for haloperidol is maintained if not somewhat increased, so surface segregation effects are minimal. It is clear that, while there is a real benefit under water cluster bombardment, there is still some way to go to lift the matrix effect entirely. This suggests that DPPC is still benefiting significantly compared to haloperidol from the extra protonation capability and that saturation of protonation species in the emission region has not been reached, so there is still more benefit to be had if cluster sizes can be increased.

### DISCUSSION AND CONCLUSIONS

The data we report makes it clear that the water cluster beam provides an approximately 10 times or more increase in ion yield for a range of molecules over the yields observed with 10 keV argon clusters of the same nuclearity (n = 1000). Under these conditions, there is also a benefit in a reduced matrix suppression effect. These observations are consistent with the experimental observations reported from earlier studies on the benefits of ice matrices and water dosing of samples that resulted in increased [M + H]⁺ yields. Furthermore, MD simulations from Garrison’s group have suggested that hydrogen species generated by a primary ion impact in the emission zone may well give rise to protonated molecules that are in a sense preformed to be sputtered out by subsequent

| compound          | static+ conditions (1 × 10¹² ions cm⁻²) | whole 1 μm² area consumed |
|-------------------|----------------------------------------|---------------------------|
|                   | argon  | water  | argon  | water  |
| arginine m/z 175  | 0.17   | 1.4    | 2.6    | 21.0   |
| haloperidol m/z 358 | 0.06  | 1.3    | 0.9    | 13.4   |
| DPPC m/z 184      | 0.033  | 0.55   | 0.51   | 6.4    |
| DPPC m/z 735      | 0.001  | 0.02   | 0.02   | 0.24   |
| angiotensin II m/z 1032 | 0.09  | 0.84   | 1.09   | 6.5    |

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primary particle impacts.\textsuperscript{32} The data presented in this paper is understandable in the light of this idea such that a water cluster impact would result in an increased density of hydrogen, protons, oxygen atoms and ions, and hydroxyl ions. The resulting ion–molecule reactions in the emission zone would be those expected to result in secondary ion formation and subsequent release. It would clearly be extremely interesting to be able to use a D\textsubscript{2}O cluster beam to clarify the mechanisms of protonation and other reactions in the emission zone. Similarly, the use of \textsuperscript{18}O enriched water could possible provide further insights, although at some expense!

Other beam systems have been investigated in the past with the aim of enhancing protonated organic molecule ion yields. Massive clusters of glycerol were successful in desorbing and detecting large peptides, much larger than can be detected in routine SIMS today,\textsuperscript{33} however, the development did not take off possibly because of the technical difficulties of handling the glycerol beam. Desorption electrospray ionization (DESI) uses a spray of charged droplets to desorb and ionize under atmospheric conditions.\textsuperscript{34} Proton exchange reactions are important in this process, but yields relative to SIMS are difficult to determine. Giant water cluster beams generated by electrospray methods\textsuperscript{35,36} have been successful in generating SIMS type spectra and would be expected to deliver similar ion yields that enable useful analysis of multiple compounds and 20 keV Ar\textsubscript{1000} data we have obtained in this laboratory, we hope that these enhanced conditions would enable the matrix effect benefits to be increased too.

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\section{REFERENCES}
(1) Vickerman, J. C.; Briggs, D. TOF-SIMS - Materials Analysis by Mass Spectrometry; IM Publications and SurfaceSpectra Ltd: Manchester, UK: 2013.
(2) Fletcher, J. S.; Vickerman, J. C. Anal. Chem. 2013, 85, 610–639.
(3) Briggs, D.; Wootton, A. B. Surf. Interface Anal. 1982, 4, 109–115.
(4) Mahoney, C. M. Mass Spectrom. Rev. 2010, 29, 247–293.
(5) Wucher, A. Appl. Surf. Sci. 2006, 252, 6482–6489.
(6) Cheng, J.; Koizumi, J.; Hengstebek, R.; Winograd, N. J. Am. Soc. Mass Spectrom. 2007, 18, 406–412.
(7) Fletcher, J. S.; Rabban, S.; Henderson, A.; Blankinopp, P.; Thompson, S. P.; Lockyer, N. P.; Vickerman, J. C. Anal. Chem. 2008, 80, 9058–9064.
(8) Heile, A.; Lipinsky, D.; Wehbe, N.; Delcorte, A.; Bertrand, P.; Felten, A.; Houssiaux, L.; Pierreux, J. J.; De Mondt, R.; Van Royen, P.; Van Vaes, L.; Airlinghaus, H. F. Surf. Interface Anal. 2008, 40, 538–542.
(9) Delcorte, A. Appl. Surf. Sci. 2006, 252, 6582–6587.
(10) Prabhakaran, A.; Yunus, S.; Wehbe, N.; Bertrand, P.; Delcorte, A. Surf. Interface Anal. 2011, 43, 74–77.
(11) Wu, K. J.; Odom, R. W. Anal. Chem. 1996, 68, 873–882.
(12) Cheng, J.; Winograd, N. Anal. Chem. 2005, 77, 3651–3659.
(13) Conlan, X. A.; Lockyer, N. P.; Vickerman, J. C. Rapid Commun. Mass Spectrom. 2006, 20, 1327–1334.
(14) Piwowar, A. M.; Fletcher, J. S.; Kordys, J.; Lockyer, N. P.; Winograd, N.; Vickerman, J. C. Anal. Chem. 2010, 82, 8291–8299.
(15) Mouhib, T.; Delcorte, A.; Poleuni, C.; Bertrand, P. J. Am. Soc. Mass Spectrom. 2010, 21, 2005–2010.
(16) Mouhib, T.; Delcorte, A.; Poleuni, C.; Bertrand, P. Surf. Interface Anal. 2013, 45, 46–49.
(17) Smith, D.; Spanel, P. Mass Spectrom. Rev. 2005, 24, 661–700.
(18) Blake, R. S.; Monks, P. S.; Ellis, A. M. Chem. Rev. 2009, 109, 861–896.
(19) Beuhler, R. J.; Friedman, L. J. Chem. Phys. 1982, 77, 2549–2557.
(20) Beuhler, R.; Friedman, L. Chem. Rev. 1986, 86, 521–537.
(21) Ninomiya, S.; Ichiki, K.; Seki, T.; Aoki, T.; Matsu, J. Nucl. Instrum. Methods Phys. Res, Sect. B 2009, 267, 2601–2604.
(22) Takaoka, G. H.; Kawashita, M. Synth. React. Inorg., Met.-Org., Nano-Met. Chem. 2008, 38, 111–117.
(23) Rabbani, S.; Barber, A. M.; Fletcher, J. S.; Lockyer, N. P.; Vickerman, J. C. Anal. Chem. 2011, 83, 3793–3800.
(24) (a) Leggett, G. J.; Vickerman, J. C.; Briggs, D. Surf. Interface Anal. 1990, 16, 3–8. (b) Leggett, G. J.; Vickerman, J. C. Int. J. Mass Spectrom. Ion Processes 1992, 122, 281–319.
(25) Leggett, G. J.; Briggs, D.; Vickerman, J. C. Surf. Interface Anal. 1991, 17, 737–744.
(26) Jones, E. A.; Lockyer, N. P.; Vickerman, J. C. Anal. Chem. 2008, 80, 2125–2132.
(27) Piwowar, A. M.; Lockyer, N. P.; Vickerman, J. C. Anal. Chem. 2009, 81, 1040–1048.
(28) Cheng, J.; Wucher, A.; Winograd, N. J. Phys. Chem. B 2006, 110, 8329–8336.
(29) Biddulph, G. X.; Piwowar, A. M.; Fletcher, J. S.; Lockyer, N. P.; Vickerman, J. C. Anal. Chem. 2007, 79, 7259–7266.
(30) Jones, E. A.; Lockyer, N. P.; Vickerman, J. C. Int. J. Mass Spectrom. 2007, 260, 146–157.
(31) Jones, E. A.; Lockyer, N. P.; Vickerman, J. C. Appl. Surf. Sci. 2006, 252, 6727–6730.
(32) Brenes, D. A.; Postawa, Z.; Wucher, A.; Blankinopp, P.; Garrison, B. J.; Winograd, N. Surf. Interface Anal. 2013, 45, 50–53.
(33) Cornett, D. S.; Lee, T. D.; Mahoney, J. F. Rapid Commun. Mass Spectrom. 1994, 8, 996–1000.
(34) Ifa, D. R.; Wu, C.; Ouyang, Z.; Cooks, R. G. Analyst 2010, 135, 669–681.
(35) Asakawa, D.; Fujimaki, S.; Hashimoto, Y.; Mori, K.; Hiraoka, K. Rapid Commun. Mass Spectrom. 2007, 21, 1579–1586.
(36) Asakawa, D.; Hiraoka, K. Rapid Commun. Mass Spectrom. 2011, 25, 655–660.