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LIQUID SECONDARY ION MASS SPECTROMETRY
I. Molecular ion intensities as a function of primary ion pulse frequency *

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A liquid matrix and high primary ion currents are employed on a time-of-flight mass spectrometer to produce molecular ions in a manner similar to that employed for fast atom bombardment (FAB) techniques on sector instruments. The primary ion beam is pulsed and the molecular yield is found to depend upon sample concentration, instantaneous primary ion current, and pulse repetition rate. The latter, in particular, indicates that a finite recovery time is required to repair radiation damage from high flux particle beams.

1. Introduction

For nearly two decades, keV ion beams have been used to desorb intact molecular ion species from nonvolatile organic compounds. When analyzed by mass spectrometry, such methods are referred to as secondary ion mass spectrometry of SIMS. In 1970, Benninghoven [1,2] introduced "static" SIMS which employed primary ion current densities on the order of 1 nA/cm^2. Low ion current densities proved neccessary to desorb intact molecules without the sample damage observed for "dynamic" SIMS which employs current densities greater than 1 μA/cm^2 [3,4], and which is more suitable for elemental analysis. Approximately five years ago, the introduction of fast atom bombardment (FAB) or liquid secondary ion mass spectrometry (LSIMS) permitted the use of current densities comparable to "dynamic" SIMS to desorb intact molecular ion species. Dissolving the nonvolatile sample in a liquid matrix (usually glycerol) before introduction into the source prevented the sample damage previously observed with high flux primary ion beams. It has been hypothesized that the sample is repaired by diffusion of new sample to the surface from the bulk of the matrix [5], or perhaps by the stripping away of successive layers of sample and matrix [6,7].

The large, continuous secondary ion currents produced by liquid SIMS has been an advantage for high mass range, sector instruments which scan the mass range and record analog signals. The time-of-flight (TOF) analyzer is also capable of high mass ranges, and its high transmission could provide increases in sensitivity when used in combination with liquid SIMS. Ordinarily, however, ions are formed on an equipotential surface in a high voltage (10–20 kV) source in TOF instruments, which would be distorted by the use of a liquid matrix. In addition, distortion of the liquid droplet by the electric field can lead to arcing. Finally, it is difficult to take advantage of the large secondary ion currents when time-to-digital conversion (TDC) techniques are used to record the ions. While this is the most common method for recording spectra on a TOF, it requires that primary ion currents be kept purposely low to prevent simultaneous formation of ions of the same mass, since these ions are recorded as only a single count. However, analog measurements have been used with laser desorption [8] TOF mass spectrometers (including LAMMA, LIMA, and MPI instruments) to accommodate large secondary ion currents. We have achieved the combination of high flux LSIMS with TOF by utilizing a pulsed Xe^+ ion gun with a grounded source, pulsed extraction TOF analyzer [9,10]. The grounded source allows liquid samples, and the pulsed TOF allows the use of long (> 1 μs), high current density ($> 1\mu$A/cm^2) primary ion pulses which provide more energy in a single ion pulse than currently is available in other TOF systems with keV sources.

We have used the ability of this instrument to vary both the frequency and instantaneous current densities (amplitudes) of the primary ion pulses to study the repair mechanism of glycerol. This type of experiment is unique to a pulsed LSIMS apparatus because it allows one to study the repair of the sample in the absence of the continuous damage caused by the beam in FAB sources. In this paper we present the intensities of MH^+ ions from various samples dissolved in glycerol as a

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function of ion pulse frequency at different instantaneous current densities and sample concentrations. A "memory effect" is observed for most samples. At higher frequencies MH^+ intensities tend to decrease, thus implying that sample repair after one pulse may not be complete before the next pulse of ions strikes the sample. The degree of this effect varies for different primary ion current densities, sample concentration, and sample identity.

2. Experimental

The high flux TOF mass spectrometer is described in detail elsewhere [9,10] so only a brief description is presented here. The TOF mass spectrometer is a CVC 2000 electron impact mass spectrometer which has been modified to accommodate a keV ion gun. The ion gun is a Kratos Minibeam I which has been modified to produce Xe^+ ion pulses. The width of the primary Xe^+ ion pulses may be varied from 1 to 10 μs (with rise and fall times of < 200 ns), and the frequency range extends from 1 Hz to 50 kHz. The current density amplitude during an ion pulse (instantaneous primary ion current density) may be varied from 1 to 200 $\mu A/cm^2$. After the primary ion pulse strikes the liquid sample, the secondary ions are removed from the source by a draw-out pulse which initiates the timing sequence. The time between the ionization pulse and the draw-out pulse is varied between 0 and 20 μs to focus the secondary ions produced in the source. This is similar to the time lag focussing developed by Wiley and McLaren [11] for electron impact TOF mass spectrometers. The secondary ions are detected by dual channel plates and the detector output is digitized and recorded by a LeCroy 3500 signal averager. Usually 1000 spectra are add/averaged at 100 Hz.

In this experiment, protonated molecular ion intensity was measured at a known frequency, instantaneous current density, and bulk sample concentration. First, the instantaneous current was adjusted to the desired value, then a sample of known concentration was applied to the probe. An intensity measurement was made by acquiring and adding 1000 spectra at a given frequency. The number of counts under the MH^+ peak was calculated after subtracting the background by computer. Intensities were measured at five different frequencies (10 Hz, 100 Hz, 1 kHz, 10 kHz, and 50 kHz), and 4 μs long ion pulses were used. To prevent instrumental effects, the mass analyzer was triggered at 100 Hz for all ion pulse frequencies greater than or equal to 100 Hz. When the ion gun was pulsed at 10 Hz, the mass analyzer was also triggered at 10 Hz. Thus the acquisition time for a single data point was constant (except at 10 Hz) at 10 s (1000 pulses at 100 Hz). For example, when the ion gun was triggered at 1 kHz, the trigger for the draw-out pulse of the mass spectrometer was divided by 10 down to 100 Hz. Therefore the secondary ions from every tenth primary ion pulse were extracted into the flight tube. This means the draw-out pulse did not remove the ions from the source after each ionization pulse. This was not a problem since the average source residence time for a mass 1000 ion is approximately 10 μs. No accumulation of ions in the source was observed from one pulse to the next.

Seven to ten intensity measurements were made at 100 Hz, 1 kHz, and 10 kHz from a single sample droplet in varying orders. A single sample was used only a short time (< 10 min) to minimize sample loss due to evaporation. The liquid sample droplet was still observable upon the probe tip after removal from the vacuum. Intensities at the highest and lowest frequencies (10 Hz and 50 kHz) were measured from a different sample droplet. The relative intensities at these two frequencies were determined by comparison with intensities measured at 100 Hz and 10 kHz.

Instantaneous current densities (D_0) were varied between 150 $\mu A/cm^2$ and 1.5 $\mu A/cm^2$. They were determined by using a Keithley 610c electrometer to measure continuous currents striking the copper probe tip when the ion beam was not pulsed. A large beam diameter was used so the entire probe tip was irradiated. These current density values were then checked by comparison with average current densities measured at different frequencies with a 4 μs ion pulse. A third check was performed by observing the current pulses from the probe with an oscilloscope. All measured values of D_0 agreed to within 20%, and the measured average currents were directly proportional to the ion pulse frequencies at which they were measured.

Gramicidin S, a small cyclic peptide of mass 1141 daltons, was the primary compound used for this study. The samples were prepared by dissolving a known amount of sample in methanol. Successive dilutions with methanol were then performed to obtain three different concentrations of Gramicidin S, each an order of magnitude more dilute than the previous one. 10 μl of each of the Gramicidin/methanol solutions were then added to 100 μl samples of glycerol and mixed. This produced three glycerol/Gramicidin S samples with concentrations of 10 nmole/μl, 1 nmole/μl, and 0.1 nmole/μl. These same samples were used for the entire experiment. Other samples used in this study were prepared in a similar manner. Gramicidin S and Bradykinin were obtained from Sigma. Oxytocin and Leu-Enkephalin were obtained from Calbiochem. Leu-Trp-Met-Arg-Phe-Ala, Leu-Trp-Met, and Arg-Phe-Ala were obtained from Research Plus Laboratories. Cyclosporin A was compliments of M. Colvin, Oncology Department, Johns Hopkins University. All samples were used without further purification and were dissolved in appropriate solvents before use.

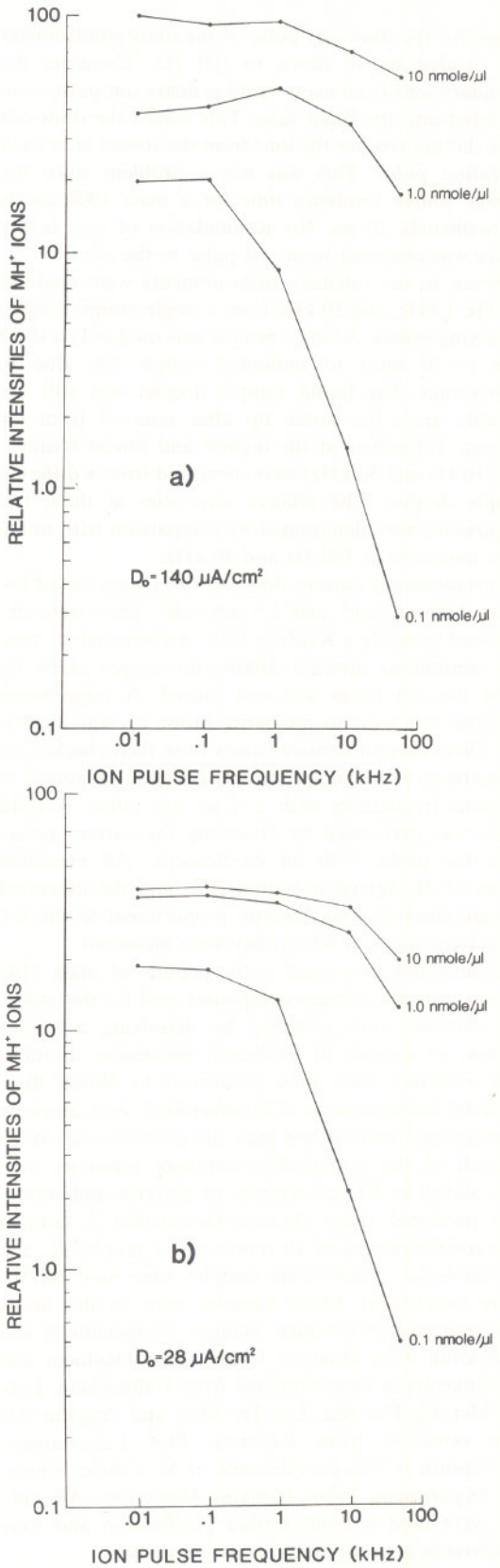

Fig. 1. Relative intensities of MH^+ ions as a function of primary ion pulse frequency for different concentrations of Gramicidin S in glycerol. Instantaneous primary ion current densities are a) 140 $\mu A/cm^2$, b) 28 $\mu A/cm^2$, c) 14 $\mu A/cm^2$.

3. Results and discussion

Figs. 1a, 1b, and 1c show the MH^+ ion intensity for Gramicidin S as a function of primary ion pulse frequency at different instantaneous current densities (D_0) and bulk sample concentrations. The most noticeable result is that in all cases, the production of MH^+ ions decreases with increasing repetition rate. Secondly, when the instantaneous current is high (140 $\mu A/cm^2$ in fig. 1a), the relative intensities of MH^+ ions reflect the bulk sample concentrations, while at lower currents, intensities of the molecular ion species are less sensitive to sample concentration (figs. 1b and 1c). Finally, molecular ion intensities are not proportional to the instantaneous current density (D_0).

We interpret these results by noting, first, that secondary ion techniques sample ions primarily from the surface layers [12]. The concentration of molecular species at the surface, while influenced by the concentration in the bulk of the solution [12], is also affected in dynamic situations by the supply of molecu-

lar species from the bulk to the surface. Supply mechanisms might include convection (stirring), migration (motion in an electric field), or diffusion (dependent upon the concentration gradient).

Rollgen [6,7] has noted that at high primary ion currents, surface layers may be "peeled away" at such rates that the secondary ion current, in fact, reflects the concentration of ions in the bulk of the solution. We suggest that this is the case when instantaneous currents of 140 $\mu A/cm^2$ (Figure 1a) are used [13]. Alternatively, large primary ion pulses result in increased stirring of the surface layers (convection), so that the surface and bulk of the solution have comparable concentrations, and the molecular ion current becomes proportional to the bulk concentration. The proportionality of the ion yields as a function of concentration for $D_0 = 140$ $\mu A/cm^2$ (fig. 1a), versus the insensitivity of yield as a function of concentration observed at $D_0 = 14$ $\mu A/cm^2$ (fig. 1c), shows that convection is most prominate at high current densities.

At a sample concentration of 0.1 nmole/μl and instantaneous ion current density of 140 $\mu a/cm^2$ (fig. 1a), the MH^+ ion decreases significantly when the repetition rate is 1 kHz (1 ms between primary ion pulses). At lower instantaneous currents, the decrease in MH^+ intensity for the same concentration occurs at higher repetition rates (figs. 1b and 1c). Since diffusion should be dependent only upon concentration (and viscosity of the glycerol solution), we suggest that charging of the sample surface by the primary ion beam inhibits ion migration to the surface. This provides the most notable effect of decreased ion intensity at higher repetition rates. This is particularly born out by the fact that the molecular ion current when $D_0 = 140$ $\mu A/cm^2$ and $f = 1$ kHz is nearly identical to that when $D_0 = 14$ $\mu A/cm^2$ and $f = 10$ kHz, i.e. when the average primary ion current is the same. At higher concentrations, the decrease in ion current occurs more gradually with increasing pulse frequency, perhaps suggesting that diffusion mechanisms become more dominant.

That the molecular ion current does in fact depend in some complex way upon the supply of sample ions from the bulk to the surface layers, is further corroborated by the molecular ion yield for pure glycerol (fig. 2), where surface and bulk concentrations are the same. The intensities are unaffected by the repetition rate, and molecular ion intensities are directly proportional to the instantaneous ion current.

The different surfactant properties of samples may effect supply to the surface as shown in fig. 3. Some compounds, such as Cyclosporin A (fig. 3, curve A) exhibit a marked decrease in intensity with increasing ion pulse frequency. Others, like Oxytocin (fig. 3, curve G), exhibit little or no effect. The fact that the "memory effect" is compound dependent implies that it cannot be explained solely in terms of convection or migration.

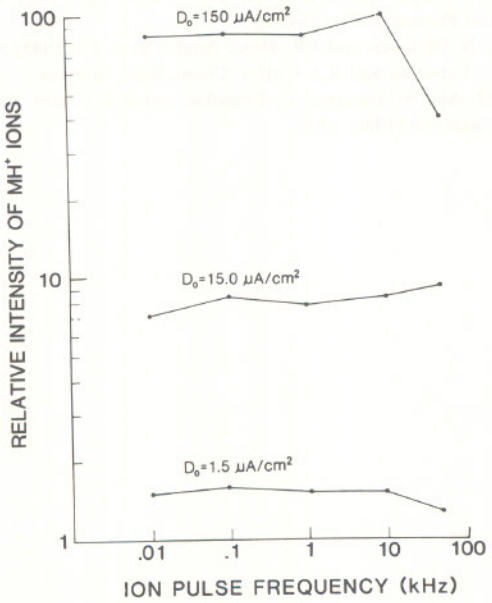

Fig. 2. Relative intensities of protonated glycerol ions from pure glycerol as a function of primary ion pulse frequency at different instantaneous current densities (D_0).

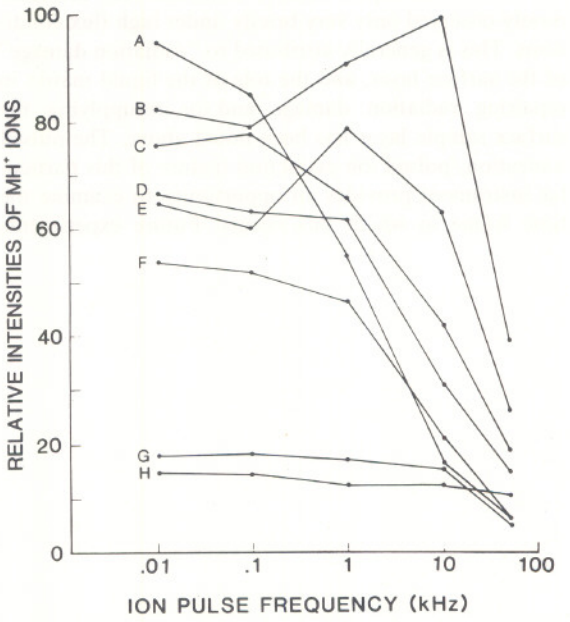

Fig. 3. Relative intensities of MH^+ ions as a function of primary ion pulse frequency for A) Cyclosporin A, B) Leu-Enkephalin, C) Gramicidin S, D) Arg-Phe-Ala, E) Leu-Trp-Met, F) Bradykinin, G) Oxytocin, and H) Leu-Trp-Met-Arg-Phe-Ala. Concentrations were 1.0 nmole/μl and the primary ion current density was 55 $\mu A/cm^2$.

We note, therefore, that at high primary ion currents, convection leads to sampling of ions which reflect the bulk concentration of the sample solution; that charging of the matrix produces a fairly long term effect proportional to average current; and that rates of diffusion of sample ions to the surface, while dependent upon sample concentration, are of the order of 100 μs.

4. Conclusions

The role of the liquid matrix in secondary ion mass spectrometry is not completely understood, while it is clear that it permits desorption of intact high mass molecular ions where high primary ion currents are used. Chemical effects are certainly apparent since thioglycerol or acidified glycerol improves the MH^+ ion current in positive ion spectra over that of pure glycerol [14]. Triethanolamine, having a high proton affinity, is similarly advantageous for the formation of deprotonated molecules, generally $(M-H)^-$, in negative ion mass spectra. And the mixture, dithioerythritol/dithiothreitol, may play a role in preserving the tertiary structure of large peptides [15]. The liquid SIMS-TOF instrument constructed in our laboratory was thus intended to exploit these chemical advantages in combination with an analyzer having high ion transmission and high mass range.

Without the liquid matrix, molecular ions are commonly observed only very briefly under high flux conditions. This is generally attributed to "radiation damage" of the surface layer, and the role of the liquid matrix in repairing radiation damage and/or resupplying the surface sample layer has been noted above. The pulsed ionization/pulsed ion extraction feature of this particular instrument provides an opportunity to examine the time frame in which this occurs. Future experiments may further distinguish sample diffusion to the surface from other repair mechanisms, such as, removal of the damaged surface layer by sputtering, charge neutralization, etc. In particular, solution viscosity should effect the rates of diffusion. A further understanding of the fundamental mechanisms involved will, in addition, improve the analytical capabilities of liquid SIMS.

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