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Analyses of Cryogenic Samples Using Ion-Induced Desorption and Multiphoton Resonance Ionization

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In this study, ion-beam-induced desorption with multiphoton resonance ionization detection of desorbed neutral molecules is used to characterize frozen aqueous solutions. This type of matrix is of particular importance since it serves as a model for biological matrices. The time-of-flight mass spectrum, obtained in this way for a millimolar tryptophan/H_2O solution, is virtually identical to that for a submonolayer of tryptophan on a silicon wafer. The tryptophan signal from a frozen solution is demonstrated to have a linear dependence on concentration by using 4,4′-biphenyldiol as an internal standard. A detection limit of 2×10^{-6} M is also demonstrated. Since our ion beam samples one layer of 0.1 cm^2 and we assume 10^{15} molecules/cm^2 of ice, this concentration corresponds to approximately 4×10^6 molecules/layer. It is also shown that the signal exhibits an exponential decay with primary ion dose due to the accumulation of primary ion damage in the near-surface molecules.

INTRODUCTION

Surface characterization of organic molecules in complex matrices is essential to the resolution of many environmental and biological research initiatives. Mass spectrometric techniques offer considerable promise as a tool to selectively identify surface molecules in the presence of a wide range of background species because of the inherent selectivity associated with mass analysis. In fact, secondary ion mass spectrometry (SIMS), where surface molecules may be desorbed directly as intact ions by kiloelectronvolt ion bombardment, is being developed for many applications.[1]

Possibly the most difficult issue to deal with in SIMS measurements involves matrix ionization effects. The ion yield of molecular species, in particular, can vary over many orders of magnitude. These yields are also strongly dependent upon the surface electronic properties. Moreover, since molecular SIMS measurements are most always performed in the static regime (dose $< 10^{13}$ ions/cm^2), it is not generally possible to utilize Cs^+ or O_2^+ ion bombardment to optimize secondary ion yields. Cationization or anionization offers only slight improvements. As a result, characterization of molecular species in an ill-defined background is problematic.

Matrix effects have, of course, limited the application of SIMS to the microanalysis of heterogeneous samples such as biological material. In one case, however, a number of organic salts have been detected by SIMS after they were dissolved in H_2O and analyzed in a frozen ice matrix.[2] This matrix is particularly important for biological studies since it acts as a model for frozen biological tissue prepared for study in vacuo by freeze fracture techniques.[3]

Since 1982, our group has focused on the use of laser techniques to postionize neutral species that desorb from the surface.[4] Using resonance-enhanced multiphoton ionization, for example, it is possible to improve sensitivity by several orders of magnitude by efficient sampling of the more abundant neutral flux. Moreover, in many cases the signal intensity of the postionized neutral species is more closely related to the surface concentration.[5]

In this paper, we demonstrate the feasibility of characterizing ion-desorbed neutral molecules dissolved in frozen liquid matrices. The results show that for two previously studied compounds, tryptophan and phenylalanine,[6] spectra are obtained from a frozen water matrix which are very similar to those obtained when these molecules are ion-desorbed from thin films on Si wafers or metal foils. Similar results are found for pyrene dissolved in a frozen benzene solution. Moreover, using an internal standard to compensate for morphological variations between samples, it is shown that the response is linearly related to the concentration of the dissolved molecules. Detection limits for tryptophan are 2×10^{-6} M, corresponding to 4×10^6 molecules in the analyzed area. These limits are even somewhat improved over the SIMS results for organic salts[3] and suggest that the MPRI approach may indeed be useful for the direct analysis of frozen biological materials. The low detection limits also provide evidence that spatially resolved information may be obtainable using this methodology.

EXPERIMENTAL SECTION

The basic experimental apparatus has been described elsewhere.[7,8] Briefly, the vacuum chamber consists of an ion-pumped Perkin-Elmer Ultek TNB-X chamber with a base pressure of 5×10^{-9} Torr. The system is equipped with a load-lock assembly pumped by a Balzers TSU170, 170 L/s turbomolecular pump. This assembly allows new samples to be inserted in less than 10 min. A schematic diagram illustrating the arrangement of the important components is shown elsewhere.[9]

During the experiment, 5.6-μs pulses of primary Ar^+ ions (30 μA, 10 keV) are produced by deflecting the continuous ion beam, generated by a Physicon Model DP10-01 duoplasmatron source, across an aperture. The primary ion beam has a spot size of 0.1 cm^2 and is incident on the target at an angle of 45°. Sample charging is not encountered when samples of the size employed here (0.25 inches in diameter) are used. Presumably, these samples are small enough that the charge from one ion pulse dissipates (via the metallic sample holder) before the next ion pulse arrives.

The laser system consists of a Quanta-Ray Model PDL-2 dye laser pumped by a Quanta-Ray Model DCR-2A Nd-YAG laser triggered at a 30-Hz repetition rate. Frequency doubling of the

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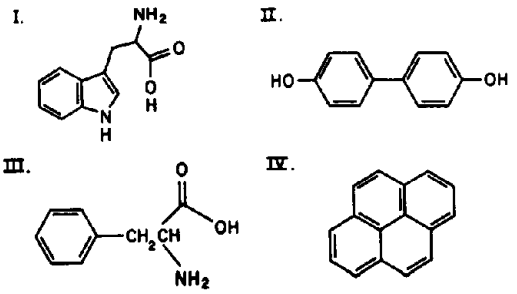

Figure 1. Structures of molecules used in this work: (I) tryptophan; (II) 4,4'-biphenyldiol; (III) 3-phenylalanine; (IV) pyrene.

dye laser output is accomplished using a Quanta-Ray Model WEX-1 wavelength extension unit. Laser energy is monitored using a Scientech Model 362 power meter.

For the molecules investigated in this work, shown in Figure 1, ionization is performed using 280-nm radiation because this wavelength has already been shown to produce good ionization efficiencies for molecules containing aromatic rings.[6,9] The 280-nm radiation is produced by doubling the output of Exciton Rhodamine 590 dye dissolved in spectroscopic grade methanol, yielding up to 17 mJ/6ns pulse. The cross-sectional area of the unfocused 280-nm laser beam is 0.38 cm^2, yielding a maximum, unfocused power density of 7.5×10^6 W/cm^2. The laser beam is passed above the sample with the nearest edge being approximately 1 mm from the sample surface. The laser is typically fired about 200 ns after the end of the ion pulse on the target.

Several experiments utilize the postionization of atomic Na to elucidate the role of ion beam damage in the decay of the signal with primary ion dose. For this case, ionization is performed using 330.3- and 660.6-nm light. The 660.6-nm light was produced with Exciton DCM dye in spectroscopic grade methanol with up to a 30 mJ/6ns pulse, and the 330.3-nm light was produced by frequency doubling some of this 660.6-nm light. In all Na experiments, the 330.3-nm power density is sufficient to saturate the excitation step. The cross-sectional area of the unfocused 660.6-nm laser beam is 0.57 cm^2, yielding a maximum, unfocused power density of 8.7×10^6 W/cm^2.

Following resonant postionization, the photoions are extracted into a reflecting TOF mass spectrometer and are detected with a Galileo Electro-Optics Corp. Model FTD 2002 dual microchannel plate (MCP) assembly. The resolution of the mass spectrometer varies depending on various parameters, such as laser beam diameter, and it is approximately 100 in the experiments presented here. Time-of-flight mass spectra are recorded by routing the analog signal from the MCP to a 100-MHz Digital Signal Processing (DSP) Technology Model 2001AS transient recorder equipped with a DSP Model 4100 averaging memory and averaging over 1024 laser pulses (34 s). Averaging over 1024 shots requires a total dose of 10^{12} incident ions into a 0.1-cm^2 area. The detection limit for tryptophan was determined while averaging over 9000 shots (5 min) since this additional averaging improves the signal to noise ratio by a factor of 3. Analog signals are digitized with 8-bit precision using the transient recorder and are transferred to a Digital Equipment Corp. MicroVax II via a CAMAC interface. The computer then manipulates and/or displays the mass spectra or calculates the areas under the peaks of interest.

An alternate method for data acquisition, used when only the intensity of a mass spectral peak is desired, involves routing the signal from the MCP to a Stanford Research Systems Model SR 250 gated integrator. This device is used as a boxcar averager with the integrated peak areas being recorded manually. The details of the pulsing/timing sequence used in the experiment have been previously reported.[7]

Sample cooling is essential for the experiments described here for two critical reasons. The first reason involves sample volatility. The molecules studied in this work have very low vapor pressures at room temperature, but due to the sensitivity of our technique, a gas-phase signal resulting from vacuum sublimation of the sample is frequently observed in the absence of any ion

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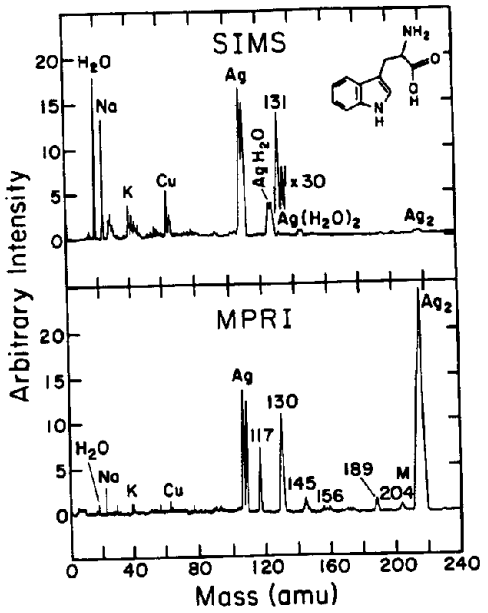

Figure 2. SIMS and MPRI (280 nm, 0.28 W), time-of-flight mass spectra of a frozen 1.1×10^{-3} M tryptophan in water solution on a silver backing.

bombardment. This gas-phase spectrum, obtained by turning off the incident ion pulse while leaving the MPRI laser on, often may be ignored, or it may be subtracted from the data when necessary. Some samples, however, have such a large gas-phase signal that the ion beam actually reduces the total signal by removing more molecules from the ionization region than it puts into it through sample sputtering. Under these conditions, computational manipulation of the data will not eliminate the interference, and the gas-phase signal must be reduced by cooling the sample. The second reason that sample cooling is important is that it makes many more samples vacuum compatible. Thus, sample cooling can make systems which are liquids at room temperature, such as aqueous solutions or biological tissues, amenable to analysis.

For experiments where sample cooling is necessary, the sample holder is conductively cooled in the load-lock and/or the chamber through contact with copper braids that are attached to liquid nitrogen (LN_2) reservoirs on LN_2 feedthroughs. An aluminum ring around the samples shields them from being irradiated by the laser which would cause noise in the form of laser ablation.

Most of the samples were prepared by depositing liquid solutions onto a precooled sample holder. The solutions were prepared using chemicals obtained from Aldrich Chemical Co. (used without further purification) and solvents (HPLC grade, water, and benzene) obtained from Fisher Scientific. Each biological substance was dissolved in HPLC grade water and was then either used directly or analytically diluted to the desired concentration. Pyrene was dissolved in benzene to a concentration of 1.1×10^{-3} M. These sample solutions were then deposited into a depression on the precooled (~−130 °C) copper sample holder in the load-lock using a Pasteur pipet. The solutions froze in 5–10 s, after which the load-lock was evacuated and the sample was transferred into the analysis chamber. The remaining samples were tryptophan pellets formed with a Parr Instrument Co. pellet press or a mixture of tryptophan and glycerol (Aldrich, 99+% pure) spread on a copper substrate. The glycerol samples were not cooled. All samples and experiments were repeated to confirm their reproducibility.

RESULTS AND DISCUSSION

The SIMS and MPRI mass spectra of a 1.1×10^{-3} M frozen aqueous tryptophan solution are shown in Figure 2. The MPRI spectrum is virtually identical to that obtained for a submonolayer of tryptophan on a silicon wafer.[6] Note that the laser postionization tryptophan signal is about 30 times larger than the corresponding SIMS signal, although the SIMS spectrum was recorded with one-tenth the dose of incident

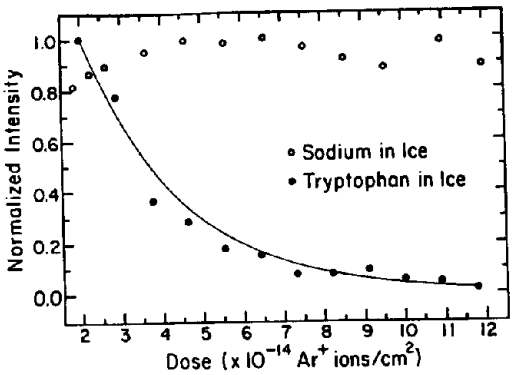

Figure 3. Exponential decay of tryptophan (130 amu) signal with incident ion dose due to the accumulation of primary ion damage in the near-surface molecules. The solid line represents an exponential fit to the data with a decay constant of 4×10^{-15} cm²/ion and a correlation coefficient of 0.987. The Na signal from a frozen NaCl solution does not exhibit such a decay.

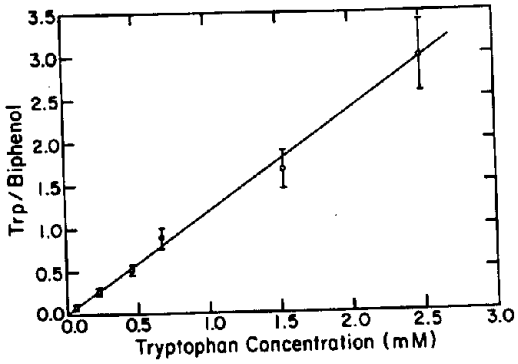

Figure 4. Tryptophan (130 amu) signal intensity as a function of concentration using 4,4'-biphenyldiol as an internal standard.

ions. The MPRI characteristic ions at mass to charge ratios of 204, 189, 145, 130, and 117 correspond to the molecular ion (M), (M – NH), (M – NH – CO_2), (M – $CHNH_2COOH$), and (M – $CHCHNH_2COOH$), respectively. The fragment ions are the result of rearrangements/fragmentations that result in the loss of one or both functional groups or part or all of the amino acid side chain. Also of interest is that the undesired signals from the sample holder (Cu, K, Na), the matrix (H_2O), and the substrate (Ag, Ag_2) are all, with the exception of the Ag_2 signal, smaller in the MPRI spectrum. The SIMS spectra have additional interfering signals resulting from Ag cationization of water clusters. The Ag_2 signal is larger in the postionization spectra probably due to an accidental Ag_2 resonance at this wavelength.

While tryptophan/ice spectra are recorded, it is apparent that the signal decays with time. This decay, shown in Figure 3, is exponential in nature and looks much like the decay observed when the sample is a submonolayer of tryptophan on a silicon wafer. In the submonolayer case this decay can be ascribed to the depletion of the tryptophan on the surface due to sputtering. However, when the sample is a frozen tryptophan solution that presumably contains tryptophan throughout the bulk, this explanation is no longer viable. While sample depletion plays a role in submonolayer samples, a more general cause of signal decay with primary ion dose is the accumulation of primary ion damage in the sample layers beneath the surface.[2] As a result, when lower layers are finally exposed, due to the erosion of the layers on top of them, the molecules that were initially present in them no longer retain their molecular identity (i.e. tryptophan molecules are now fragments of indeterminate origin).

In order to test this hypothesis, a frozen 1.0×10^{-3} M NaCl/H_2O solution was analyzed for Na content under the same conditions as were used for the tryptophan experiments,

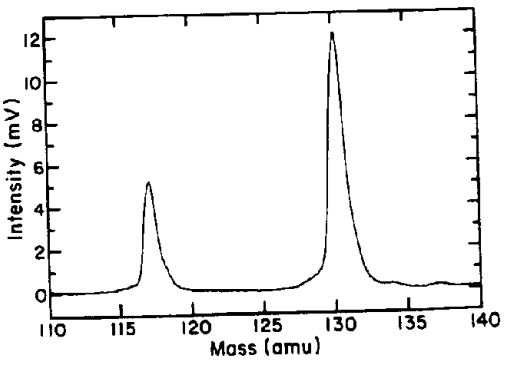

Figure 5. Representative tryptophan 130 amu peak (280 nm, 0.42 W) from one of three 1.35×10^{-4} M samples used to determine the detection limit. The incident ion dose is 3×10^{13} ions/cm².

except the ionization was performed using 330.0- and 660.6-nm light. This analysis, as seen in Figure 3, yields a constant Na signal as long as there is sample remaining. This result indicates that atomic species do not exhibit this decay and that the solute is present throughout the bulk of the sample.

In a second set of experiments, the postionized tryptophan signal is observed as a function of primary ion dose from pressed tryptophan pellets and from tryptophan dissolved in glycerol. The tryptophan signal from the pellet exhibits an exponential decay even when there is plenty of sample remaining. Since the entire sample is tryptophan, sample damage must indeed be accumulating in the surface layers. Similar results have been observed for secondary ions emitted from thick layers of organic films[2,10] and from molecules in ice matrices.[2] For the glycerol samples, the measured ion signal is observed to be constant with time as long as there is sample remaining. Glycerol is a liquid which allows fresh tryptophan from deep within the sample (out of range for primary ion damage) to diffuse to the surface, thus continually replenishing the surface with intact molecules. Both of these experiments support the hypothesis that the decay of the signal as a function of primary ion dose is the result of the accumulation of sample damage.

A concentration gradient in the sample could also result in a diminishing signal as lower layers are exposed. Such a concentration gradient could be formed by solute segregation on the surface during the freezing process. A plausible mechanism for this segregation is precipitation, when the saturation limit of the solute (1.1 mM) at 0 °C[11] is exceeded. This could result in a sample surface consisting of a thick tryptophan film whose concentration does not increase with further increases in sample solution concentrations. We do find that the tryptophan signal intensity (normalized to primary ion current and laser power) is no longer linear with concentration above values of 1.1 mM where it reaches a maximum value. While this might explain the observed signal decay with primary ion dose at high concentrations it cannot explain why it is also observed at low concentrations.

Molecular depth profiling may be difficult due to the observed accumulation of primary ion damage, although there is evidence that some molecular species remains even after sputtering to steady state.[2] It may be possible to improve on this procedure with the aid of an auxiliary sample erosion source such as a laser. Laser evaporation of frozen aqueous tryptophan solutions with resonant postionization has been demonstrated with little (30%) reduction in tryptophan signal after 13 000 laser shots (100 μs, 3.3×10^4 W/cm², 1064 nm).[12]

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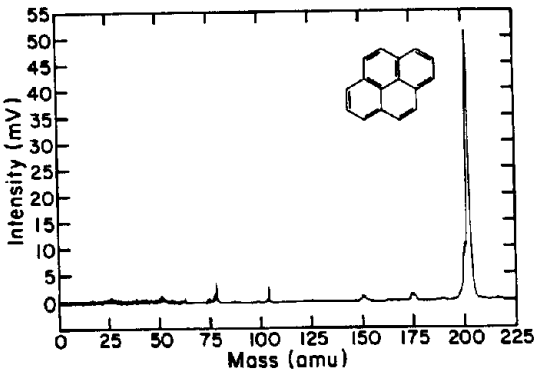

Figure 6. MPRI (280 nm, 0.23 W), time-of-flight mass spectrum of a frozen 1.1×10^{-3} M pyrene (MW = 202) in benzene (MW = 78) solution. The incident ion dose is 2×10^{12} ions/cm^2.

The combination of laser ablation and ion beam desorption might allow molecular depth profiling while maintaining of the spatial resolution available with ion beam desorption.

Due to the imprecise nature of the sample deposition, the samples exhibit greatly varying morphologies. These variations can cause fluctuations in sputter yields, differences in the overlap between the sputtered plume and the laser, and different extraction efficiencies. Although it is possible to obtain a linear relationship between the measured signal and concentration, precise analyses are facilitated by the use of an internal standard, 4,4′-biphenyldiol. Due to the similarity between the internal standard and the analytes, the internal standard also compensates for variations in primary ion current and laser power, so the data are easily analyzed after collection. For these experiments, equal amounts of analyte solution and internal standard solution are mixed to produce the sample solutions. While the analyte concentrations are varied, the internal standard concentration is held constant for all calibration curve samples. The resultant calibration curve is shown in Figure 4. As can be seen in Figure 4, the calibration curve is linear over the 3 orders of magnitude investigated. Likewise, a linear calibration curve was obtained for 3-phenylalanine, again using 4,4′-biphenyldiol as the internal standard. Therefore, it seems that the use of an internal standard is a promising methodology for experiments of this nature. A similar approach has recently been successfully utilized in SIMS studies of cyclosporin.[13]

In order to determine the detection limit for tryptophan in ice using this technique, replicate 1.35×10^{-4} M samples were analyzed under optimized conditions that include the pulsed extraction field as described by Pappas et al.[7] for reducing noise, relatively high laser power, and extended counting times. Due to the signal decay discussed above, extending the counting times beyond 9000 shots (5 min) is not liable to provide any additional benefit. The 130 amu (base) peak of one of the samples used to determine the detection limit is shown in Figure 5. Three identical samples were analyzed, using a dose of 3×10^{13} Ar^+ ions/cm^2 in each case, and the resulting signal to noise (S/N) ratios were averaged. When this average S/N ratio is extrapolated to 2, a detection limit of 2×10^{-6} M is obtained. Assuming a density of ice of 0.93 g/cm^3 at −130 °C, there should be about 10^{15} molecules/cm^2 for a dilute tryptophan solution. This corresponds to 4×10^6 molecules of tryptophan in the region sampled by the ion beam (0.1 cm^2) when the mole fraction of tryptophan in the samples is taken into account.

In order to demonstrate the generality of analyzing frozen solutions, frozen 1.1×10^{-3} M pyrene in benzene solution was examined. The resulting time-of-flight mass spectrum is shown in Figure 6. This spectrum was produced under essentially the same conditions as the tryptophan MPRI spectrum in Figure 2. The base peak in this spectrum (202 amu, 50+ mV) is the pyrene molecular ion. The signal from the matrix (benzene) at 78 amu is less than 10% of the pyrene signal even though there are about 10 000 benzene molecules to every pyrene molecular in the sample. Again, these results show that a frozen solution provides a spectrum similar to that obtained for the same molecule on a different substrate. It also shows that no special sample preparation is required, as a nonpolar system works as well as a polar one.

CONCLUSIONS

Not only are MPRI measurements frequently more sensitive and more easily quantitated than SIMS measurements, but also the detection of the neutral species also makes special sample preparation unnecessary. As a result, ion-induced desorption with MPRI allows the examination of many species in situ, thereby maximizing the amount of available information. For these reasons, the postionization of ion-desorbed molecules is a valuable addition to surface analysis techniques. This sample flexibility is even greater when cryogenic sample handling is available. Sample cooling can make systems which are liquids at room temperature, such as aqueous solutions or biological tissues, and many volatile species amenable to analysis. This sample flexibility has been demonstrated by analyzing samples in matrices that are not specially suited to this technique (frozen water, glycerol, and frozen benzene) and by analyzing a number of molecules that sublimate in vacuum (tryptophan, 4,4′-biphenyldiol, phenylalanine, and pyrene). Elsewhere, similar conclusions are reached through the analysis of particulates which are covered with somewhat volatile contaminants.[14]

The work presented here demonstrates that the analysis of molecules desorbed from frozen solutions can be performed efficiently. As noted earlier, the detection limit of 4×10^6 molecules in the region sampled by the ion beam suggests that if these molecules were packed together on the surface, they would cover an area of about 1 μm^2. This indicates that molecular imaging with a liquid metal ion source is feasible, especially since submicron imaging of atomic species[15] and micron imaging of molecular species[16,17] have already been accomplished with scanning microprobe SIMS. In addition, preliminary imaging experiments have been performed using liquid metal ion sources coupled with laser postionization.[18] Spatial resolution of 100 nm has been reported using MPRI of atomic species.[19] Most recently, atomic and molecular systems have been imaged with micron resolution using a femtosecond laser[20] for postionization. One exciting application of these techniques would be to map the chemistry of individual biological cells.

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