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Investigations into the interactions of a MALDI Matrix with organic thin films using C_{60}^+ SIMS depth profiling

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Molecular depth profiling of multilayer organic films is now an established protocol for cluster SIMS. This unique capability is exploited here to study the ionization mechanism associated with matrix-enhanced SIMS and possibly matrix-assisted laser desorption/ionization. Successful depth profiling experiments were performed on model bi-layer systems using 2,5-dihydroxybenzoic acid as the matrix with dipalmitoylphosphatidylcholine or phenylalanine. The interaction between the matrix and organic analyte is monitored at the interface of the films. Tri-layer films with D_2O as a thin film sandwiched between the matrix and organic layers are also investigated to determine what role, if any, water plays during ionization. The results show successful depth profiles when taken at 90 K. Mixing is observed at the interfaces of the films due to primary ion bombardment, but this mixing does not recreate the conditions necessary for ionization enhancement. Copyright © 2014 John Wiley & Sons, Ltd.

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Introduction

Adding matrices to an organic sample to enhance molecular ion signal can prove useful when analyzing low-concentration analytes. A variety of matrices have been shown to generate more molecular ion signal with SIMS.[1–4] Matrix-assisted laser desorption/ionization matrices and their ability to enhance the signal for a variety of organic molecules make them a viable resource for signal enhancement purposes. In conjunction with matrix/analytic combinations, application methodology of the matrices for SIMS purposes has also been thoroughly studied.[5–7]

The two common benefits of matrix-enhanced SIMS (MeSIMS) are increased ion signal and decreased fragmentation. The mechanisms behind these benefits are still being scrutinized;[8] however, more mechanistic information is necessary to fully exploit matrices and better tune them for SIMS experiments. This study aims to interrogate a simplified matrix-analyte mixture in an attempt to recreate and investigate the ionization enhancement mechanism.

The model system utilized for this experimentation employs a bi-layer of matrix deposited on top of an organic analyte and also a tri-layer that incorporates a thin layer of D_2O between the matrix and organic layers. The matrix chosen is 2,5-dihydroxybenzoic acid (DHB) because of its broad SIMS ion enhancement abilities.[1,4] Either dipalmitoylphosphatidylcholine (DPPC) or phenylalanine (PHE) serves as the organic layer. This experiment utilizes depth profiling analysis, which serves two purposes: the first of which is to monitor the formation of ions during the experiment via SIMS. The second purpose is to cause mixing of the matrix layers with the subsequent organic layers underneath. The intentional primary-ion-bombardment-induced mixing of unique layers in depth profiling has been exploited previously.[9] It is known that the matrix/analyte ratio is crucial to the ionization behavior in matrix-enhanced SIMS (MeSIMS) experiments and utilizing primary-ion-induced mixing of the matrix/analyte system may produce ideal stoichiometric conditions for ionization enhancement in the depth profile analysis. Should the ideal conditions be met, an increase in ion signal from the organic molecule may be observable in the plotted depth profile.

Materials and methods

Thin film preparation

Dipalmitoylphosphatidylcholine was obtained from Avanti Polar Lipids (Alabaster, AL, USA), PHE from Sigma Aldrich (St. Louis, MO, USA), and D_2O from Acros Organics (Geel, Belgium). DPPC films were prepared by spin coating a 5 µl aliquot of 20 mg/ml DPPC in chloroform onto a $5 \times 5\,mm^2$ Si wafer (Ted Pella, Redding, CA, USA) at 3500 rpm for 30 s yielding 100 nm-thick films. PHE samples were created by physical vapor desorption (PVD) onto a Si wafer. The PVD chamber has been employed previously for PHE thin film preparation.[10] Samples were stored in a dessicator until use.

After the initial sample preparation, the wafers were mounted onto a Cu sample holder and inserted into the mass spectrometer, which contains the PVD chamber. The sample block/wafer was cooled in the sample stage to 90 K and then inserted into PVD chamber where heated DHB was already subliming. After a minute of deposition, a 200-nm film of DHB was deposited onto the wafer/organic film. Immediately following deposition, the samples were inserted into the cooled stage in the analysis chamber and analyzed.

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Me-SIMS comparison spectra

Spectra were obtained from neat DPPC samples and mixed DPPC/DHB samples for comparative purposes. The analytes were dissolved in solutions of 1 : 1 acetonitrile : water (ACN : H_2O), where either pure water or water containing trifluoroacetic acid (TFA) was used. The 0.5 M DHB and 0.001 M DPPC in ACN : H_2O and ACN : (0.1%TFA)H_2O were prepared according to literature.[11] A 5 μl aliquot of 0.001 M DPPC in the ACN/H_2O solution was spotted onto a pre-cleaned Si wafer for use as a DPPC reference. 2.5 μl aliquots of DPPC solutions were mixed with 5 μl of corresponding DHB or DHB with TFA solutions and then spotted onto wafers. A total of three spectra from many unique points across each sample were collected.

D_2O films

D_2O was incorporated into the model system by means of a leak valve. D_2O was purified by five freeze–pump–thaw cycles. The leak valve was mounted in a separate chamber of the mass spectrometer, which is connected to the analysis chamber by a butterfly valve. Base pressure in the chamber was typically 2×10^{-9} torr. With the leak valve open, the pressure was adjusted to 1×10^{-7} torr. A sample, pre-cooled to 90 K, of either DPPC or PHE was exposed to D_2O while static SIMS spectra were constantly being obtained. Once the D_2O^+ signal reached the same intensity as $[M + H]^+$ ion signal from DPPC or PHE, the leak valve and the valve between the chambers were closed, and the vacuum system was allowed to equilibrate.

Film characterization

Film thicknesses were monitored with atomic force microscopy (AFM) profilometry on a Nanopics 2100 AFM profilometry (KLA Tencor, Milpitas, CA, USA). Crater thicknesses of DPPC and PHE films were recorded. However, thicknesses of DHB films could not be accurately recorded because DHB readily sublimes at room temperature under UHV conditions.

SIMS characterization and depth profiles

All depth profile analyses were collected at 90 K. Static SIMS spectra and depth profiles were recorded on a Bio-ToF mass spectrometer previously described.[12] A 20-keV C_{60}^+ source (Ionoptika IOG-C60, Warrior Park, UK) was utilized for sputtering and analysis. Depth profile spectra were obtained from a $200 \times 200\ \mu m^2$ analysis area in a $350 \times 350\ \mu m^2$ etch area. The primary ion beam typically measured 100–300 pA in d.c. mode. For analysis, a 60 ns pulse-width beam with a repetition rate of 3 kHz was used to collect the 100 000 summed spectra per cycle.

Results and discussion

Reference samples

In the reference spectra comparing DPPC signal with and without applied matrix, only a weak DPPC molecular ion was observed for any sample;[13] however, the common 184 headgroup ion was observed as a high-intensity peak in all spectra. It is known that the formation of the m/z 184 ion requires at least 1 proton from its surroundings, so DHB's role as a possible proton donor may be exploited.[13] An increase in signal of ~60% was observed for the 184 ion in both DHB/DPPC and DHB(0.1%TFA)/DPPC. A low-intensity peak at m/z 224 is observed and shows similar enhancement behavior.

Bi-layer films

Successful depth profiles were obtained for the DHB/DPPC samples at 90 K (Figure 1). A decreasing steady state and lateral mixing were observed when the samples were prepared and analyzed at higher temperatures. These effects were overcome by cooling the samples during DHB sublimation and analysis as evident by consistent steady-state values and distinct interface in the depth profile data. This observation is consistent with recent C_{60}^+ depth profiling experiments using amino acids where successful depth profiles at room temperature could not be obtained.[14] Unique ions at m/z 155 for $[M_{DHB} + H]^+$ and at 184 for the $[C_5H_{15}NPO_4]^+$ DPPC headgroup ion are observed. Both ions are present for a time at the interface of the films; however, no significant enhancement of the 184 ion is observed.

Tri-layer films

Water has the ability to lend protons during ionization and is an ubiquitous species during sample preparation and inside mass spectrometers. Water, in the form of D_2O, was added to investigate its role in ionization enhancement in this MeSIMS system. Samples of both DHB/D_2O/DPPC and DHB/D_2O/PHE were analyzed successfully with SIMS depth profiling. Ions from all three components of the respective films are present at the interface of the depth profile in Figure 2. The DPPC headgroup ions with an abstracted deuteron at m/z 185 are observed; however, their abundance is very low. This is not believed to be due to enhancement in the sense of matrix enhancement; rather, there is an abundance of free deuterons that are able to act in non-enhanced ionization pathways. In cases with thick D_2O layers, thick enough such that no mixing is observed between DHB and DPPC during depth profiling, deuterated molecules are observed at higher intensities (2× the ^{13}C steady state) showing a relationship between D_2O and DPPC, owing no

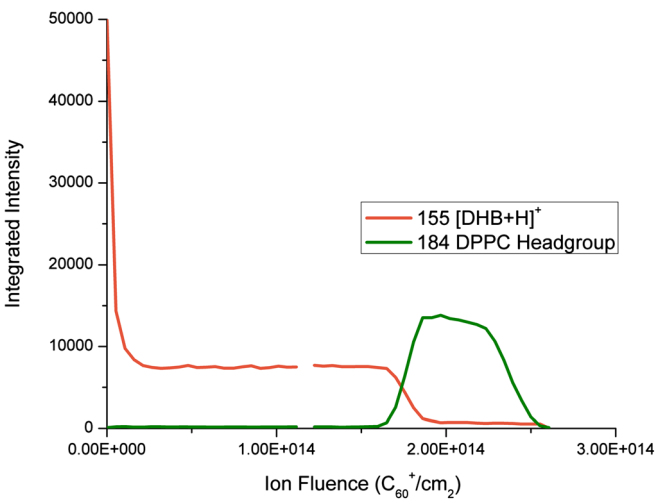

Figure 1. Depth profiles through the 2,5-dihydroxybenzoic acid (DHB)/dipalmitoylphosphatidylcholine (DPPC) films.

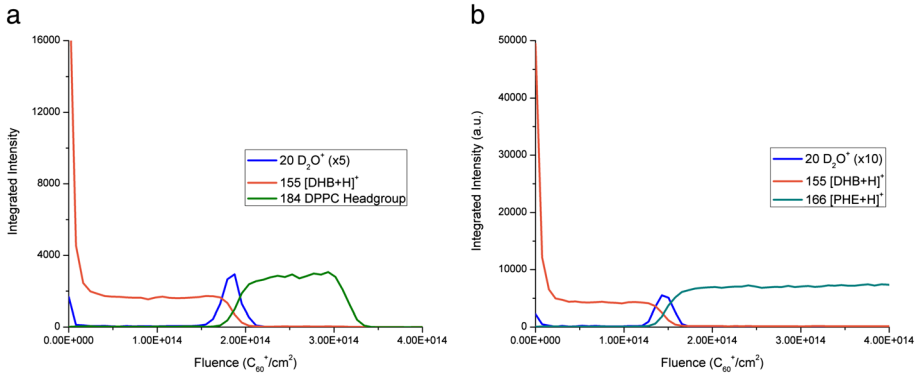

Figure 2. Depth profiles of systems with D_2O incorporated. The system in Figure 2a shows no enhancement of the 184 signal of dipalmitoylphosphatidylcholine (DPPC) for 2,5-dihydroxybenzoic acid (DHB)/DPPC films. Figure 2b shows no enhancement of the phenylalanine (PHE) signal in the DHB/DPPC films.

matrix enhancement on behalf of DHB or a combination of D_2O and DHB (Figure 2). This type of behavior has been exploited in previous studies[9] and leads the researchers to believe that the presence of water or D_2O in conjunction with DHB does not yield ionization enhancement in this study.

Conclusions

In this work, we have constructed a multilayer organic system aimed to mimic the MeSIMS environment and have acquired successful depth profiles on these constructs. The motivation for this experiment is to create an ion-beam-induced mixed interface where ionization enhancement may be observed. As is clear from Figure 1, however, no enhancement effects are present. Thus, we have not created the correct environment to promote ionization enhancement. Moreover, even the addition of a thin D_2O layer to the interface does not appear to influence the ionization probability in any measurable fashion.

This very well-defined layered structure would seem to be a model that allows conditions to be controlled in a systematic way. Perhaps the enhancement effects we are looking for might be found by choosing molecules that exhibit a larger MeSIMS enhancement effect or by using an organic molecule with a higher gas-phase basicity in order to more readily accept excess protons.

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