MALDI OF LAYERED POLYMER FILMS

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Introduction

Previous Small Angle Neutron Scattering (SANS) measurements of MALDI (matrix-assisted laser desorption and ionization) samples have shown limited mixing from conventional sample preparation methods such as electrospray or other methods of deposition from evaporation of dissolved components.[1] Therefore, molecular dispersions of polymer molecules in which each analyte is completely surrounded by polymer molecules are not present in typical MALDI preparations. This finding is consistent with the results of the solvent-free sample preparation method[2] which involves physical grinding of a polymer/matrix/cationization agent mixture. This physical mixing method suggests that limited mixing takes place on a molecular level. We have developed a layered preparation method to generate structured targets with well-defined size and composition. This probes the effect of the location of individual polymer molecules in micrometer-sized layers of pure polymer on the strength of their MALDI signals. The variation of MALDI signal intensity with the analyte's location within the aggregates suggests a potential biasing effect of molecular mass determinations.

Layering of MALDI samples has been examined previously in different applications. Layering of pure matrix and matrix/polymer mixtures has been used to improve signal intensity, but the matrix/polymer layer itself has a two-phase morphology.[3] A layering procedure has been described in which the polymer and matrix are sequentially spotted without previous mixing.[4] However, this procedure often uses the same solvent for both solutions, so redissolving and mixing cannot be ruled out. The sequential layering of different polymers on top of a matrix using selective solvents has not been described to the best of our knowledge.

Experimental[*]

Materials: Deuterated Polystyrene (DPS) and conventional hydrogenous Polystyrene (HPS) with matched molecular masses of approximately 7000 g mol^{-1}, were used in this study. They have nearly identical thermodynamic properties such as solubility and can be easily distinguished by SANS and MALDI. Layered samples are made by successive applications of matrix, HPS, and DPS to the MALDI sample using selective solvents to prevent mixing of the components. In the first set of samples, the 2,5-dihydroxybenzoic acid (DHB) matrix containing 1 % by mass fraction silver trifluoroacetate (AgTFA) dissolved in tetrahydrofuran was electrosprayed on the target and the HPS and DPS solutions in cyclohexane were hand spotted on top. Cyclohexane in a non-solvent for the matrix so that no mixing of the matrix and the polymers can occur. In a second set of samples, HPS and DPS were spin coated onto a silicon wafer for measurement of any mixing between the sucessively applied layers. In a third set of samples, tri-α-naphthyl benzene (TαNB) was used as a matrix.[5] It forms a glassy surface that creates smooth films when applied by spin-coating or hand spotting techniques. The adsorption of the matrix at the irradiation wavelength of 337 nm can be increased by addition of mass fraction 25 % 1-4 diphenyl butadiene. Layers of HPS or polyethylene glycol (PEG) were applied from solutions of varied concentration to control the total thickness. In the fourth series of samples, sequential layers were applied by using measured amounts of the HPS, DPS, or PEG on top of the previous layers. By using selective solvents (cyclohexane for PHS or DPS and acetone for PEG), application of new layers would not dissolve previous polymer layers.

Instrumentation: Neutron reflectivity was used to measure the density profile of layered samples using the NCNR NG7 instrument. The mass spectrometry was performed on a Bruker REFLEX II instrument in reflectron

mode using delayed extraction. Ions were generated using a 337 nm wavelength nitrogen laser.
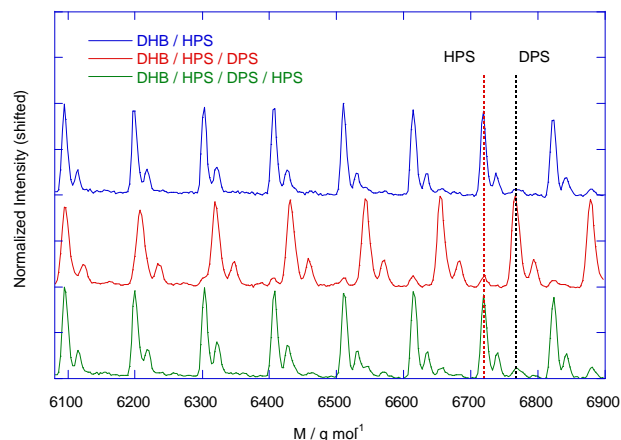

Figure 1. MALDI from layered samples. First layer, electrosprayed DHB/AgTFA; second layer, HPS, third layer DPS.

Results and Discussion

DHB-HPD-DPS MALDI: The MALDI of samples having successive layering of electrosprayed DHB and several applications of HPS and DPS are shown in figure 1. Cyclohexane is a non-solvent for the components of the matrix layer so mixing of the components of the matrix and successive layers was prevented. Figure 1 shows that HPS and DPS are easily distinguished by MALDI. At the left hand side of the figure, peaks of HPS and DPS coincide, but at the right hand side, the peaks are cleanly separated and the relative amounts of the two layers can be easily discerned.

The predominant signal is from the top-most layer, suggesting that biasing may occur depending on the location of the analyte. There are complicating factors in the analysis of this system, however. First, electrosprayed surfaces can have a roughness of micrometer size scale, which is similar in magnitude to the thickness of the HPS and DPS layers. Secondly, the application of the polystyrene layers may involve some mixing of HPS and DPS due to similar solubility. The hand spotting method involves relatively slow evaporation of subsequent layers, which may allow redissolution of the previous layer.

HPS-DPS Neutron Reflectivity: Another valuable property of HPS and DPS combinations is the ability distinguish them by neutron scattering and reflectivity. The zone of mixing between successive layers can be measured by neutron reflectivity. Figure 2 shows a fit of the density profile of two successive layers spin coated onto a molecularly smooth silicon wafer. The first application is HPS and the second is DPS. The total thickness of the two nearly identical applications is approximately 0.17 μm. The zone of mixing between the two layers is approximately 0.03 μm. The rapid drop at the right of the distribution
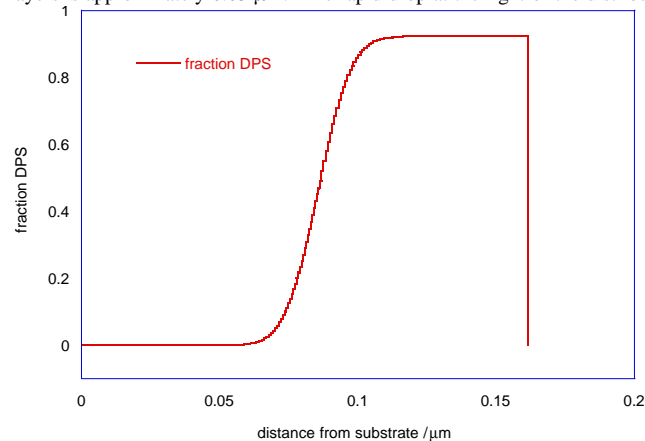

Figure 2. Neutron reflectivity from sequential spin cast layers of HPS and DPS. Fit of density profile.
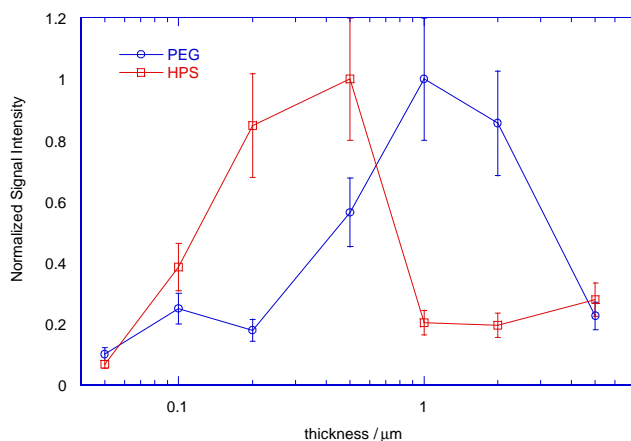
Figure 3. Normalized intensity for layered PEG or HPS on TaNB for films of varied thickness.

represents the polymer-air interface. As can be seen, the successive spin coating of two layers involves solvent evaporation that is sufficiently rapid to prevent disappearance of the individual layers. Hand spotting, however, involves a slower evaporation. To eliminate this problem with HPS and DPS, spin coating is being developed as a MALDI preparation method.

TαNB Matrix: To address the problem of the rough surface that results from the electrospray process, TαNB is used as a matrix. Its smooth, interpenetrateable surface produces a more uniform structure. It has a glass transition temperature that is well above ambient (75 °C) so films are rigid and stable. Also, solvents can be chosen such that the solution being deposited does not dissolve the components of the layer below.

A series of films were made from HPS or PEG with a wide range of thicknesses from approximately 0.05 µm to 5 µm. Figure 3 shows the relative signal intensities of HPS and PEG as a function of polymer thickness. Both pass through a maximum. Therefore, over this size range, the thicker films cannot be sampling all of the analyte. The error bars are one standard deviation of multiple measurements and are taken as an estimate of the standard uncertainty.

TαNB-HPS-PEG-DPS.: Figure 4 shows the MALDI signal from a four layer sample. Both the second layer (HPS or DPS) and the third layer (PEG) can be seen, but the top layer (HPS or DPS) is absent. The location of cationization agents can also be controlled by the layering process. The example shown in figure 4 had the AgTFA cationization agent added to the TαNB layer only. Therefore, the uppermost polystyrene layer was completely isolated from the cationization agent and the lower polystyrene layer was in contact with the AgTFA only at the interface between the first and second layers.

Conclusions

A multi-polymer layering technique is described that uses specific solvents
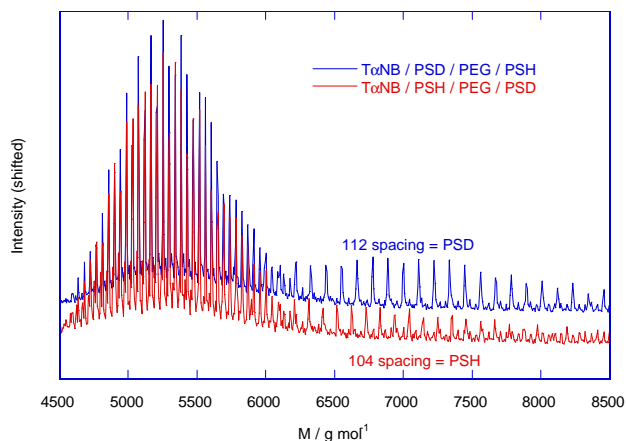

Figure 4. MALDI from layered films. First layer, TαNB; second layer, (HPS or DPS); third layer PEG, fourth layer, (DPS of HPS).

to isolate the polymers. It is similar to previous multi-layer deposition techniques[3-4] but employs additional methods of isolating the successive layers. By using selective solvents for a polymer or matrix material that will not redissolve the previous layer, the mixing is minimized and the layer components are isolated. Some combinations such as HPS and DPS do not have selective solvents that could isolate them, as is the case with the combination of HPS and PEG. However, the amount of mixing can be minimized by use of spin coating which promotes very rapid solvent evaporation. Neutron reflectivity shows that a layered structure is formed even for low molecular mass polymers. The separate MALDI signals can be used to probe the effect of cationization agent–matrix–polymer proximity on signal strength.

References

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