MALDI of Structured Polymer Particles

Barry J. Bauer and Kathleen M. Flynn

National Institute of Standards and Technology, Gaithersburg, MD, USA

Introduction

The solvent-free sample preparation method[1] involves physical grinding of a polymer/matrix/cationization agent mixture. This physical mixing method suggests that limited mixing takes place on a molecular level. Small Angle Neutron Scattering (SANS) has been used to measure the size and level of dispersion of synthetic polymers in MALDI (matrix-assisted laser desorption ionization) samples on a nanometer size scale[2] and it has shown limited mixing from conventional sample preparation methods such as electrospray or other methods of deposition from evaporation of dissolved components. Therefore, molecular dispersions of polymer molecules in which each analyte is completely surrounded by polymer molecules are not present in typical MALDI preparations. We have developed a layered preparation method to generate structured targets with well-defined phase size and composition. This will probe the effect of the location of individual polymer molecules in micrometer-sized phases of pure polymer on the strength of their MALDI signals. The variation of MALDI signal intensity with the analyte's location within the aggregates may suggest 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.

Methods

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 identical thermodynamic properties such as solubility and can be easily distinguished by SANS and MALDI TOF MS. 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. 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. MALDI easily distinguishes the repeat units of (104 and 112) g mol^{-1} for HPS and DPS.

Tri-α-naphthyl benzene (TαNB) is a glassy matrix that forms smooth films.[5] TαNB and AgTFA in toluene were placed in a circular mold that confined the area covered. The thickness was controlled by using an appropriate concentration. Sequential layering was performed by placing measured amounts of the HPS or DPS on top of the previous layer. Poly(ethylene glycol) (PEG) was dissolved in acetone and was used in the layering experiments. The cyclohexane and acetone were selective solvents and did not re-dissolve the previous layers.

Results

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 HPS and DPS layers. Secondly, the application of the polystyrene layers may involve some mixing of HPS and DPS due to similar solubility.

To eliminate these problems, TaNB is used as a matrix. Its smooth, interpenetrate able surface produces a more uniform structure. Also, solvents were chosen such that the solution being deposited does not dissolve the components of the layer below. Figure 2 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.

Figure 3 shows the MALDI signal from a three layer sample. Both the second PEG layer and the third HPS layer can be seen. It is notable that both figures 1 and 3 show signals from polystyrenes that were physically separated from the AgTFA cationization agent and therefore could not have been charged in the solid state.

In conclusion, a new multi-polymer layering technique is described that uses specific solvents to isolate the polymers. The separate MALDI signals can be used to probe the effect of cationization agent–matrix–polymer proximity on signal strength.

References

1. Trimpin, S.; Rouhanipour, A.; Az, R.; Rader, H. J.; Mullen, K. *Rapid Communications in Mass Spectrometry* **2001**, *15*, 1364-1373.
2. Bauer, B. J.; Byrd, H. C. M.; Guttman, C. M. *Rapid Communications in Mass Spectrometry* **2002**, *16*, 1494-1500.
3. Schriemer, D. C.; Li, L. *Analytical Chemistry* **1996**, *68*, 2721-2725.
4. Meier, M. A. R.; Schubert, U. S. *Rapid Communications in Mass Spectrometry* **2003**, *17*, 713-716.
5. Bauer, B. J.; Guttman, C. M.; Liu, D. W.; Blair, W. R. *Rapid Communications in Mass Spectrometry* **2002**, *16*, 1192-1198.

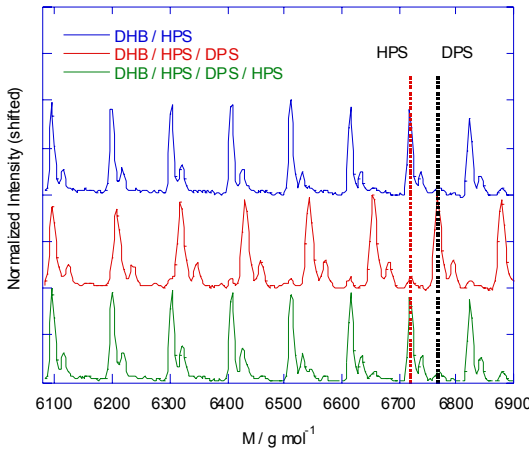

Figure 1. Normalized MALDI from electrosprayed DHB/AgTFA with layered HPS and DPS.

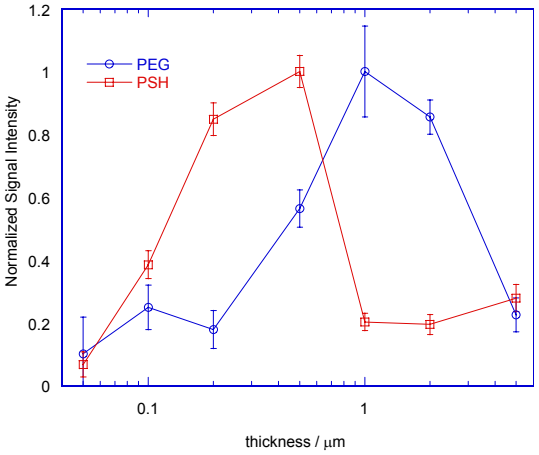

Figure 2. Normalized intensity for layered PEG or HPS on TαNB for varied thickness'.

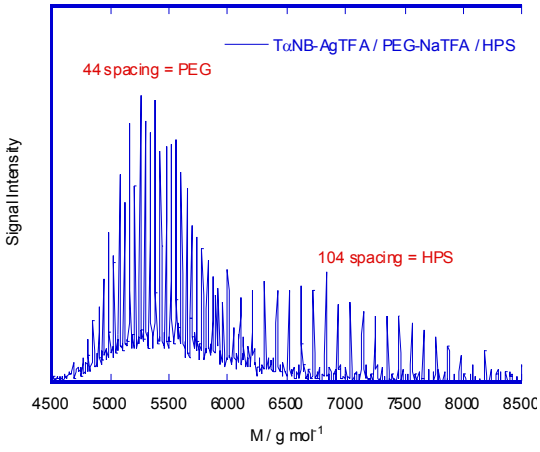

Figure 3. MALDI from layered TαNB / PEG / HPS.