

# RESPONSE SATURATION OF POLYSTYRENE IN MALDI-TOF-MS

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## Introduction

For synthetic polymer samples, matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometry (MS) can yield qualitative information about end groups and repeat units, and also quantitative information, such as molecular masses and molecular mass distributions. Accurate quantitative measurements require equimolar responses for all sample species in the mass range over which a sample is distributed, or at least a predictable variation in response. Presently, quantitative results from MALDI are considered reliable only for narrowly distributed samples; i.e., those with a polydispersity index (PD, where  $PD = M_w/M_n$ ) below about 1.2 [1,2]. There are instrumental and sample-related factors that can influence the relative responses of sample species, but understanding is not complete [3-7]. There is not a definitive explanation for the difficulties MALDI has with more broadly distributed samples, and it has not been established whether or not the currently recognized polydispersity limits can be extended by adjustments in sample preparation or by instrumental developments.

Improvements in understanding of MALDI mechanisms and of the factors that influence polymer response in MALDI perhaps can extend the range of samples to which MALDI can successfully be applied. It should also help to guide sample preparation and mass spectrum acquisition procedures. In this work we examine the dependence of signal strength on polymer loading and how it is affected by selected sample preparation factors. As with any quantitative analytical technique, there is a limited range over which signal level increases with analyte concentration. Work with low molecular mass poly(ethylene glycol) (PEG) samples indicates that with the matrix 2,5-dihydroxybenzoic acid (DHB), signal versus concentration curves reach a plateau, rather than a peak [8]. This also appears to be true for PS with the matrix retinoic acid.

We refer to the analyte level at which signal strength reaches a plateau as the saturation level. It could conceivably be influenced by several factors, such as the availability of metal cations, the degree of isolation achieved by the analyte in the matrix, and the laser fluence used. Which factor is limiting may depend upon the particular set of experimental conditions used. For the PEG/DHB system it has been shown that the saturation level decreases with increasing molecular mass of the polymer [8]. This may have implications for the analysis of samples with broad molecular mass distributions. For example, suppose a polymer is present at a level such that the highest mass portion of its distribution is above its saturation level but the lower mass portions are not. Work with the PEG/DHB system suggests that it may still be possible to obtain an accurate representation of the distribution, but only under a limited set of values of tested factors that influence polymer response [8]. Experiments with other polymer and matrix systems are necessary for a broader view of the effects of response saturation in MALDI.

## Experimental

Experiments were performed on a Bruker Reflex II MALDI-TOF Mass Spectrometer operated in linear mode, with a dual microchannel plate detector and a 3 ns pulse width nitrogen laser [9]. All mass spectra were acquired using delayed extraction. A narrow molecular mass distribution polystyrene (PS) sample, PS7900, was used, where the number refers to the nominal molecular mass in g/mol. The matrix used was retinoic acid. Tetrahydrofuran (THF) was used as solvent and silver trifluoroacetate (AgTFA) was used as a cationizing agent in all experiments. Matrix, PS, and AgTFA solutions were prepared separately and mixed prior to deposition on the sample target. The matrix, PS and AgTFA amounts given in the text refer to the amounts in the final, mixed solutions, not to the amounts deposited on the target. Hand-spotted samples were deposited by pipette and allowed to dry in ambient air. Electrospray sample deposition was performed from a 0.15 mm i.d. stainless steel syringe needle at 5 kV, with a spray tip to target distance of (2 to 3) cm and a flow rate of 4  $\mu\text{L}/\text{min}$ .

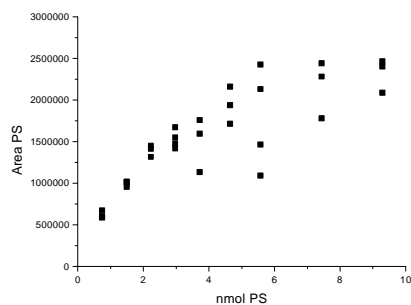
## Results and Discussion

Figures 1 through 4 contain plots of the integrated PS7900 area response versus amount of PS7900 under selected conditions. Matrix and AgTFA levels were held constant. Ions below  $m/z$  2500 were suppressed using the instrument's ion deflector. In Figures 1 and 2, sample deposition was by the "dried droplet" method. THF solutions tend to spread over the target surface and dry quickly. With THF solvent and the matrix retinoic acid the uniformity of sample appearance is usually much better than for biological MALDI samples dried from aqueous solutions. Still, regions of different sample appearance (e.g. crystal size, sample thickness) can result from a single sample application, and PS signal strength can noticeably change on moving from one region to another. In obtaining mass spectra for Figures 1 and 2, we attempted to acquire from regions that appeared uniform, relatively thin, and with fine crystals. These tended to give the strongest signal level for a given sample application. Mass spectra for Figure 1 were acquired at a laser fluence about 25% higher than the threshold for detecting PS7900 ions, and those for Figure 2 at a laser fluence about 50% higher than threshold. With the variation in signal level achieved it is difficult to specify the saturation levels, but they appear to be similar in the two cases. Thus under the specified set of conditions the laser fluence is not the limiting factor. At all levels of PS7900 the AgTFA level is in excess; however, either an inhomogeneous distribution of AgTFA or competition for silver from the matrix could possibly lead to silver availability being the limiting factor. A limitation to the amount of PS7900 isolated in the matrix could also account for the observed saturation level.

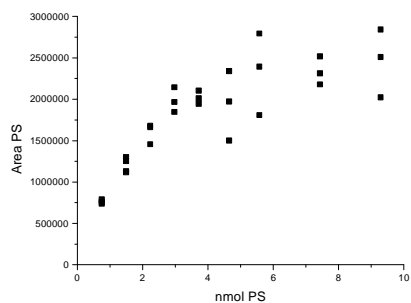
In Figures 3 and 4 sample deposition was by electrospray. The laser fluence levels in Figures 3 and 4 correspond to those in Figures 1 and 2, respectively. The saturation level in Figure 3 is in a similar range to that in Figures 1 and 2, and possibly a little higher. The saturation level in Figure 4, however, is two times or more as high. Thus under the conditions of Figure 3, laser fluence appears to be the limiting factor. Note that the signal levels in Figures 3 and 4 are lower than the corresponding ones in Figures 1 and 2. This could perhaps be related to sample thickness (thicker in the electrosprayed samples) or to differences in crystal characteristics. In the electrosprayed samples, apparently, laser energy is not converted as efficiently into the channels needed for desorption or ionization of PS as it is in the regions sampled from the dried droplet samples.

Improved dispersion of analyte has been demonstrated for electrospray of biological samples in MALDI, compared to dried droplet deposition [10]. Some improvement is likely also for polymer samples. Electrospraying produces fine, rapidly drying droplets, so that separation of sample components that can occur during crystallization should be minimized. Thus improved isolation of analyte in the matrix must be suspected as the reason for the increased saturation level in Figure 4. Another possible influence is that, due to uneven dispersion of PS, the effective matrix-to-analyte ratio in the sampled regions of the hand-spotted samples is lower than the nominal matrix-to-analyte ratio. Some effect of electrospraying on cation attachment to PS can also not be ruled out.

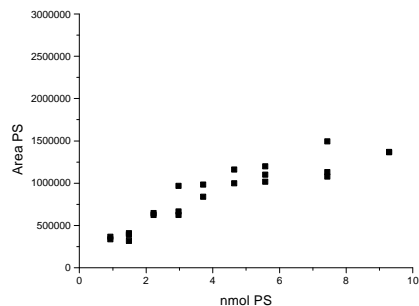
The precision of measurements of signal strength in MALDI is generally considered poor. Electrospray sample deposition has been found to improve precision considerably [10]. In this work the variance of the measurements from the electrosprayed samples appears constant over the range of PS levels tested. An estimate of the standard deviation of a single measurement obtained by pooling over all measurements is 100,000 area units. For the hand-spotted samples, the variance at higher PS levels is clearly greater than that at lower PS levels. A pooled estimate of the standard deviation of a single measurement for the lowest four PS levels is 70,000 area units, and for the upper five PS levels a pooled estimate is 250,000 area units. Note that since sampling from the hand-spotted samples was restricted, these estimates are likely to be lower than an estimate obtained from unrestricted sampling.



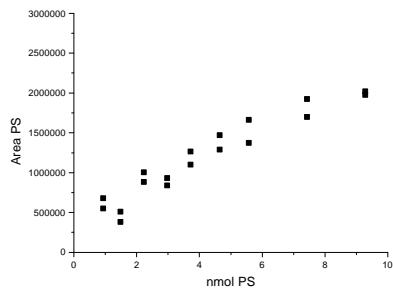
**Figure 1.** Integrated area versus amount PS7900 in final sample solution. Hand-spotted samples using low laser fluence level. The matrix level is 10,000 nmol and the AgTFA level is 33 nmol at all PS levels.



**Figure 2.** Integrated area versus amount PS7900 in final sample solution. Hand-spotted samples using high laser fluence level. The matrix level is 10,000 nmol and the AgTFA level is 33 nmol at all PS levels.



**Figure 3.** Integrated area versus amount PS7900 in final sample solution. Electrospayed samples using low laser fluence level. The matrix level is 10,000 nmol and the AgTFA level is 33 nmol at all PS levels.



**Figure 4.** Integrated area versus amount PS7900 in final sample solution. Electrospayed samples using high laser fluence level. The matrix level is 10,000 nmol and the AgTFA level is 33 nmol at all PS levels.

## Conclusions

Electrospray sample deposition can increase the saturation level of PS in MALDI. It is suspected that this is due to a greater degree of isolation of PS oligomers in the matrix. There is also some difference between electrospayed samples and hand-spotted samples in how efficiently PS ions are detected at a given laser fluence, at least for the particular regions of the hand-spotted samples from which mass spectra were acquired. The effects of other matrices, the molecular mass of PS, and cation amount will be probed in other experiments. These experiments should contribute to understanding of the factors that influence polymer response in MALDI and may be relevant to the analysis of samples with broad molecular mass distributions.

## References

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- (9) Certain commercial materials and equipment are identified in this paper in order to specify adequately the experimental procedure. In no case does such identification imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the items identified are necessarily the best available for the purpose.
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