

**Surface Morphology Variations in MALDI-TOF-Mass Spectrometry  
Samples Prepared by Various Techniques Examined by  
Scanning Electron Microscopy**

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## **Introduction**

Believing that the relationship of the polymer molecules and matrix crystals in a sample preparation had a significant influence on the evolution of charged polymer species when the target was ablated by UV laser, we investigated the use of non-crystalline, UV absorbing matrix compounds. If the random crystallization of the matrix produces inhomogeneous sample surfaces, eliminating the matrix crystallization event from the sequence of events occurring during sample preparation seem to hold promise for improved sample homogeneity. Using a series of specially synthesized glassy azodyes as matrix material and electro spray sample deposition, we generated replicate MALDI spectra with integrated peak area variations as low as 4.4%. However, due to solubility problems, the applicability of the azodyes was limited to relatively low molecular mass poly(ethylene glycol) polymers. Additionally, the lack of commercial availability of the azodyes limits their use by others.

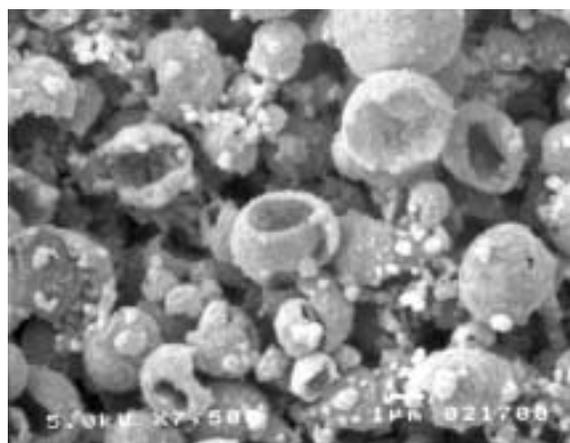
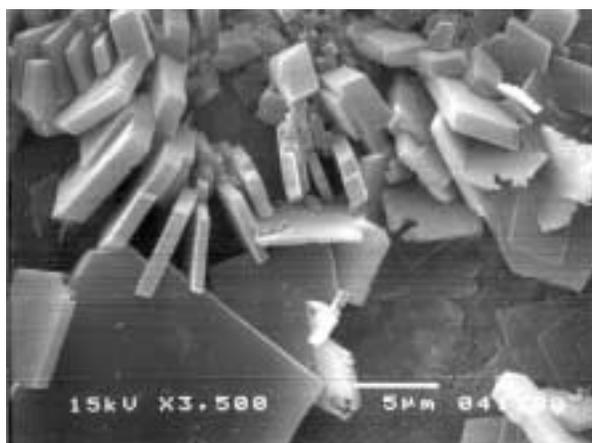
During the above investigations, we discovered that the electro spray sample preparation technique could, by itself, provide a significant improvement in the consistency of peak signal integrals. This effect was not realized if the electro sprayed sample surface coating was thin, prepared using only 2 to 4  $\mu\text{L}$  of matrix/polymer solution. Improvement in the statistical variation of the peak intensities occurred when the electro sprayed coating was made thick. Lacking a technique to measure the thickness of our samples, they were designated as thick or thin by the volume of matrix/polymer solution sprayed onto the target surface. A solution volume of 30 to 40  $\mu\text{L}$  was sprayed to produce a thick sample. An attempt to measure target coating thicknesses by focusing a conventional light microscope on the target base and then on the coating surface indicated a coating thickness of approximately 170  $\mu\text{m}$ .

## **Experimental Methods**

To gain further understanding of the MALDI sample preparation process, we have examined hand spotted and thick electro sprayed targets prepared with a glassy azodye, dithranol and 2,5-dihydroxybenzoic acid (DHB) by scanning electron microscopy (SEM). An electro spray apparatus was assembled from the following components: A Spellman High Voltage Electronics Corp. (Hauppauge, NY 11788) adjustable high voltage DC power supply, a Harvard Apparatus (Holliston, MA 01745) Model 22 syringe pump and a manually adjusted x-y-z positioning movement (Edmund Scientific, Barrington, NJ 08007) to hold the MALDI target. All these components were mounted on a small (8" x 24") optical bench plate (Edmund Scientific, Barrington, NJ 08007) to provide easy, reproducible alignment of the syringe needle and MALDI target. For SEM examination, samples were given a light gold coating. A JEOL model 5300 electron microscope was used for all SEM microscopy. MALDI analyses were performed on a Bruker Reflex II MALDI-TOF mass spectrometer<sup>1</sup>.

## Data and Results

The SEM micrographs below both show a dithranol, silver trifluoroacetate, and polystyrene 10,200 MW polymer preparation. The illustration on the left was prepared by hand spotting. The one on the right is a thick electro sprayed sample. The more uniform structure of the electro sprayed sample, apparently composed primarily of hollow spheres of 2 to 3 micrometers diameter, may be a significant factor in the generation of replicate MALDI analyses with small variations in integrated peak area intensities.



If, as we believe, a synthetic polymer is held at the surface of the organic matrix crystal or in the crystal interstices, one would expect the polymer to be more homogeneously distributed if the matrix forms many small crystals and provides numerous interstices. By making a thick sample coating, abundant polymer signal is typically seen well before laser power has been increased to a level high enough to ablate all the way through the sample to the underlying stainless steel. Consequently, the amount of polymer going into the gas phase is controlled by laser power, which typically varies by 4 to 5 per cent over 150 to 200 laser shots. Replicate analyses with small standard deviations in total integrated peak area can be obtained.

Azodye/PEG 1470		DHB/PEG 5000	Dithranol/PS 7900
Repeat #	peak area	peak area	peak area
1	3465547	2643232	822008
2	3578749	2844063	912473
3	3417726	3102621	948421
4	3398540		1055399
5	3657623		
RSD =	3.2%	RSD = 8.0%	RSD = 10.3%

## References

1. Certain commercial items are identified in this paper in order to adequately specify the experimental procedure. This does not imply endorsement by N.I.S.T.