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The Optimization of MALDI-TOF-MS for Synthetic Polymer Characterization by Factorial Design*

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One of the most significant issues in any analytical practice is optimization. Optimization and calibration are key factors in quantitation. In matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS), one of the limitations restricting quantitation is instrument optimization. Understanding which parameters are most influential and the effects of these parameters on the mass spectrum is required for optimization. This understanding is especially important when characterizing synthetic polymers by MALDI-TOF-MS due to the complex nature of synthetic polymers.

The two matrixes studied were *all-trans* retinoic acid (RA) and dithranol. The polymer concentrations studied initially were 1:75:1, 1:150:1 and 1:225:1 by mass of polystyrene (PS):RA:silver trifluroacetate (AgTFA) and 1:40:1, 1:80:1 and 1:120:1 by mass of PS:dithranol:AgTFA. The spectra were taken from random spots on the target. Each mass spectrum was the sum of 100 laser shots. The signal-to-noise ratio was calculated for each polymer distribution using the central peak in the polystyrene molecular mass distribution (MMD) at a mass of approx. 8900 u.

A 2^{5-1} fractional factorial design was employed to study the optimization of MALDI-TOF-MS. This is an orthogonal design, which is a highly efficient design. Orthogonal designs yield precise effect estimates with minimal bias in addition to the parameter effects and interaction effects of any factorial design. The 2^{5-1} design is beneficial due to its ease in interpreting the results. Because there are only two options at which each parameter is run, it is easy to interpret parameter effects.

The optimization of a 9 ku polystyrene is different than for a low mass polymer. Isotope peaks are not resolvable at 9 ku in our MALDI-TOF-MS. As a result we chose to consider only signal-to-noise as a response variable. At lower mass both signal-to-noise and resolution can be considered in optimization. Our five factors in our design are all instrument parameters. Laser energy and delay time are factors that influence how much sample is desorbed and cationized in the MALDI process. The lens and extraction voltages influence the ion envelope. The detector voltage increases the sensitivity of the detector, so that all the ions reaching the detector are detected, but if the detector voltage is too high, a loss in signal-to-noise may result. Two sample preparation parameters were also considered in this analysis; the matrix used in the analysis, as well as the polymer concentration. These have both been shown to influence the measured molecular mass distribution in synthetic polymers.

In a factorial analysis, mean plots are used to represent the data and give a first look at the parameter effects. Figure 1 shows the plot of the overall means. A first look at the data reveals that the most influential instrument parameters on the signal-to-noise are detector voltage and the time length of the delayed extraction. Each of the instrument parameters has three data points. Each point represents a mean signal-to-noise for the factors at each of the values were data was collected. Since higher signal-to-noise values represent optimization of the instrument, a higher detector voltage yields a more optimized mass spectrum. The longer delay time also yields higher signal-to-noise values. Laser energy, extraction voltage and lens voltage change slightly in the range of values considered, but their effects are small relative to the detector voltage and the delay length.

The last three plots in Figure 1, test, matrix and polymer concentration, represent means determined by the experimental design. The test represents all six sample sets, three polymer concentrations in either dithranol or retinoic acid. The first three test data points represent data obtained in dithranol, and the last three test points are means of signal in RA. The matrix data reveals higher signal-to-noise values in RA, and the polymer concentration yields higher mean values at the highest polymer concentration.

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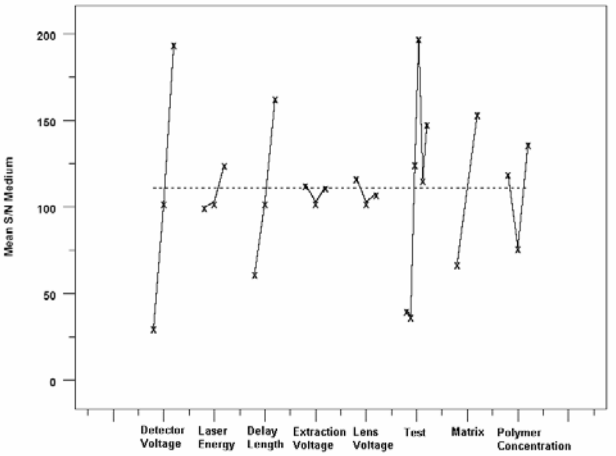

Figure 1. The mean effects plot for each factor.

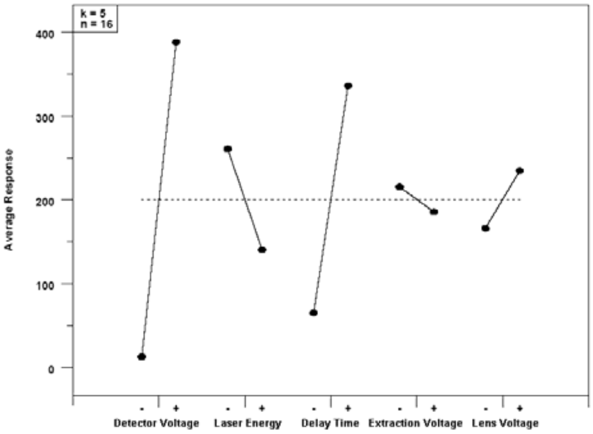

Figure 2. Main effects plot for test 4, polystyrene in RA at 1:5:1 polymer concentration.

The mean plots for each test reveal the effects of the instrument parameters within each matrix and polymer concentration. From these plots, it can be seen that in both dithranol and retinoic acid, the highest polymer concentration (1:5:1, PS:Matrix:AgTFA) yields the highest mean signal-to-noise. Overall, the mass spectra obtained in retinoic acid provide higher mean signal-to-noise values than those run in dithranol. A more in-depth analysis was performed on Test 4, where the highest signal-to-noise was obtained in each matrix. Test 4 is polystyrene run in RA at the highest polymer concentration. The further analysis reveals the effects of the factors on the response, as well as interaction effects.

The main effects plot for Test 4 is shown in Figure 2. The main effects plot reaffirms that the detector voltage and delay time are the most influential parameters on signal-to-noise. The maximum delay time and detector voltages yielded higher signal-to-noise values. Laser energy also influences the signal to noise, although lower laser energies resulted in better signal-to-noise.

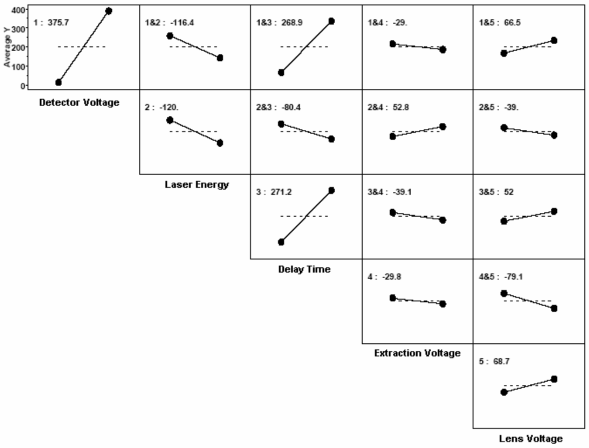

Figure 3. Interaction Effects Matrix for Test 4.

Interactions occur when the effect of one factor on a response depends on the level of another factor. The interaction effects matrix for Test 4 is shown in Figure 3. The interaction effects matrix shows the effect of each individual factor as well as the interaction of each factor with every other factor. Figure 3 shows the highest effects for the detector voltage. Delay length is the next highest effect and the interaction between the detector voltage and the delay length is also high. The laser energy and the interaction between laser energy and detector voltage influence the signal-to-noise to a lesser extent.

We found that the detector voltage and the time delay were the most influential of the instrument parameters for polystyrene; longer delay times and higher detector voltages cause the signal-to-noise to increase. Both the matrix and the polymer concentration influenced the optimization as well. *All-trans* retinoic acid yielded higher signal-to-noise values than dithranol. And higher polymer concentrations increased the signal-to-noise as well. Factorial design is a promising technique for understanding and optimizing MALDI-TOF-MS.