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Numerical optimization of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry: Application to synthetic polymer molecular mass distribution measurement☆

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ABSTRACT

A novel approach is described for the selection of optimal instrument parameters that yield a mass spectrum which best replicates the molecular mass distribution of a synthetic polymer. The application of implicit filtering algorithms is shown to be a viable method to find the best instrument settings while simultaneously minimizing the total number of experiments that need to be performed. This includes considerations of when to halt the iterative optimization process at a point when statistically-significant gains can no longer be expected. An algorithm to determine the confidence intervals for each parameter is also given. Details on sample preparation and data analysis that ensure stability of the measurement over the time scale of the optimization experiments are provided. This work represents part of an effort to develop an absolute molecular mass distribution polymer Standard Reference Material.

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1. Introduction

There has been sustained interest in the use of matrix-assisted laser desorption/ionization (MALDI) mass spectrometry as a means to measure quantitatively the molecular mass distribution (MMD) of synthetic polymers ever since Tanaka et al. [1] first demonstrated the technique on poly (ethylene glycol) and poly (propylene glycol) almost 20 years ago. As early as the second paper in the field in 1992 Wilkins and coworkers [2] pointed out the importance of the proper choice of ion-extraction delay time following the laser ablation event and its possible role in mass discrimination. In the same year Hillenkamp and coworkers [3] investigated the mass discrimination that arises from the loss of detector sensitivity for higher mass oligomers, which often results in an underestimation of the moments of the polymer MMD. From that time forward the central role of instrument tuning on the accurate determination of the MMD has been a widely recognized. McEwen et al. [4] in the context of wide-polydispersity polymers were the first to examine systematically the specific role of instrumentation in the measurement of the MMD. They concluded that instrumental effects play as significant a role as do laser desorption/ionization effects. In 1999 Vitalini et al. [5] explored the interplay between ion-extraction delay time

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and ion-extraction voltage on the measured peak resolution and mass accuracy of mixtures of poly (ethylene glycols). They discovered that highest resolution and mass accuracy could not be obtained across all parts of a wide-polydispersity polymer, only parts of the spectrum could be optimized for any given machine settings. Vitalini et al. conducted their experiment by systematically varying each of the two instrument parameters to map out in full the two-dimensional parameter space. This necessitated taking hundreds of spectra. If they had added a third parameter and sought the same coverage of the parameter space their effort would easily have expanded into thousands of spectra. Beyond three parameters the experiments necessary would have become prohibitively time consuming. To reduce the number of experiments that need to be performed when studying the effects of more than two parameters, Wetzel et al. [6] used an orthogonal experimental design to isolate the effects of five instrument parameters (and two sample parameters) on the signal-to-noise ratio of polystyrene MALDI mass spectra. The use of an orthogonal experimental design allowed for a large number of parameters to be studied but did not require complete coverage of the parameter space. While the work of Wetzel et al. revealed which parameters played the greatest role in determining signal-to-noise ratio it did not explicitly provide a means to optimize that ratio.

A variety of mathematical methods exist that allow the experimentalist to optimize instrument settings without performing an exhaustive search of the parameter space. Broadly classified, these methods are all forms of *numerical optimization*. When the topology of the search space is very complex, for example when it has great sensitivity to one or more parameters (as mass spectrometers often do), the methods used are part of the field of *non-linear programming* [7]. They are called non-linear because some (or all) of the instrument parameters do not have a linear relationship between parameter value and measurement response, that is, the derivative of the parameter-value versus measurement-response curve is not a constant. A simple example is laser intensity and its effect on signal-to-noise ratio where a relatively sharp threshold is observed experimentally. When the measurement outcomes (which in the present case are mass spectra) contain random noise the mathematical methods are termed *stochastic numerical optimization* [8,9]. Stochastic methods are important in mass spectrometry because all mass spectra have noise, this noise varies as the instrument parameters are adjusted, and the noise will often change across the spectrum. In this case, we will use the word noise to mean uncorrelated, random variations in the spectrum as a function of instrument parameters. Measurement noise presents a significant challenge to any optimization method especially for cases where signal-to-noise is not the measurand to be optimized. Nevertheless, numerical optimization methods offer experimentalists a way to tune the instrument parameters to achieve the desired goal without having to search all possible parameter combinations.

A type of numerical optimization known as *genetic search* has been applied recently by Kell and coworkers [10] to the optimization of electrospray ionization (ESI) mass spectrometry in the study of proteins. They optimized 14 instrument settings to achieve the highest simultaneous detection efficiency for five proteins in an equimolar mixture. They found that the optimal settings for any one protein are not the optimal settings for the mixture of proteins. Furthermore, some instrument settings have several optimal (or near-optimal settings) while others show an unambiguous single optimal value. In a more recent paper Kell and coworkers [11] have gone on to show how genetic algorithms can be used to automatically (that is, without operator intervention) optimize a gas-chromatography–mass spectrometer (GC–MS) over nine instrument variables. Using mass spectrometry and working to identify the various metabolites in human serum and yeast fermentations they define a mulitobjective optimization function where not only the signal-to-noise ratio but also the total number of peaks and the total run time of the chromatographic separation were optimized simultaneously.

To measure the absolute molecular mass distribution of a synthetic polymer, it would be ideal to locate a region in parameter space where the instrument response function was uniform across the entire mass range. Finding the instrument response function is necessary to calibrate the intensity axis of the mass spectrum, that is, to go from mass spectrum to molecular mass distribution. If the instrument response function is uniform then the relative peak areas in the mass spectrum correspond directly to the relative abundances of individual *n-mers* in the sample. A uniform instrument response function would be a line of zero slope, that is, it would have a derivative of zero. If not uniform, the instrument response function could slowly vary across the mass range, preferably linearly with mass. The optimal conditions are those that give the simplest (or flattest) instrument response function, that is, the one with the smallest derivative.

To determine the instrument response function, a gravimetric mixture was made consisting of three low-polydispersity polystyrenes that were very close in average molecular mass. The optimal instrument settings were those that provided the closest match between the total integrated peak intensity of each of the three polymers in the mass spectrum with the known gravimetric ratios. Note that there is no guarantee (or even assumption) that the optimal instrument settings that give the flattest instrument response function will also yield optimal signal-to-noise ratios. In fact there is no reason to believe that a search for the instrument settings that optimize the gravimetric ratios will not lead into a region where the mass spectra become so noisy as to make peak integration impossible. Thus, to find the optimal instrument settings we used *stochastic gradient approximation methods* [8,9]. These methods have proven to be extremely robust in cases where the measured data are very noisy.

2. Experimental methods

2.1. *Polymer samples*

Instrument optimization was performed using a gravimetric mixture of three polystyrene samples custom synthesized by Scientific Polymer Products, Inc. (Ontario, NY) [12]. The samples where made by anionic polymerization using either *n*-butyl lithium or *n*-octyl lithium as initiators. Each was terminated with a proton. Their molecular mass distributions were