

The Influence of Laser Energy and Matrix of MALDI on the Molecular Mass Distribution of Poly(ethylene glycol)

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INTRODUCTION

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) has the potential to provide not only end group and branching information for synthetic polymers, but also molecular mass data.¹ But the characterization of synthetic polymers by MALDI-TOF-MS has yielded inconsistent results, indicating that the MALDI-MS determined molecular mass distribution (MMD) is sensitive to instrumental and sample preparation parameters used to obtain the polymer mass spectrum.²⁻⁶ The disparity of these results produces questions as to the accuracy and repeatability of the MALDI-TOF-MS method for characterizing synthetic polymers, therefore, creating a need to define the parameters that affect the molecular mass distribution of the synthetic polymer as determined by MALDI-MS. We considered the effects of laser energy and matrix on the molecular mass distribution of several polymers of varying stability. This paper will focus on a study of the MMD of poly(ethylene glycol) (PEG).

EXPERIMENTAL

The polymer mass spectra were obtained using a Bruker (Billerica, MA)⁷ Reflex II MALDI-TOF-MS equipped with dual micro channel plate detectors for both linear and reflectron modes. Delayed extraction was employed during the collection of all data. A nitrogen laser operating at 337 nm and a 3 ns pulse width was utilized. An attenuator was altered to vary the laser energy from 1 μ J to 8 μ J. The laser energy was measured with a LaserProbe Rm-3700 Universal Radiometer (Laser Probe, Inc. Utica, NY) placed after the optics and before the last mirror prior to the source. The laser energy range measured and used for PEG in a particular matrix was defined by the ability to obtain measurable signal. Three MALDI mass spectra were obtained at each laser energy.

Each mass spectrum is the total number of ions from 100 laser shots, as the laser was moved over a site on a stainless steel MALDI target. The laser was in constant motion to prevent possible biases in the molecular mass distribution due to hot spots. An estimated standard uncertainty of M_n obtained by MALDI-TOF-MS of 50 u.

The polymer moments of each MMD obtained by MALDI-TOF-MS were calculated. The polymer mass distributions were also divided by mass into six equally spaced bins. The first and last bin may contain a greater mass range, because these bins contain all of the data remaining in the tails of the MMD. The moments and bins were used to describe effects on the MMD.

RESULTS

Polyethylene glycol was analyzed in *all trans*-retinoic acid (RA), 2,5-dihydroxybenzoic acid (DHB), and dithranol. The mass spectra of PEG in these matrixes are shown in Figure 1. The secondary peak series seen throughout the mass spectrum obtained with dithranol as the matrix is due to PEG molecules ionized by adventitious potassium ions. This series is not seen in the RA and DHB data. The laser energy used to obtain the mass spectra of PEG ranged from 2.0 μ J to 7.5 μ J in DHB, between 1.5 μ J and 5.0 μ J in dithranol and from 1.0 μ J to 1.8 μ J in RA.

The distributions of the mean bins of the MMD of PEG in RA, DHB and dithranol are shown in Figure 2. The bin distributions reveal

that bin 1, the lowest mass bin of the PEG distribution analyzed in DHB, contains a greater percentage of the MMD than the bins of the MMD analyzed in RA and dithranol. This result indicates that there is greater fragmentation of the PEG occurring when DHB is the matrix. In Figure 1, besides the main peak series, a secondary series can be seen in the low mass tail, bins 1 and 2 of the PEG mass spectrum obtained in DHB.

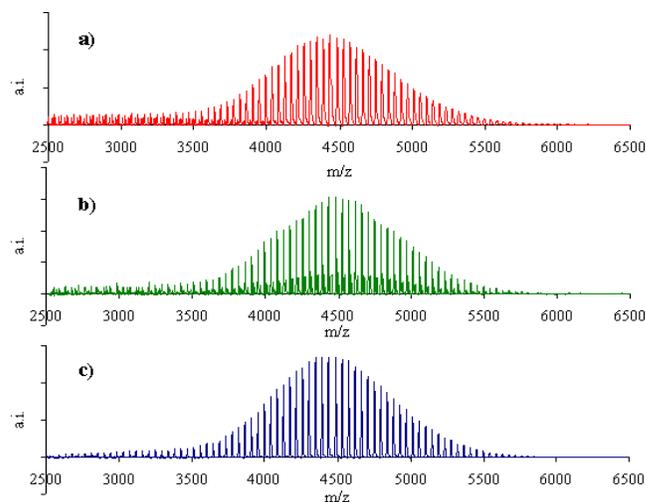


Figure 1. MALDI mass spectra of PEG in a) DHB, b) dithranol, and c) retinoic acid.

In DHB and in dithranol, the moments obtained as a function of the laser energies were found to vary significantly. In DHB the number-average molecular mass (M_n) decreases as laser energy is increased, while in dithranol the M_n increases as laser energy is increased. The moments of PEG analyzed in RA were not found to vary significantly as implied by an ANOVA test. These results are reflected in the moments shown in Table 1. Both DHB and dithranol have lower mean moments than RA.

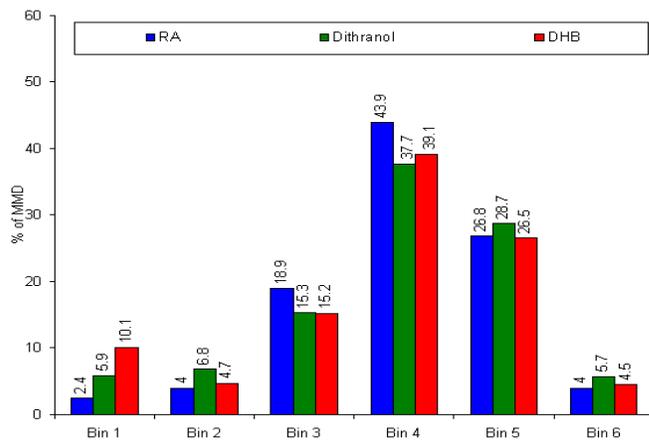


Figure 2. The histogram represents the distribution of the bins of PEG in RA, dithranol and DHB. The standard uncertainty of the bin percentages obtained by MALDI-TOF-MS is 3 %.

The ANOVA results of the bin data indicate that in DHB the fraction of polymer in the low mass bins increases with increasing laser energy. This is supported by the M_n data found in Table 1 and the appearance of the low mass secondary series seen in Figure 1. The M_n of PEG in DHB is lower than that of PEG in RA. In dithranol, the high mass bins of PEG increase with increasing laser energy suggesting that more high mass polymer is being ablated into the gas phase as laser energy increases. In RA no effects of laser energy on the MMD of PEG are seen. The PEG samples analyzed in dithranol

and RA appear to show few signs of fragmentation in the analysis of the moments and bins. These results indicate that effects of laser energy are seen for both the PEG analyzed in DHB and dithranol. If the effect of laser energy were independent of the matrix, the expected effect of the laser energy would be the same for each matrix, when the laser energy ranges overlap. However, these results show vastly different effects when PEG was analyzed in DHB and dithranol at the same laser energies. An effect of laser energy exists, but it is matrix dependent.

Table 1. The Mean M_n and Standard Deviation for the Initial PEG Mass Distributions and the With Fragmentation Peaks Subtracted

Matrix	M_n	SD	Corrected M_n	SD
	(u)	(u)	(u)	(u)
RA	4350	60	4390	60
Dithranol	4080	140	4210	130
DHB	4210	140	4420	60

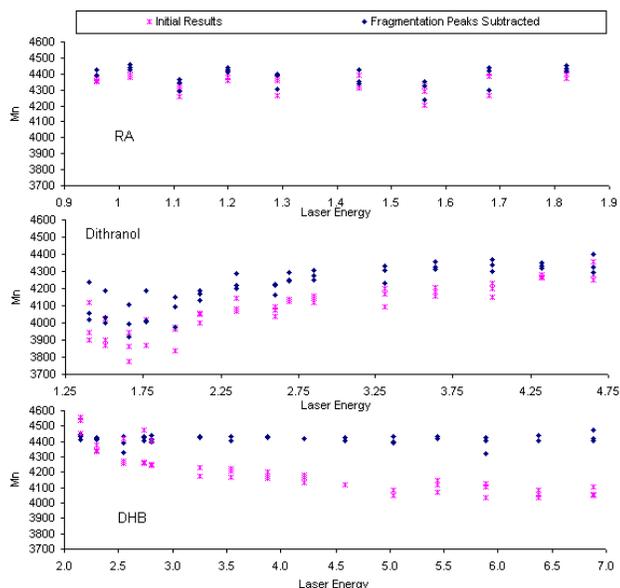


Figure 3. Plots of the moments of PEG, both the original data and the moments after the fragmentation peaks were subtracted, as laser energy increases in each matrix.

DISCUSSION

PEG produces a secondary series of peaks arising from the fragmentation of the polymer. This is a result of the fragmentation occurring at the oxygen-carbon bond in the repeat unit of the PEG molecule.⁵ Each polymer molecule fragmentation produces two discernible fragment molecules. One PEG fragment molecule will have the same end groups as the original polymer molecule, and the second fragment ion will have a different end group mass. This secondary peak series is shifted sixteen mass units from the main series in the mass distribution. If the assumption is made that the PEG molecule is equally likely to fragment anywhere along the polymer chain, then the distribution of the fragmentation peaks with no end group change should be the same as the distribution of the fragment ions with an end group change.

The PEG mass spectra were integrated into peak values using Polymerix (Sierra Analytics, Modesto, CA) analysis software. This software has the ability to identify individual peak series and calculate separate distribution information such as moments and polydispersity for the secondary series. Using this software the PEG data were analyzed and then the fragmentation peaks were subtracted from the mass spectrum. The resulting mass spectra were then analyzed and compared with the original PEG data.

The molecular mass moments of the original data and those calculated after the fragmentation peaks were subtracted from the

molecular mass distribution shown in Table 1 are averaged over all laser energies for each matrix. Regardless of matrix, all the moments increase after the fragmentation is subtracted. Changes to the effect of laser energy on the molecular mass distribution due to the subtraction of the fragment peaks are considered in Figure 3. Each matrix seems to have a different effect on the molecular mass distribution of PEG. When PEG was analyzed in RA, no effect of laser energy on the molecular mass distribution is seen. The fragmentation and change in the M_n for the PEG in RA is the same for all laser energies.

When PEG is analyzed in DHB, the effect of laser energy is different from that in RA after the fragmentation peaks are subtracted. The M_n of PEG in DHB decreased as laser energy increases. However, when the fragmentation peaks are subtracted from the mass distribution, the M_n remains constant, independent of laser energy. Therefore the effect of changing laser energy on the PEG MMD in DHB can be attributed to fragmentation.

The PEG in dithranol has a different result from the other two matrices. The original data revealed an increase in M_n as laser energy increases. This trend in the data remains after the fragmentation peaks are subtracted from the PEG molecular mass distribution. There appears to be a uniform amount of fragmentation at all laser energies. This indicates that although there is an effect of matrix on the PEG mass distribution, it is not due to fragmentation. The increase in the M_n that is observed as laser energy increases is due to an increase in the number of high mass molecules getting into the gas phase. The analysis of the bins indicates that a higher number of high mass molecules are present at higher laser energies.

CONCLUSIONS

The analysis of PEG reveals that the matrix used in the MALDI sample preparation has an effect on the polymer mass distribution. The data analysis also reveals an influence of laser energy on the molecular mass distribution. But the effect of laser energy is matrix dependent. A more intense fragmentation pattern is seen in the PEG spectrum when analyzed in DHB. Little or no fragmentation is seen when PEG is analyzed with RA or dithranol as the matrix. This effect of matrix is not due entirely to the laser energy required for ablation. The results of the analysis reveal that the matrix plays a greater role in the desorption/ionization process than just the energy needed for the desorption of intact polymer into the gas phase.

PEG shows more fragmentation as laser energy increases when analyzed in DHB. The moments of PEG in dithranol increase as laser energy increases, indicating that more high-mass polymer ablates into the gas phase as laser energy increases. The polymer structure, which is already known to influence ionization in MALDI, also influences the effect that the laser energy has on the molecular mass distribution of MALDI-TOF-MS.

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