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The influence of electrospray deposition in matrix-assisted laser desorption/ionization mass spectrometry sample preparation for synthetic polymers[†,‡]

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Although electrospray sample deposition in matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) sample preparation increases the repeatability of both the MALDI signal intensity and the measured molecular mass distribution (MMD), the electrospray sample deposition method may influence the apparent MMD of a synthetic polymer. The MMDs of three polymers of differing thermal stability, polystyrene (PS), poly(ethylene glycol) (PEG), and poly(propylene glycol) (PPG), were studied by MALDI time-of-flight (TOF) MS as the electrospray deposition voltage was varied. The MMDs obtained using the electrospray deposition method were compared with those obtained for hand-spotted samples. No change was observed in the measured polymer MMD when the electrospray deposition voltage was varied in the analysis of PS, but those of PEG and PPG changed at higher electrospray voltages due to increased ion fragmentation. It was also shown that the fragmentation in the hand-spotted samples is dependent on the matrix used in sample preparation. Copyright © 2004 John Wiley & Sons, Ltd.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS) is a powerful tool for synthetic polymer characterization.[1–4] MALDI-MS yields not only the molecular mass distribution of the polymer, but also resolves individual oligomeric species for low mass polymers. End group and branching information can also be gained from the MALDI mass spectrum. However, many questions remain about the MALDI process itself.

Sample preparation methods greatly influence the apparent molecular mass distribution (MMD) of synthetic polymers as obtained by MALDI-TOFMS.[5–9] Matrix, ionizing salt, and matrix-to-polymer ratio are some of the principal sample preparation factors that influence the polymer mass spectrum. Previous work by our group has shown some of these parameters to be more influential on less thermally stable polymers.[10] In a study that varied the matrix and the laser energy, the number-average molecular mass (M_n) of polystyrene, a thermally stable polymer at low temperatures,

changed little when the matrix and the laser energy were altered. However, both the matrix and laser energy were shown to cause changes in the M_n of poly(tetrahydrofuran), a polymer which is thermally unstable at relatively low temperatures.[10]

Another influential factor in the sample preparation for MALDI-MS of synthetic polymers is the manner in which the analyte, ionizing salt, and matrix are applied to the sample target. Generally, two common approaches are used to deposit sample onto the target surface, namely, the hand-spotting sample deposition method and electrospray sample deposition. In hand-spotting sample deposition, or the 'dried-droplet' technique, 0.5–2 μL of solution of a polymer, matrix, and salt mixture is deposited directly onto the target plate. The solvent is allowed to evaporate, often aided by using a fan, by heating, or by drawing the pipette tip across the plate to spread the solvent out.

The same solutions that are used for hand-spotting can be used in the electrospray sample deposition technique. After mixing, these solutions are drawn into a syringe that is placed into a syringe pump. The needle of the syringe is held at a potential of between 3–9 kV against the sample target at ground.[11] When the solution is sprayed at 2–20 μL/min, a fine mist of charged droplets is delivered out of the needle. The sprayed solvent evaporates from the droplets and the polymer/salt/matrix mixture is deposited on the sample plate nearly dry. This procedure keeps the crystals of the matrix small (ca. 2–5 μm diameter) and the polymer matrix and salt in an intimate mixture.[12]

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Better repeatability is seen for electrospray sample deposition than for hand-spotted samples.[11–13] Hand-spotting usually causes large crystals of matrix to form, suggesting that the polymer sample is not homogeneously distributed throughout the matrix. Hand-spotted samples can have large signal variations across the target plate; in some regions, the sample gives large polymer signals ('sweet spots'), and in other regions the sample yields no polymer signal. The advantage of this method is that it requires little additional equipment. The increased repeatability found with electrospray sample deposition is a result of smaller matrix crystals.

The conditions used for electrospray sample deposition are similar to those for electrospray ionization. In electrospray ionization mass spectrometry (ESI-MS), a cone voltage is applied in the atmospheric pressure ionization (API) interface region to strip off solvent molecules clustered to the sample ions, and this can lead to fragmentation of the analyte.[14–18] For example, an analysis of heavy aromatic petroleum fractions revealed that higher cone voltages improved signal intensity, but also caused the sample to fragment.[14] Higher cone voltages have also been shown to fragment other materials, and have been used, e.g., to cause fragmentation in β-cyclodextrins.[15–18]

Polystyrene (PS), poly(ethylene glycol) (PEG) and poly(propylene glycol) (PPG) were used in the present study. These polymers vary in their thermal stabilities; PS has the highest ceiling temperature, and PPG has the lowest.[19] These polymers have all been previously characterized by MALDI-MS; the present polystyrene sample was studied in the NIST inter-laboratory comparison for MALDI-MS of synthetic polymers.[20–23] PEG and PPG have been shown to fragment during MALDI-TOFMS.[24–26] The PEG and PPG backbones break at an oxygen atom, creating an end group mass difference from the precursor ion of about 16 u.[10] The resulting secondary ion series in the mass spectrum makes these polymers ideal for studying fragmentation in the mass spectrometer. Polystyrene is a thermally stable polymer and does not fragment during MALDI-TOFMS under typical conditions.[10,19]

The three polymer sample solutions were prepared in each of the three matrices, and deposited both by hand-spotting and by electrospray deposition onto the MALDI target at several electrospray voltages. Three MALDI mass spectra were obtained for each sample preparation at each of three different laser energies. The polymer distributions and fragmentations were then analyzed to determine the effects of electrospray voltage on the measured polymer MMD.

EXPERIMENTAL

Samples and reagents

MALDI-TOFMS analysis was performed on three synthetic polymer samples: (1) a 7000 u polystyrene (PS) sample (NIST SRM® 2888); (2) a 5000 u poly(ethylene glycol) (PEG) sample (American Polymer Standards Corp., Mentor, OH, USA); and (3) an 8000 u poly(propylene glycol) (PPG) (American Polymers Standards Corp.). Matrices used in these experiments were all-*trans*-retinoic acid (RA), dithranol, and 2,5-dihydroxybenzoic acid (DHB), all purchased from Aldrich Chemical Co. (Milwaukee, WI, USA) and

used as received. Salts used in these experiments were sodium trifluoroacetate (NaTFA) and silver trifluoroacetate (AgTFA) purchased from Aldrich and used as received. The solvent used was tetrahydrofuran (THF) stabilized with butylhydroxytoluene (BHT) (Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA).

MALDI-TOFMS

The polymer mass spectra were obtained using a Bruker (Billerica, MA, USA) Reflex II MALDI-TOF mass spectrometer equipped with dual micro-channel plate detectors. The acceleration voltage was +25 kV and ions were measured in the reflectron mode. Delayed extraction was optimized for signal-to-noise for the necessary mass range and the medium delay setting (500 ns) was employed for the collection of all data. A nitrogen laser operating at 337 nm and a 3 ns pulse width was utilized. The applied laser energy was focused over a spot size of $200 \times 50\,\mu m$. The standard uncertainties, based on one standard deviation of the M_n and the weight-average molecular mass (M_w) obtained by MALDI-TOFMS, were estimated to be 50 and 40 u, respectively. Three MALDI mass spectra were obtained for each polymer sample at each electrospray deposition voltage. The resolution of the peaks near molecular mass of 5000 u at FWHM for 100 laser shots was 500.

Sample preparation

The polymer samples were analyzed by MALDI-TOFMS using RA, dithranol, and DHB. The PS, PEG and PPG samples in RA were solutions, 1:150:1 by mass, of PS/matrix/AgTFA in THF. PS, PEG and PPG sample solutions in dithranol and DHB were prepared by mixing polymer, matrix and NaTFA in a 1:100:1 ratio by mass in THF. The resulting solutions of PS were both hand-spotted and electrosprayed at varying voltages onto a MALDI probe.

Sampling method

The MALDI mass spectra were obtained in a manner designed to minimize bias due to sample preparation and application. The sample solution containing matrix, salt and polymer was applied over the entire probe surface. The samples were either hand-spotted or were electrosprayed at varying voltages onto the sample probe. Each mass spectrum represents the accumulated data from 100 laser shots as the laser spot was moved over a sample site on a stainless steel MALDI target.

For the data analysis three spectra were obtained for each sample deposition method and for each of three different laser energies. The spectra were obtained from different sites on the 26-site MALDI target to reduce the possibility of bias. Matrix type has a large influence on the laser energy required to obtain a MALDI mass spectrum. For each matrix the data were obtained at randomized laser energy intervals within that range, not in order of increasing or decreasing laser energy. The polymer mass spectra were collected at laser energies of 0.85, 1.1, and 1.35 µJ for RA; 1.4, 1.8, and 2.5 µJ for dithranol; and 2.5, 3.4, and 4.7 µJ for DHB.

Data analysis methods

M_n and M_w were calculated from each polymer mass spectrum obtained by MALDI-MS. Polymerix (Sierra Analytics,

Modesto, CA, USA) analysis software was used to integrate the MMD, obtain the moments, and separate the different peak series. For PEG and PPG, the secondary and sometimes tertiary peak series were used to obtain an estimate of extent of fragmentation. The Polymerix software calculates the percent of the total distribution that can be attributed to each peak series identified. The moments of the MMD and the percent of the peaks attributed to the fragmentation series were used to observe changes in the polymer mass distribution due to the different matrices and different electrospray voltages.

Analysis of variance (ANOVA) was used to determine whether the measured polymer MMD moments were influenced by laser energy. The significance level of the ANOVA (α) was chosen to be 0.05. ANOVA compares the variance at a given parameter value with the variance among parameter values to determine if there is a significant influence of the parameter on the polymer distribution.[27] A parameter is said to have a significant variation when the variance between parameter values is greater than a multiple (depending on α) of the variance within parameter values.

RESULTS AND DISCUSSION

Polystyrene

In a previous study we observed little variation in the measured PS MMD at different laser energies,[10] indicating the general overall stability of PS in the MALDI-TOFMS process. Polystyrene was run in RA, DHB and dithranol, although DHB is an inferior matrix for polystyrene yielding poor signal-to-noise. The PS spectra obtained using dithranol and RA showed good repeatability. The M_n values for PS at the various electrospray voltages, averaged over all three laser energies, are shown in Table 1.

No measurable fragmentation can be seen in the PS spectra for both RA and dithranol. This conclusion is inferred from the lack of the appearance, or an increase in the intensities, of lower mass oligomers in the PS mass spectra as the electrospray voltage is increased. Figure 1 shows the mass spectrum of PS in RA at an electrospray deposition voltage of 5 kV. The M_n of the PS sample in RA showed little change as a function of electrospray voltage, as shown in Fig. 2. The ANOVA analysis of the M_n values revealed no significant variation as a result of varying electrospray voltage for PS in RA and DHB. In dithranol the PS MMD showed a small increase in M_n as electrospray voltage increased. This may be due to a morphological change in the sample.

The hand-spotted samples for PS in RA, dithranol, and DHB yielded M_n values comparable to those obtained for PS using electrospray deposition, though the values obtained in RA and dithranol are both somewhat lower than the corresponding values for the electrospray deposition method. When using DHB, no significant difference was seen between the data for the hand-spotted sample and the electrospray-deposited sample.

Poly(ethylene glycol)

The effect of the electrospray deposition voltage on MALDI spectra of PEG differs from that for PS. Figure 3 shows the PEG spectra in RA, dithranol and DHB at a 5 kV electrospray deposition voltage. A secondary ion series is observed, shifted by about 16 u to lower masses from the main series. The secondary series peaks are taken to indicate fragmentation at the carbon–oxygen bond, resulting in different end groups for the two resulting polymer fragments.[25] Figure 4 shows the structures of PEG and its probable fragments; the observed secondary series represents the **A** fragments in Fig. 4. Other possible fragment ions exist for PEG; the fragment **A** end group could be either ethyl (as shown in Fig. 4) or vinyl, but the resolution of our instrument can not distinguish these structures. For the purposes of this study, it is unnecessary to fully characterize the fragments, since both configurations similarly influence the apparent molecular mass distribution obtained by MALDI-TOFMS. PEG fragmentation has been studied extensively by Lattimer.[25] The PEG spectra reveal fragmentation peaks when all three matrices (RA, dithranol and DHB) are used, to varying degrees based on the laser energy and electrospray deposition conditions. The PEG M_n values obtained here for the various sample preparation methods are shown in Table 1.

The ANOVA analysis of the M_n values obtained for PEG reveals that the variation due to the electrospray deposition voltage is significant for all matrices. The M_n values of PEG in dithranol and DHB also show significant variation with laser energy, in agreement with previous work.[10] In RA and dithranol, the M_n of the PEG distribution decreases with increased electrospray deposition voltage. Figure 5 shows the graph of the PEG M_n in RA as the electrospray voltage is increased. No trend with electrospray voltage from 3–9 kV is seen for the M_n of PEG in DHB, although the fragmentation series is clearly seen in the MALDI spectra of PEG analyzed in DHB. The variance in the M_n of PEG in DHB is greater than the variance of the M_n of the PEG MMD in both RA and dithranol.

Table 1. M_n values $\pm$ one standard deviation are given for the three polymers in each matrix at each electrospray deposition voltage and the hand-spotted samples. These M_n values are averages of the data obtained at all three laser energies

|		5 kV	7 kV	9 kV	Hand-spotted
PS	RA	6570 ± 20	6570 ± 15	6570 ± 20	6550 ± 40
	Dithranol	6450 ± 35	6490 ± 35	6530 ± 40	6430 ± 40
	DHB	6150 ± 150	6140 ± 70	6110 ± 70	6110 ± 200
PEG	RA	4470 ± 10	4410 ± 50	4330 ± 25	4300 ± 60
	Dithranol	4460 ± 40	4350 ± 45	4110 ± 100	4170 ± 200
	DHB	3980 ± 60	4150 ± 80	4020 ± 70	3640 ± 300
PPG	RA	4680 ± 100	4610 ± 100	4370 ± 125	4710 ± 150
	Dithranol	4550 ± 50	4300 ± 100	4070 ± 150	4460 ± 150
	DHB	3830 ± 150	3820 ± 120	3870 ± 150	3920 ± 65
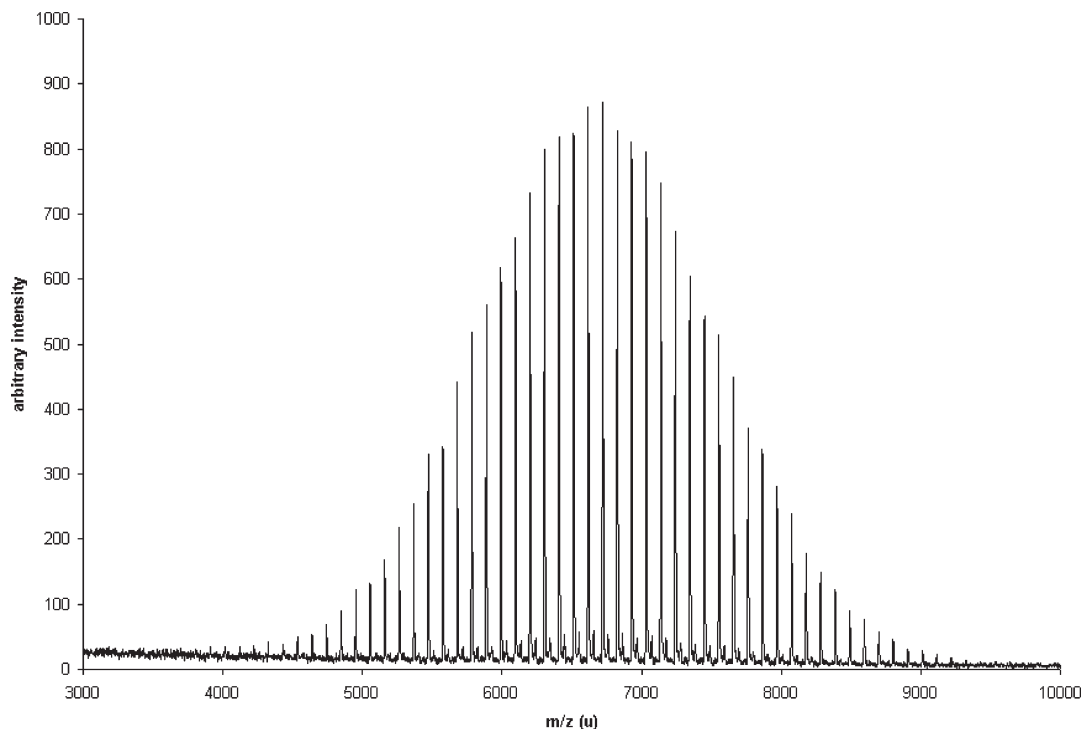
Figure 1. MMD of PS in RA at an electrospray deposition voltage of 5 kV. The secondary peak series represents matrix adducts.

The data analysis for PEG in RA shows an increase in the percent of the PEG distribution represented by the secondary (fragmentation) ion series as electrospray deposition voltages increases. This secondary series does not represent all of the PEG fragmentation, only the fragment **A** (Fig. 4), which is offset from the main series. In the PEG distribution, if there is no preference for which carbon–oxygen bond breaks, the total fragmentation would be twice that of the secondary series. Though the secondary series does not represent all of the fragmentation, it does yield an estimate of the amount of fragmentation for comparison of the sample preparation methods. At an electrospray deposition voltage of 3 kV, 4% of
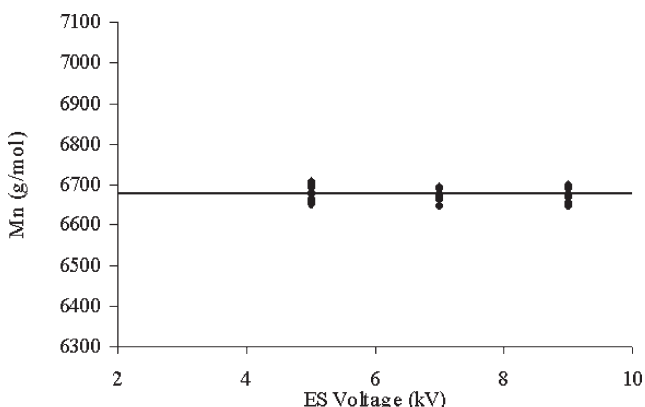

Figure 2. The effect of electrospray deposition voltage on the values obtained for M_n of PS in RA. The M_n does not change with increasing electrospray sample deposition voltage. The standard uncertainty in the M_n of MALDI-TOFMS is estimated to be 50 u (the line is drawn to aid the reader's eye).

the distribution or peak area consists of the fragmentation series. The percentages of the distributions representing fragmentation at electrospray deposition voltages of 5, 7 and 9 kV are 3, 5, and 8%, respectively. These percentages of the PEG MMD represented by the fragmentation series are shown in Table 2. A similar trend exists for PEG in dithranol; fragmentation ranges from approximately 2.5% at 5 kV to 10% at 9 kV (see Table 2). Greater fragmentation of PEG is seen in DHB than for either RA or dithranol, but the ANOVA shows that the variation among electrospray voltages is not significant, indicating that no trend exists. On average 10% of the mass distribution is represented by the fragmentation series for all the electrospray voltages and the hand-spotted sample preparations.

The PEG MMDs obtained from the electrosprayed samples were compared with those for the hand-spotted samples to determine the influence of electrospray deposition on the MMD. No fragmentation is detected in the mass spectra of the hand-spotted samples run in RA. The hand-spotted samples run in both DHB and dithranol showed fragmentation. This indicates that the electrospray sample preparation method is the ultimate source of PEG fragmentation in RA. The hand-spotted samples of PEG in all three matrixes analyzed by MALDI-TOFMS resulted in lower M_n than for the electrosprayed samples, indicating that, although there is increased fragmentation due to the electrospray deposition technique, a higher M_n actually results.

Poly(propylene glycol)

PPG was analyzed in RA, dithranol and DHB. The mass spectrum of PPG obtained in DHB is shown in Fig. 6. Unlike PEG, the substituents on either side of the PPG carbon–oxygen bonds are not the same (Fig. 7). The C–O bond (**I**) closest to
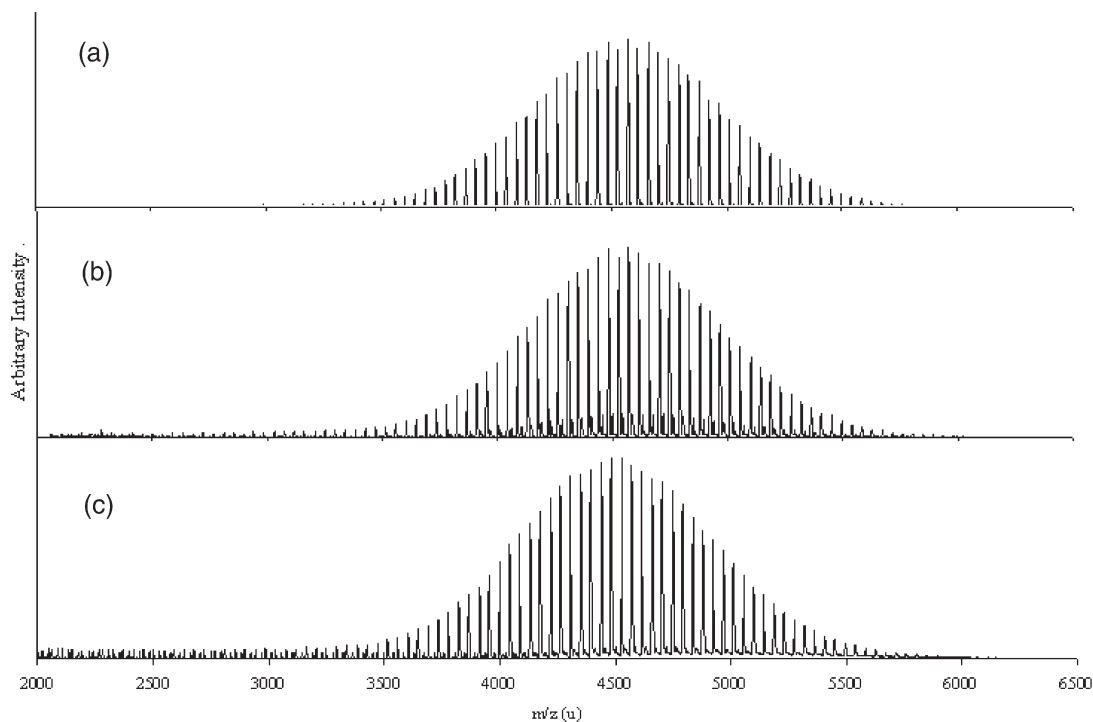
Figure 3. The MALDI mass spectra of PEG in all three matrices: (a) RA; (b) dithranol; and (c) DHB, at 5 kV electrospray deposition voltage.
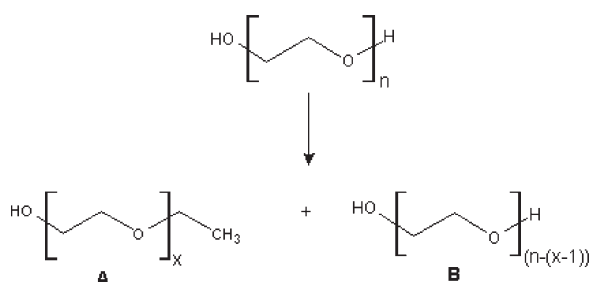

Figure 4. Structures of PEG and its possible products due to fragmentation in the MALDI source.
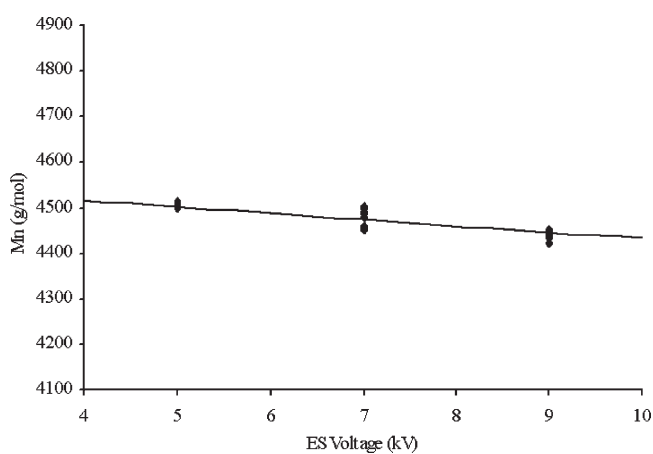

Figure 5. The effect of electrospray voltage on the M_n calculated for PEG in RA. The M_n is clearly decreasing with increasing voltage (the line is drawn to aid the reader's eye).

the methyl group on the chain requires less energy to break. This pathway results in fragments with one hydroxyl-terminated fragment like the precursor ion (**B**), and fragments (**A**) with a propylene end group. The higher energy pathway (**II**) also yields fragments with no end group change (**B**), and fragments with an ethylene end group (**C**). The ethylene-terminated fragmentation is likely a result of cleaving by the second pathway (**II**), followed by a methyl elimination, similar to those observed for the thermal degradation pathways.[26] More pathway **II** fragmentation is seen at higher electrospray deposition voltages and higher laser energies. Other possible fragment ions exist for PPG; these have been studied by Barton et al.[26] The resolution of our instrument cannot distinguish the 2 u difference in some of these structures nor, of course, isomers. For the purposes of this study, it is unnecessary to fully characterize the fragments, since both configurations similarly influence the apparent MMD obtained by MALDI-TOFMS.

The ANOVA analysis of the M_n values obtained for PPG reveals that in RA the M_n varies significantly with changing electrospray voltage, but the variation due to laser energy is

Table 2. Percent of spectrum represented by fragmentation ion series. The standard deviation of the percent of fragmentation is 2.5%

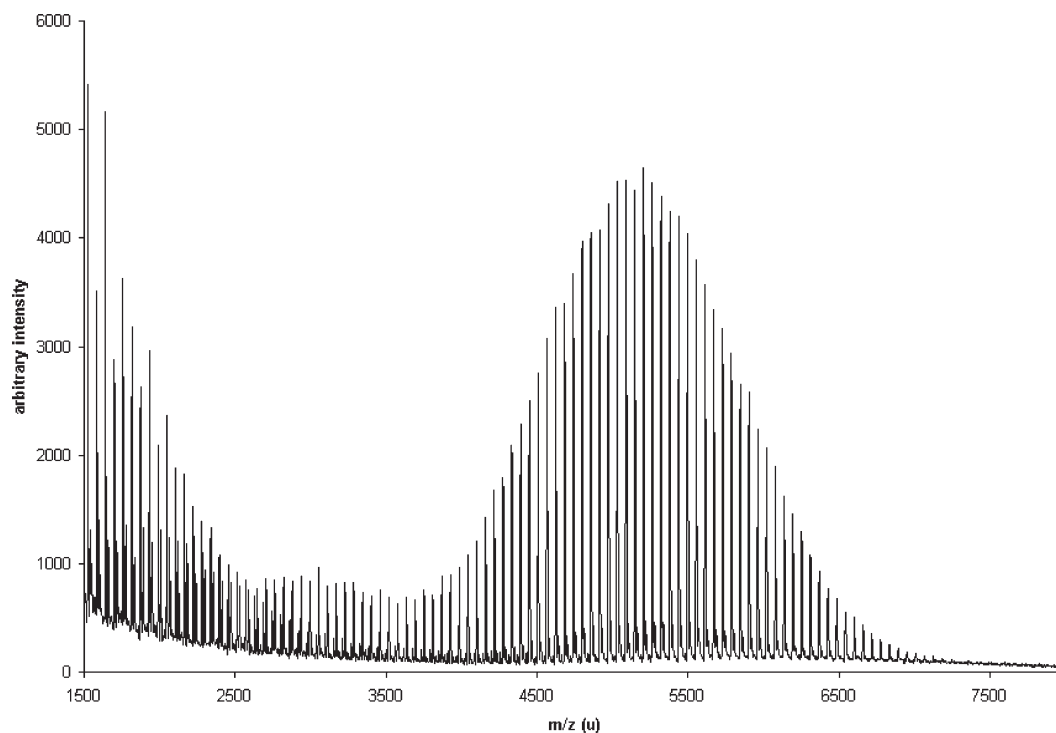|		5 kV	7 kV	9 kV	Hand-spotted
PEG	RA	3.8%	4.5%	7.4%	0.3%
	Dithranol	2.1%	5.1%	9.0%	10.0%
	DHB	11.4%	7.2%	9.6%	13.9%
PPG	RA	10.7%	14.3%	15.8%	10.6%
	Dithranol	13.1%	18.9%	28.7%	18.5%
	DHB	27.2%	26.8%	25.1%	24.4%

Figure 6. MALDI mass spectrum of PPG in DHB at an electrospray deposition voltage of 5 kV.

not significant. The M_n of PPG in RA decreases with increased electrospray deposition voltage (Fig. 8). The M_n values for the PPG MMDs are shown in Table 1. The M_n of PPG in dithranol varies significantly with both electrospray deposition voltage and laser energy. The M_n of PPG in dithranol also decreases with increased electrospray deposition voltage. In DHB no
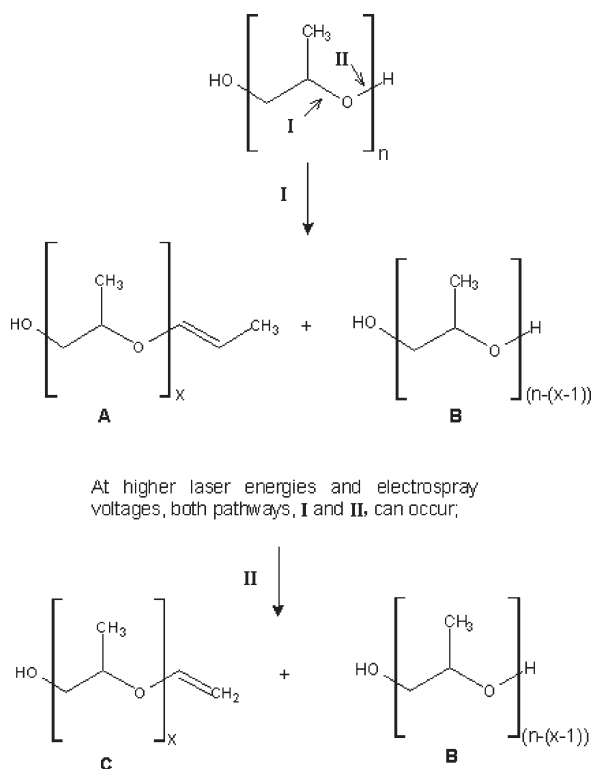
trend is evident in the MMD of PPG with respect to electrospray voltage. The ANOVA of the M_n of PPG in DHB shows significant variation due to laser energy, but no significant variation as a result of electrospray deposition voltage.

The percent of the measured PPG MMD represented by fragmentation is shown in Table 2. The percent of the MMD represented by the secondary series only includes the fragments that are significantly mass-shifted from the main peak series. The fragmentation of PPG in RA increases from 12% at 5 kV to 16% of the MMD at 9 kV electrospray deposition voltage. This fragmentation is a much greater fraction of the MMD than was seen for PEG. For PPG in dithranol, the fragmentation increases from 14% at 5 kV to 27% of the MMD at 9 kV. The mass spectra of PPG in dithranol at varying electrospray deposition voltages are shown in
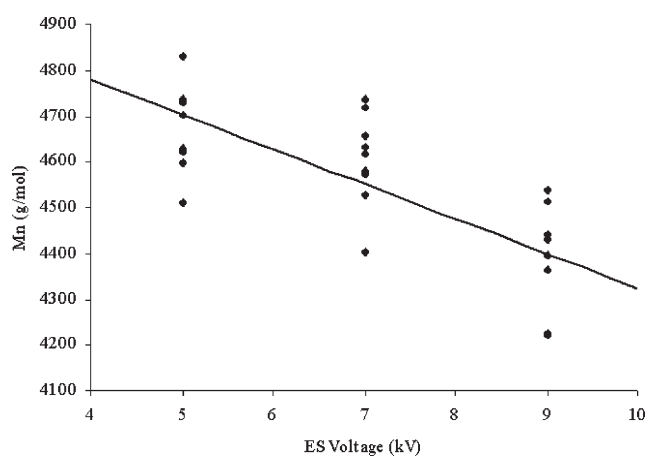

At higher laser energies and electrospray voltages, both pathways, **I** and **II**, can occur;

Figure 7. Structures of PPG and its possible fragments that occur in the MALDI-TOFMS process.

Figure 8. Effect of electrospray deposition voltage on the M_n obtained for PPG in RA (the line is drawn to aid the reader's eye).
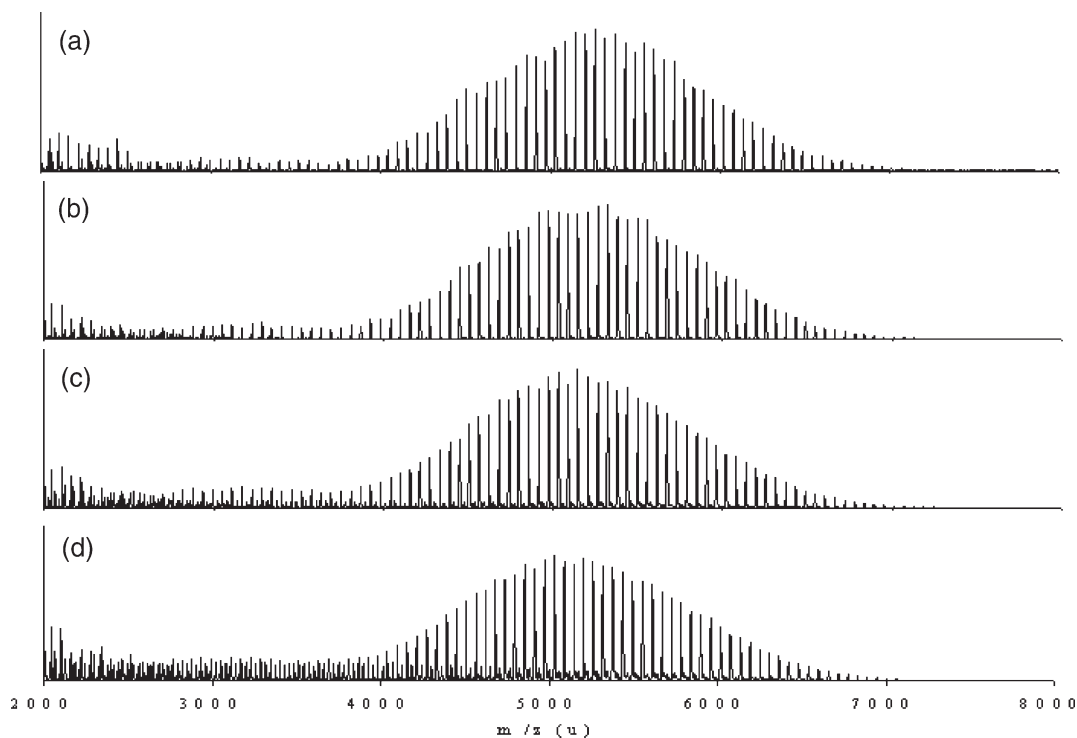
Figure 9. Mass spectra of PPG in dithranol using different deposition methods; (a) hand-spotted; the remaining spectra were obtained using electrospray deposition, (b) at 5 kV, (c) at 7 kV, and (d) at 9 kV.

Fig. 9. The fragmentation of PPG in DHB is about 26% of the MMD for all the electrospray voltages used.

The hand-spotted samples of PPG were also compared with the electrospray-deposited samples. The hand-spotted samples in RA, dithranol and DHB yielded higher M_n values than the electrospray-deposited samples. The hand-spotted samples in DHB had a lower variance than the electrosprayed samples and were more repeatable. Fragmentation was seen in the PPG spectra in RA, DHB and dithranol for the hand-spotted sample preparation, unlike PEG which does not fragment for the hand-spotted method in RA. These results confirm that PPG is less stable than PEG in the MALDI-MS process.

CONCLUSIONS

Both PEG and PPG show increased fragmentation with increased voltage for the electrospray deposition, whereas the PS MMD does not indicate any fragmentation or fragmentation changes. Fragmentation is seen for PEG and PPG in all of the matrices examined when the samples are electrosprayed. The PEG and PPG fragmentation in DHB does not increase with increased voltage, but greater fragmentation is seen for both PEG and PPG in DHB than is seen for dithranol and RA.

The comparison of the results obtained using electrospray deposition reveals a polymer dependence. For PS the M_n values from the hand-spotted and the electrosprayed methods were comparable. The M_n values from the hand-spotting method for PEG were lower than those for electrospray deposition. In PPG, the values of M_n were higher for the hand-spotting method. The variability in comparison of the hand-spotted and electrosprayed samples may be an effect of sample morphology. Further studies are needed to better understand these results.

The M_n values for all three polymer samples reveal effects of matrix on the polymer MMDs. All of the M_n values for the polymer MMDs in DHB are lower than for those of RA and dithranol. These results are consistent with previous studies.[10] The matrix has a large effect on the MALDI technique and further studies are needed to fully understand how the matrix influences the MALDI process.

Our results again show that thermal stability is a good predictor for polymer fragmentation in MALDI-TOFMS. PPG is less thermally stable than PEG, and shows greater fraction of peak area attributed to fragmentation. PS, which is the most thermally stable of the polymers studied, does not show fragmentation. Greater fragmentation is seen overall for PPG than for PEG in MALDI-MS. Electrospray deposition increases the integration of polymer and matrix and improves the repeatability of MALDI-MS. However, the thermal stability of the polymer and possible fragmentation should be considered when using the electrospray deposition technique.

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