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Poly(ethylene glycol) Limits of Detection Using Internal Matrix-assisted Laser Desorption/Ionization Fourier Transform Mass Spectrometry

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Detection limits of poly(ethylene glycol) were examined in the mass range 2000–6000 Da. Using an aerospray sample deposition technique, highly uniform sample surfaces were produced. This method allows signal averaging of spectra from up to 400 shots on the same sample spot. It is found that, as the material available for desorption is decreased, the overall average sample consumption per shot is decreased. Experimentally determined detection limits of 40 and 280 fmol (based on the average molecular masses of 2000 and 6000) were found for PEG 2000 and PEG 6000, respectively. The sample spectra show oligomer distributions in agreement with their higher concentration counterparts. However, at the lowest signal-to-noise levels, oligomers at the extremes of the distribution are no longer detected, making the polymer distribution appear to be narrower in mass range. © **1998 John Wiley & Sons, Ltd.**

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INTRODUCTION

Mass spectrometric detection limits for peptides and proteins have been of interest for a number of years. This stems from the fact that bioanalytical techniques are moving to the sub-cellular level, and mass spectrometry can be a fast and highly sensitive method. In fact, femtomole detection by time-of-flight (TOF) and Fourier transform mass spectrometry (FTMS) is not uncommon, with attomole detection possible in favorable cases.[1–6] However, there is only limited information available regarding mass spectral detection limits for polymers. Most recent studies have focused on the equally important issue of analyzing polymers without mass discrimination. There are few cases when the amount of polymer material analyzed is mentioned. In that research, sample sizes included 100 ng of polymer by quadrupole mass spectrometry,[7] a 2 pmol polymer sample [of which less than 1% was consumed using matrix-assisted laser desorption/ionization (MALDI)TOF],[8] 100 pmol of polymer (on a probe tip) using MALDI/TOF[9,10] and 0.1 nmol of polymer (on a probe tip) using MALDI/TOF.[11,12] Hence, there is a need for more definitive studies of sample quantities required for mass spectral polymer analysis.

With the recent increased interest in the study of polymers by mass spectrometry, MALDI in particular, researchers have been able to provide much information of use to those investigating polymer synthesis strategies. For example, polymer molecular mass distributions, end-groups and even structural information is now readily available by MS, providing chemists with new insight into creating polymers for specific applications. Because exploratory synthetic strategies sometimes yield limited sample quantities for evaluation, it is necessary to use more sensitive analytical methods or smarter sample preparation strategies.

A difficulty with polymer analysis is that the mass spectral signal is spread out over an oligomer distribution rather than concentrated into a few ions, as is the case for pure compounds not occurring in mixtures. Thus, a polymer with a low polydispersity and containing 30 oligomers within its distribution will show relatively lower ion abundances than a single pure compound of similar concentration. Furthermore, polymer spectra are often complicated by the presence of ion sequences resulting from both sodium and potassium adduction. FTMS has been established to be capable of polymer analysis including both polar and non-polar polymers,[13–16] without mass discrimination.[13,17,18] The FTMS trapped ion technique allows for a multitude of experimental possibilities including ion dissociation and high resolution analysis. Here detection limits for a popular class of polar polymers [poly(ethylene glycols)] are examined.

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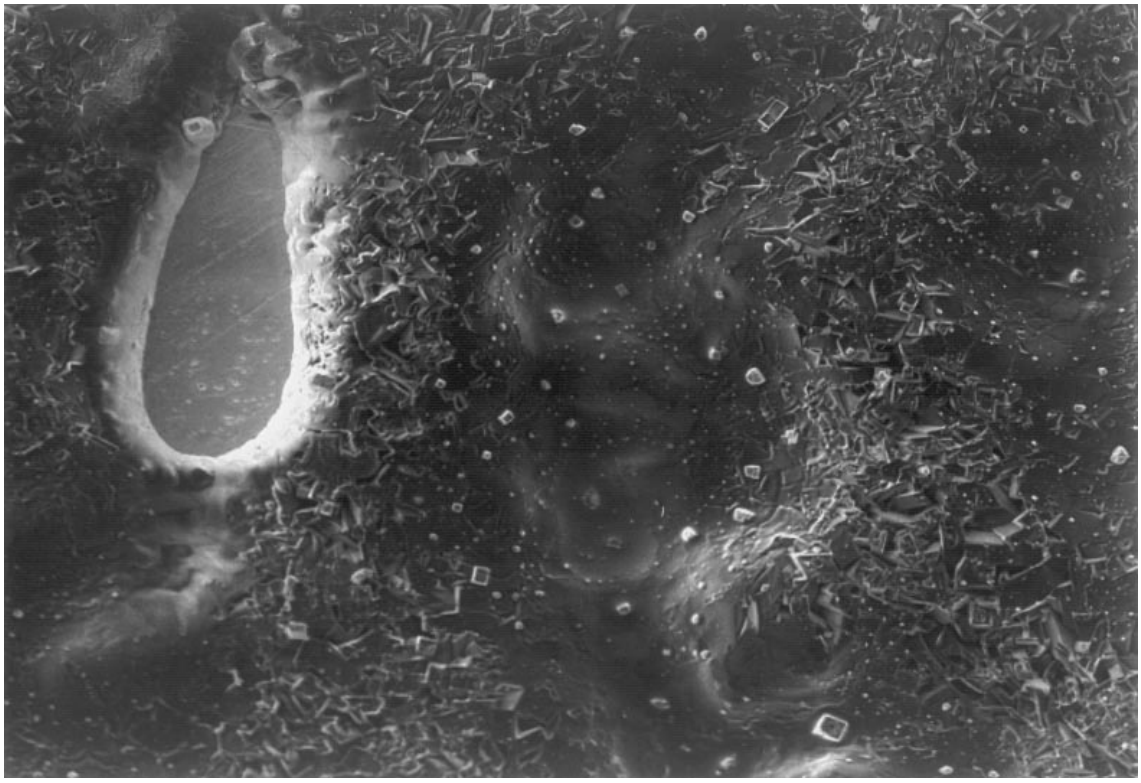

Figure 1. Scanning electron microscope image of a PEG 2000 sample. The image shows microfine particles produced by the aerospray technique. Laser spot size, 80 × 210 μm.

EXPERIMENTAL

Instrumentation

A Fourier transform mass spectrometer (Finnigan, Madison, WI, USA) equipped with a 7.05 T superconducting magnet (Oxford Instruments, Oxford, UK) was used for all experiments. As described previously,[13] internal MALDI ion generation was accomplished using a 337 nm nitrogen laser (PTI Canada, London, ON, Canada) and associated optics. Samples were placed on a 16.5 mm removable stainless-steel probe tip that was positioned 4 mm on the outside of the cubic source cell trapping plate via a manual insertion probe. This internal MALDI arrangement eliminates potential discrimination from ion transport to the analysis cell and gives maximum ion throughout. The background pressure during the experiments described here was 8×10^{-8} Torr (1 Torr = 133.3 Pa), achieved by the use of two Edwards diffusion pumps (Diffstak Model 100, Edwards High Vacuum Crawley, West Sussex, UK). Experimental control and data were acquired using a Sun Sparc data station running Odyssey program software version 3.1 (Finnigan).

Sample preparation

For the poly(ethylene glycol) 2000 (PEG 2000) sample (Fluka, Buchs, Switzerland) of average molecular mass 2000 and the PEG 6000 sample (Fluka) of average molecular mass 6000, 5.1 and 10.8 mg, respectively, were weighed out and mixed in a 1:400 (analyte:matrix) ratio with 2,5-dihydroxybenzoic acid (DHB) (Fluka). These samples were each dissolved in 1 ml of methanol. From each stock solution, a 200 μl aliquot was aerosprayed on to the rotating sample probe tip. For detection limit studies, subsequent samples were successively diluted from more concentrated samples by 1/10 dilution of the previous sample. For calculation of molar quantities, the average masses of the two polymer samples were used. Thus, before analysis, the PEG 2000 sample probe tips had 5.1×10^{-7}, 5.1×10^{-8}, 5.1×10^{-9} and 5.1×10^{-10} mol of material loaded and the PEG 6000 sample probe tips had 3.6×10^{-7}, 3.6×10^{-8} and 3.6×10^{-9} mol of polymer loaded. Calculations for detection limits assume that there was no significant overspray of the sample solution.

The aerospray technique for sample deposition has been described previously[19] and was first reported as an alternative sample preparation technique as early as 1992.[14,15] Because the solution is sprayed, the result is microfine crystals with a highly uniform surface. This permits excellent sample shot-to-shot reproducibility. As an alternative, poly(ethylene glycol) was mixed with the matrix and water as the solvent, and it was deposited in the channel of a grooved probe tip of fixed geometry. Crystals resulted from evaporation of this solvent mixture.

Experimental sequence

For consistency, with the exception of deceleration time, the same experimental conditions were used for all analyses in the present study. The signal for PEG 2000 was optimized prior to the concentration study. MALDI ions were decelerated for 115 μs (PEG 2000) and 170 μs (PEG 6000) using a gated trapping tech-

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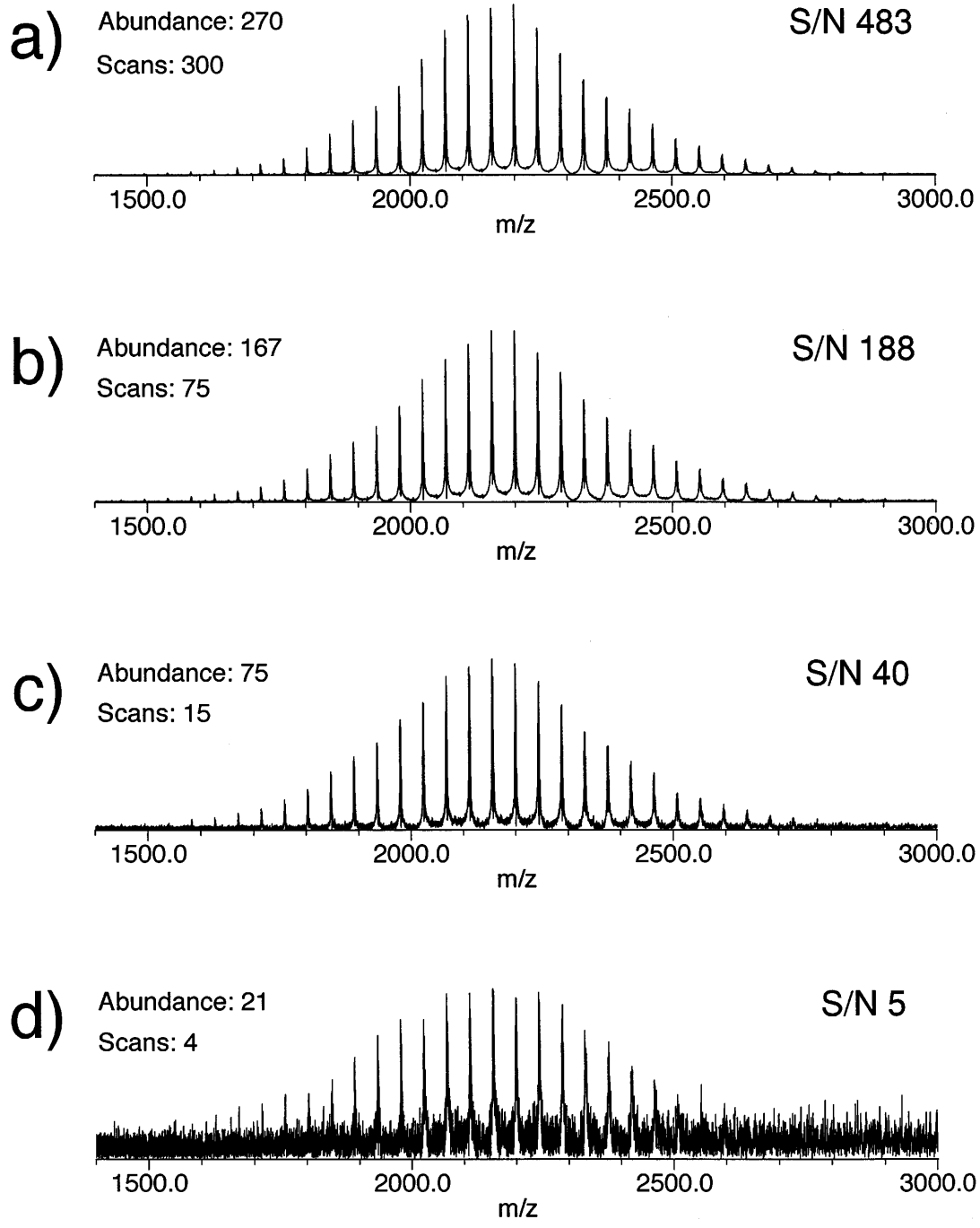

Figure 2. Example spectra of 5.1×10^{-7}, 5.1×10^{-8}, 5.1×10^{-9} and 5.1×10^{-10} mol of PEG 2000 loaded on to the probe tip. The material present on the probe allowed for signal averaging in the following amounts: (a) 400, (b) 100, (c) 20 and (d) 4 shots.

nique.[20,21] Following ion deceleration, a 100 ms delay was imposed to allow sufficient sodium cationization and ion relaxation. For spectral observation, ions were excited to a radius of *ca.* 1.0 cm using the SWIFT excitation procedure. A higher radius produced distortions within the distribution and eventual loss of ions; a lower radius produced lower signal levels. A PEG 6000 sample confirmed the use of a 1.0 cm radius for best results in obtaining near-Gaussian molecular mass distributions and maximum signal intensity. Detection was done at a bandwidth of 400 kHz with 64K data points and 9 dB of attenuation. A 1.0 s quench ended each measurement, and there was a 100 ms delay before each subsequent experiment. All mass spectra were measured with the trapping plates at 1.0 V.

Scanning electron microscopy

A Philips Model XL30 scanning electron microscope (Philips Electron Optics, Eindhoven, The Netherlands) with a field emission gun was used. An accelerating voltage of 5 kV was applied. Samples were mounted on the specimen holder using adhesive carbon tape. No coating was applied to the sample. Imaging was done using a secondary electron detector.

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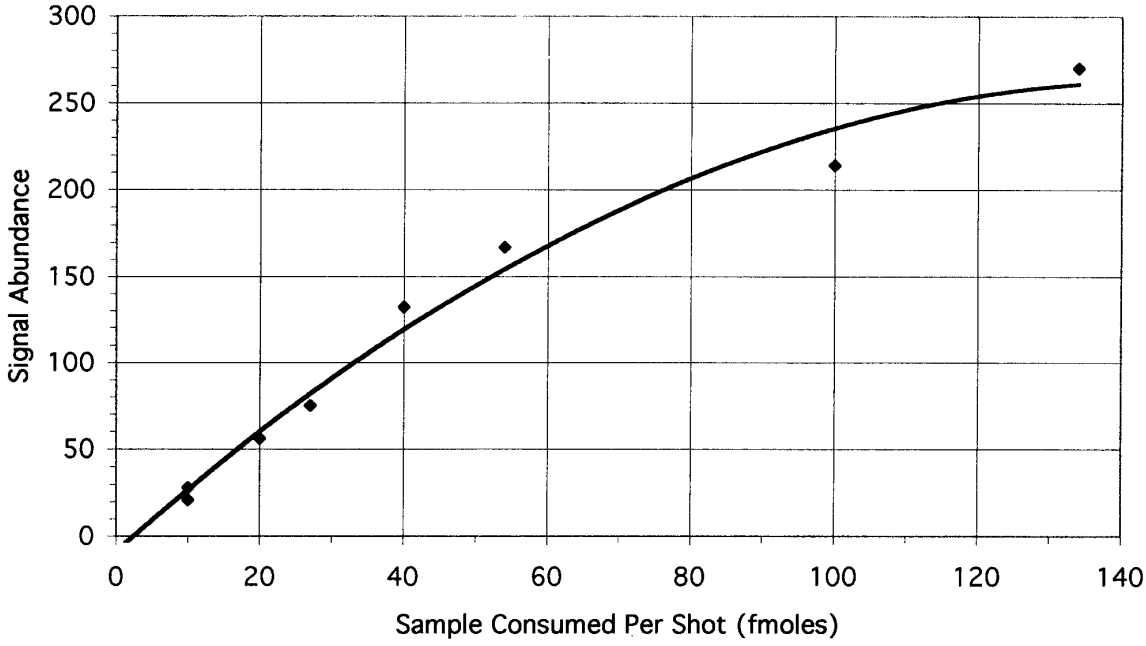

Figure 3. Plot of signal intensity *vs.* sample consumed per shot (in femtomoles) for PEG 2000.

RESULTS AND DISCUSSION

Aerospray *vs.* dried-drop method

The benefits of using the aerospray technique for sample deposition over the dried-drop method are quicker sample preparations, more homogeneous surfaces and smaller crystal formation. An example sample preparation is shown in Fig. 1. Microfine crystals formed are clearly visible, as is the laser spot (80 × 210 μm). Solution sprayed from the aerospray quickly evaporates solvent and leaves the crystals to self-stack. Slight valleys and elevated regions are visible where the surface is built upwards.

It was found that the dried-drop method produced larger crystals in selected areas of the probe tip. For example, the channel probe tip suffered from crystal formation along the top edge of the groove when a volatile solvent such as methanol was used. Also, because the crystals grow by association, the laser desorption mass spectral signal can fluctuate from shot to shot as one searches for a 'good spot.' Thus, for reproducibility reasons and because of the desire to signal average on a single spot, the dried-drop method was abandoned in favor of the aerospray technique for polymer deposition.

Additional benefits of the aerospray technique may be good analyte crystallization with the matrix. A topic of debate has been the spatial location of the analyte, matrix and metal salts within the crystal, so called Marangoni effects.[22–24] In addition, depending on when ionization occurs, before the laser pulse or immediately after and close to the surface, the proximity of the analyte to the cationizing agent could prove crucial in obtaining usable signal intensities.

Experimental detection limits

Laser desorption mass spectra of PEG normally show cationization with sodium. However, this can easily be converted to cationization with any other metal by addition of the appropriate metal salts to the matrix. Figure 2 shows spectra of sodiated oligomers from a PEG 2000 sample that was acquired using MALDI/FTMS. In Fig. 2(a) is a near-Gaussian distribution that extends from 1500 to almost 3000 Da. Figure 2(a)–(d) are spectra obtained from samples where 5.1×10^{-7}, 5.1×10^{-8}, 5.1×10^{-9} and 5.1×10^{-10} mol of material were loaded on to the probe tip. Signal averaging on the same spot was done to determine the relative amounts of material present after each dilution step. For example, the probe tip was 5.1×10^{-7} mol of PEG 2000 loaded [Fig. 2(a)] could be subjected to 400 laser shots on the same spot until the signal was essentially gone. This is in contrast to the probe tip with only 5.1×10^{-10} mol loaded [Fig. 2(d)], which produced signals only for the first four laser shots. Hence the number of laser shots required to deplete the sample is related to the sample thickness, since a fixed aliquot of 200 μl of solution was deposited in each case. Higher sample concentrations produced thicker sample preparations, allowing more laser shots per spot.

A scanning electron microscope was used to determine the laser spot size of 80 × 210 μm. Knowing the probe tip's circular dimensions and assuming that all sample solution was deposited on the probe tip without significant overspray, a scaling factor of 12 700 was determined. This means that ablated material from the nitrogen laser produces a hole that is 1/12 700 of the total material available for analysis. The maximum number of shots available with each sample preparation was determined experimentally. From these data, the amount of material ablated per shot was determined.

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Table 1. Summary of data for PEG 2000

Material on probe (mol)	Probe/laser spot ratio	Material on spot (mol)	No. of scans	Material per shot (fmol)	Average signal per shot	Signal-to-noise ratio for added spectra
5.10×10^{-7}	12 700	4.02×10^{-11}	300	134	270	483
5.10×10^{-7}	12 700	4.02×10^{-11}	400	100	214	404
5.10×10^{-8}	12 700	4.02×10^{-12}	75	54	167	188
5.10×10^{-8}	12 700	4.02×10^{-12}	100	40	132	184
5.10×10^{-9}	12 700	4.02×10^{-13}	15	27	75	40
5.10×10^{-9}	12 700	4.02×10^{-13}	20	20	56	34
5.10×10^{-10}	12 700	4.02×10^{-14}	4	10	21	5
5.10×10^{-10}	12 700	4.02×10^{-14}	4	10	28	6
5.10×10^{-10}	12 700	4.02×10^{-14}	4	10	28	9

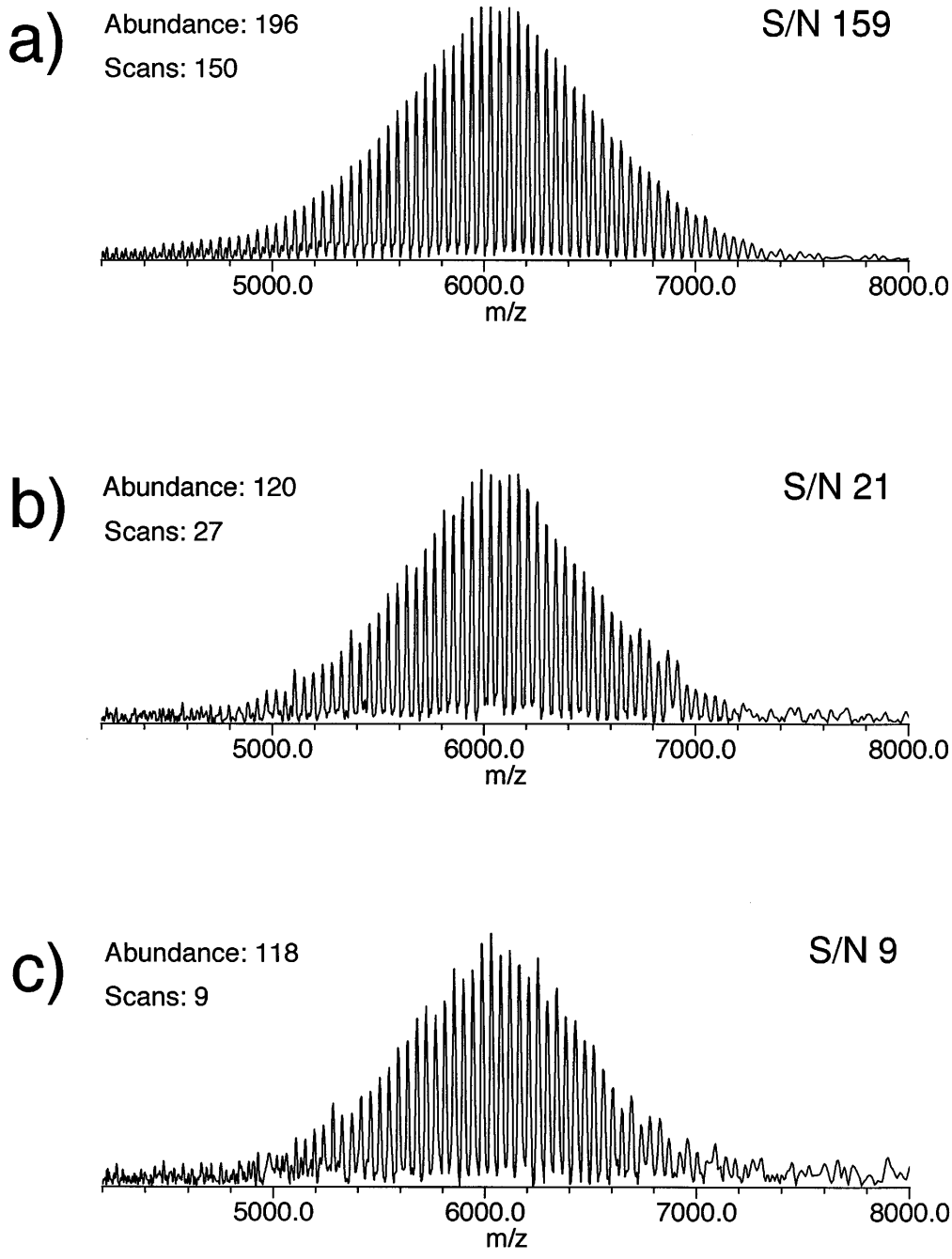

Figure 4. Example spectra of 3.6×10^{-7}, 3.6×10^{-8} and 3.6×10^{-9} mol of PEG 6000 loaded on to the probe tip. The material present on the probe allowed for signal averaging in the following amounts: (a) 200, (b) 36 and (c) 9 shots.

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The average of all laser shots, until the sample shot was depleted, was plotted to yield a representative detection limit. Figure 3 is a plot of the signal abundance *vs.* sample consumed for PEG 2000 from the data in Table 1. The plot shows that when sample is plentiful, more can be consumed on average than in a sample limited situation such a 'bad spray' or thin film application. The result is higher signal intensities and better signal-to-noise ratios. Figure 3 also shows the lowest sample consumption per shot to be 10 fmol for PEG 2000. When spectra resulting from four shots are signal averaged (i.e. 40 fmol consumed), the mass spectrum in Fig. 2(d) is obtained. To obtain signal-to-noise ratios between 5 and 9, consumption of 40 fmol appears to be the lowest detection limit possible for this m/z range. Although it is worthwhile to know that the average molecular mass distribution can still be calculated at this low polymer concentration, the poor signal-to-noise ratio makes it impossible to obtain the true polydispersity of the polymer.

The net result of not generating enough ions per laser shot is lowered signal intensities. This is a major limitation for analyzing high-mass polymers by a trapped ion technique. In the case of MALDI, ions are desorbed with significant translational energies which pose experimental difficulties for trapping. Use of a higher power laser and larger spot sizes would increase ion abundances. However, the energetics of the ion formation process limit mass ranges. Wilkins and coworkers[14,25] have shown that singly charged MALDI PEG oligomers up to m/z 14 000 can be observed using a gated trapping technique and a 7 T FTMS system. O'Conner and McLafferty[26] have demonstrated that PEG oligomers with masses up to 23 000 Da can be analyzed by electrospray FTMS.

In a second series of experiments, PEG 6000 was examined to see the effect of higher mass range on the detection limits of PEG polymers. Figure 4 shows spectral plots of (a) 3.6×10^{-7} (b) 3.6×10^{-8} (c) and 3.6×10^{-9} mol of PEG 6000 loaded on the probe tip. When analyzed as for the PEG 2000 studies, this corresponded to average detection limits per shot of 190, 100 and 31 fmol, respectively. Because the mass spectrum shown in Fig. 4(c) resulted from averaging the spectra from nine laser shots to produce the signal-to-noise ratio of 9 shown, the ultimate detection limit for this PEG mass range is *ca.* 280 fmol (i.e. 9×31 fmol). Again, it is found that when more sample is present on the probe tip, the average consumption per shot is increased, leading to higher signal intensities.

CONCLUSION

A study of MALDI/FTMS detection limits for two poly(ethylene glycol) polymers (PEG 2000 and PEG 6000) was carried out. It was found that, as the amount of material applied to the sample probe tip was decreased, the overall sample material consumed per laser shot was diminished. At low signal-to-noise ratios of between 5 and 9, the total sample consumed was 40 fmol for PEG 2000 and 280 fmol for PEG 6000. The spectra acquired under these conditions exhibit accurate average molecular mass distributions and M_p values. However, they suffer from a loss of end oligomer information owing to lower signal-to-noise ratios, making the distribution appear narrower. Owing to the symmetry of the distributions, these losses do not have a substantial effect upon the calculated averages.

The use of an aerospray technique greatly facilitates the analysis of all polymer materials because of the increased reproducibility from shot to shot from a more uniform surface over the dried drop method. At the highest sample concentration depositions, spectra from up to 400 laser shots could be signal averaged from the same probe spot.

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