

Utilising time-lag focusing ultraviolet-matrix-assisted laser desorption/ionisation-mass spectrometry for the end group analysis of synthetic polymers

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Time-lag focusing matrix-assisted laser desorption/ionisation-mass spectrometry (MALDI-MS) has been employed to analyse synthetic polymers with average molecular weights between approximately 3500 and 12500 Da. Spectra are shown that demonstrate the improvement in resolution and sensitivity when time-lag focusing is used instead of prompt extraction techniques. A resolution of almost 9000 (FWHM) was obtained, in the reflectron mode of operation, for a poly(methyl methacrylate) (PMMA) sample with a relatively narrow dispersion. The data for PMMA polymers of industrial relevance easily enables the differentiation of many end groups as a consequence of the improved signal-to-noise and good mass accuracy when time-lag focusing is employed.

MALDI-MS, originally used almost exclusively for biopolymer applications [1,2], has recently been shown to be a very useful tool for the analysis of synthetic polymers [3–5]. The technique has been shown to generate data for narrow dispersion polymers that are representative of the sample [6–11]. Many groups have reported that there are limitations for systems which are more polydisperse (>1.3) with the observed average molecular weight values being lower than that generated from gel permeation chromatography (GPC) data [9–12]. Montaudo et al. have recently shown, however, that reliable average molecular weight values may be generated in some cases by an off-line procedure of processing MALDI spectra from polydisperse polymers [13].

The ability of MALDI-MS to differentiate between oligomeric species in polymer samples of relatively low molecular weights (approximately < 20 kDa) has enabled the determination of end groups [8,14–16]. MALDI-MS can be a very powerful tool for end group analysis, especially when used in conjunction with NMR spectroscopy which is commonly used for determination of their structures. It has also recently been shown that the masses of end groups may be inferred from the fragment ion peaks in the MALDI-collision induced dissociation spectra of polymers [17]. The principles of time-lag focusing were originally reported by Wiley and MacLaren in 1955 [18] and were applied to MALDI-

time-of-flight (TOF) by Cotter et al. [19]. Time-lag focusing-MALDI-MS has recently been used for end group determination of low molecular weight poly(ethylene glycol)s [20,21] and has been shown to increase both the observed resolution and sensitivity in spectra of polymers [22]. This paper shows some time-lag focusing MALDI data for synthetic polymers with molecular weights of less than 15 kDa and indicates the potential for end group analysis of these systems.

Experimental

Mass spectrometry

The time-lag focusing UV-MALDI data were obtained using a Tofspec E (Micromass, Manchester, UK) TOF mass spectrometer, operated in linear or reflectron mode at an accelerating voltage of 25 kV. A hybrid microchannel plate-electron multiplier detector was used and was sampled at 500 MHz. A VSL-337i nitrogen laser (Laser Science Inc.) was employed, operating at 337 nm with a pulse width of 4 ns and a maximum energy output of 180 μJ.

For the data shown here an external multi-point calibration was employed giving a mass accuracy of approximately 0.03% in linear and 0.01% in reflectron mode. The same mass of polymer was used as that for the experiments [22]. PMMA A, PMMA B, PMMA C and PMMA D were the calibration compounds for MALDI-MS analysis of the methacrylate polymers. Average molecular weight data (M_n , M_w and polydispersity) was generated using the PC based software called Poly (Micromass) using the formulae shown below.

$$M_n = \frac{\sum M_i N_i}{\sum N_i}$$

$$M_w = \frac{\sum M_i^2 N_i}{\sum M_i N_i}$$

$$\text{Polydispersity} = \frac{M_w}{M_n}$$

Sample preparation

All polymer standards were purchased from Polymer Laboratories (Church Stretton, UK), except poly(styrene) E and poly(styrene) F which were from Aldrich Chemical Co. (Gillingham, UK) and were used without further

purification. Dithranol (1,8-dihydroxy-9[10H]-anthracenone) and 1,1,1,3,3,3-hexa-fluoro-iso-propanol (HFIP) were purchased from Sigma Chemical company (St. Louis, MO). Silver tri-fluoroacetate was from Aldrich Chemical Company, sodium iodide from BDH Ltd. (Poole, UK), analytical grade acetone from Fisher Scientific (Loughborough, UK) and analytical grade tetrahydrofuran from Fisons Scientific Equipment (Loughborough, UK).

The most probable mass or peak average molecular weight (MP) and polydispersity values of the polymers, which are estimated by the manufacturer using GPC, are 2010 and 1.10 for PMMA A, 5270 and 1.06 for PMMA B, 10300 and 1.06 for PMMA C, 20200 and 1.06 for PMMA D, 1700 and 1.06 for poly(styrene) A, 3250 and 1.04 for poly(styrene) B, 7000 and 1.04 for poly(styrene) C and 11600 and 1.03 for poly(styrene) D. The mean molecular weight generated by viscometry, also estimated by the manufacturer, is 24150 for poly(styrene) E. The structure of the end groups of PMMA E, PMMA F, PMMA G, PMMA H and PMMA I were determined by nuclear magnetic resonance spectroscopy (data not shown). Average mass data was not available for these polymers.

Calibration

All solutions were prepared at a concentration of 10 mg/mL. The samples (poly(styrene) A, poly(styrene) B, poly(styrene) C, poly(styrene) D and poly(styrene) E), matrix (dithranol) and salt (silver tri-fluoroacetate) were separately dissolved in tetrahydrofuran for analysis of mixtures of poly(styrene)s. A ratio of 50:[5:5:5:5]:2 matrix solution:sample solution:salt solution (v/v) was employed and approximately 0.5 μ L of this mixture was deposited on the sample disk. Each separate component of poly(styrene) was made up with an equal weight of sample. A layer of sodium iodide (from approximately 0.5 μ L of a solution in acetone) was applied to the target in the PMMA case. The samples and corresponding matrix (dithranol) were separately dissolved in HFIP. The ratios used were 10:[1:1:1:1] matrix solution to sample (PMMA A, PMMA B, PMMA C and PMMA D) solutions (v/v) and approximately 0.5 μ L of this mixture was applied to the sample stage. Equal weights of each of the components were used, as for the mixture of poly(styrene) standards.

Single components

Solutions of poly(styrene) F, matrix and silver salt were mixed in a 1:10:1 ratio (v/v) before application of approximately 0.5 μ L to the sample stage. The alkali metal salt was again applied to the sample disk before deposition of the sample and matrix for analysis of PMMA E, PMMA F, PMMA G, PMMA H and PMMA I. The separate matrix and sample solutions, each at a concentration of 10 mg/mL, were mixed in a 10:1 ratio (v/v).

Results and discussion

Instrument performance

The basic parameters of mass range, resolution and mass accuracy need to be established, as is the case with all new

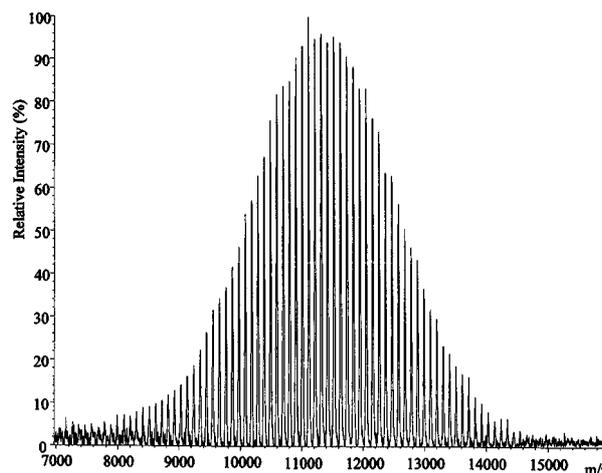


Figure 1. Time-Lag Focusing UV-MALDI Spectrum of Poly(styrene) F obtained in the linear mode of operation.

mass spectrometry techniques. This is particularly important in the case of polymer characterisation as these materials are typically complex mixtures. Additional problems, in terms of the dynamic range of the experiment, are imposed by the need for quantification of the many oligomers, often over wide mass ranges, that are typically present. The oligomeric adduct ions that are observed in the MALDI spectra should reflect the relative intensities of the respective species in the original sample.

Figure 1 shows a time-lag focusing UV-MALDI spectrum of poly(styrene) F with an approximate molecular weight of 12.5 kDa. The distribution of oligomeric adduct ions is resolved to baseline. The average molecular weights, calculated from the MALDI data for polystyrene F, are shown in table I. The use of time-lag focusing significantly improves the resolution and signal-to-noise that is observed. A spectrum of a sample of similar average molecular weight was obtained by means of FD-MS [23]. The ions observed in the FD-MS spectrum were molecular ions, M^+ [23], whereas adducts with silver ($[M+Ag]^+$) are seen by means of MALDI-MS. These spectra indicate that there is no significant mass dependence of the detector over this mass range. The advantage of the MALDI-MS experiment is the short time-scale of analysis. MALDI spectra can be acquired in seconds whereas FD spectra take in the region of one-to-two hours. Spectra of polymers of even higher molecular weight may be acquired successfully by means of MALDI-MS [24] but the attainable resolution is, as yet, not sufficient in general to differentiate the oligomer structure above approximately 30 kDa, depending on the mass of the repeat unit.

End group characterisation

One of the most important areas of polymer characterisation in which MALDI-MS has a significant contribution to make is in the characterisation of end groups. The ability of the technique, in this case, to be able to establish the mass to approximately 1 in 5000 Da means that information regarding the molecular mass of end groups can be established within these experimental limits.

Table I.

Sample	Number Average Molecular weight (M_n)	Weight Average Molecular Weight (M_w)	Polydispersity
Poly(styrene) F	13081	13239	1.01
PMMA E	3831	4045	1.06
PMMA F	4731	5838	1.24
PMMA G	4164	5000	1.18
PMMA H	5760	6194	1.08
PMMA I	7901	8679	1.10

Poly(methyl methacrylate)s are well studied by MALDI-TOF-MS [3,5,9,25,26] since the molecular weight range is often amenable to study and the end groups are of considerable practical importance. The UV-MALDI spectrum of PMMA E, acquired in reflectron mode, is shown in figure 2. The spectrum is dominated by one series of ion peaks which have m/z ratios that are consistent with the proposed structure 1 (Fig. 3) for the polymer, as determined by NMR spectroscopy. The calculated M_n , M_w and polydispersity values are shown in table I. The observed resolution (see inset to Fig. 2) is approximately 8900 (FWHM) which would easily enable the differentiation of end groups differing in mass by 2 Da in this molecular weight range. This would be the case, for example, when a sample has a mixture of both a saturated and unsaturated terminating group at one end of the polymer chain. A similar resolution was observed in the Fourier-transform ion cyclotron resonance-MS spectrum of a poly(ethylene glycol) sample with a similar average molecular weight [14].

Figure 4 shows the MALDI spectra, acquired in the linear mode of operation, of some PMMA samples with unusual end groups. The peak molecular weight, as observed by mass spectrometry, (M_m) values [27] are approximately

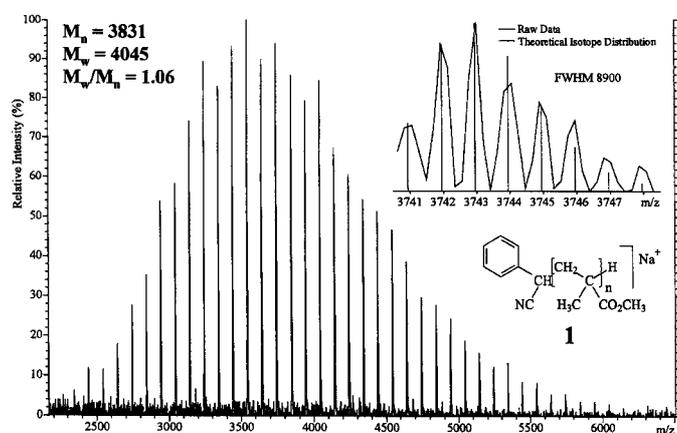


Figure 2. Time-Lag Focusing UV-MALDI Spectrum of PMMA E obtained in the reflectron mode of operation (Inset-expansion of the molecule adduct ion ($[M+Na]^+$) peak region for the 36-mer of PMMA E and the theoretical isotope pattern).

4500, 4500 and 6000 Da respectively (see Tab. I for M_n , M_w and polydispersity values). The main series in each of the spectra have the proposed structures 2, 3 and 4 (Fig. 3) but other species are also observed, including those with cyclic and hydrogen end groups (structures 5 and 6, Fig. 3). These spectra indicate the importance of being able to resolve the ion peaks for different oligomers in the mass range to be studied and the requirement for good mass accuracy.

The UV-MALDI spectrum of a novel PMMA sample of higher molecular weight is shown in figure 5. The calculated average molecular weight data for this polymer are shown in table I. Two intense series of peaks are observed in the spectra and the proposed end groups for these series

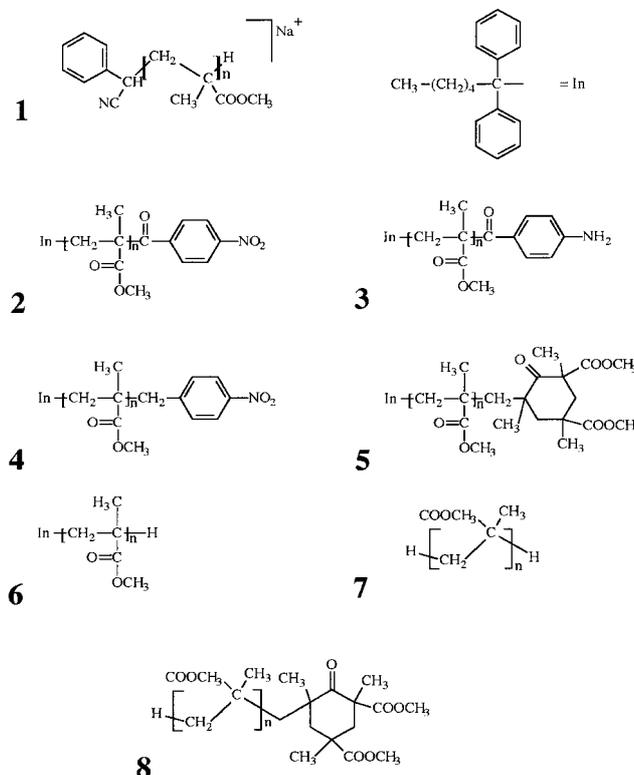


Figure 3. Polymer and end-group structures.

are shown (structures 7 and 8, Fig. 3). The two distributions are of distinctly different shape and have been assigned as species with hydrogen end groups (7) and a cyclic end group (8). Other series of low intensity are observed in the spec-

trum as a consequence of the high resolution and signal-to-noise ratio. These species were not observed in the MALDI-MS spectrum obtained without time-lag focusing, due to the lower signal-to-noise and resolution.

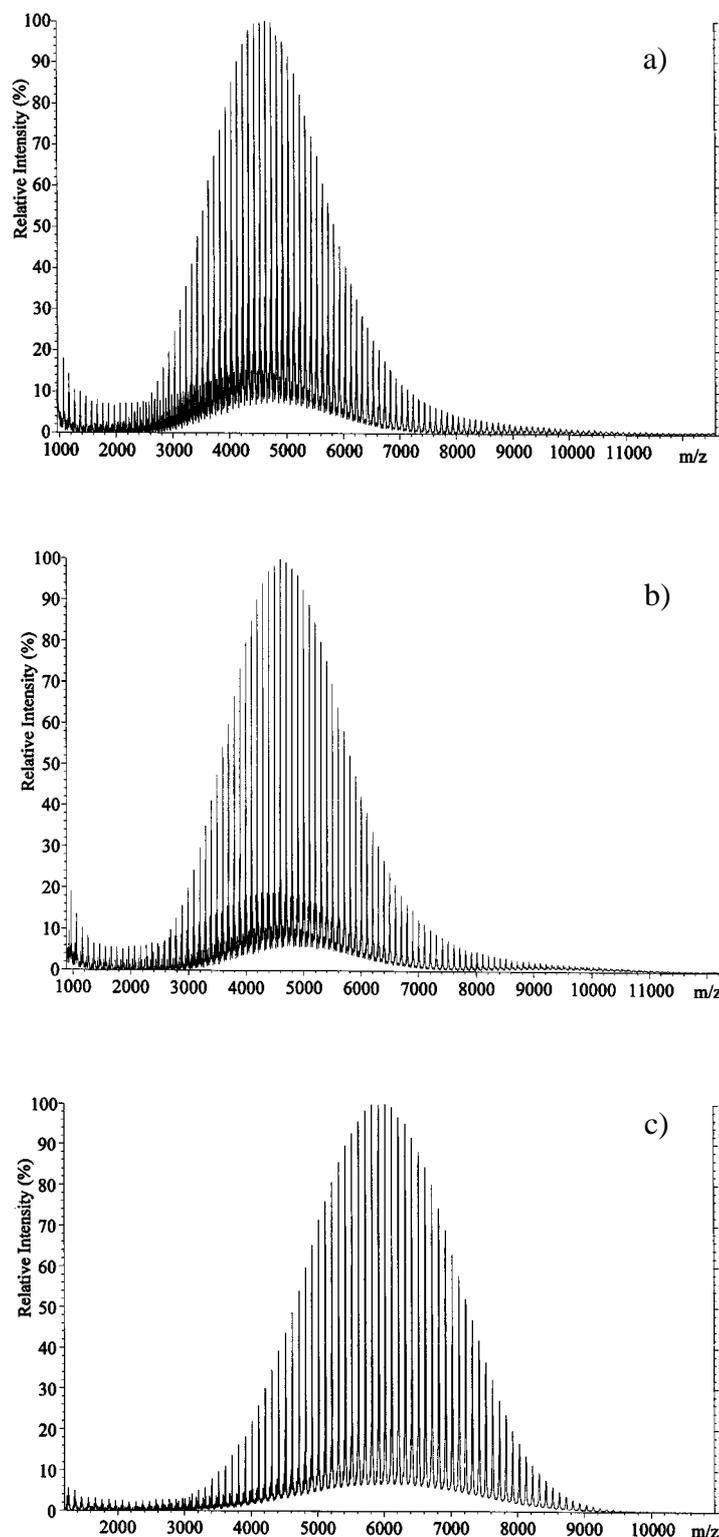


Figure 4. Time-Lag Focusing UV-MALDI Spectra of (a) PMMA F; (b) PMMA G and (c) PMMA H obtained in the linear mode of operation.

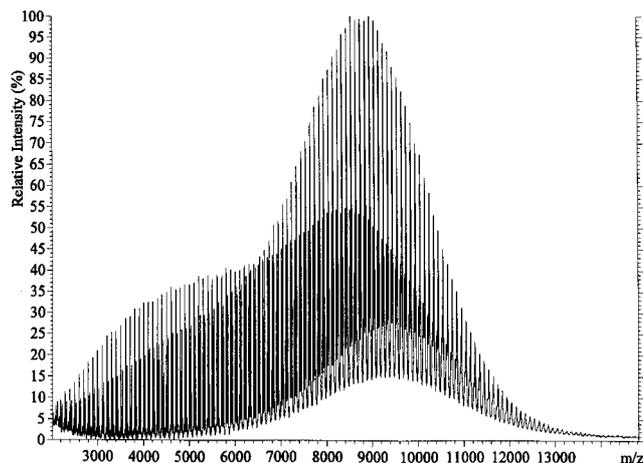


Figure 5. Time-Lag Focusing UV-MALDI Spectrum of PMMA I obtained in the linear mode of operation.

Conclusions

Time-lag focusing-MALDI has been shown here, and in other reports [20–22], to significantly increase the signal-to-noise ratio and resolution that is observed in spectra of synthetic polymers. This is especially important for the determination of the end groups, especially those of low abundance which may not be seen when prompt extraction is employed. This technique, in conjunction with other improvements in instrumentation such as detector performance, should mean that MALDI-TOF has an important role to play in the analysis of these compounds in the future. More experiments are needed, however, to determine whether the quantitative determination of the relative proportions of different end groups in polymers is possible by MALDI-MS.

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