Polymer Mass Spectrometry

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High-Resolution Ion Mobility Spectrometry–Mass Spectrometry on Poly(methyl methacrylate)**

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Synthetic polymers are produced in industry to serve a global market across a wide range of areas.[1a–c] Increasingly complex polymeric structures have been developed to provide desirable properties and functions.[1d] The performance of these products depends on many factors such as endgroup composition, molecular weight distribution (MWD), and 3D conformation.[1e,f] Various analytical methods have been developed to obtain information about these properties. Conventional analytical techniques to study polymer systems include gel-permeation chromatography (GPC),[2a] Fourier-transform IR (FTIR),[2b] NMR spectroscopy,[2c] and differential scanning calorimetry (DSC).[2d]

The development of "soft" ionization methods such as elecrospray ionization (ESI)[3a,b] and matrix-assisted laser desorption/ionization (MALDI)[3c,d] allowed mass spectrometry (MS) to become one of the most promising analytical methods for the analysis of polymeric systems. MS has the ability to characterize a dispersed polymer containing oligomers with different structures such as isomers or isobaric molecular weights. The combination of liquid chromatography (LC) and MS reduces the effects of ion suppression that may occur in an infusion MS analysis and provides an extra dimension of separation.[4] However, a relatively long separation time (> 30 min for HPLC, around 10 min for UPLC) is needed and a complex elution system using a variety of solvents has to be developed to suit a specific polymeric system.

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The combination of ion mobility spectrometry (IMS) and ESI-MS has been developed to analyze biomolecules and biopolymers.[5] Ion mobility describes how fast an ion in the gas phase moves through a drift cell that is filled with a carrier buffer gas under the influence of an electric field. More compact ions with a smaller collision cross-section will drift more quickly than expanded ions. The time-scale for separations in IMS is 100 μs to 10 ms, which is ideally suited for interfacing with an MS instrument. The extra dimension of separation based on drift time (t_D) provided by IMS is also highly complementary to the information obtained by MS. Although some studies on IMS-MS measurements of blends of disperse macromolecules, for example, poly(ethylene glycol) (PEG), have been reported, studies using IMS-MS on complex synthetic polymer systems are still limited.[6]

Here, we demonstrate the power of using high resolution IMS-MS to study a poly(methyl methacrylate) (PMMA) synthesized by radical polymerization using peroxide initiator (*tert*-butyl peroxy-3,5,5-trimethylhexanoate) in solvent. Comprehensive studies on acrylic polymer produced by radical polymerization using MS have been done in the past decade.[7] It has been proven that high-resolution MS can discriminate between the effects of various polymerization mechanisms such as β-scission, chain transfer to solvent, radical transfer to solvent from the initiator, etc.[7] A system with complex endgroup combinations is expected since various initiation and termination reactions may occur. We show that by using the IMS-MS combination, detailed endgroup information and discrimination of molecules with same nominal masses were achieved without the need of a preceding time-consuming LC separation.

Figure 1 shows the results of a typical IMS-MS experiment with a 2D analysis of the PMMA polymer, t_D along the *x*-axis

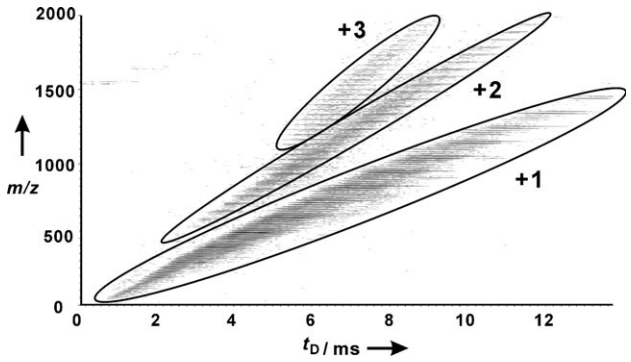

Figure 1. Plot of m/z vs. t_D (drift time) for PMMA polymerized by free radical polymerization on IMS-MS. Sodiated species with charge states up to +3 series were observed.

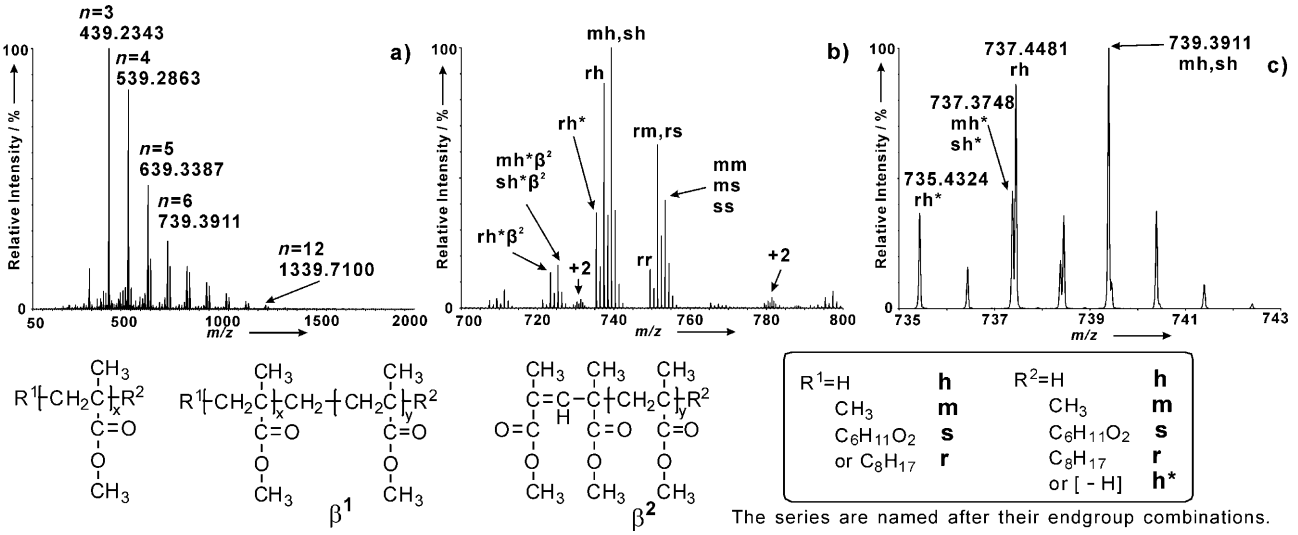

Figure 2. Averaged mass spectra of PMMA: a) the entire scan range *m/z* 50–2000, b) expanded mass spectra (*m/z* 700–800) with series assignment, and c) further expanded mass spectra (*m/z* 735–743) with series assignment. Differences of ca. 72 mDa are clearly shown.

and mass-to-charge ratio (*m/z*) along the *y*-axis. Clear distributions of sodium cation adducts of PMMA according to the size and shape of individual components with up to triply charged ion peaks were observed. The triply charged ion peaks ranging from *m/z* 1000 to *m/z* 2000 demonstrate the presence of PMMAs with molecular weights of up to 6000 Da, which is consistent with our GPC data (M_n of 3300 Da, data not shown). Although the abundance of the high-molecular weight components in the PMMA is relatively low, IMS-MS is still capable of the analysis. Compared to the LC-MS work on poly(*n*-butyl acrylate)s (PBAs) of comparable average molecular weight (M_n of 3800 Da) reported elsewhere,[7f] in which components were only observed up to 2000 Da, IMS-MS can be used independently for the detailed investigation of the entire MWD of the PMMA sample.

Averaged IMS-MS spectra of PMMA are presented in Figure 2a. Several series of peaks start at *m/z* 339 continuing to greater than 1339 with a separation of 100 Da between each group. The 100 Da mass difference is attributed to the mass of MMA ($C_5H_8O_2$; $m_{theo} = 100.0524$ Da). Figure 2b is an expanded averaged spectrum showing one monomeric mass range (*m/z* 700–800).

The elemental compositions of the endgroups appearing in the polymer series were identified using a linear regression method.[8] The naming system used here is similar to that used in previous reports,[7a,f] that is, the series are named after their endgroup composition (Figure 2, [-H] indicating an unsaturated endgroup resulting from disproportionation.) The most intense series of peaks sh (or mh, which is one degree of polymerization (DP) higher than sh, but has the same elemental composition and therefore has the same mass as sh), $m/z\ 439 + (n-3) \times 100$, contains a butyl acetate endgroup (or methyl) at one end of the chain and is terminated with a H-abstracted endgroup at the other end. The monomer mass calculated from the accurate mass data is 100.0523 Da ($\Delta =$ 0.0001 Da) and the residual mass of this series is 139.0770 Da ($\Delta = 0.0039$ Da). The correlation coefficient (R^2) of the calculation is 1.0000. Details of the procedure are presented in the Supporting Information. Although the results obtained by IMS-MS are not as accurate as those obtained from a FTICR MS or Orbitrap MS, which have higher resolution and mass accuracy, the accuracy of the results still allows determination of the endgroup elemental compositions.

Further expanded mass spectra (*m/z* 735–743) are presented in Figure 2c. Two resolved peaks with 0.0733 Da difference, *m/z* 737.3748 ($C_{38}H_{66}O_{12}$) and *m/z* 737.4481 ($C_{36}H_{58}O_{14}$), are observed in the spectra. This mass difference is attributed to the difference in elemental composition of two endgroups, exchanging C_2H_8 for O_2 (the theoretical mass difference is 0.0728 Da). The first peak (*m/z* 737.3748) is attributed to sh* or mh* and the second peak (*m/z* 737.4481) is attributed to rh. It is very likely that both mh* and sh* are present because both endgroups were observed by LC-Orbitrap MS in a PBA sample that was prepared under similar polymerization conditions.[7f] This small exact mass difference would not be detected in a low-resolution mass spectrometer, resulting in a single, unresolved peak with *m/z* 737.4.

Within this subset of the IMS-MS data, the aforementioned substances can also be differentiated by their different t_D. Figure 3 presents the ion mobilograms of the peaks in the two series (sh* or mh* and rh) described above (with 0.0728 Da difference). Within the same DP, molecules in series sh* and mh* have shorter t_D than the molecules in series rh. This demonstrates that although the backbones of the molecules in the two series are the same, the subtle differences in endgroups determine the size and space conformation differences which can be discriminated in IMS-MS. An explanation for this observation may be that the molecules in series rh have a more extended octyl endgroup from the initiator than the relatively short methyl endgroup or butyl acetate endgroup (that is similar to the polymer backbone).

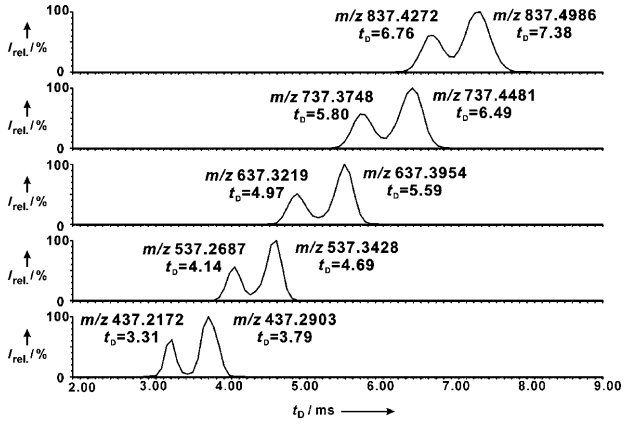

Figure 3. Extracted (extraction window 200 mDa) ion mobilogram of (sh* or mh*) and rh PMMA oligomers. The *m*/*z* difference between (sh* or mh*) and rh peaks is 0.0728(±0.0014) Da.

Therefore molecules in series rh have a longer t_D. To confirm this hypothesis, modeling of the gas-phase molecular structures would be required.

Well-resolved *m*/*z* peaks of the two series (sh* or mh* and rh) were obtained in this IMS-MS study even up to a nominal mass of *m*/*z* 1037. Peaks at higher *m*/*z* were not very well resolved; a higher resolving power would be required. Drift time separation alone could not resolve the differences of the two peaks at higher masses either. A combination of t_D and *m*/*z* separation, however, allowed the discrimination of these peaks at higher degrees of polymerization. Figure 4 is a 3D representation of the partial data set of the same IMS-MS experiment on PMMA. The t_D range displayed is 8.5–11.5 ms and the *m*/*z* range is 1137–1138. The inserts represent projections on the t_D axis (Figure 4a) and the *m*/*z* axis (Figure 4b). At this DP neither the t_D separation nor the mass spectrum alone allowed the separation of the two peaks. In the 3D display, however, two well separated peaks are observed. The 3D representation avoids the congestion of the data on either dimension. The extra dimension of separation brought by IMS in addition to the *m*/*z* information generated by MS increases the Euclidean distance between the peaks in the dataset and thereby facilitates the discrimination of nominal isobars.

Figure 5 shows a 3D representation of a part of the data of the same IMS-MS experiment on PMMA. The t_D range displayed is 6–7.5 ms and the *m*/*z* range displayed is 730–760.

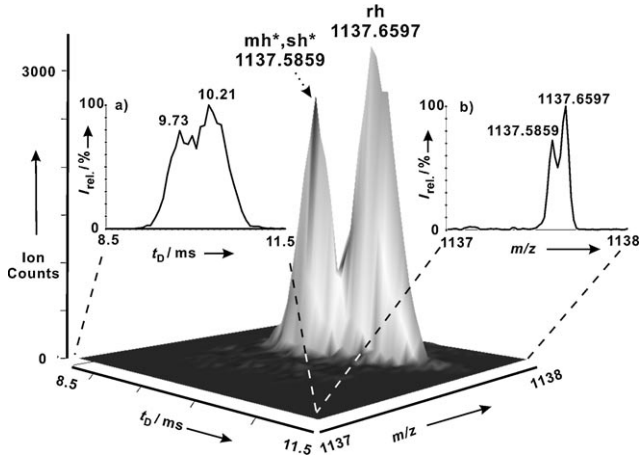

Figure 4. 3D representation of the IMS-MS data obtained on the PMMA sample. Selected t_D range 8.5–11.5 ms, selected *m*/*z* range 1137–1138. Inserts represent projections of a) the average ion mobiligram and b) the average mass spectrum.

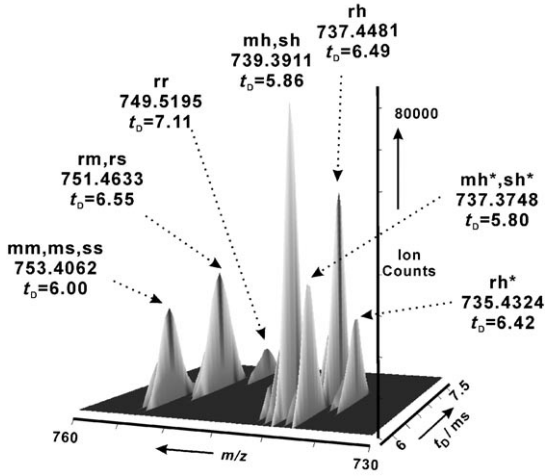

Figure 5. 3D representation of a typical IMS-MS experiment output with assignment of accurate masses, series, and drift times.

All the series were well separated based on their t_D and *m*/*z*. Interestingly, some products with larger molecular weight were observed to have shorter t_D. For example, the peak at *m*/*z* 753.4062 in series mm (or ms or ss) has a t_D of 6.00 ms. A lighter pseudomolecular ion at *m*/*z* 735.4324 from series rh* has a longer t_D of 6.42 ms. The cause of this phenomenon can be attributed to the different compositions of their endgroups. The lighter molecule, belonging to the series rh* has a more extended endgroup, octyl, originating from the radical initiator. The heavier molecule, on the contrary, has a more compact methyl endgroup. This results in different drift times. It also proves that IMS does not only offer separation based on molecular weight but also on size and conformation of PMMAs. Similar observations have been made in IMS-MS of biomolecules,[5] but they have not yet been reported on a real polymerization mixture with detailed mapping of endgroups and to further allow confirmation or elucidation of polymerization mechanisms.

In the very short time-span of the experiment, in the tens of milliseconds range, IMS-MS offers full separation and identification of the components of the very complex PMMA system studied here across its MWD. A similar result can be achieved using HPLC-MS or UPLC-MS technique but with a much longer experimental time (see Supporting Information for the HPLC-MS experiment). These chromatographic techniques require that either a gradient or isocratic elution system is available or developed for every specific polymer system.

The development of multidimensional IMS-MS strategies is likely to aid the characterization of more complex polymer systems such as copolymers. As subtle structural differences can be noticed by applying IMS-MS, a separation of isomers would be achieved by LC-IMS-MS or IMS-MS/MS. The 2D and 3D visualization of the data facilitates extraction of structural information reflecting differences in mass and size, and/or conformation of the molecules. Furthermore, information such as branching in polymers which normally cannot be acquired by MS study alone can be investigated using IMS-MS.[9]

Experimental Section

The PMMA polymer was prepared by radical polymerization in butyl acetate as solvent under relatively high temperature (160 °C) using *tert*-butyl peroxy-3,5,5-trimethylhexanoate as initiator. It was diluted to 10 $\mu g\,mL^{-1}$ in methanol for direct infusion experiments on a Waters Synapt G2 HDMS mass spectrometer. The system was used in positive ionization electrospray mode with "resolution" set to 20 kDa. The m/z was calibrated using sodium formate. A solution of leucine enkephaline at 2 $ng\,mL^{-1}$ was used for the lock mass signal. Reference scans were taken every 10 s. Source parameters as well as mobility settings were optimized using the sample solution of PMMA. The most relevant parameters were the following: Capillary voltage: 3.7 kV; cone voltage: 60 V; source temperature: 100 °C; desolvation gas flow: 500 $L\,min^{-1}$; desolvation temperature: 350 °C; helium cell gas flow: 180 $mL\,min^{-1}$; IMS gas flow: 85 $mL\,min^{-1}$; IMS wave velocity: 600 ms; IMS wave height: 40 V. Nitrogen was used as carrier buffer gas. Data were obtained and processed using Waters MassLynx 4.1 SCN 779 and DriftScope 2.1 software. The scan time was 1 s with an inter-scan delay of 24 ms. A total of 100 scans taken in the range m/z 50–2000 were averaged for data processing.

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