Application of mass spectrometry to the characterization of polymers
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Recent advances in soft ionization techniques for mass spectrometry of polymeric materials make it possible to determine the mass of intact molecular ions exceeding $1 \times 10^6$ Da. Developments in high resolution mass spectrometers have additionally led to impressive advances in our ability to characterize polymers. The entire molecular mass distribution of a polymer sample can be accurately measured. From the molecular mass, the molecular formulae and information regarding polymer composition and end-groups can be deduced. The two techniques which have received the most attention are matrix-assisted laser desorption/ionization and electrospray ionization. In recent work, these techniques have been combined with chromatographic separations, and a series of mass spectra are acquired for each fraction of the distribution. This simplifies the analysis by reducing the number of components present in each mass spectrum, and additionally improves quantitation.

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Abbreviations
ESI electrospray ionization
FT-MS Fourier transform ion cyclotron resonance mass spectrometer
MALDI matrix-assisted laser desorption/ionization
SEC size exclusion chromatography
TOF time of flight

Introduction
The characterization of polymers presents particular problems for analytical techniques. In addition to their high molecular weights, most polymers are polydisperse and cover an extremely wide range of molecular weights, with hundreds or thousands of different molecular weight species. Furthermore, additional distributions in composition, end-groups and topology, are superimposed on the molecular weight distribution.

Mass spectrometry can be used to accurately measure molecular masses, however, the difficulty in applying this technique to polymers has been volatizing high molecular mass polymeric ions without fragmentation. Although analysis of the spectra of fragmented polymers provides considerable information about the polymer, it does not allow the chain length, and chain length distribution, to be measured. Recent advances in soft ionization mass spectrometry, in which only intact molecular ions are observed with minimal fragmentation, affords both the chain length distribution and the chemical composition at every chain length, which may be deduced from the mass. The complete distribution of chains (length, monomers, end-groups) can ideally be identified and quantified in a single experiment which usually takes under ten minutes to perform, and uses only sub-microgram quantities of materials. The mass spectrometry data are also predictable as both the exact mass (monoisotopic) and isotope patterns can be computed prior to analysis if the composition is known. In addition, on-line databases, such as the Science and Technology Network (STN) [1], can facilitate identification and retrieve patent information by searching the formula weight or molecular formula furnished by mass spectrometry. This is particularly useful for industrial chemists.

Molecular weight distributions of polymers with molecular weights exceeding a million Daltons (Da) have recently been reported [2]. In this case the polymers were ionized using matrix-assisted laser desorption/ionization (MALDI) and the ions were mass separated on a time-of-flight (TOF) analyzer. The area of biopolymers boasts the most successful high molecular mass characterization in which 10^6 Da DNA strands were ionized using electrospray ionization (ESI) and the mass separation was performed on an ion cyclotron resonance mass spectrometer [3]. In conjunction with developments in ionization capabilities, developments in the capabilities of time-of-flight analyzers and Fourier transform ion cyclotron resonance mass spectrometers (FT-MSs) have greatly increased the resolution available in polymer mass spectrometry. Recently, McLafferty and coworkers reported unit resolution, that is the separation of molecular ions differing by 1 Da, for chondroitinase, a 112 kDa protein using FT-MS [4].

The two techniques, MALDI and ESI, mentioned above are the latest accomplishments in the characterization of synthetic polymers and hold the most promise, therefore we will focus our discussion on these techniques. In particular, we discuss recent work where these MS methods are combined with a condensed phase polymer separation technique, such as size exclusion chromatography (SEC).

It should be noted, however, that in addition to MALDI and ESI there are a variety of analytical mass spectrometric methods currently available which furnish intact molecular ions from polymeric materials up to about 5000 Da.
This molecular weight range is of major importance in coatings technology, for example, and many of the building blocks used in the synthesis of architecturally designed polymers also fall into this mass range [5]. Some of the more successful methods applied to the analysis of polymeric materials include field desorption [6,7], secondary ion mass spectrometry [8-10], fast atom bombardment [11-13], desorption chemical ionization [14], potassium ionization of desorbed species [15,16] and direct laser desorption [17,18]. For a comparison of these methods in the analysis of phenol formaldehyde oligomers see [19].

**Ionization**

**Electrospray ionization**

Electrospray ionization mass spectrometry is a soft ionization technique that has been widely used in the biological arena. ESI is a method for transforming ions that are present in solution into the gas phase for mass spectral analysis. A sample solution is sprayed or nebulized under the influence of a high electric field. The resultant aerosol is desolvated using a combination of heat and vacuum, and a perpendicular flow of inert gas. The ions formed, usually with the addition of sodium or potassium, are frequently multiply charged due to the size of the polymer molecule. Because mass spectrometers separate ions based upon the mass-to-charge ratio, the multiple charging inherent in this technique, extends the application of this technique to higher mass polymers [20]. Unfortunately, this means that each species of a given molecular formula can yield a charge distribution envelope, thus further complicating an already complex polymer mass spectrum. However, the mass spectrum possessing several charge distributions can be deconvoluted to give the correct distribution for simple low molecular weight homopolymers [21]. Alternatively, multiple charge distribution envelopes can be resolved using the ultrahigh resolution of FT-MS [22]. Another approach, discussed below, is to reduce the number of components entering the ESI source by performing a condensed phase separation, such as gel permeation chromatography, prior to mass spectrometry [23,24,25*,26*].

**Matrix-assisted laser desorption/ionization**

Matrix-assisted laser desorption ionization, which was also originally developed and applied to biological molecules, has recently found widespread application in polymer characterization. MALDI can produce intact molecular ions in the gas phase with molecular weights up to and beyond 10^6 Da. The polymer in solution is mixed with a light absorbing matrix material, such as dihydroxybenzoic acid, dissolved in the same solvent. The ratio of analyte to matrix is generally 1:1000. The mixture is deposited and dried on a laser target surface. The incident laser energy is absorbed by the matrix solution leaving the desorbed macromolecular ions, generally singly charged, in the gas phase. The application of MALDI has recently been extended to the characterization of hydrocarbon polymers, which are difficult to ionize because of their chemical inertness, using time-of-flight [27] and Fourier transform mass spectrometers [28]. In the following section we shall focus on two areas in which MALDI and ESI have been applied to polymer analysis: accurate measurement of molecular weight distribution and chemical composition and end-group and topology determination.

**Applications**

**Molecular weight distribution and quantitative measurements**

Ideally, mass spectrometry provides a quantitative measure of the molecular weight distribution of the polymer sample as well as composition and end-group distributions. The accuracy of these distributions, and the molecular weight averages derived from them, depends upon uniform measurement efficiency across the relevant molecular weight range. The measurement efficiency for a given polymeric species in a distribution depends on a number of factors, including the efficiency of dissolving the sample in the matrix, sample vaporization, ionization, ion transmission and finally detection. Considerable work has been carried out to disentangle the effects of these different factors on the resultant distribution. MALDI-TOF produces results in good agreement with molecular weight distribution data from traditional measurements, such as size exclusion chromatography when the samples have low polydispersity, typically M_w/M_n < 1.4 (after the correct conversion from a weight fraction logarithmic distribution to a number fraction distribution [29,30,31*]). For broader distributions, the polydispersity calculated from the MS data is frequently underestimated. In the case of copolymers, or homopolymers with different end-groups, there is the additional concern that the measurement efficiency may not be the same for compositionally different molecules even if the molecular masses are similar.

One cause of variation in the measured spectra is in the technique used to prepare the polymer sample in the matrix. Using an aerosol spray to deposit the sample and matrix solution creates a homogeneous layer of equally sized crystals and has been shown to lead to improved shot-to-shot, and spot-to-spot, reproducibility, as well as increased signal intensity, in the spectra obtained [32]. Measurements on mixtures of isolated methacrylate oligomers by MALDI [33*], electrospray [21] and measurements on mixtures of narrow polydispersity PMMA (poly(methyl methacrylate) standards [34] showed no molecular weight discrimination and the detectors gave a molar response over the molecular weight range studied. This suggests that low values for molecular weight averages obtained by MALDI-TOF are not the result of mass discrimination or fragmentation. In addition, for a polydisperse PMMA sample, the high molecular weight tail of the distribution observed by SEC measurements, could not be observed by MALDI-TOF. Only when the more abundant lower mass ions were deflected before reaching the detector were the higher mass ions observed. These results suggest that the errors in the measured molecular weight dis-
Contributions by MALDI-TOF are in part due to instrumental limitations rather than ionization limitations. Detector saturation caused by the continuous stream of particles impacting the detector causes a loss of sensitivity at high molecular masses. This may also explain the observation that the spectra vary with laser power [35].

Composition, end-group and topology determination
In order to separate the many species in a polymer molecular weight distribution extremely high resolution is required. For example, a poly(methyl methacrylate) macromolecule made up from 1000 monomer units, with a molecular weight 100,000 Da, differs in mass from the next macromolecule in the distribution, with 1001 monomer units, by only 100 Da or 0.1% of its total mass. In the structural characterization of copolymers the resolution requirements are more severe than for homopolymers. This is because, for a random copolymer, each n-mer with a given degree of polymerization has a wide distribution of different possible compositions, giving a distribution in molecular weights at each degree of polymerization within the overall distribution of degrees of polymerization. In addition, for both homopolymers and copolymers there can be distributions in the end-groups, and all these differences have to be distinguished from the isotope distribution for each species.

Mass spectrometry can be used to determine end-groups from the mass of the end-group, which may be calculated by subtracting the appropriate number of monomer mass units (and the mass of the cation) from the mass of a given molecular ion. Alternatively, both the repeat unit molecular weight and the end-group of a homopolymer can be obtained from a linear regression analysis on the measured molecular weights of an isotopically resolved series of component polymer molecules as a function of the degree of polymerization. For copolymers, the comonomer unit masses can, generally, also be obtained from a series of polymer molecules with different degrees of polymerization, in many cases using computer-based algorithms for the calculation [36,37]. The first method, where the monomer mass is known, provides greater accuracy in the end-group mass determination. The limitation to this approach is the high-resolution required to separate different high molecular weight species, and the overall mass accuracy of the measurements. MALDI-TOF has typically lacked the resolution necessary to provide oligomeric resolution for a wide mass range of a given polymer, and this is particularly true for compositionally complex copolymers. However, the recent development of MALDI-TOF systems based on the time-lag focusing principle, in which there is an equilibrating delay between ion desorption and acceleration, have greatly extended the mass range. For example, Jackson et al. [38,39] have shown how time-lag focusing in MALDI-TOF can significantly increase the resolution and signal-to-noise ratio in the measured spectra. Figure 1 is the MALDI spectrum for polystyrene with molecular weight of about 12.5 kDa using time-lag focusing, showing baseline separation of the oligomers. A resolution of 8900 (FWHM) for a PMMA sample with number-average molecular weight of 3800 was obtained, enabling the separate identification of end-groups differing in mass by 2 Da. This would enable the differentiation of samples with either a saturated or unsaturated terminating group at one end of the chain. For example, vinyl-terminated macromonomers, used as precursors to grafted polymers, could be distinguished from nonfunctional chains. The mass accuracy was approximately 0.03% and 0.01% in linear and reflection mode, respectively. Li and coworkers [40] demonstrated similar improvements using time-lag focusing on poly(ethylene glycol) and polystyrene, but cautioned that, although instrumental resolution is improved, the apparent resolution is still dependent on the matrix and sample preparation. They also presented an extensive study [41] on several copolymers designed to illustrate the analytical merits of time-lag focusing MALDI-TOF in the analysis of complex polymeric systems. Detailed structural information can be obtained from a copolymer mass spectrum providing that both repeat unit and end-group masses are known, or can be determined. The amount of information obtained is dependent upon both sufficient resolution and mass accuracy.

Characterization of topologically different polymers, such as linear and cyclic species, is also possible using MS. Montaudo et al. [42] and Pasch et al. [43] both demonstrated how MALDI-TOF could be used to separate and identify the distributions of linear and cyclic species in condensation polymers. Barton et al. [44] used electrospray ionization MS to characterize linear and cyclic polyethylene glycols. Figure 2 shows the mass spectrum of a nylon-6 sample reacted with adipic acid. The expanded spectrum in the
MALDI-TOF mass spectrum of nylon-6 reacted with adipic acid. The expanded spectrum in the inset shows the peaks corresponding to cyclic (peaks 1 and 2), dicarboxy-terminated (peaks 3 and 5) and amino- and carboxy-terminated (peaks 4 and 7) chains. Peak 6 was not identified. Reproduced with permission from [42*].

Greater mass accuracies and increased resolution can be obtained by using a Fourier transform ion cyclotron resonance mass spectrometer. This approach was used [45*] to analyze end groups of native, perdeuterated methylated, propylated and acetylated polyethylene glycol samples in the mass range 500 to 5000 Da. Over the entire mass range an accuracy better than 10 ppm was obtained. This is sufficient to identify the elemental composition of end-groups in unknown polymer samples.

Although soft ionization mass spectrometry yields a simplified spectrum based upon the molecular formula of the ions formed, no direct structural information is furnished. One must rely on other techniques or manufacturing specifications for this information. Even though copolymers with monomers differing in mass by a mere 0.036 Da have been resolved by high-resolution FT-MS [26*], copolymers containing monomers with identical molecular formulae such as methyl methacrylate and ethyl acrylate (C₅H₈O₂) cannot be resolved by single-stage soft ionization mass spectrometry. Fortunately, tandem mass spectrometry (MS/MS) is able to provide structural information. Although this approach is common place for the characterization of peptides and proteins, polymer science is just beginning to enjoy the benefits. The first step in MS/MS studies is the selection of the precursor parent molecular ion. All other molecular ions are removed from the experiment so as not to complicate the data. An excess of internal energy is deposited to the remaining parent molecular ion resulting in fragmentation. Careful analysis of the fragments allows one to propose or verify structures. MALDI-TOF has been combined with collision-induced dissociation (CID) [46] and with liquid secondary-ion-tan-
dem mass spectrometry [47] to determine information about the polymer structure and end-groups. End-group information was obtained from the fragmentation spectra of the polymer ions for poly(methyl methacrylate), poly(ethylene glycol) and poly(ethylene terephthalate). Mixed polyesters have also been fragmented, affording both repeat unit and end-group identification [48,49].

**Combining size exclusion chromatography with mass spectrometry**

In order to overcome the mass sensitivity of detector efficiency, and also in order to simplify the mass spectra obtained for complex polymers, mass spectrometry can be combined with size exclusion chromatography. SEC is a form of liquid chromatography that separates a polymer sample in solution by the size of the polymer chains. At a given elution time, the solvent eluting from the packed column contains a nearly monodisperse fraction of the total polymer molecular weight distribution. These fractions can be collected off-line and, because of the narrow molecular weight distribution in each fraction (typically $M_w/M_n < 1.05$), are amenable to analysis by MALDI-TOF. Montaudo [50–52] has demonstrated how the measured molecular mass of each fraction collected from the chromatograph can be used to construct an SEC calibration curve of molecular weight against elution volume for the particular polymer under analysis, the SEC concentration detector can then be used for the concentration of each molecular weight. In addition to obtaining the molecular weight distribution, and associated averages, the MALDI-TOF spectra of the lower molecular weight fractions show the oligomers as mass resolved signals, permitting the assignment of each peak to a specific oligomer, and allowing the composition and end-group to be determined. This procedure was used to estimate the composition distribution of a copolyester. In the case of a poly(dimethylsiloxane), so analyzed, it was possible to determine the SEC calibration curves for both linear and cyclic species and so measure these two distributions independently.

In another approach, Fei and Murray [53**] combined SEC directly with aerosol MALDI-TOF MS. The effluent from the SEC column was combined with matrix solution and sprayed directly into a TOF MS. Ions were formed by irradiating the aerosol particles with 355 nm radiation from a Nd:YAG (yttrium aluminum garnet) laser. Results from poly(ethylene glycol) and poly(propylene glycol) with average molecular weights of about 1000 were presented and demonstrated the feasibility of this approach.

Electrospray ionization mass spectrometry is easier to interface with size exclusion chromatography because the polymer solution eluting from the chromatograph can flow directly into the electrospray ionization interface to the mass spectrometer. As described above, SEC separates the polymer solution by hydrodynamic size and so simplifies the polymer distribution entering the spectrometer at a given time allowing small fractions of the total distribution to be measured one at a time. In addition, it avoids many of the problems of overlapping peaks caused by multiple charging. The number of oligomers observed has been shown to be influenced by the cone voltage setting and the concentration of cationization salt, however, the approach has been demonstrated to overcome both the problem of unreliable calibration in SEC, and the problem of measuring accurate molecular weight distributions using mass spectrometry [25*]. Figures 3 and 4 illustrate the application of SEC/ESI to the characterization of polyester copolymers.

**Conclusions**

The ability to generate macromolecular ions in the gas phase from a polymer sample and acquire high-resolution mass spectra, enables the determination of information about the compositional and molecular weight distributions of a polymer. The difficulties that exist in the analysis are largely due to the broad and complex distribution of species with different molecular mass. Because of this complexity, higher resolutions are required in time-of-flight mass spectrometers in order to further separate different high molecular weight species. Delayed extraction has significantly improved resolution for TOF mass spectrometers. In addition, the extremely broad distribution leads to errors in quantification of the different species as a function of molecular weight and composition. Improvements in sample preparation and in experimental techniques have greatly improved the reproducibility of

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*Figure 3*

Electrospray mass spectrum for an isophthalic acid (IA), adipic acid (AA) and butanediol (BD) copolyester. The spectrum was obtained by summation of successive scans as the polymer eluted from the size exclusion chromatograph. All of the observed ions can be attributed to specific structures. For example, the group of peaks clustered around 530 Da is due to pentamers. The main peak at 533 Da is due to the [BD-IA-BD-AA-BD]N⁺ oligomer. Substitution of AA (148 Da) by IA (166 Da) increases the molecular mass by 20 Da, forming the 553 Da ion. Similarly replacement of IA by AA explains the 513 Da ion. The inset shows the total ion chromatogram as a function of elution time. Reproduced with permission from [54].
Figure 4

Molecular weight SEC calibration curve constructed from the SEC/ESI/MS data on the polyester in Figure 3. Oligomers with a high aromatic content (isophthalic acid rich) elute at a later retention volume than oligomers of the same degree of polymerization but with a higher adipic acid content, because they have a smaller hydrodynamic volume. Reproduced with permission from [53**].

molecular weight distribution measurements from MALDI-TOF and further refinements can be expected. The combination of SEC with MS addresses both of the problems above. By analyzing the polymer mass distribution, one fraction at a time, the resultant spectra are greatly simplified. Furthermore, SEC is complementary to MS. The SEC produces accurate and precise quantitation but the molecular mass is often unknown or is only relative, in MS the mass is known but the quantification may be unreliable. In addition, the SEC provides information about the size of the molecule in solution which can be used to deduce information about branching and molecular conformation. The use of MS as a detection method can easily be extended to condensed-phase separations other than SEC. For example, MS can also be coupled with liquid chromatographic separation of polymers, providing a separation by chemical composition prior to the MS mass analysis.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest
** of outstanding interest

2. Schriemer DC, Li L: Detection of high molecular weight narrow
   This paper presents data for the highest molecular weight polymer analyzed by MALDI to date.
   This paper illustrates the ultra-high resolution capabilities of FTMS applied to biological molecules.
This paper demonstrates the molar response of MALDI-TOF for isolated methyl methacrylate oligomers with no fragmentation. This paper addresses the relationship between molecular weight and broad theoretical distributions. This paper shows what information can be obtained using MALDI-TOF to characterize condensation polymers. This paper provides a good summary of the utility of MALDI-TOF for characterizing complex copolymers and the issue of obtaining the necessary quantitative information for complete characterization. This paper shows a good overview of the capabilities and limitations of SEC/ESI performed on a quadrupole MS.

First report of coupling SEC with FTMS through an electrospray interface for methacrylate polymers.


Comparison of most probable peak values as measured for polymer distributions by MALDI-TOF and by SEC in detail for narrow and broad theoretical distributions.


This paper addresses the relationship between molecular weight distributions measured by MALDI-TOF and by SEC in detail for narrow and broad theoretical distributions.


This paper provides a good summary of the utility of MALDI-TOF for characterizing complex copolymers and the issue of obtaining the necessary quantitative information for complete characterization. This paper shows what information can be obtained using MALDI-TOF to characterize condensation polymers. This paper provides a good summary of the utility of MALDI-TOF for characterizing complex copolymers and the issue of obtaining the necessary quantitative information for complete characterization. This paper shows what information can be obtained using MALDI-TOF to characterize condensation polymers.


This paper describes the methods for determining the elemental composition of polymer end-groups using MALDI and FT-MS.