Characterizing Synthetic Polymers by MALDI MS

MALDI MS is a powerful tool for determining molecular-weight distributions and structures of synthetic organic polymers.

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Synthetic polymers play increasingly important roles in everyday life and are ubiquitous in products ranging from automobile components to drug delivery devices. For example, poly(ethylene glycol) in pharmaceuticals and polystyrene in major household appliances, contact lenses, fabrics, and housewares are based on synthetic polymers.

Different polymer formulations possess unique chemical, physical, and mechanical properties. Small variations in the structure of a given polymer formulation can significantly change its characteristics, resulting in new functions and applications. For example, polyvinyl alcohol is a water-soluble synthetic polymer made by alcoholysis of polyvinyl acetate. Its chemical and physical properties depend on the degree of polymerization and the percentage of alcoholysis. Water solubility increases as molecular weight decreases; however, properties such as viscosity, strength, and flexibility improve with increasing molecular weight. Therefore, it is important to control and monitor the parameters that affect the final properties during the development and manufacture of polymers for specific applications.

Numerous techniques are available for characterizing synthetic polymers, such as pyrolysis GC, size-exclusion and gel-permeation chromatographies, nuclear magnetic resonance (NMR) and Raman spectroscopies, thermogravimetric analysis, and MS techniques. In this Report, we will focus on using matrix-assisted laser desorption/ionization time-of-flight (MALDI TOF) MS to characterize synthetic organic polymers.

Overview of MALDI
MALDI is a "soft" ionization process that produces (quasi)molecular ions from large nonvolatile molecules, such as proteins, oligonucleotides, polysaccharides, and synthetic polymers, with minimum fragmentation. The first attempt to use organic matrices to facilitate laser desorption and ionization of nonvolatile amino acids and peptides was reported by Karas, Bachmann, and Hillenkamp in 1985 using nicotinic acid as the organic matrix (1). Hillenkamp’s group further developed this approach and produced molecular ion mass spectra of proteins with masses in excess of 10,000 Da (2). Laser desorption of intact polymer molecular ions was first reported by Tanaka et al. in 1988 for masses up to 22,000 Da using glycerol/polymer mixtures containing fine metallic particles as the laser-absorbing matrix (3).

The use of organic matrices has become routine for MALDI, which is one of the most powerful tools for mass analysis of high-molecular-weight compounds. (A recent article by Fenselau on MALDI MS and strategies for protein analysis is recommended for interested readers [4].) The growth of this technique has been further accelerated by innovative MS methods for polymer analysis, including new matrices for analyzing high-mass compounds (5-7); reproducible sample preparation procedures (8); improved instrumentation, including postsource decay (9); delayed extraction (10-13); and enhanced ion optics and improved data-processing software.

Tanaka’s first experiments generated a distribution of sodium-cationized oligomer ions from poly(propylene glycol) (4 kDa) and poly(ethylene glycol) (20 kDa) (3). These experiments were followed by the work of Danis et al., who reported the analysis of the industrial polymers poly(acrylic acid) and poly(styrene sulfonic acid) (masses up to 300 kDa) using standard MALDI sample-preparation methods (14). The list of compounds analyzed by MALDI has grown steadily, and the technique has become a viable tool for detailed characterization of synthetic polymers.

**Ion desorption mechanism**

MALDI generates high-mass ions by irradiating a solid mixture of an analyte dissolved in a suitable matrix compound with a pulsed laser beam. As the name implies, the laser pulse desorbs and indirectly ionizes the analyte molecules. A short-pulse (a few nanoseconds) UV laser is typically used for desorption. Different wavelengths such as IR have been investigated recently as alternatives.

In practice, MALDI analysis consists of two steps: sample preparation and mass spectral analysis. The key to a successful MALDI analysis depends primarily on uniformly mixing the matrix and the analyte. Samples are typically prepared in the concentration ratio of $1:10^4$ analyte:matrix in a suitable solvent such as water, acetone, or tetrahydrofuran. A few microliters of this mixture is deposited onto a substrate and dried, and the solid mixture is then placed into the mass spectrometer.
To initiate an effective desorption and subsequent ionization process, MALDI matrices should have strong absorption coefficients at the chosen laser wavelength. Many organic compounds that absorb strongly in the UV range have been evaluated as potential MALDI matrices, but only a small number function well as MALDI matrices. Although it has been 10 years since the birth of MALDI, the search for efficient matrices is still very often carried out on a trial-and-error basis.

The actual desorption and ionization event can best be understood by visualizing each step. To begin, the matrix serves as a solvent for analyte molecules and separates them from each other, thereby reducing strong intermolecular forces (matrix isolation) and minimizing analyte cluster formation.

Upon irradiation by the laser pulse, the matrix molecules absorb most of the laser's energy. The high matrix-to-analyte concentration ratio ensures that most of the photon energy is absorbed by the matrix and minimizes direct irradiation of the analyte. The energy absorbed by the matrix molecules is transferred into electronic excitation of the matrix within the solid sample mixture, creating an instantaneous phase transition from a solid phase to the gas phase.

The amount of material removed by each laser pulse is estimated to be approximately 10-100 nm in diameter (laser spot size) and a few hundred nanometers (tens of monolayers) deep. A dense gas cloud is formed and expands supersonically (at about Mach 3) into the vacuum. It is believed that analyte ionization occurs in the expanding plume as the direct result of collisions between analyte neutrals, excited matrix ions, and protons and cations such as sodium and silver.

The analyte ions are introduced into the mass spectrometer and analyzed. MALDI mass spectra are most often acquired using TOFMS techniques that determine \( m/z \) ratios by measuring ion drift times between points of formation and detection.

**TOFMS**

Various mass spectrometer systems are used for MALDI, such as TOF, Fourier transform, magnetic sector, Paul trap, and quadrupole. Among them, MALDI TOFMS and MALDI FTMS are commercially available--the majority of the MALDI instruments are equipped with the TOFMS. Figure 1 is a schematic of a MALDI TOFMS system showing a sample surface to which a voltage is applied, a drift region of a certain length, an ion detector, and associated electronics. The laser pulse that initiates desorption and ionization is focused onto the sample surface. Each pulse of laser light generates a pulse of ionization comprising matrix ions, analyte molecular ions, and, possibly, ionic fragments of the analyte species. The ions formed by the laser pulse are accelerated from the sample surface and pass through a much longer drift region; they are then detected by an ion-detection assembly. The intensities and flight times of each ion pack are registered and converted into a mass spectrum.
The kinetic energy of the ions is determined by the voltage applied to the sample. The kinetic energy is equal to $1/2 \, m \, v^2$, in which $m$ is the mass of the ion and $v$ is the ion velocity. The lower the ion mass, the greater its velocity.

In the early days of MALDI, a simple linear TOF spectrometer was used; mass resolution was suitable but far from satisfactory. Subsequent hardware improvements included sophisticated ion mirrors or reflectrons (15) as well as electrostatic energy analyzers (16), both of which compensate for differences in ion flight times as a function of ion kinetic energy. Time-lag focusing (pulsed extraction) has been resurrected and has provided significant mass-resolution enhancement in modern-day MALDI TOF mass spectrometer instrumentation (17).

**Synthetic polymer analysis**

One of the key advantages of MALDI MS for synthetic polymer analysis is that the absolute molecular weights of oligomers can be determined as opposed to obtaining relative molecular weights by chromatographic techniques. MALDI polymer analysis does not require polymer standards to assign molecular weights to oligomers, and the technique permits accurate determination of molecular weights from narrowly distributed polymers (polydispersity <1.2). Using submilligram amounts of sample material, the actual analysis can be accomplished in a few minutes. Therefore, the speed and information obtained by MALDI are significantly greater than with other conventional molecular-weight-determination techniques.

In addition, MALDI can determine molecular weight independent of polymer structure. For example, rigid-rod polymers such as tetrahydropyrene are a challenge to analyze by conventional polymer analysis methods such as gel permeation chromatography (GPC) because these polymers have a linear sticklike geometry (18). Most synthetic
polymers have structures with flexible backbones that form random coil conformations in solution. These polymers are readily analyzed by GPC methods, which are based on correlating the hydrodynamic volume of the coiled polymer chains with molecular weight. However, the hydrodynamic volume of rigid-rod polymers does not correlate with polymer molecular weight; hence, molecular weights determined by GPC analysis of these structures often deviate strongly from the true values.

Thus, MALDI MS can be used to analyze materials that are difficult to characterize by GPC methods, because of either solvent or column incompatibilities. In addition, GPC does not perform well in low-molecular-weight regions (masses less than a few kilodaltons), whereas MALDI works very effectively in this mass range. Polymer characteristics that can readily be determined by MALDI MS include molecular weight averages (including the number \( M_n \) and weight \( M_w \) averaged molecular weights), polydispersity, mass of repeat units, and end-group mass structure.

Molecular weight and polydispersity data can be used to verify synthetic pathways, study degradation mechanisms, look for additives and impurities, compare product formulations, and provide QC data on batch-to-batch compositional variations. End-group and chemical structure data are critical to understanding structure-property relationships of polymer formulations. MALDI MS provides a direct method of determining end-group mass and composition as well as the mass and composition of oligomer repeat units.

The successful characterization of synthetic polymers by MALDI MS is strongly dependent on the chemical structure of the polymer. It is important to point out that not all synthetic polymers are readily analyzed by MALDI MS, and polymers with different chemical structures may require different sample preparation for a successful analysis.

From a practical standpoint, analyzable polymers can be classified into the four groups (5): water-soluble polymers, such as poly(acrylic acid) and poly(ethylene glycol) (PEG); polar organic-soluble polymers, such as acrylics and poly(methyl methacrylate) (PMMA); nonpolar organic-soluble polymers, such as polystyrene (PS), polyvinyl chloride, and polyethylene; and low-solubility polymers, such as cured polyimide. This last class includes polymers soluble in solvents that are not compatible with matrix materials, and these are the most difficult to analyze by MALDI because a homogeneous mixture of matrix and analyte is not easily prepared.

Sample preparation for the first three classes of polymers is fairly well established (6). Matrices for these polymers include 1,8,9-anthracenetriol (or dithranol), 2,5-dihydroxybenzoic acid, trans-3-indoleacrylic acid, and 2-(4-hydroxyphenylazo)benzoic acid. These matrices are often used in conjunction with alkali metal salts (LiCl, NaCl, KCl) or silver salts such as silver trifluoroacetate (AgTFA) to form matrix-cationization agent mixtures. The use of common solvents for matrix and polymer permits direct mixing and promotes polymer-matrix interaction.
Ionization by cationization is further enhanced by adding metal salts. In some instances, the selection of metal salts is more critical than the matrix itself. Because different polymers can have drastically different ionization or cationization efficiencies, there is no universal matrix-cationization agent combination applicable to all polymers in these classes. The new electrospray sample deposition method for preparing MALDI samples has demonstrated uniform surface-analyte distribution and increased the reliability of quantitative analysis (19). Nonsoluble polymers are generally not analyzable by MALDI because a mixture of matrix/analyte cannot be created because of the lack of a common solvent.

**Molecular-weight analysis**

PS, a nonpolar hydrocarbon polymer soluble in several organic solvents, is used in a wide range of commercial products. Initial MALDI MS analyses of PS oligomers were hampered by their nonpolar nature and lack of an effective site for ionization. However, sample preparation methods have recently been developed for analyzing hydrocarbon polymers, and PS and styrene-based derivatives are now readily analyzable (5, 6).

Figure 2 shows a positive-ion MALDI mass spectrum of a PS 29k standard (a mass calibration standard for GPC measurements with a molecular weight of 29,000). The spectrum shows an envelope of PS oligomers with well-resolved styrene oligomer peaks. Polymer distributions are typically characterized by $M_n$ and $M_w$, which are calculated from
The sample was prepared by mixing a dithranol matrix, AgTFA, and ~1 mg/mL PS in chloroform.

\[ M_n = \frac{\sum (N_i M_i)}{N_i} \]

and

\[ M_w = \frac{\sum (N_i M_i^2)}{N_i M_i} \]

in which \( N_i \) and \( M_i \) are the abundance and mass of the \( i \)th oligomer, respectively. The molecular-weight averages can be calculated directly from the spectrum in Figure 2: \( M_n = 26,250 \), \( M_w = 26,500 \), and polydispersity \( (D = M_w/M_n) = 1.01 \).

Direct measurement of molecular-weight distributions and structure characterization are the two most frequent objectives of MALDI MS in polymer characterizations. It is important to point out that \( M_n \) and \( M_w \) values determined by MALDI MS are in qualitative agreement with chromatographic values only for polymers with narrow molecular-weight distributions \( (D \leq 1.2) \) (20).

MALDI mass spectra from polydisperse polymers indicate that high-mass components are generally underrepresented with respect to lower mass
oligomer peaks. This mass discrimination effect is caused by a combination of several factors, including sample preparation, mass-dependent desorption/ionization processes, and mass-dependent detection efficiency. Caution must be exercised when interpreting average molecular weights and molecular-weight distribution values from MALDI data, especially for highly polydisperse polymers. Although MALDI cannot yet provide reliable molecular-weight parameters for polymers having large polydispersities, the combination of GPC and MALDI MS does provide a useful method for determining molecular-weight distributions of polydisperse polymers.

The resolved repeat units shown in Figure 2 are separated by 104 mass units, which confirms that the analyte is a styrene-based polymer. Detailed analysis of the peak at \( m/z = 25,578 \) reveals that this peak is a PS with 244 repeat styrene units (mass = 25,413 Da). The difference of 165 mass units (25,578 - 25,413) is made up by the addition of 108 and 57, in which 108 is the average mass of the silver atom as a result of silver-cationized PS ions. It has been suggested that silver cationization is favored for those analytes having unsaturated bonds, such as PS and poly(dimethyl silicones) (6). Mass 57 is the butyl group. This PS sample was prepared anionically with a \( t \)-butyl lithium initiator; therefore, it is expected to be terminated with a butyl group.

Thus, the MALDI results provide good confirmation for the structure. Although a single MALDI spectrum does not always provide enough information to uniquely identify an unknown polymer compound, the technique can accurately determine the masses of oligomers, demonstrating that MALDI is a viable tool for end-group analysis as well as product confirmation.

PEG is a very biocompatible polymer (21). In aqueous solutions, PEG is a very mobile, conformationally flexible, random coil. Every ethylene oxide repeating unit of PEG is known to bind 2-3 water molecules. These properties, combined with its very low toxicity, lack of immunogenicity, and ease of excretion from living organisms, make PEG unique and popular as a biomaterial.

When grafted onto a surface, PEG causes exclusion, or steric stabilization (22), which is repulsion of proteins and particulates from the surface. This property is considered to be very valuable for any material that comes in contact with blood plasma. The steric stabilization property is thought to be directly related to the high chain mobility and conformational flexibility of the polymer in aqueous solutions. PEG is widely used for preparing bioconjugates with various biologically relevant molecules, including proteins, peptides, lipids, oligonucleotides, and low-molecular-weight drugs. These conjugates are currently under development for drug delivery and medical device applications.

Because of the low dispersity of commercially available PEG and the ability of the polyether units of PEG to form positive oxonium ions, MALDI is very valuable for characterizing reactive PEG derivatives and their conjugates (23). Typically, MALDI spectra of PEG derivatives or
conjugates can be recognized by a characteristic distribution of spectral lines equally spaced at 44 mass units apart, corresponding to the molecular weight of one ethylene oxide repeat unit (24). Both $M_w$ and $M_n$ can be accurately deduced from the spectrum.

In the case of PEG-modified proteins, MALDI spectra unambiguously determine the type of conjugate species present in the preparation and can distinguish various methoxy-PEG-protein conjugates. This type of information is often difficult to obtain by alternative means. Recently three-component conjugates containing the lipid DSPE, a heterobifunctional PEG-2000 derivative, and a biological ligand were used to prepare liposomes presenting these ligands at the periphery of the grafted PEG chains. The ligands, an oligosaccharide (sialyl Lewis X) and a peptide (YIGSR), were conjugated and then characterized (25). The spectra in both cases confirmed the theoretical molecular weight of the conjugates.

MALDI and GPC

Although MALDI MS has been used extensively to provide molecular weight and structural and compositional information of synthetic polymers, one limitation of the technique is that it fails to provide correct molecular-weight values for polydisperse polymers ($M_w/M_n > 1.2$). Numerous factors--including sample preparation, mass-dependent desorption/ionization effects, and instrumental configurations--contribute to significant mass discrimination effects in analyzing polymers with high polydispersity (26, 27).

A direct experimental procedure has been developed to overcome this limitation (28). The concept involves combining MALDI with chromatography: The intensity of the polymer present (the relative abundance of the polymer distribution) is determined by the refractive index response of GPC, and the mass of the eluant is then measured by a mass spectrometer. Analytically, the method couples the output of GPC to the MALDI MS system by collecting GPC fractions and performing MALDI analysis off-line. The average molecular weight of each fraction is then determined, allowing calibration of the GPC curve against absolute molecular weight. The calibrated GPC trace can then be used to compute average molecular weight and molecular-weight distribution of the unfractionated polymer samples (29, 30).

Figure 3 is an example of GPC and MALDI molecular-weight analysis with a polybutylene adipate (PBA) 4k standard as the test compound. An interesting comparison can be made with MALDI spectra for several of the fractions collected from the PBA. Each spectrum represents a narrow PBA distribution and corresponds to a given GPC elution volume (or time). The GPC calibration line can then be accurately determined without standards. Once the calibrated GPC trace is established, the average molecular weight and molecular-weight distribution of the unfractionated polymers can then be calculated. Coupling GPC and MALDI MS can provide a way to determine absolute molecular weights
for polydisperse polymers. Although the speed of analysis is not an advantage for this combination, it is a valuable supplemental method. The methodology has particular advantages for analyzing synthetic polymers for which GPC standards are not available.

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Figure 3. GPC and MALDI MS of a 4k PBA standard.

(a) Gel-permeation chromatogram of 18 fractions collected over the course of the elution. (b) MALDI spectra from four fractions collected at
To sum up

Molecular weights measured by MALDI MS are in qualitative agreement with those obtained from other conventional methods such as GPC and low-angle light scattering. Compositional information (end group and structure) that is sometimes difficult to obtain by NMR and IR spectroscopies can be derived from mass spectra. Ease of analysis, speed, high mass accuracy, as well as the structure-independent nature of MS are the key advantages of MALDI for polymer analysis. The inability to analyze highly polydisperse polymers is currently a major shortcoming. However, this limitation can be overcome by using MALDI to calibrate GPC profiles.

Although effective matrices and reproducible sample preparation protocols mean that many polymers can now be routinely analyzed by MALDI, numerous technologically important synthetic polymers, such as polyurethane, polyimide, and Teflon, still cannot be characterized by MALDI. Future developments in sample preparation and new matrices are the major tasks for advancing MALDI MS in synthetic polymer analysis.

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