

MALDI Mass Spectrometry of Synthetic Polymers

Review

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マトリックス支援レーザー脱離イオン化質量分析法による 合成高分子の分析

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Abstract

This paper reviews the application of matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) to the analysis of synthetic polymers, and includes data obtained by Toyota CRDL. In MALDI-MS of synthetic polymers, the selection of a matrix and cationizing agent, as well as the sample preparation techniques, are very important to obtaining a successful spectrum. MALDI-MS makes it possible to measure the absolute molecular weight and molecular weight distribution of synthetic polymers having narrow polydispersity. For most synthetic polymers with wide polydispersity, on the other hand, a technique combining size-exclusion chromatography and MALDI-MS has been proposed to determine the absolute molecular weight distribution in order to overcome the problem with mass

discrimination whereby the high molecular weight components are hard to detect, such that the molecular weight distribution cannot be obtained accurately. Additionally, MALDI-MS makes it possible to characterize the molecular structure of the products of degradation caused by light or heat, as well as insoluble samples such as polycyclic aromatic hydrocarbon.

It is expected that MALDI-MS will significantly contribute to the progress of materials development and degradation analysis. We believe that many interesting avenues of research remain unexplored in the field of MALDI-MS of synthetic polymers. These include the search of a universal matrix that can ionize any synthetic polymers, sample preparation techniques, and so on.

Keywords

Matrix-assisted laser desorption/ionization, Synthetic polymers, Molecular weight, Molecular characterization

要 旨

本報ではマトリックス支援レーザー脱離イオン化質量分析法 (MALDI-MS) の合成高分子への適用について、当社で得られたデータとともに概説する。

合成高分子のMALDI-MSではマトリックスやカチオン化剤の選定および試料調製法が良好なスペクトルを得るのに重要である。単分散に近い合成高分子の場合には絶対的な分子量や分子量分布がMALDI-MSにより得られる。一方、多分散度の大きい一般の合成高分子の場合にはマスディスクリミネーションのために高分子量成分が検出しにくくなり、MALDI-MSにより正確な分子量分布

が得られないため、サイズ排除クロマトグラフィーとMALDI-MSを組合せた方法が合成高分子の絶対的な分子量分布を解析する方法として提案されている。さらに、光や熱による劣化生成物や多環芳香族炭化水素のような溶媒に不溶な物質の分子構造をMALDI-MSにより解析できる。

今後、MALDI-MSは材料開発や劣化解析に大いに貢献するものと期待される。また、種々の高分子をイオン化できる普遍的なマトリックスや試料調製法の探索など、合成高分子のMALDI-MSでは非常に興味深い研究機会がまだ十分に残されている。

キーワード

マトリックス支援レーザー脱離イオン化, 合成高分子, 分子量, 分子構造解析

1. Introduction

Mass spectrometry is a powerful tool for analyzing organic materials thanks to its ability to determine the mass of compounds. A mass spectrometer consists of an ion source for ionizing the samples, a mass analyzer for separating the resulting ions according to their mass-charge ratio (m/z), and a detector for counting the separated ions. Above all, the most important part of the process is the ionization of samples in an ion source. Various ionization techniques have been developed depending on the purpose. For example, electron impact ionization (EI) and chemical ionization (CI) are suitable for relatively low-mass samples such as gasses and liquids. Field desorption ionization (FD) is appropriate for oligomer samples with a molecular weight of up to around 10000, and in most cases below 5000. The recently developed electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) are suited to high-mass polymer samples. The latter MALDI technique was developed simultaneously by Tanaka et al.¹⁾ and Karas et al.²⁾ Nowadays, MALDI mass spectrometry (MALDI-MS), which often draws on both the MALDI technique and a time-of-flight (TOF) mass analyzer, is an essential and commonly used tool for the structural determination of biopolymers such as peptides and proteins. In addition, MALDI-MS is becoming a promising analytical tool for synthetic polymers.

This paper reviews the application of MALDI-MS to the analysis of synthetic polymers, and includes data obtained by Toyota CRDL.

2. Principle of MALDI

MALDI is a soft ionization technique that involves

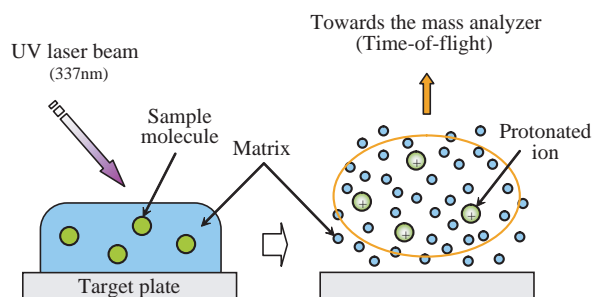


Fig. 1 Principle of MALDI.

the use of a laser beam to irradiate a large amount of matrix and the sample. **Figure 1** shows the principle on which MALDI is based. At first, the matrix molecules are excited by the laser beam such that they evaporate rapidly. The sample molecules are evaporated together with the matrix molecule and then ionized by protonation. Nowadays, since ultraviolet laser beams (wavelength: 337 nm) are generally used, organic compounds having an aromatic ring and/or conjugating double-bond that can effectively absorb the laser beam energy are generally selected as the matrix material. **Table 1** shows typical MALDI matrices. In general, the matrix/sample molar ratio ranges from about 100 to 1000. It is thought that the effective ionization of a sample molecule will be accomplished provided each sample molecule is completely surrounded by matrix molecules. And, the sample ions formed by the MALDI process are accelerated by a high voltage and separated according to their mass-charge ratio in a mass analyzer.

3. Analysis of synthetic polymers

3.1 Selection of matrix and cationizing agent

In MALDI-MS of biopolymers, a good mass spectrum can be obtained by using α -cyano-4-hydroxycinnamic acid, sinapinic acid, or 2,5-dihydroxybenzoic acid as the matrix (as shown in Table 1). In the case of synthetic polymers, on the other hand, it is very important to select an

Table 1 Typical matrices for MALDI.

<u>α-Cyano-4-hydroxycinnamic acid</u>	<u>Sinapinic acid</u>
<u>2,5-Dihydroxybenzoic acid</u>	<u>Dithranol (1,8,9-Trihydroxyanthracene)</u>
<u>2'-(4-Hydroxyphenylazo)benzoic acid</u>	

appropriate matrix in order to obtain a good mass spectrum, because synthetic polymers have a variety of molecular structures and different polarities ranging from hydrophilic (e.g., poly(ethyleneglycol)) to relatively hydrophobic (e.g., polystyrene). An excellent and comprehensive review of the MALDI-MS of synthetic polymers was published by Nielen.³⁾ He summarized suitable matrices for synthetic polymers having a variety of molecular structures and polarities. For example, 2,5-dihydroxybenzoic acid, dithranol and 2'-(4-hydroxyphenylazo)benzoic acid are recommended as matrices for poly(ethyleneglycol), polystyrene and polyamide, respectively. If, however, we were to try to analyze a novel synthetic polymer by means of MALDI-MS, which to date has not been reported in the literature, we would have to attempt to find an appropriate matrix by means of trial and error. Therefore, we can say that the search of a suitable matrix is an ongoing issue in the case of the MALDI-MS of synthetic polymers.

And, because sample molecules of synthetic polymers are usually less easy to ionize by protonation than those of biopolymers in the MALDI process, it is necessary to add a cationizing agent to aid the ionization in sample preparation. Therefore, the selection of a cationizing agent is also important to the MALDI-MS of synthetic polymers. In general, most synthetic polymers having heteroatoms such as polyethers, polyacrylates, polyesters, and polyamides are easy to ionize by the simple addition of sodium or potassium salts. And, synthetic polymers with relatively low polarities, such as polystyrene, polybutadiene, and polyisoprene are easy to ionize by the addition of silver or copper salts, which interact with the double-bonds of these polymers. Unfortunately, polyolefines such as polyethylene and polypropylene are very difficult to ionize by the MALDI process because of the absence of heteroatoms and double-bonds in their molecular structures.

Additionally, because interference peaks derived from the matrix will inevitably appear in the MALDI-MS spectrum, it is often difficult to analyze a low-mass sample with around several hundred such peaks, as in the case of biopolymers or synthetic polymers. Recently, some studies have set

out to eliminate matrix interference by using meso-tetrakis(pentafluorophenyl)porphyrin with a high molecular weight⁴⁾ and carbon nanotubes^{5, 6)} in place of the conventional matrix. Another interesting technique for the desorption/ionization of the sample molecules on porous silicon, which was made by galvanostatic etching, has also attracted considerable attention.⁷⁾

3. 2 Sample preparation techniques

After the selection of an appropriate matrix and cationizing agent for the sample being examined, it is important to prepare the homogeneous cocrystallization of the matrix, sample, and cationizing agent. The dried-droplet method is widely used as the most popular one. In this method, the three solutions are mixed by volume. Then, about 1 μ l of the mixture is applied to the target plate and then dried at room temperature. To homogeneously cocrystallize and minimize the segregation among the matrix, sample and cationizing agent during the evaporation of the solvent, it is preferable to use the same solvent for all three solutions. If, however, the solvents for the matrix, sample and cationizing agent are different, rapid crystallization is necessary to minimize the segregation. For rapid crystallization, methods using a vacuum chamber or a stream of nitrogen have been proposed.^{8, 9)}

3. 3 Determination of average molecular weight and molecular weight distribution

It is well known that synthetic polymers have a molecular weight distribution. In the past, the molecular weight distribution of synthetic polymers has been measured by size-exclusion chromatography (SEC). From the molecular weight distribution, the weight-average molecular weight (Mw), the number-average molecular weight (Mn) and polydispersity (Mw/Mn) are calculated. However, the Mw, Mn and Mw/Mn values, as obtained by SEC, are relative to the calibration standard polymer (e.g., polystyrene). On the other hand, because MALDI-MS makes it possible to measure the absolute molecular weight of a sample without a standard polymer, it is expected to be a powerful tool for evaluating absolute molecular weight distributions. MALDI-MS has been used to analyze some synthetic polymers.^{10, 11)} Through

these studies, it has become clear that MALDI-MS can be used to obtain the absolute molecular weight distribution for synthetic polymers with a narrow polydispersity ($M_w/M_n < 1.1$). **Figure 2** shows the MALDI mass spectrum of commercially available poly(ethyleneglycol)monododecyl ether, as obtained by measurement at Toyota CRDL. The main intense peaks are observed at the repeat unit mass of the C_2H_4O interval ($m/z = 44$) and it was found that this sample has a molecular weight distribution with a center at $n = 21$ (n represents the repeat unit number) and a range from $n = 10$ to 40. This result agrees with the manufacturer's value (n is about 25). **Figure 3** shows the MALDI mass spectrum of narrow polydispersity polystyrene ($M_w/M_n = 1.01$) for the SEC calibration standard. The main intense peaks are observed at the repeat unit mass of the styrene monomer interval ($m/z = 104$). From this data, we can calculate the values of M_w , M_n and M_w/M_n , giving 17670, 17570 and 1.006, respectively. This result corresponds well to the manufacturer's value ($M_w = 17200$), as measured using the light scattering method.

Unfortunately, most synthetic polymers have a widely polydisperse molecular weight distribution. With MALDI-MS of synthetic polymers, mass discrimination occurs with a widely polydisperse polymer sample such that the high molecular weight components are hard to detect and, as a result, the

molecular weight distribution is not obtained accurately. To overcome this problem, a combination of SEC and MALDI-MS has been proposed. The absolute molecular weight distributions of some synthetic polymers were determined by this method. After the polydisperse sample was fractionated by SEC, those fractions with a narrow polydispersity ($M_w/M_n < 1.1$) were analyzed by MALDI-MS.^{12, 13)}

3.4 Characterization of molecular structure

In the past, the analysis of the degradation of synthetic polymers as caused by light or heat was performed by infrared spectrometry (IR), nuclear magnetic resonance spectrometry (NMR), SEC, and the like. It has not been easy, however, to characterize the molecular structure of degradation products in detail, even with a combination of conventional analytical tools, because the quantities of the degradation products are often small and their molecular structure is complicated. MALDI-MS offers the potential to obtain other information in addition to the molecular weight. It can be used to determine the end groups on a polymer by measuring the exact mass of a sample with a high mass resolution, depending largely on the progress of the MALDI and TOF instruments. To date, many MALDI-MS studies of polyamide-6,¹⁴⁾ polyamide-66,¹⁵⁾ polycarbonate,¹⁶⁾ polyester¹⁷⁾ and suchlike have been performed in order to characterize the molecular structure of the degradation products.

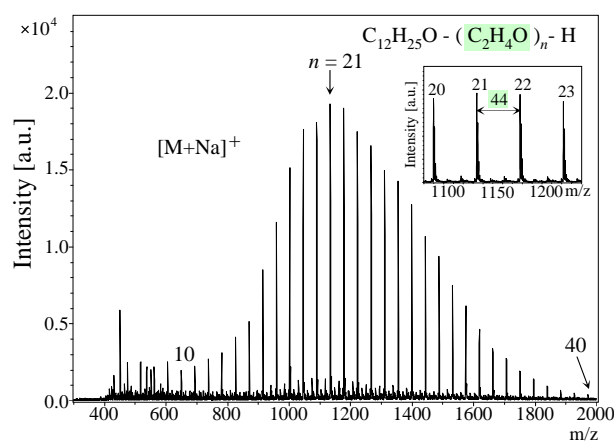


Fig. 2 MALDI mass spectrum of poly(ethyleneglycol) monododecyl ether obtained using 2,5-dihydroxybenzoic acid as the matrix and NaCl as the cationizing agent. The inset shows the expanded spectrum of the region between m/z 1080 and 1240.

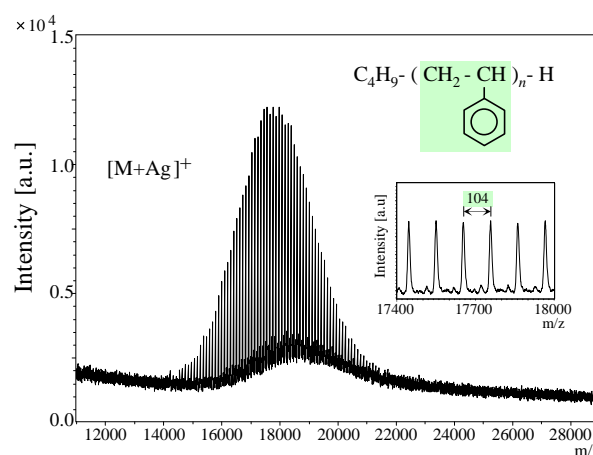


Fig. 3 MALDI mass spectrum of narrow polydispersity polystyrene ($M_w = 17200$) obtained using dithranol as the matrix and silver trifluoroacetate as the cationizing agent. The inset shows the expanded spectrum of the region between m/z 17400 and 18000.

Figure 4 shows the MALDI mass spectra of polyamide-6 before and after heating in air at 150 °C for 7 days, as measured at Toyota CRDL. In the pre-heating spectrum, the main intense peaks (●) are observed at the repeat unit mass of the polyamide-6 interval ($m/z = 113$). On the other hand, some other peaks appear in the spectrum after heating. **Figure 5** shows the MALDI mass spectra expanded region between m/z 1580 and 1780. In the pre-heating spectrum, in addition to a series of main intense peaks marked $[An]$ (n represents the number of repeat units), two series of weak peaks (marked $[Bn]$ and $[Cn]$) are observed when m/z is 18 and 40 higher than $[An]$, respectively. On the other hand, in the spectrum after heating, another three series of peaks (marked $[Dn]$, $[En]$ and $[Fn]$) are observed

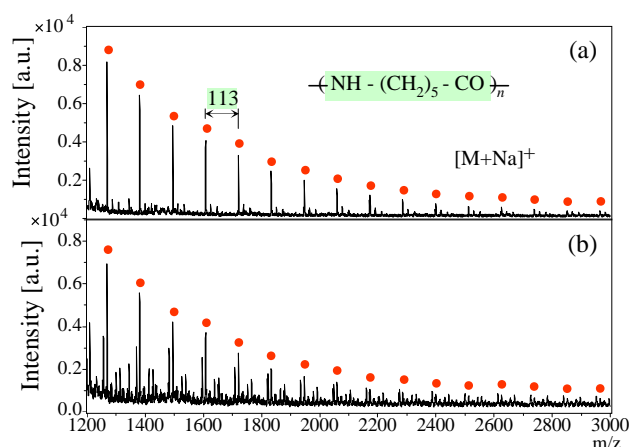


Fig. 4 MALDI mass spectra of polyamide-6 obtained using 2'-(4-hydroxyphenylazo)benzoic acid as the matrix and sodium trifluoroacetate as the cationizing agent: (a) before and (b) after heating in air at 150 °C for 7 days.

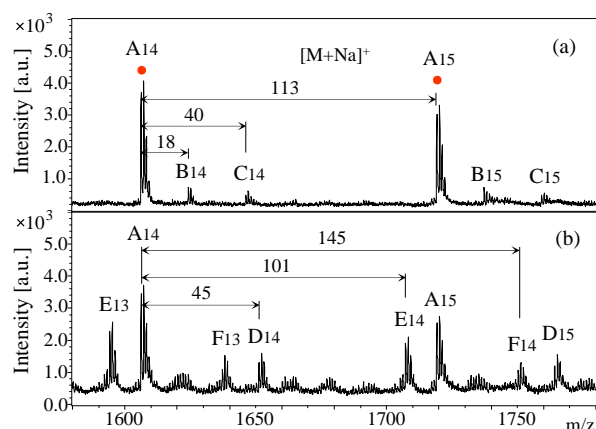


Fig. 5 MALDI mass spectra of polyamide-6 expanded region between m/z 1580 and 1780: (a) before and (b) after heating in air at 150 °C for 7 days.

when m/z is 45, 101 and 145 higher than $[An]$, respectively. **Table 2** lists the structural assignments of peaks in Fig. 5. MALDI-MS makes it possible to characterize the molecular structure in detail.

In the past, it was not easy to analyze the molecular weight of an insoluble sample. MALDI-MS, however, makes this possible.¹⁸⁾ For example, as shown in **Fig. 6**, polycyclic aromatic hydrocarbon $C_{78}H_{26}$ yielded by the cyclodehydrogenation of oligophenylene $C_{78}H_{54}$ is insoluble in any solvent. Therefore, it is impossible to characterize this sample by NMR or SEC. **Figure 7** shows the MALDI mass spectra for oligophenylene $C_{78}H_{54}$ and polycyclic aromatic hydrocarbon $C_{78}H_{26}$. The mass difference between $C_{78}H_{54}$ and $C_{78}H_{26}$ was found to be m/z 28. This result indicates that the cyclodehydrogenation reaction of oligophenylene has occurred successfully. MALDI-MS makes it possible to characterize the molecular structure of an insoluble sample by measuring the exact mass with high mass resolution.

4. Conclusion

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) is widely used to

Table 2 Structural assignments of peaks observed in Fig. 5.

Peaks	Structure
A_n	$\left[\text{NH} - (\text{CH}_2)_5 - \text{CO} \right]_n$
B_n	$\text{H} - \text{NH} - (\text{CH}_2)_5 - \text{CO} - \text{OH}$
C_n	$\text{H} - \text{NH} - (\text{CH}_2)_5 - \text{CO} - \text{ONa}$
D_n	$\text{H} - \text{NH} - (\text{CH}_2)_5 - \text{CO} - \text{NH} - \text{CHO}$
E_n	$\text{CH}_3 - (\text{CH}_2)_3 - \text{CO} - \text{NH} - (\text{CH}_2)_5 - \text{CO} - \text{NH}_2$
F_n	$\text{HOOC} - (\text{CH}_2)_4 - \text{CO} - \text{NH} - (\text{CH}_2)_5 - \text{CO} - \text{NH}_2$

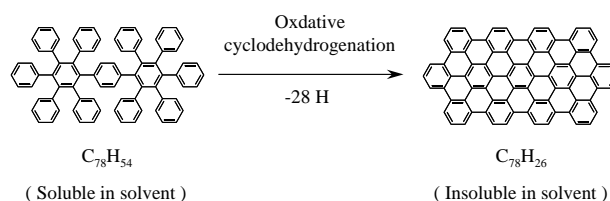


Fig. 6 Cyclodehydrogenation of oligophenylene $C_{78}H_{54}$ yielding to polycyclic aromatic hydrocarbon $C_{78}H_{26}$.

characterize the molecular structure of biopolymers such as peptides and proteins. This paper has reviewed the application of MALDI-MS to the analysis of synthetic polymers, including data obtained at Toyota CRDL.

In MALDI-MS of synthetic polymers, the selection of the matrix and cationizing agent, as well as sample preparation techniques, are very important to obtaining a successful spectrum. And, MALDI-MS makes it possible to measure the absolute molecular weight and molecular weight distribution for synthetic polymers with narrow polydispersity. On the other hand, for synthetic polymers with a wide polydispersity, the combination of size-exclusion chromatography and MALDI-MS is proposed to determine the absolute molecular weight distribution in order to overcome the problems of mass discrimination where the high molecular weight components are hard to detect, and where the molecular weight distribution is not obtained accurately. Additionally, MALDI-MS makes possible the detailed characterization of the molecular structure of degradation products, as caused by light or heat, as well as insoluble samples, by measuring the exact molecular mass with a high mass resolution.

As mentioned above, MALDI-MS is a very useful and promising analytical tool for characterizing synthetic polymers. Therefore, it is expected that MALDI-MS will significantly contribute to

materials development and degradation analysis in the future. We also believe that there are several avenues of research still to be explored in the field of MALDI-MS of synthetic polymers: a universal matrix that can ionize any synthetic polymers, sample preparation techniques, and so on.

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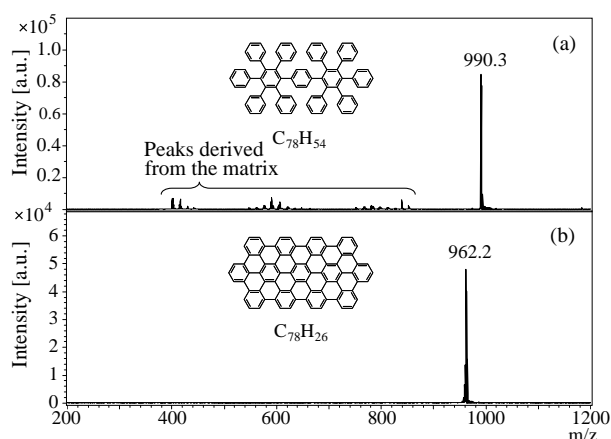


Fig. 7 MALDI mass spectra of oligophenylene $C_{78}H_{54}$ and polycyclic aromatic hydrocarbons $C_{78}H_{26}$: (a) $C_{78}H_{54}$ obtained using 9-nitroanthracene as the matrix and (b) $C_{78}H_{26}$ obtained without the matrix.



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