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Technical Report

Improvement of Ionization Efficiency and Application of Structural Analysis for MALDI-TOFMS by Derivatization of Polyacrylic Acid

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Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS) is a suitable method for polymer analysis. MALDI is a soft ionization technique that can generate mainly singly charged ions. Therefore, the polymer's molecular weight distribution is easy to analyze, facilitating the calculation of the number average molecular weight and weight average molecular weight and polydispersity. However, there are polymers that are difficult to detect by MALDI-TOFMS. For example, polyacrylic acid includes carboxylic acid in the main chain, which is difficult to measure due to its low ionization efficiency. As a solution, the ionization efficiency was improved by methylation. In this technical report, we introduce a method to utilize derivatization to determine the degree of polymerization by accurate mass spectrometry (MS). Furthermore, the structures of both ends of the polymers were estimated by tandem time-of-flight MS.

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1. INTRODUCTION

The primary structure of the polymer consists of repeating units of monomer with end groups on both sides. Various polymers exist depending on the combination of repeating units and end groups. Polymers are characterized by having a molecular weight distribution depending on the number of monomers called the polymerization number. Obtaining detailed information on polymer structure is essential for understanding the function of polymers, the synthesis process, quality control, and degradation conditions. Many mass spectrometric methods, such as pyrolysis gas chromatography–mass spectrometry (MS), liquid chromatography–mass spectrometry (LC–MS), and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS), are used for polymer analysis. In particular, MALDI is a soft ionization method that can generate singly charged ions of intact polymers. Although limited to polydispersity <1.3 where mass discrimination is less affected, the molecular weight distribution (*e.g.*, average molecular weight and polydispersity) of the polymer can be easily interpreted from the mass spectrum, even in mixtures of different polymers.[1–3] In contrast, electrospray ionization, the typical ion source of LC–MS, generates multiply charged ions in a large molecular weight range so that the different charge states of the same polymer series often overlap in the mass spectrum and make it difficult to interpret. The MALDI ion source works well in combination with TOFMS, which can analyze the ions distributed in a wide mass range. Using high mass-resolution TOFMS, the accurate mass of each polymer peak is obtained. By successively subtracting the mass of the repeating unit, the total mass of the both end groups and adduct ions can be estimated. However, the polymerization number cannot be decided by accurate mass. The elemental composition of the end group will have an ambiguity that is an integral multiple of the elemental composition of the repeating unit. Tandem time-of-flight mass spectrometry (TOF-TOF), which can analyze the end groups of polymers by MS/MS analysis using high-energy collision-induced dissociation, is also useful for analyzing polymer end groups. However, some polymers are difficult to analyze with MS without any pretreatment. For example, polyacrylic acid has low ionization efficiency due to its high polarity caused by carboxylic acids in its main chain. To improve the ionization efficiency of polyacrylic acid, derivatization methods, such as trimethylsilylation and methylation,[4–7] have been applied.

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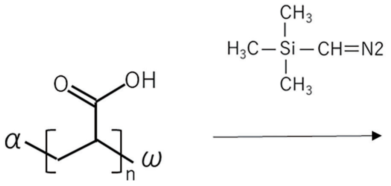

Fig. 1. Methylation reaction process for the formation of polymethyl acrylate.

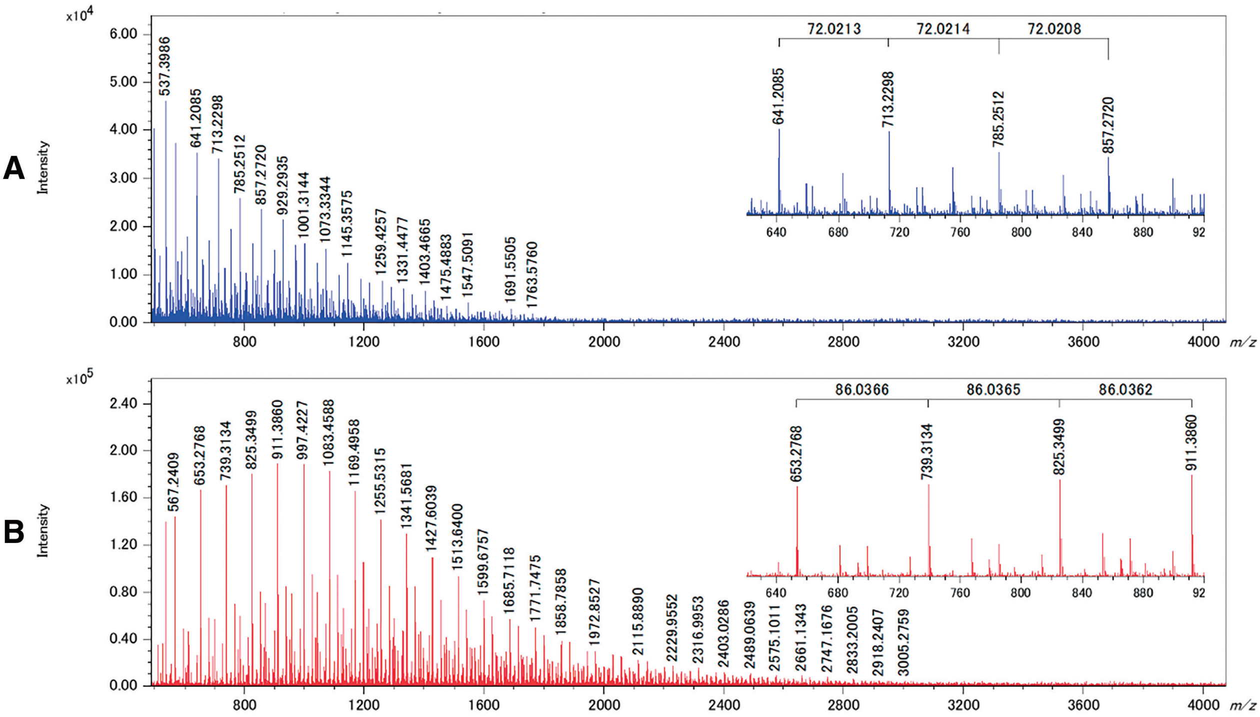

Fig. 2. Mass spectra of the original polyacrylic acid (A) and methylated polyacrylic acid (B).

Derivatization methods, such as trimethylsilylation and acylation,[8,9] were used to determine the number of OH groups in the end group of poly(alkylene glycol)s.

In this technical report, we introduce a method using derivatization to estimate the structures of polyacrylic acid with unknown end groups while simultaneously enhancing the ionization efficiency. The methylation is considered to be suitable compared to the trimethylsilylation because of the following reasons: (i) COOH groups will be selectively derivatized by methylation, (ii) the total mass shift of methylation is smaller than trimethylsilylation because the dozens of repeat units will derivatized in this experiment, and (iii) the isotope patterns will be broadened because the silicon will be included in each repeat unit in trimethylsilylation. The improvement of the ionization efficiency will enhance the quality of TOF-TOF spectra for end-group analyses.

2. EXPERIMENTAL METHOD

Polyacrylic acid with an average molecular weight (Mw) of 1800 (Merck & Co., Inc., Rahway, NJ, USA) was dissolved in methanol to 10 mg/mL. The derivatization reagent for carboxylic acid methylation was (trimethylsilyl)diazomethane hexane solution (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan). The polyacrylic acid solution, the derivatization reagent solution, and toluene were mixed at a ratio of 20 µL:10 µL:10 µL, respectively. Derivatization was performed at room temperature for 1 hour. 2,5-Dihydroxybenzoic acid (FUJIFILM Wako Pure Chemical Corporation) was prepared at 20 mg/mL in methanol, and sodium trifluoroacetic acid (Merck & Co., Inc.) was prepared at 1 mg/mL in methanol. The methylated sample, matrix, and cationizing agent solutions were mixed at a ratio of 1:10:1 (v/v/v); dropped on a stainless plate; and air dried. The mass spectra were acquired by the positive ion mode of high-resolution MALDI-TOFMS (JMS-S3000; JEOL Ltd., Tokyo, Japan).[10,11]

3. RESULTS AND DISCUSSION

The methylation reaction process of polyacrylic acid is shown in Fig. 1. The mass spectra of the original polyacrylic acid and methylated polyacrylic acid after the methylation reaction are shown in Fig. 2. The polymer distribution was observed up to *m/z* 1600 in the mass spectrum of the original polyacrylic acid (Fig. 2A). The methylation improved the ionization efficiency, which could observe the mass distribution up to *m/z* 4000 (Fig. 2B). The original polyacrylic acid repeating unit showed that a 72 u peak interval was changed to an 86 u interval due to methylation of the carboxylic acid in the main chain. We confirmed that 99% of the carboxylic acids were methylated by LC–MS (see Supplemental Figs. S1 and S2). This methylation reaction process is a simple and rapid method, but can methylate the main chain of polyacrylic acid

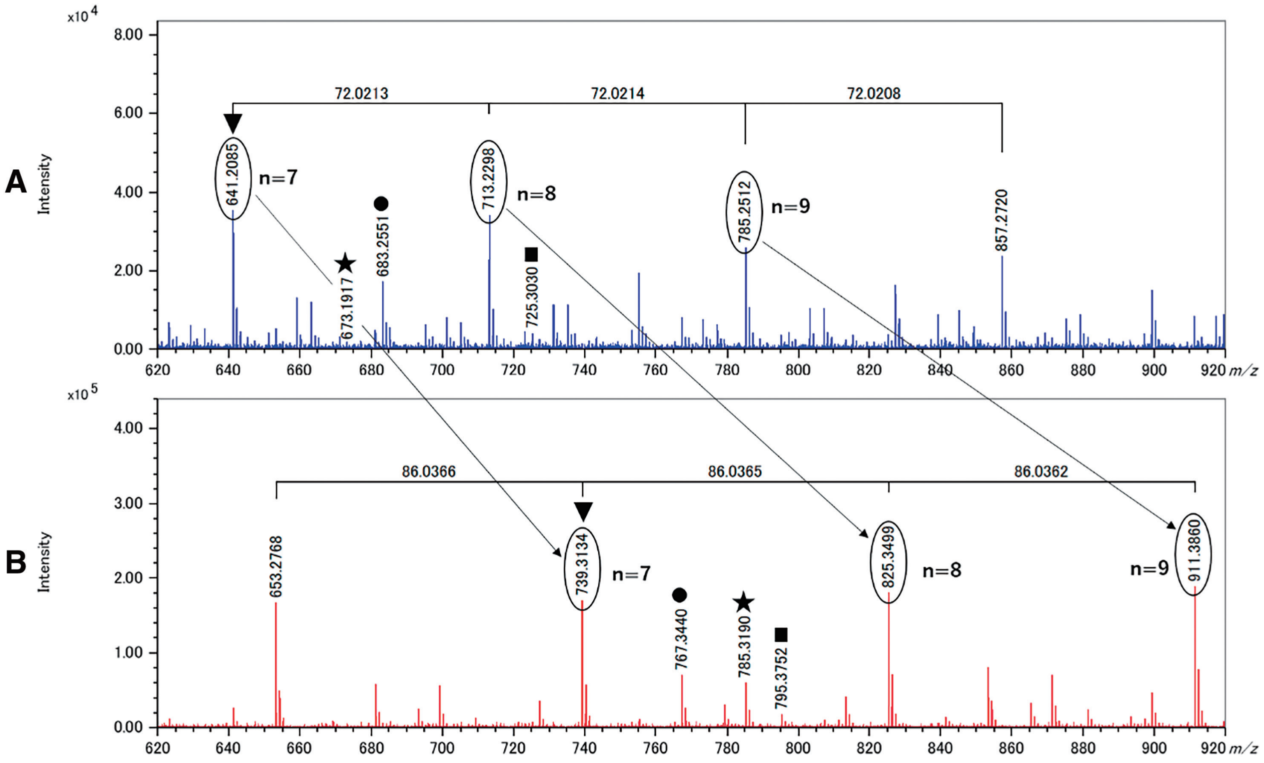

Fig. 3. Enlarged mass spectra from *m/z* 620 to 920 of the original polyacrylic acid (A) and methylated polyacrylic acid (B).

Table 1. Expected *m/z* value after methylation of the three peaks in the major polymer series of polyacrylic acid.

Number of polymerization after methylation	5	6	7	8	9
641.2085 (original polyacrylic acid)	711.2868	725.3024	739.3181	753.3337	767.3494
713.2298 (original polyacrylic acid)	783.3081	797.3237	811.3394	825.3550	839.3707
785.2512 (original polyacrylic acid)	855.3295	869.3451	883.3608	897.3764	911.3921

and enhance the ionization efficiency. We further applied this method to structural analysis of polyacrylic acid.

The polymerization number was investigated as follows by comparing the original polyacrylic acid with after-methylation mass spectra. When the COOH group of the carboxylic acid in the main chain is methylated, a mass shift of $n \times 14$ u occurs according to the degree of polymerization n. Figure 3 shows the enlarged mass spectrum of Fig. 2 at *m/z* 620–920. Three peaks in the major polymer series of the original polyacrylic acid were observed at *m/z* 641, 713, and 785. Table 1 shows the expected mass of these peaks after methylation if the polymerization number is five to nine. In Fig. 3B, *m/z* 739, 825, and 911 were all observed, so the polymerization number of *m/z* 641 was confirmed to be seven.

Next, the structural analysis of end groups was performed by TOF-TOF analysis. The sum mass of both end groups can be calculated from the following equation.

$$\text{Mass of end groups} = \text{Observed } m/z \text{ value} - \text{mass of adduct ion} - (\text{mass of repeating unit} \times n) \qquad (1)$$

As mentioned above, the polymerization number of methylated polyacrylic acid at *m/z* 739 was confirmed as $n=7$. The product ion mass spectrum of *m/z* 739 is shown in Fig. 4A. Observation of the peak at *m/z* 23 (Na^+) confirmed that the precursor ions were $[M+Na]^+$. According to Eq. (1), the molecular weight of the sum of the end groups at *m/z* 739.3134 was calculated as 114.0667. The elemental composition of the sum of both end groups was estimated to be $C_6H_{10}O_2$. To evaluate the elemental composition at each end of the polymer, a reminder of Kendrick mass (RKM) analysis[12)] was performed on the product ion spectrum at *m/z* 739 using msRepeatFinder (JEOL Ltd.). The RKM plot is shown in Fig. 4B by setting methyl acrylate (86.04) as the base unit. Five series were identified at 86 u intervals from the RKM plot. Series 3 was started from *m/z* 23 (Na^+) to *m/z* 625, which is −114 u from the precursor ion mass. In contrast, series 2 was started from *m/z* 136 (=23+113) up to *m/z* 739, which is the *m/z* of precursor ions. These results indicated that the end groups of each side of the polymer chain were $C_6H_9O_2$/H. Furthermore, the number of derivatized repeat units of *m/z* 739 in Table 1 and the observed repeat units in the series 2 and 3 were both seven; the end-groups were not derivatized. The estimated structure and fragmentation pathways are shown in Fig. 5. In the same way, the elemental compositions of the end groups of each polymer series were estimated as listed in Table 2. Regarding end groups of *m/z* 795, the elemental composition of the sum of both end groups was estimated to be $C_{18}H_{30}O_6$, but due to the large mass

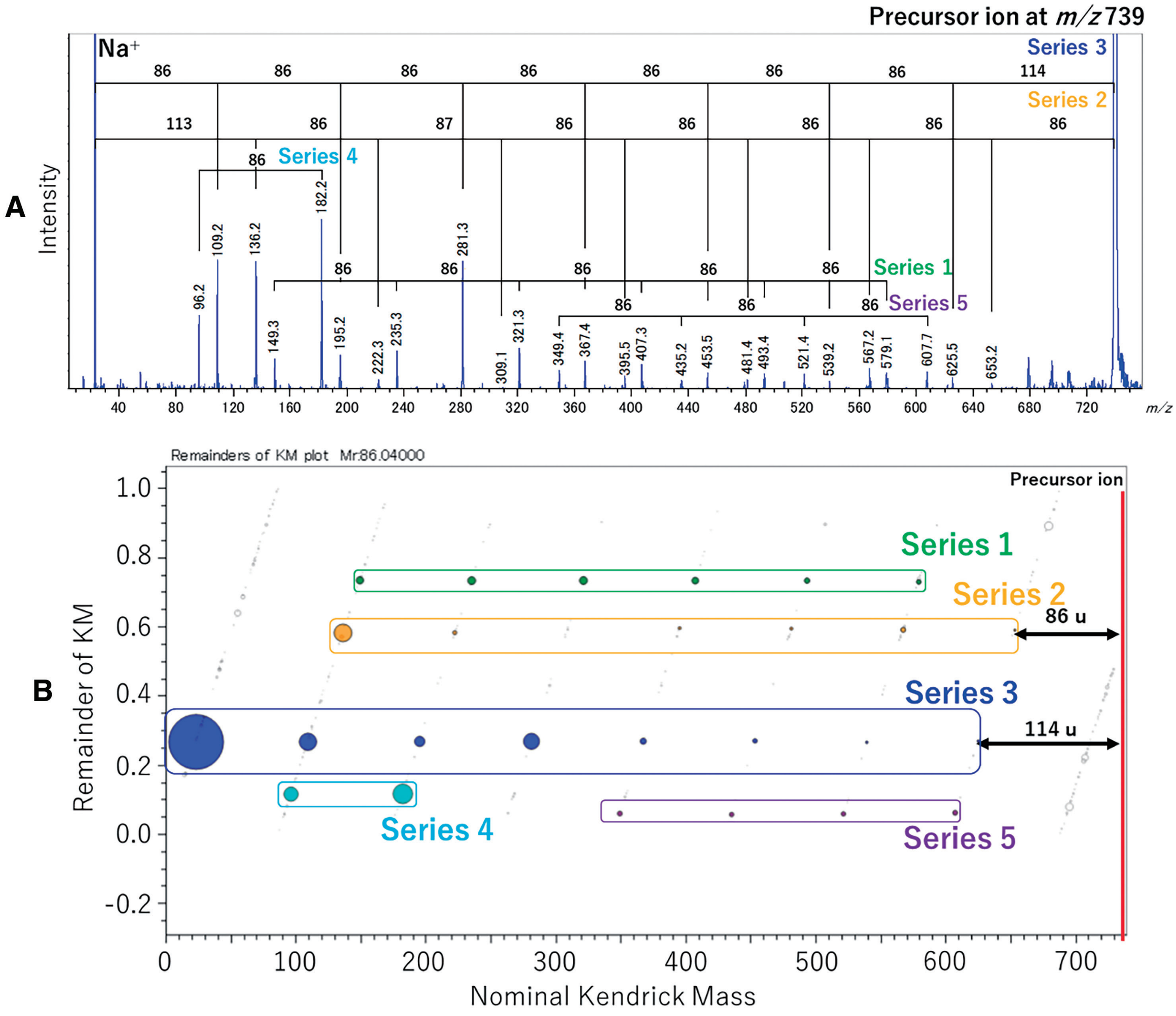

Fig. 4. Product ion spectrum of *m/z* 739 in methylated polyacrylic acid (A) and its RKM plot (B). RKM, reminder of Kendrick mass.

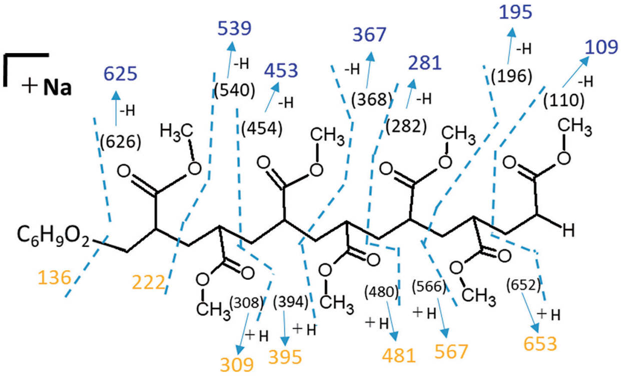

Fig. 5. The structure of *m/z* 739.

Table 2. Estimated end group of each peak series.

Observed *m/z* value of original polyacrylic acid	Observed *m/z* value of methylated polyacrylic acid	Degree of polymerization	Elemental composition of end groups
m/z 641.2085	*m/z* 739.3134	7	$C_6H_9O_2$/H
m/z 683.2551	*m/z* 767.3440	6	$C_6H_9O_2$/ $C_6H_{11}O_2$
m/z 673.1917	*m/z* 785.3190	8	$C_3H_5O_2$/H
m/z 725.3030	*m/z* 795.3752	5	$C_{18}H_{30}O_6$

of end groups (342 Da), it was not possible to narrow down each end group candidate from MS/MS measurement.

4. CONCLUSION

The methyl derivatization of carboxylic acids in the main chain of polyacrylic acid improved ionization efficiency. Comparing the original and methylation mass spectra helped identify the degree of polymerization. In addition, it will simplify the structural analysis of the product ion spectrum using TOF-TOF. This method can be applied to other polymers, including carboxylic acids in the repeating unit.

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