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Review

말디토프 질량분석을 이용한 고분자의 특성분석

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Analysis of Polymer Characteristics Using Matrix-assisted Laser Desorption/Ionization Time-of-flight Mass Spectrometry

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초 록

최근에, 질량분석기술의 폴리머 분석에의 응용은 MALDI-TOF MS 개발 이후 급속도로 발전하였다. 이 리뷰 논문은 현재까지 연구된 MALDI-TOF MS의 폴리머 특성분석에의 응용에 관한 최신 논문을 정리하였다. MALDI-TOF MS는 바이오 폴리머와, 합성 폴리머의 평균분자량 분석, 폴리머의 시퀀스 분석을 통한 구조의 해석, 모노머의 조성분석에까지 이용되고 있다. 엔드그룹의 특성과 농도를 분석하는 연구도 많이 진행되었고, 복잡한 폴리머의 분자량의 분석에는 SEC와 MALDI-TOF MS를 연결한 분석법을 추천한다. MALDI에 tandem MS를 결합한 분석기술이나, 이온 모빌리티를 응용한 질량분석기, TOF-SIMS, MALDI-TOF-Imaging 기술도 급격히 발전하고 있으며, 이의 폴리머 특성분석에의 응용은 별도의 분리기술이 필요 없어 앞으로 더 많이 이용될 것으로 생각된다. 분자량, 시퀀스, 그리고 모노머의 조성을 정확하게 계산해주는 소프트웨어와 고분자량(> 100 kDa)의 분석을 가능하게 해주는 기술이 개발된다면, 폴리머를 연구하는 과학자들에게 MALDI-TOF MS의 이용은 문제점을 해결하고, 목적하는 폴리머를 합성하는 데 중요한 수단이 될 것이다.

Abstract

The application of mass spectrometry to polymer science has rapidly increased since the development of MALDI-TOF MS. This review summarizes current polymer analysis methods using MALDI-TOF MS, which has been extensively applied to analyze the average molecular weight of biopolymers and synthetic polymers. Polymer sequences have also been analyzed to reveal the structures and composition of monomers. In addition, the analysis of unknown end-groups and the determination of polymer concentrations are very important applications. Hyphenated techniques using MALDI-tandem MS have been used for the analysis of fragmentation patterns and end-groups, and also the combination of SEC and MALDI-TOF MS techniques is recommended for the analysis of complex polymers. Moreover, MALDI-TOF MS has been utilized for the observation of polymer degradation. Ion mobility MS, TOF-SIMS, and MALDI-TOF-imaging are also emerging technologies for polymer characterization because of their ability to automatically fractionate and localize polymer samples. The determination of polymer characteristics and their relation to the material properties is one of the most important demands for polymer scientists; the development of software and instrument for higher molecular mass range (> 100 kD) will increase the applications of MALDI-TOF MS for polymer scientists.

Keywords: *polymer characteristics, MALDI-TOF MS, determination of molecular weight, chemical structure, composition of monomers, concentration of end groups*

1. Introduction

Polymer characteristics determined using mass spectrometry include the molecular weight, chemical structure, the composition of monomers, and end groups. For the preparation of useful synthetic polymers,

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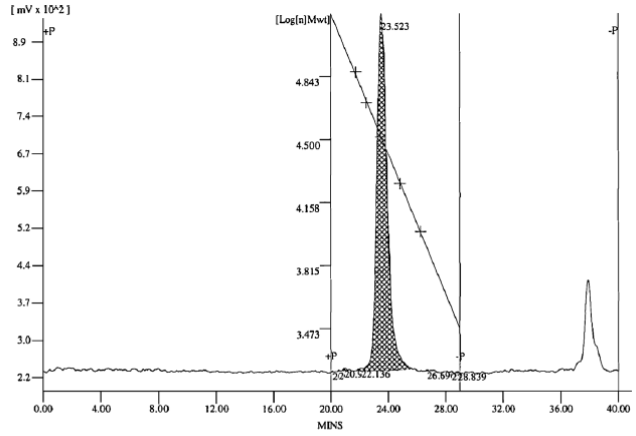
many researchers have tried to control the polymer functionality and characteristics, and the ability to prepare well-defined polymers with designed functionalities is the dream of polymer scientists. The determination of the polymer molecular weight can be carried out using gel permeation chromatography (GPC) or by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS)[1-17].

The application of mass spectrometry to polymer science has increased rapidly since the development of MALDI-TOF MS[18-24]. MALDI-TOF MS has been applied to analyze the average molecular weight of biopolymers[17,25,26] and synthetic polymers[8,12,27-30]. Furthermore, polymer sequences have been analyzed to determine the composition of monomers and the structures of synthetic polymers [22,23,31-37]. Unknown end-group analysis and characterization are also very important to polymer scientists, and hyphenated techniques using MALDI-tandem MS have been used for fragmentation and end-group analysis[13,38-40]. Liquid chromatography (LC)[41-44] and gas chromatography (GC-MS)[45-48] are separation techniques that simplify the complex mixture of polymers and are advantageous because they cause extensive fragmentation, yielding vital information for structural characterization. However, the extensive fragmentation of polymers makes the determination of the intact polymer molecular weight challenging. The soft ionization of MALDI-TOF MS is, therefore, superior for this task. Moreover, hyphenated MALDI-TOF techniques with collision-induced dissociation (CID) and accurate mass spectrometry also give accurate and detailed information concerning the monomer compositions and the concentration of end-groups[49-51]. The extreme sensitivity of MALDI-TOF MS is also advantageous for polymer analysis.

MALDI[18,24,52] is ionization method achieved in two steps: the first step is the absorption of strong laser light by a matrix of small organic molecules. The laser induces ionization of the matrix, producing heat. The second step is the transfer of heat to the analytes, followed by their ionization. To date, the ionization mechanism remains poorly understood. The suggested mechanisms are “gas-phase photo-ionization”, “excited-state proton transfer”, and “ion-molecule reactions”. This review summarizes the current methods developed for polymer analysis using MALDI-TOF MS. In addition, because the application of MALDI-TOF MS to polymer analysis has become very popular (because of its soft ionization and the possibility of detecting both of intact molecules and structural fragments), a detailed discussion of techniques for the analysis of chemical composition and structure is included.

2. Use of MALDI-TOF MS for the Estimation of Polymer Molecular Weight

The variation in the molecular weights of synthetic polymers is very broad. Furthermore, the average molecular weight is sometimes quite different from the median molecular weight distribution or the highest peak among the broad range of analyzed polymer when the dispersity is very large. Size exclusion chromatography (SEC) or gel permeation chromatography (GPC) is the most commonly used techniques for the characterization of molecular weight parameters, and there are several



Peak Molecular Weight Report

Peak	Component	RT	Area	%Area	PMwt	Mw	Mn	Mw/Mn	StartMw	EndMw
1		20.117	17188.0	0.048	145525.5	146112.8	146055.2	1.0004	153735.6	138292.5
2		20.545	23970.1	0.067	120636.5	120285.3	120196.4	1.0007	128555.7	113320.3
3		20.958	75140.9	0.210	100678.3	101218.5	101028.2	1.0019	113320.3	90882.7
4	73.4 kDa	22.136	100782.8	0.281	60088.2	62720.0	62526.5	1.0031	74622.7	57293.2
5	32.6 kDa	23.523	35508227.2	99.110	32729.3	31761.5	30747.4	1.0330	57293.2	12520.6
6	10.0 kDa	26.696	20705.0	0.058	8152.5	8230.8	8223.9	1.0008	9180.5	7705.0
7		28.405	35357.0	0.099	3856.6	3850.0	3846.6	1.0009	4157.6	3566.6
8		28.839	45844.0	0.128	3188.4	3234.6	3229.8	1.0015	3553.6	2971.6

Figure 1. Chromatogram and polydispersity calculations of a reference standard of known polydispersity[3].

different definitions of molecular weight for polymers. The number-average molecular weight (M_n) is determined by simply dividing the sum of molecular weight by the number of molecules:

$$Mn = \frac{\sum NiMi}{\sum Ni} \quad (1)$$

The weight-average molecular weight (M_w) is calculated by using a weighting proportional to the population of polymer molecules with the same molecular weight:

$$Mw = \frac{\sum NiMi^2}{\sum NiMi} \quad (2)$$

The broadness of molecular weight distribution is determined by M_m/M_w and gives information about the polydispersity (PD). In contrast to M_n, M_w considers the contributions of the molecular weight to the molecular weight average. The higher the molecular weight, the greater is the contribution of the molecular weight to M_w. M_p is the peak molecular weight defined as:

$$Mp = molecular\ weight\ of\ the\ highest\ peak \quad (3)$$

SEC/GPC can be used to measure M_n, M_w, and M_p at the same time by measuring the entire weight distribution of the polymer.

As shown in Figure 1, the measurement of M_n, M_w, and M_p of polyethylene glycol (PEG) by SEC with corona-charged aerosol detection (CAD) allows the determination of the molecular mass distributions and calculation of M_w/M_n[3]. In this example, the 32.6 kDa polymer occupies 99.110% of the area of the eight identified peaks. The average molecular weights by SEC detected by CAD or evaporative light

Table 1. Polydispersity Data for Seven PEG Samples Determined by CAD and ELSD with the Turbo-SEC Software[3]

PEG reagent Lot #	By CAD			By ELSD		
	PD	M_w	M_n	PD	M_w	M_n
1	1.025	32264	31474	1.015	34270	33748
2	1.033	32171	31147	1.015	33332	32854
3	1.034	31904	30867	1.023	32880	32136
4	1.038	32065	30887	1.016	34252	33706
5	1.192	28924	24265	1.020	36493	35790
6	1.195	30357	25398	1.018	33170	32574
7	1.375	25039	18210	1.033	31666	30651

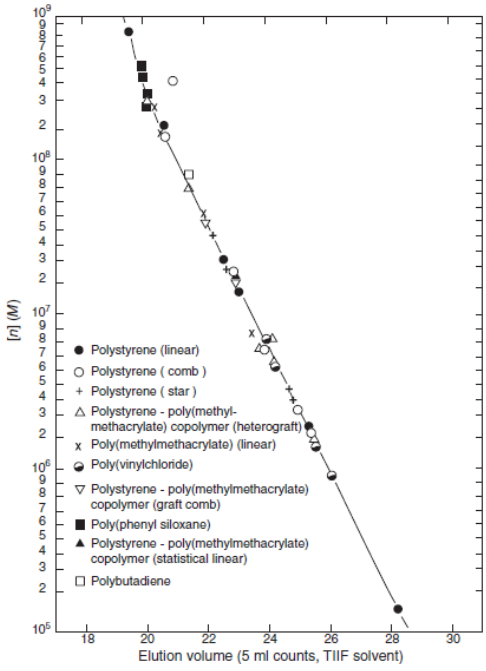


Figure 2. The universal reference standards for SEC measurement of the polymer[4].

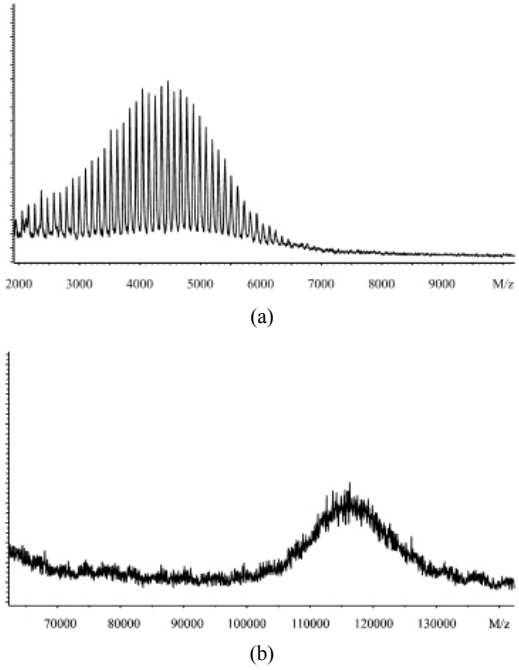


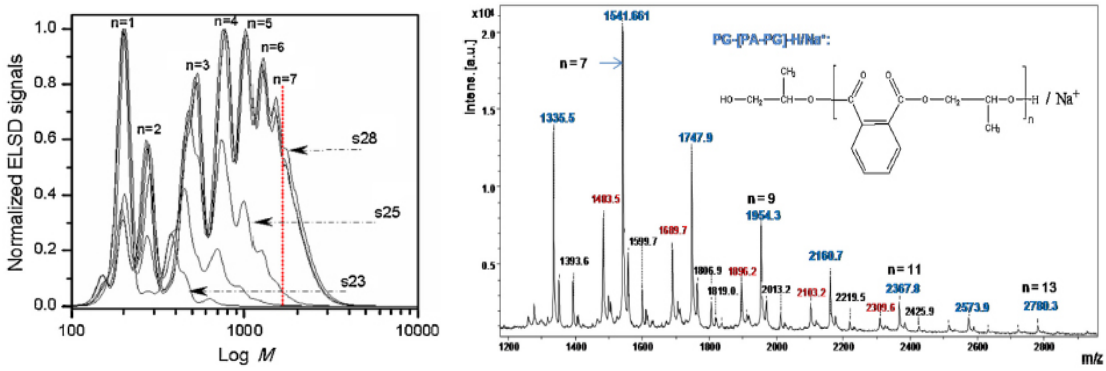
Figure 3. MALDI molecular weight spectra for polystyrene (PS) : (a) M_w = 4700, PD = 1.05, and PSp = 21, and (b) M_w = 116,400, PD = 1.01, and PSp = 154[5].

scattering detector (ELSD) are a little different from those detected by mass spectrometry[8,9,14]. The differences in the average molecular weight measured by SEC or MALDI-TOF MS arise from the different polymers formed in the SEC buffer. The PD data for seven different PEG samples, determined by CAD or ELSD using the program Turbo-SEC, are shown in Table 1[3]. CAD is a new type of universal detector, developed in 2004, and ELSD measures the light scattered by the particles after the eluent has been nebulized and evaporated. Both SEC-CAD and SEC-ELSD require reference standards for the determination of the molecular weight of polymers and the PD. Typical universal reference standards are shown in Figure 2[4]. The exact calibration using universal references allows the accurate determination of the average molecular weights of polymers.

Among mass spectrometry techniques, MALDI-TOF MS is most frequently used for the estimation of the molecular weight of polymers. In the late 1980s, the soft ionization of MALDI was first introduced for the analysis of biopolymers[18,20]. Then, MALDI-TOF MS was extended very rapidly to analyze synthetic polymers and dendrimers [27,53-56]. As mentioned, the MALDI matrices are usually organic compounds, and, in UV-MALDI, which is most widely used for the analysis of synthetic polymers, the matrix is an aromatic organic compound carrying oxo, hydroxyl, and/or carboxyl groups. Commonly selected matrices are 2,5-dihydroxybenzoic acid (DHB), 2-(4-hydroxyphenylazo)-benzoic acid (HABA), α-cyano-4-hydroxycinnamic acid (αCHCA), sinapinic acid, trans-3-indoleacrylic acid (IAA), dithranol, and all-trans retinoic acid[57,58]. For m/z separation with a TOF analyzer, the ions produced by the ion source are first accelerated through a potential difference to acquire kinetic energies in the keV range. The flight time depends on the ion velocity, which, in turn, is dependent on m/z ratio[21,24]. Overall, the time taken by an ion to reach the detector is proportional to the square root of the m/z value. Based on this relationship, larger ions (higher masses) take longer to arrive at the detector. MALDI-TOF MS has an advantage being rapid and accurate

Table 2. Theoretical Calculations *M* [calc.] vs. SEC Results for the Molecular Weight Determination of the PA-PG (Excess of 30 mol% PG) Polyester Samples. Polystyrene Standards were Used for the SEC Calibration. The Calculated Values are Based on the Most Basic Ideal Structure of HO-[PA-PG]$_n$-H, where n Equals the Targeted Degree of Oligomerization[6]

Sample	*n* values for HO-[PA-PG]$_n$-H	M [calc.] (g mol^{-1})	M_n [SEC] (g mol^{-1})	M_w[SEC] (g mol^{-1})	PD
S23	1	224	135	216	1.60
S24	2	430	184	464	2.52
S25	3	636	283	837	2.96
S26	4	842	553	1470	2.65
S27	7	1460	530	1450	2.75
S28	8	1666	569	1520	2.67



(a) (b)

Figure 4. (a) Molar mass distribution of the PA-PG polyester batch obtained from SEC (PS equivalents). The proposed degree of polymerization, n, for different oligomeric species found within each sample is indicated. The higher molecular weight fraction collected for mass spectrometry analysis is illustrated by the dotted red line. (b) MALDI-TOF mass spectrum of the high molecular weight fractions of s28. The matrix solution used was dithranol in 1, 4-dioxane (10 mg ml^{-1}) and no additional ionizing agent was added. The highlighted peak distribution is related to the sodium adducts of the structure Na^+/PG-[PA-PG]$_n$-H[6].

in the determination of molecular weight, and no reference standards are required.

Figure 3 shows the MALDI-TOF MS spectra of polystyrene (PS) which has two different average molecular weights[5]. The PD values for the two PS samples were similar (1.05 and 1.01), but the polymer spread values (PSp) were quite different for each polymer (21 and 154) [5,9,59]. PSp is the width of the spectrum, increases as the molecular weight increases, and is only applicable for symmetric distributions. The combination of SEC and MALDI-TOF MS is also used for the analysis of model polyesters based on complex phthalic anhydride-1, 2-propylene glycol (PA-PG polyester)[6]. Due to the complex molecular and chemical nature of polyester resins, characterization using LC is less frequent. Hyphenated techniques provide the pre-separation of complex mixtures by SEC and detection by sensitive and accurate mass spectrometry[14,49,50]. Only low molecular weight polymer has been detected by MALDI-TOF MS among the water insoluble model polyesters because of ionization problems[19,50,60].

Figure 4 shows molecular weight distribution of the PA-PG polyester batch obtained from SEC (PS equivalents)[6]. The proposed degree of polymerization, *n*, for the different oligomeric species found within each sample is indicated. Table 2 shows the theoretically calculated vs. SEC-detected molecular weight determination of the PA-PG polyester samples. The higher molecular weight fraction collected for mass spectrometry analysis is limited to the dotted red line without pre-separation by SEC. For the accurate mass determination of water insoluble polymers by MALDI-TOF MS, pre-separation of the mixtures was useful, as shown in Figure 4a, where the peak distribution with the highest intensity is *m/z* 1541.662 (*n* = 7). The dendronization of dihydroxy PEG has also been observed by MALDI-TOF MS. The exact masses of the synthesized polymers were determined by MALDI-TOF MS, and the structures were further analyzed by NMR (Figure 4b). The mass range can be detected by different techniques, such as osmometry and cryoscopy, although these techniques have very narrow detection ranges. Ultracentrifuge and light scattering are not adequate to detect the lower molecular mass ranges, whereas intrinsic viscosity and SEC have the widest dynamic range for polymer analysis. Mass spectrometry is limited to measure extremely high molecular weight polymers (as shown in Figure 4a, where the red dotted line I is the upper limit). MALDI-TOF MS has also been proved to be an invaluable and rapid tool for monitoring polymer modification reactions. For the analysis of polymers by MALDI-TOF MS, the regulation of laser energy is also very important and influences the detection range of molecular mass distribution[56,60]. Consequently, the selection of the matrix is a crucial factor and must be chosen on a

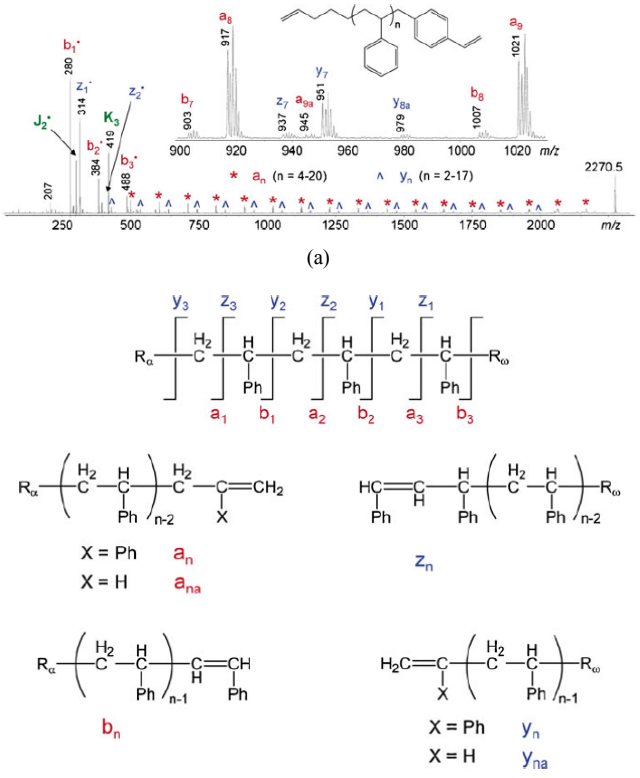


Figure 5. (a) MALDI-MS^2 spectrum of a silverated 19-mer of α-4-pentenyl-ω-(ρ-vinyl benzyl) polystyrene (m/z 2270.5) and (b) the fragment nomenclatures[35].

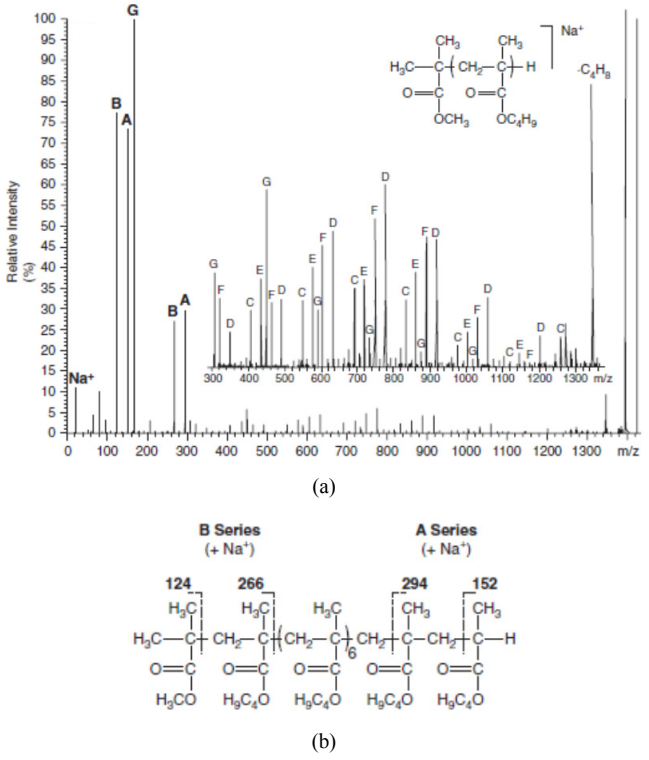


Figure 6. (a) MALDI-CAD spectrum of $[M+Na]^+$, m/z 1403, of the 9-mer of PBMA A. Seven homologous series of fragment ions (A, B, C, D, E, F, and G) are observed. All experiment ions contain Na^+. (b) Proposed fragmentation mechanism for the formation of series A and B[36].

case-by-case basis[60,61].

3. Sequencing of Copolymer and Analysis of Polymer Structure

The sequencing of copolymers, and an understanding of the relation between the polymer sequence and the material properties, is two of the most important demands of polymer scientists. The concept of copolymer sequence is often different for biopolymers and synthetic polymers. In biopolymers, such as proteins and nucleic acids, the monomer units are restricted in 20 amino acids or nucleotides with phosphodiester bonds of 4 different bases and sugar with phosphate groups. The sequence of amino acids in a protein is invariant, known as the "primary" structure of the protein. For example, in natural proteins, the amino acids are variable, with 20 different monomers. In contrast, in synthetic copolymers, the sequence of co-monomer units present in each copolymer chain is not constant, and the variation is infinite. Furthermore, we can define only an average sequence length of the monomers. The average number of identical repeating units is related to the composition, mechanism of copolymerization, and reactivity ratios and polymers have different chain lengths composed of various copolymers, as well as the chemical composition. Consequently, the structures and their mass spectra are extremely complex and difficult to interpret. Polymer sequences are often defined as A or B, if two different monomers were used for the synthesis of the polymer, as shown below. The counterparts of A and B are Z and Y, respectively.

MALDI-TOF MS and other mass spectrometry can be applied to determine the structure of cyclic polymers. Figure 5a shows the MALDI-MS^2 spectrum of the silver-ion-attached 19-mer of the di-vinyl-terminated polystyrene[35]. This spectrum includes the typical fragments observed from the metal ion adducts of linear, chain-end functionalized polystyrenes. The most abundant radical ions carrying either the α or ω chain end (b1・, b2・, z1・) and internal ions (J2・, K3) in the low-mass region. The two homologous series of relatively large fragments (an, yn) across the medium- and high-mass region each contain one original end group and one newly produced methylene end group. This fragmentation pattern arises from random, charge-remote homolytic C-C bond cleavage along the polymer backbone, which creates charged radicals that may decompose further. Figure 5b explains the nomenclature used and summarizes the structures of the fragments generated from linear polystyrenes.

Figure 6 shows the MALDI-CAD spectrum of $[M+Na]^+$ of the 9-mer of poly(butyl methacrylate), PBMA A, measured on an electron beam evaporation (EBE)-TOF instrument[36]. In this sector-orthogonal acceleration-TOF hybrid, the EBE and TOF sections are used for precursor ion selection (MS-1) and fragment ion analysis (MS-2), respectively. The CAD spectrum contains several series of fragments separated by 142 Da (repeat unit). Series A and B arise by charge-remote direct

Table 3. Characterization Results of the Investigated PEtOxs[64]

Compounds	Electrophile	Nucleophile	R1	R2
P1	*p*-Toluene-sulfonic acid	Water	H	OH
P2	Methyl-*p*-toluene sulfonate	Water	CH_3	OH
P3	Propargyl-toluene sulfonate	Water	$CHCCH_2$	OH
P4	Allyl toluene sulfonate	Water	CH_2CHCH_2	OH
P5	9-(Chloromethyl)-anthracene	Water	$AnCH_2$	OH
P6	Methyl-*p*-toluene-sulfonate	Anthracene-9-carboxylic acid	CH_3	AnCOO
P7	Methyl-*p*-toluene-sulfonate	t-Butyl-1-Piperazine-carboxylate	CH_3	Piperazine
P8	Methyl-*p*-toluene-sulfonate	Sodium azide	CH_3	N_3

cleavages at either end. Series C/D and E/F correspond to charge-remote H-rearrangements via six-membered rings with concomitant loss of $HCO_2C_4H_9$ and charge retention at either side of the polymer chain. For example, *m/z* 266 (B_2) - 23 (Na^+) - 142 × 1 (one repeat unit) = 101 Da; similarly, *m/z* 294 (A_2) - 23 - 142 × 1 = 129 Da. The fragmentation patterns and repeat units of the various fragment series may also enable the characterization of block vs. random copolymers, linear vs. branched structures, and unsaturated vs. cyclic oligomers. The sequence analysis gives valuable information about the structure of polymers[31-33,37]. The different ionization methods give different mass patterns, and, therefore, the interpretation of the spectrum is also very important[42,45,62]. Nowadays, the software for the calculation of monomer compositions and sequences are provided by the MS manufacturer[63].

4. Polymer Degradation and Analysis of End-groups

Polymers can have a variety of structures, including linear and branched chains, copolymers with different sequences, and star polymers with different numbers of arms[7,9,35]. Because of the variety of possible structures, the analysis of a polymer must proceed step by step and satisfy many criteria. The first step deals with the determination of the chemical structure of the backbone. The second step consists in finding out if the chains possess branching points and determining the degree of branching. The third step involves finding out which end-groups lie at the chain ends. The last step entails the detection of cyclic oligomers that may be present in a linear (or branched) polymer sample. By using MS to elucidate the polymer structure, one can obtain the chemical structure of the backbone and detect the end-groups and the cyclic oligomers simultaneously. In fact, polymers possessing different structures yield mass spectra that are different from the position (mass numbers) at which MS peaks appear.

End-group analysis can be achieved by soft ionization methods (ESI, APCI, and MALDI)[59,63]. Table 3 shows the various structures of poly(2-ethyl-2-oxazoline)s (PEtOxs) with different end-groups determined via three soft ionization methods (ESI, APCI, and MALDI) in a systematic manner to decide which method is best suited for the characterization of these polymers[64]. The average molecular weights and PD results shown in table 4 varied according to the analyzed methods

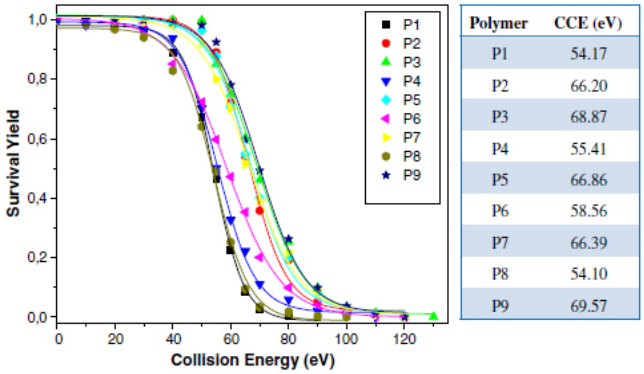


Figure 7. Survival yield curves of various sodiated PEtOx species with different end-groups (P1−P9)[64].

(NMR, SEC, ESI, APCI, and MALDI). Next, for the molecular weight determination, all three ionization methods were applied to analyze the composition, i.e., the determination of end-groups. The different survival yield (SY) curves of various sodiated PEtOx species with different end groups were obtained; these values can be obtained from the characteristic collision energy (CCE)-MS, as shown in Figure 7[64]. The collision energy necessary to promote fragmentation was also investigated for PEtOx with different end-groups (P1-P9). A comparison of the SY curves obtained showed that one group of polymers required more energy to undergo fragmentation (CCE > 66 eV) : P2, P3, P5, P7, and P9. Among the four other polymers P1 and P8 have the lowest CCE values. Thus, the varying ω end-groups can be categorized according to their CCE values and bond energies: ester < azide < amine < hydroxyl.

NMR techniques are very often used for end-group determination [65-67]. The method is quite general because it does not depend on a kind of end-group, although the detection of OH end groups often presents a problem in ^{1}H-NMR. However, the NMR determination of end-groups is limited to compounds with low molecular weight. As the size of the macromolecular chain increases, the signal due to the polymer backbone becomes larger and the intensity of the signal due to end-groups in the NMR spectrum becomes buried in the noise. Another disadvantage of NMR is that cyclic oligomers do not have end-groups. Therefore, only signals from the polymer backbone are seen in the NMR spectrum. The development of hyphenated MS techniques and software increase the opportunity to analyze the end-groups of polymers easily.

Table 4. Molar Masses and PD Values of the Investigated PEtOxs Determined by Different Methods[64]

Compounds	DP [NMR]	M_n [NMR] (g mol^{-1})	M_n [SEC] (g mol^{-1})	PD [SEC]	M_n [ESI] (g mol^{-1})	PD [ESI]	M_n [APCI] (g mol^{-1})	PD [APCI]	M_n [MALDI] (g mol^{-1})	PD [MALDI]
P1	12	1.210	1.650	1.25	1.270	1.12	830	1.07	1.475	1.23
P2	19	1.910	2.200	1.1	2.050	1.06	1060	1.08	2.095	1.05
P3	20	2.040	2.600	1.08	1.940	1.09	1055	1.05	2.455	1.06
P4	20	2.040	2.600	1.07	1.965	1.06	1075	1.12	1.935	1.08
P5	18	1.990	2.500	1.11	2.170	1.06	1020	1.07	2.020	1.06
P6	10	1.230	1.600	1.11	1.230	1.05	960	1.05	1.305	1.05
P7	21	2.285	2.700	1.07	2.295	1.02	995	1.14	2.205	1.03
P8	22	2.240	2.600	1.11	2.430	1.04	870	1.09	2.475	1.03

Moreover, MALDI-TOF MS has been utilized to analyze the degradation of polymers[68-70]. In summary, ion mobility MS[71,72], TOF-secondary ion (SI)MS[73], and MALDI-TOF-imaging[74,75] are also emerging technologies for polymer characterization because of automatic fractionation and localization.

5. Conclusions

The application of MALDI-TOF MS to polymer analysis will be expanded, and the frequency of its use is expected to increase rapidly. The other soft ionization techniques, ESI and APCI, are alternatives to characterize synthetic polymers. The main issue for polymer analysis using MS-based technique is the development of appropriate software. The complexity of molecular weight distribution and fragmentation patterns requires careful interpretation using optimized algorithms. All the reviewed MS and MS/MS analysis results for polymer characteristics show the successful determination of average molecular weight, sequences, structures, and monomer analysis. The weak point of MALDI-TOF MS for the analysis of synthetic polymers is the upper limit of the molecular weight range. The instrument should be developed that can measure molecular weights higher than 100 kDa. Continued utilization of MS will provide opportunities to overcome the problems and improve the protocols for synthesizing polymers as polymer scientists' desire.

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