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Block Length Determination of a Poly(ethylene oxide)-*b*-poly(*p*-phenylene ethynylene) Diblock Copolymer by Means of MALDI-TOF Mass Spectrometry Combined with Fragment-Ion Analysis

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ABSTRACT: Post-source decay (PSD) experiments using a matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometer have been applied to the structural determination of a poly(ethylene oxide)-*b*-poly(*p*-phenylene ethynylene) diblock copolymer (PEO-*b*-PPE). After desorption of the intact copolymer molecules, chains with a defined mass are selected and undergo fragmentation. By use of appropriate experimental conditions, the fragment-ion mass spectra reveal a main cleavage of the copolymer chain between the two blocks. The length of each block thus can be clearly determined. This is especially important in the case of copolymer chains with nearly the same nominal masses but different copolymer compositions, which can be analyzed unambiguously by their fragment ions. This investigation is the first reported structural determination of a diblock copolymer by means of fragment-ion analysis carried out with MALDI, and even more complicated polymer structures can be expected to be determined that way.

Introduction

Since matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry allows gentle desorption and ionization of very large molecules, it has been applied in the past few years with increasing success for the analysis of synthetic polymers[1,2] and in some cases also for copolymers.[3−8] Even though polymers with a molecular weight of several hundred thousand daltons can be characterized by MALDI,[9−13] most of the investigations with this technique focus on the mass range where single polymer chains are resolved.[2] The resolved mass range depends on the molecular mass of the repeating units and on the resolution of the mass spectrometer. Using a MALDI ion source equipped with time-lag focusing,[14−16] single polymer chains can be resolved up to about 50 kDa.[17] From the absolute mass of each signal of the polymer distribution, the polymer composition can be deduced directly in favorable cases. This is often true for homopolymers where the peaks are separated by the mass of the repeat unit. Alternatively, when more than one monomer is used in the preparation of the polymer, the spectra are more complicated, and the compositional information is much more difficult to extract.

Fragmentation of a copolymer chain may supply additional data and contribute to the elucidation of its structure. Fragment-ion analysis can be performed with a MALDI-TOF mass spectrometer by making use of the so-called “post-source decay” (PSD).[18] Despite a gentle desorption and ionization, the analyte ions can be constrained to undergo a fragmentation after extraction of the ion source. An electronic gate installed in the drift tube of the mass spectrometer enables then to select the mother ion to be investigated and to acquire exclusively its fragment-ion mass spectrum. By this means, it can be assured that the detected fragment ions result from a defined polymer ion selected out of the polymer distribution. Investigations of homopolymers with fragment-ion analysis, which is usually rather applied in biochemistry,[19] have already demonstrated the potential of this technique for the analysis of synthetic polymers.[20−23] It allows for instance the determination of complex end group distribution, which cannot be elucidated by a conventional MALDI-TOF mass spectrum.[23] In this paper, we describe the application of the fragment-ion analysis to the compositional determination of a poly(ethylene oxide)-*b*-poly(*p*-phenylene ethynylene) (PEO-*b*-PPE) diblock copolymer. Owing to the ester function between the two blocks of the copolymer, a selective fragmentation of the molecules in the gas phase seems to be feasible which predestines this type of copolymer for the following investigations.

Experimental Section

Materials. 1,8,9-Trihydroxyanthracene (dithranol), lithium, sodium, and potassium trifluoroacetate were obtained from Aldrich (Steinheim, Germany). HPLC grade THF was obtained from Fluka (Deisenhofen, Germany). The investigated PEO-*b*-PPE diblock copolymer was synthesized in our group and published elsewhere.[24]

MALDI Sample Preparation. Dithranol was used as matrix for all PSD-MALDI measurements. Lithium and sodium trifluoroacetate were used as cationization reagents. Matrix and cationization reagent were dissolved in THF at a concentration of 0.1 and 0.01 mol/L, respectively. Solutions of PEO-*b*-PPE (**3**) and of PPE (**2**) were prepared by dissolving 0.2−0.4 mg in 500 μL of THF. For the measurement of PEO (**1**) with the “matrix-suppression effect”, a relatively concentrated PEO solution was used: 1.5 mg was dissolved in 200 μL of THF. For all measurements, the sample solution was mixed with the matrix solution and the cation solution in a volume ratio of 10:10:1 prior to deposition of 0.5 μL on the probe tip. For the measurement of PPE (**2**) no cation was added. The drop was allowed to dry, and the sample holder then introduced into the ion source of the mass spectrometer.

MALDI-TOF Mass Spectrometer. MALDI-TOF mass spectra were recorded using a Bruker Reflex II mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany) equipped with a N_2 laser ($\lambda = 337$ nm) operating at a pulse rate of 3 Hz. The ions were accelerated with pulsed ion extraction (PIE

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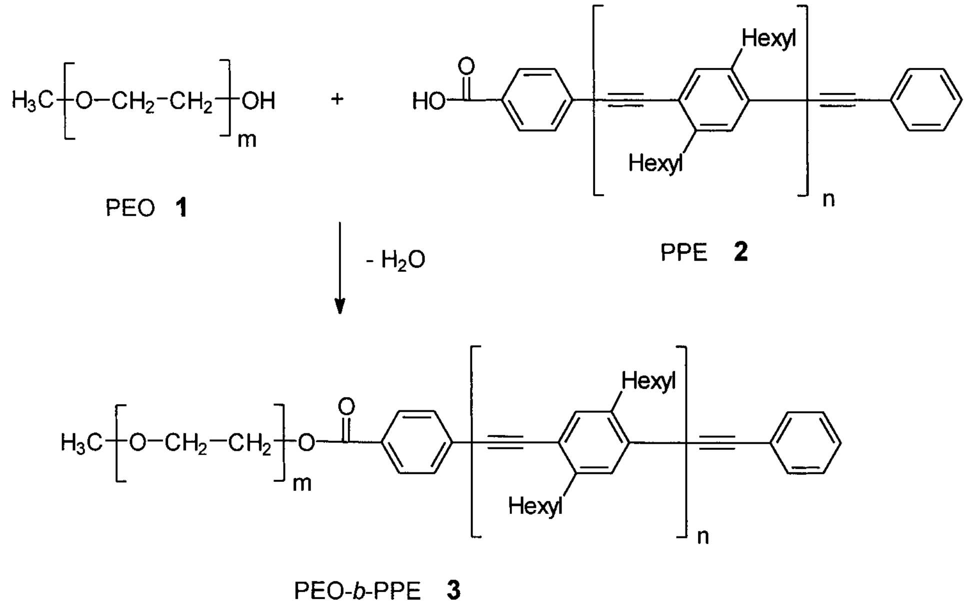
Scheme 1. Synthesis of the Investigated PEO-*b*-PPE Diblock Copolymer (3)[24]



designed from Bruker) after a delay of 50 ns by a voltage of 28.5 kV. The analyzer was operated in the reflectron mode, and the ions were detected using a microchannel plate detector. The mass spectrometer was calibrated prior to measurement with a polystyrene standard of appropriate molecular mass. For collection of PSD data, precursor ions were selected by the ion gate situated in the linear tube after the ion source, and the reflectron voltage was decreased in 15 steps from 30 to 0.95 kV. The segments acquired for each reflectron voltage are pasted together to form the complete PSD-MALDI spectrum. Pasting and calibration of the segments were processed by means of the FAST software from Bruker. A total of 200 single spectra were added in the acquisition of each segment. The calibration procedure was performed with ACTH-clip (18−39) according to the description given in the Bruker Reflex user guide.

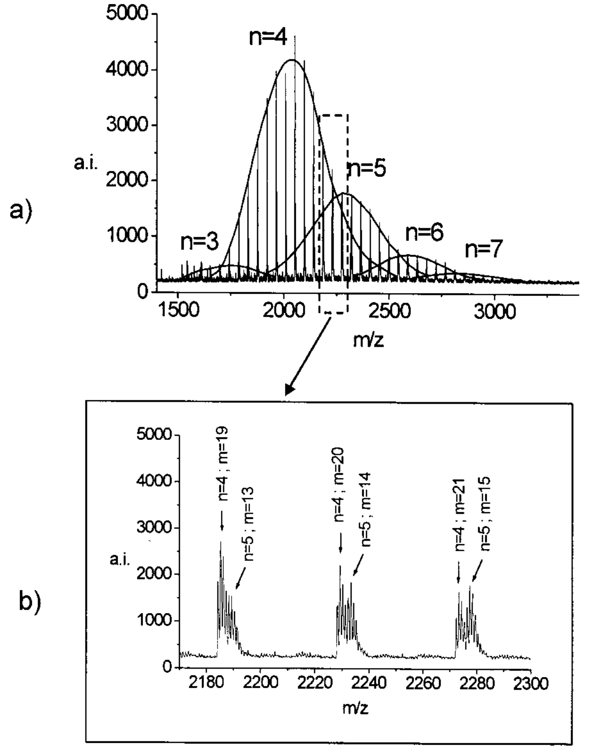


Figure 1. (a) MALDI-TOF mass spectrum of PEO-*b*-PPE (**3**) with emphasized PEO distributions in dependence of the PPE chain length *n*. (b) Expanded region of the copolymer distribution for $n = 4$ and $n = 5$. Each signal represents an overlap of two different copolymer compositions. The peaks are labeled with the number of repeating units *n* of the PPE block and *m* of the PEO block.

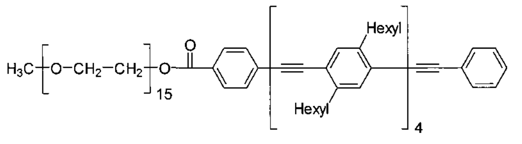
Results and Discussion

The investigated poly(ethylene oxide)-*b*-poly(*p*-phenylene ethynylene) (PEO-*b*-PPE) diblock copolymer (**3**) (for structure see Scheme 1) is a so-called "rod−coil" diblock copolymer and consists of a rodlike, hydrophobic poly(*p*-phenylene ethynylene) block (PPE) and a flexible, hydrophilic poly(ethylene oxide) block (PEO). It was obtained via condensation of the two monofunctionalized homopolymer blocks **1** and **2** (see Scheme 1).[24]

Solid-state segregation in domains on a nanometer scale are expected for the rod−coil copolymer **3**, and it should be promising for electronic or electrooptical applications.[25] The MALDI-TOF mass spectrum of the PEO-*b*-PPE (**3**) is depicted in Figure 1 and shows the overlap of several signal distributions. This is typical for the resolved mass distribution of a copolymer, since copolymers have, in comparison with homopolymers, two types of heterogeneity. Like homopolymers, they have a heterogeneity of the degree of polymerization, corresponding to a distribution of the chain length. Additionally, there is a heterogeneity of the chemical composition. The superposition of several peak distributions is emphasized in Figure 1a by the drawing of traces, which regroup signals of copolymer chains having a defined PPE block length *n* but PEO blocks of varying length *m*. The detected signals are, after subtraction of the mass of the cationization reagent, in agreement with the expected masses of the copolymer chains, which can be calculated according to eq 1:

$$M_{\mathrm{copo}} = 15 + m \times 44 + 120 + n \times 268 + 101 \quad (1)$$

where 15, 101, and 120 are the masses of the end groups and the spacer, 44 and 268 the masses of the ethylene oxide and *p*-phenylene ethynylene repeating units, respectively, and *m* and *n* the numbers of these monomers in the chain. Considering this equation, it can be noticed that the mass of the *p*-phenylene ethynylene



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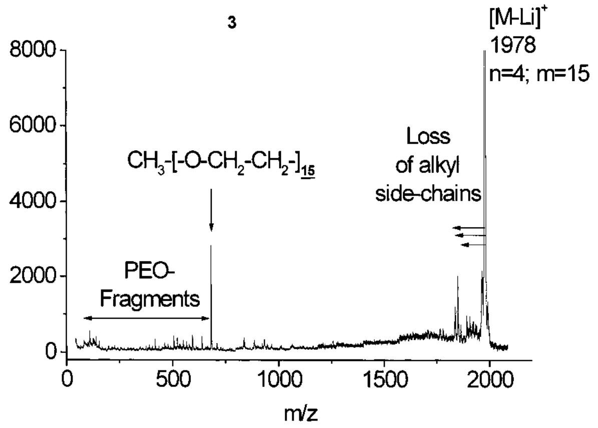

Figure 2. PSD-MALDI-TOF mass spectrum of PEO-*b*-PPE (**3**) with the composition $n = 4$, $m = 15$. The most intense fragment peak corresponds to the intact PEO block.

monomer (268 Da) is almost equal to 6 times the mass of the ethylene oxide monomer ($6 \times 44 = 264$ Da). This justifies why copolymer chains with different compositions (e.g., $n = 4$, $m = 20$, and $n = 5$, $m = 14$) are detected by almost the same masses (2188 and 2192 Da). This particularity is stressed on the expanded region of the MALDI-TOF mass spectrum in Figure 1b. (Each copolymer chain is cationized with a potassium cation and therefore detected at a *m*/*z* value 39 Da above the theoretical mass.) The resolution is in this case high enough to allow the distinction of the partially overlapping signals. However, the determination of the copolymer composition will be hindered if the molecular weight of the copolymer is higher and the resolution of the mass spectrometer is not sufficient to enable the separation of two neighboring signals or if the mass of one repeating unit is exactly a multiple of the other one, so that two copolymer compositions would have strictly the same mass. Under these conditions, the structure of the copolymer could not be directly determined from the conventional MALDI-TOF mass spectrum. The aim of our investigation is therefore to elucidate the potential of fragment-ion analysis for the compositional determination of a diblock copolymer.

At first, a copolymer chain with the composition $n = 4$, $m = 15$ and cationized by lithium was selected by the ion gate, and the corresponding PSD-MALDI-TOF mass spectrum was recorded (see Figure 2). All the detected fragments have retained the lithium cation. Fragments with a relatively high molecular weight are formed by the loss of alkyl side chains from the PPE block. The most intense fragment signal of the spectrum results however from a cleavage of the ester function situated at the junction between the PPE and the PEO blocks: the entire PEO block is detected by this signal. Fragments with a lower molecular weight than this main signal correspond to a further fragmentation of the PEO block. Hence, the structure of the copolymer chain can be clearly determined from this relatively simple fragment ion mass spectrum. The chain length *m* of the PEO block can be inferred from the most intense fragment signal, and the PPE block length *n* can then be deduced as well, by subtraction of the mass of the PEO block from the mass of the whole copolymer chain.

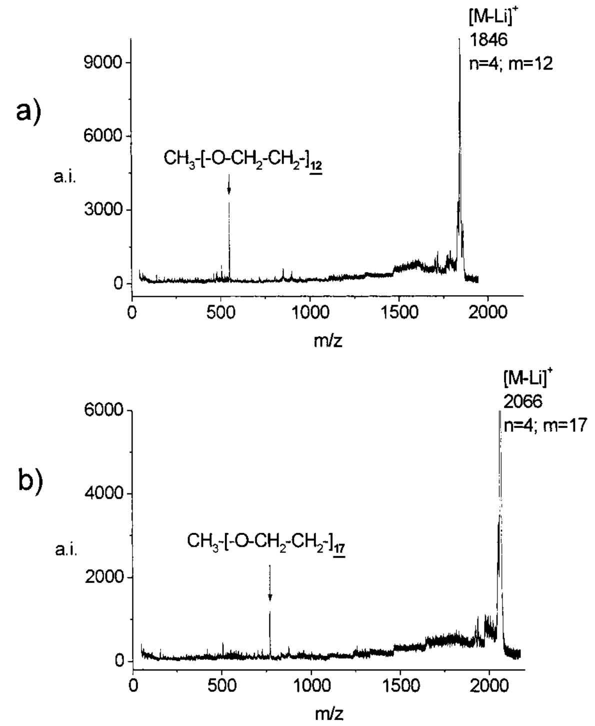

Figure 3. PSD-MALDI-TOF mass spectra of PEO-*b*-PPE (**3**) from mother ions with same PPE block length *n* but different PEO block lengths *m*: (a) $n = 4$, $m = 12$; (b) $n = 4$, $m = 17$. The PEO block length *m* can be inferred from the most intense fragment peak.

To verify the reliability of this fragment-ion analysis of the PEO-*b*-PPE diblock copolymer, PSD-MALDI-TOF mass spectra of chains with different compositions were also acquired. Figure 3a,b shows the PSD-MALDI-TOF mass spectra of copolymer chains having the same PPE block length ($n = 4$) but varying PEO block length ($m = 12$ and $m = 17$, respectively). In both spectra and in agreement with the former investigated fragment-ion mass spectrum, the PEO block is detected by far as the most intense fragment signal, allowing the composition of the copolymer chain to be determined unambiguously.

As already mentioned, the compositional information that can be gained from the fragment-ion analysis should be particularly helpful, in the case of overlapping signals. Instead of selecting only one composition for the copolymer chain, as demonstrated so far, fragment-ion mass spectra were also recorded by selecting simultaneously two neighboring signals, separated by only 4 Da and corresponding to different copolymer compositions. Figure 4 shows the PSD-MALDI-TOF mass spectrum of the copolymer chains with the compositions $n = 4$, $m = 20$ and $n = 5$, $m = 14$. The fragment-ion mass spectrum is dominated by two intense fragment ions: the PEO block with $m = 14$ and the other PEO block with $m = 20$. This means that two copolymer chains, having almost the same molecular weight but different compositions and generating overlapping signals in the conventional MALDI-TOF mass spectrum, can be clearly distinguished in the fragment-ion mass spectrum.

As part of this investigation of the PEO-*b*-PPE diblock copolymer, other useful results are worth mentioning. They concern on one hand the choice of the cationizing reagent and on the other hand the fragment-ion analysis

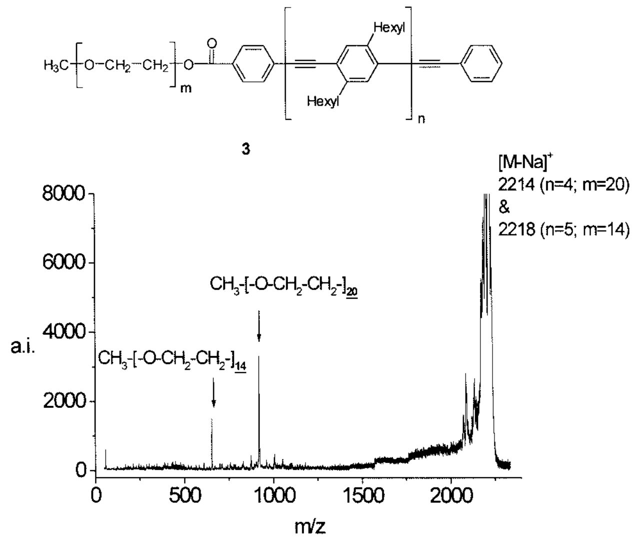

Figure 4. PSD-MALDI-TOF mass spectrum of PEO-*b*-PPE (**3**) from mother ions with different compositions having almost the same molecular weight 2214 Da ($n = 4$; $m = 20$) and 2218 Da ($n = 5$; $m = 14$). Both mother ions were selected together. The PEO block length m from each composition can be inferred from the clearly separated fragment peaks.

of the homopolymers. As already noticed for the fragment-ion analysis of polycarbonates,[23] the choice of the cationizing reagent is determining for the acquisition of a PSD-MALDI-TOF mass spectrum. By measuring lithium cationized PEO-*b*-PPE chains, a PSD-MALDI-TOF mass spectrum could be recorded even by irradiating the sample with a relative high laser fluence. From a sodium cationized copolymer chain, a fragment-ion mass spectrum can only be acquired if the laser fluence is cautiously selected. We assume that this can be explained as follows: the laser fluence used for the PSD measurement must be sufficient to induce fragmentation of the copolymer chain but not too high, to avoid dissociation of the sodium cation from the copolymer chain. In the case of potassium cationized PEO-*b*-PPE chains, no appropriate laser fluence could be found, which would allow the acquisition of a fragment-ion mass spectrum. The somewhat higher laser fluence necessary to induce the fragmentation of the copolymer chain is already sufficient to provoke dissociation of the potassium cation from the copolymer. The resulting PSD-MALDI-TOF mass spectrum reveals only the mother ion and the bare potassium cation. As already speculated elsewhere,[23] we explain these results by the structure of the cationized copolymer chain in the gas phase. The cation is supposed to be surrounded by the polymer coil in such a way that as many heteroatoms as possible interact with it. Considering the chemical composition as well as the flexibility of the PEO-*b*-PPE diblock copolymer, the cation is most probably situated in the PEO coil rather than in the environment of the PPE rodlike block. Owing to its relatively high charge density, Li^+ has the strongest interaction with the polymer coil, so that the adduct ion does not dissociate under the conditions used for PSD experiments. With increasing mass of the cation, the binding energy between cation and polymer decreases, so that dissociation of the cation from the polymer competes with fragmentation of the polymer. For the sodium adduct ion the laser fluence can still be optimized, to avoid dissociation, whereas the potassium adduct ion completely dissociates.

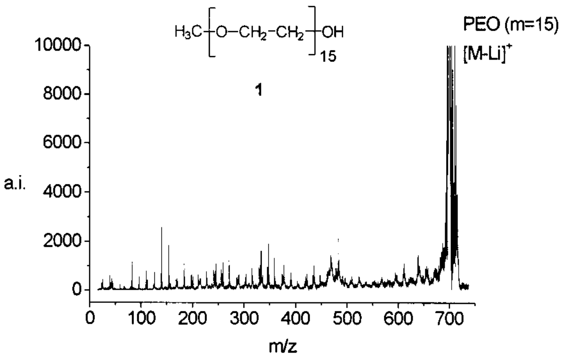

Figure 5. PSD-MALDI-TOF mass spectrum of PEO homopolymer (**1**).

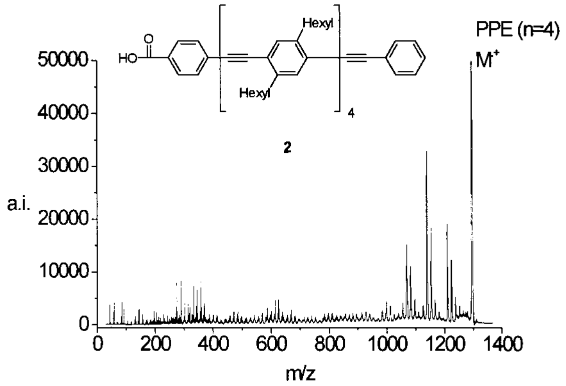

Figure 6. PSD-MALDI-TOF mass spectrum of PPE homopolymer (**2**).

Beside fragment-ion analysis of the PEO-*b*-PPE copolymer (**3**), PSD-MALDI-TOF mass spectra of the homopolymers **1** and **2** were also recorded. Figure 5 shows the fragment-ion mass spectrum of the PEO oligomer (**1**) containing 15 repeating units. This mass spectrum exhibits a rather complicated fragmentation pattern in comparison with that of the PEO-*b*-PPE diblock copolymer (**3**). All the fragment ions of the lithium cationized PEO (**1**) retain the lithium cation, and the detected masses are in agreement with the fragmentation mechanism already proposed by investigations of PEO with FAB-MS/MS[26] and CID-MALDI-MS/MS.[20] Merely cleavage of the PEO chain as well as cleavage accompanied by rearrangement reactions of the polymer chain is involved in this fragmentation mechanism. Considering Figure 5, it can be pointed out that the complicated fragment-ion mass spectrum of PEO allows indeed to confirm the structure of the homopolymer **1,** but it would be very difficult to determine, the other way round, unknown end groups from it. The PSD-MALDI-TOF mass spectrum of the PPE oligomer (**2**) with four repeating units is depicted in Figure 6. The mother ion and the fragment-ions are detected as radical cations, since PPE, in contrast to most of the synthetic polymers, does not form adduct ions during ionization but is expected to be photoionized. The photoionization of PPE can be explained by an absorption at the wavelength of the nitrogen laser (337 nm), which is used in the MALDI mass spectrometer. As for PEO, a much more complicated mass spectrum is recorded for the PPE homopolymer than for the PEO-*b*-PPE diblock copolymer (**3**). The fragment ions with relatively high molecular weights (>900 Da) are formed by cleavage of the alkyl side chains. Fragment ions involving cleavage of the PPE backbone are detected in the low mass range (200−400 Da). Their formation cannot be explained by a simple cleavage of the PPE but seems to involve rearrangement reactions. The fragment-ion mass spectrum of the PPE homopolymer is indeed characteristic for the polymer structure and could be referred to as “fingerprint”; however, information on unknown end groups would be difficult to extract from this complicated mass spectrum. On the contrary, the most important structural information (block lengths) can be reliably extracted from the fragment-ion mass spectrum of the PEO-*b*-PPE diblock copolymer. The cleavage of the ester function linking the two blocks in the diblock copolymer dominates the fragment-ion pattern and leads fortunately to the most intensive signal. Thus, the investigation of the fragment ion mass spectra of the PEO and PPE homopolymers is in this case not absolutely necessary for extracting the main structural information from the fragment-ion mass spectrum of the diblock copolymer; nevertheless, it contributes to the assignment of the less intense fragment-ion signals.

Conclusion

Unlike the homopolymers, which exhibit complex fragmentation patterns, the structure of the PEO-*b*-PPE diblock copolymer can be unambiguously inferred from its PSD-MALDI-TOF mass spectrum. The main fragmentation corresponds to the cleavage of the ester function situated at the junction between the two blocks, and the entire PEO block is detected as the by far most intense fragment-ion signal. The PEO block length can be directly determined, and the complementary PPE block length can then be inferred. In the case of the investigated PEO-*b*-PPE diblock copolymer, these structural data were already delivered by the conventional MALDI-TOF mass spectrum. However, this study demonstrates the capacity of the fragment-ion analysis. In more complex conditions, when the compositional information cannot be extracted from the MALDI-TOF mass spectrum directly, the fragment-ion mass spectrum is then the ultimate method to determine the block lengths of the diblock copolymer. Conditions complicating the conventional MALDI-TOF mass spectrum are easy to conceive. If two or more compositions of the copolymer can be calculated for a given molecular weight and if the resolution of the mass spectrometer does not allow separation of the signals, or if one monomer is exactly a multiple of the other one, so that different compositions generate strictly the same signals, the structure of the diblock copolymer cannot be inferred from the absolute mass of the chain. A sample containing a copolymer terminated by different end groups is obviously another example, for which a direct structure determination from the conventional MALDI-TOF mass spectrum would be hindered. In these cases, only the PSD-MALDI-TOF mass spectrum could deliver unequivocal structural data. Moreover, it should be outlined that the measured fragment-ion mass spectra of the diblock copolymer are characteristic for the distribution of the monomers along the chain. Other copolymer structures, such as alternating or random copolymers, should generate rather different fragmentation patterns. This means that the PSD-MALDI-TOF mass spectrum allows in principle to distinguish copolymers constituted by a different sequence of the monomers along the chain, whereas these structures are indistinguishable on the conventional MALDI-TOF mass spectrum, since they lead to the same absolute mass for the whole copolymer chain. Finally, PSD-MALDI-TOF mass spectrometry is expected to have the potential to elucidate the structure of even more complex polymers such as for instance triblock copolymers, starlike polymers, or multicomponent systems.

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