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Molecular Level Structure of (*R*,*S*)-3-Hydroxybutyrate/(*R*,*S*)-3-Hydroxy-4-ethoxybutyrate Copolyesters with Dissimilar Architecture

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ABSTRACT: New copolyesters with designed architecture were obtained via anionic ring-opening copolymerization of β-butyrolactone with β-ethoxymethyl-β-propiolactone. The resulting copolyesters contained predominantly linear macromolecular chains terminated by acetate end groups derived from the used initiator regardless of the type or the composition. Similar behavior of β-alkoxy-substituted-β-lactone and β-alkyl-substituted-β-lactone (including side reactions leading to unsaturated end groups) was observed under the conditions of anionic ring-opening copolymerization. The application of ion trap ESI-MS/MS analysis for structural studies and investigation of the fragmentation product patterns of the individual molecular ions showed significant differences in the molecular structure at the level of individual molecular chains of random and diblock copolyesters. Thus, the sequence distribution of diblock and random copolyester was determined based on the investigation and comparison of the fragmentation product patterns of the individual molecular ions selected from the both types of studied copolyesters.

Introduction

Thermoplastic biopolymers represent an interesting group of environmentally friendly materials for various medical and technical applications. Regardless of the variety of biodegradable polymeric materials available on the market, until now, there is no unique biodegradable polymer which can fulfill all of the expected requirements needed for specific applications in e.g. health care. Therefore, there is a continuous require for the development and detailed characterization of new polymer materials. Recently, successful investigations of catalytic synthesis of β-lactones offer opportunity for the synthesis of new analogues of poly(3-hydroxyalkanoate)s with desired architectures and controlled properties.[1−3] Despite the constant improvement of the synthesis methods, synthetic polymers may show a structural heterogeneity related to topology (linear, cyclic, branched), composition, and comonomers composition distribution (different functional groups, sequence of monomers). This makes the structural and molecular analysis of synthetic polymers quite a complex analytical task.[4,5] In recent years, matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI) have become a routine analytical tool for structural analysis of polymers,[4−9] complementing NMR, and other traditional techniques, e.g., size exclusion chromatography, light scattering, and infrared spectroscopy.[10−13,3] MALDI-TOF-MS and ESI-MS are suitable for polymer analysis because they are sensitive and nonaveraging techniques that provide detailed structural information about the individual molecules in a polymer sample.[14−16] These techniques are also widely used with increasing success for characterization of synthetic copolymers. In addition to the homopolyesters, the composition of the copolyesters and sequences distribution were estimated based on their mass spectra.[5,8,12,17−20] Furthermore, deeper insight into the structure of homopolyesters and copolyestrs can be achieved by means of mass spectrometric fragmentation techniques. The principal advantage of using this technique is that more information is available than can be obtained by direct analysis using conventional (single stage) mass spectral methods. Several successful applications of the ESI-MS and MALDI-TOF-MS techniques for determination of the structural architecture of individual (co) polyester macromolecules including their topology, composition, chemical structure of the end groups, location of functional groups, and comonomer composition distribution were recently reported.[14−29] Mass spectrometry was also recently applied for the structural characterization of water-soluble degradation product of aliphatic copolymers.[30,31]

The aim of this work was to determine and compare the molecular structure and heterogeneities present in different type of aliphatic copolyesters. For purpose of this study a new model of copolyesters of β-butyrolactone with β-ethoxymethyl-β-propiolactone with different architecture (diblock and random) were synthesized via anionic ROP. The resulting copolyesters were preliminary characterized by FT-IR, SEC, and NMR.

My hypothesis was that the type of comonomeric units distribution along the individual macromolecular chains present in the studied copolyesters should influence their fragmentation product patterns formed during the ion trap ESI-MS/MS experiments. Thus, the application of ion trap ESI-MS/MS analysis for the structural studies could show differences in the structure at the individual chains level of random and diblock copolyesters studied.

Experimental Section

Materials. Racemic β-(ethoxymethyl)-β-propiolactone (EMPL) was prepared at Cornell University by carbonylation of respective oxirane using the catalyst and conditions discussed in ref 1 and characterized using the reported data in ref 2. (*R*,*S*)-β-Butyrolactone (β-BL) (Aldrich) was distilled over CaH_2, and the fraction boiling at 56 °C (9 mmHg) was collected. Tetrabutylammonium acetate ($AcNBu_4$, Fluka) was used as received.

Synthesis of Poly[(*R*,*S*)3-hydroxy-4-ethoxybutyrate-*co*-(*R*,*S*)-3-hydroxybutyrate)] Random Copolyester. Poly[(*R*,*S*)-3-hydroxy-4-ethoxybutyrate-*co*-(*R*,*S*)-3-hydroxybutyrate)] copolyester was synthesized by anionic ring-opening copolymerization

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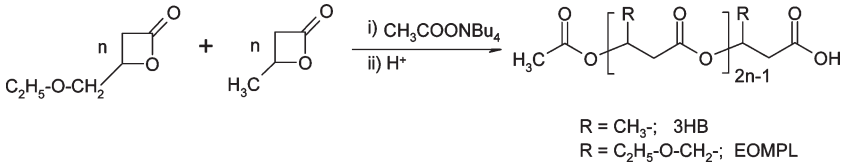
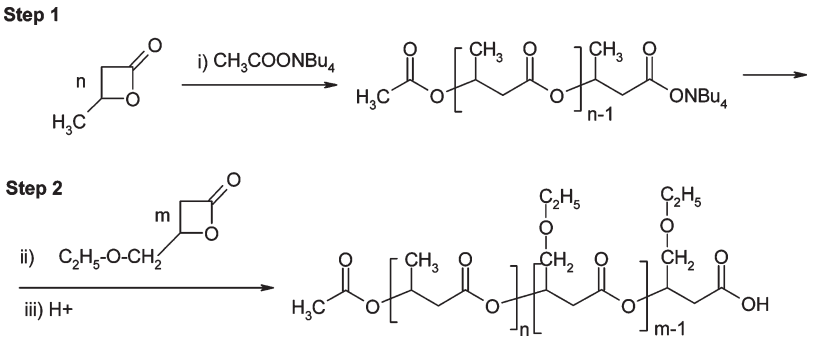
Scheme 1. Synthesis of Poly[(*R*,*S*)3-hydroxy-4-ethoxybutyrate-*co*-(*R*,*S*)-3-hydroxybutyrate)] Random Copolyester



Scheme 2. Synthesis of Poly[(*R*,*S*)-3-hydroxybutyrate)-*block*-(*R*,*S*)-3-hydroxy-4-ethoxybutyrate)] Diblock Copolyester



(ROC) of racemic β-(ethoxymethyl)-β-propiolactone (EMPL) with racemic β-butyrolactone (β-BL) initiated by tetrabutylammonium acetate ($AcNBu_4$). The copolymerization experiments were carried out in round-bottom flasks equipped with magnetic stirring bars at temperature of 23 °C. First the initiator ($AcNBu_4$) was weighed into the flask, and then the monomers (EMPL and β-BL) solution in THF was added into the flask under an argon atmosphere. The mixture was stirred by magnetic stirring bar until the monomers reacted nearly completely. The progress of the copolymerization reactions was followed by FTIR spectroscopy, basing on the measurements of the relative intensities of carbonyl group bands of respective lactones (at 1830 cm^{-1}) and the formed polyesters (1740 cm^{-1}). After completion of the polymerization reactions the resulting copolymers were precipitated in cold hexane and dried under vacuum.

Synthesis of Poly[(*R*,*S*)-3-hydroxybutyrate)-*block*-(*R*,*S*)-3-hydroxy-4-ethoxybutyrate)] Diblock Copolyester. The poly-[(*R*,*S*)-3-hydroxybutyrate)-*block*-(*R*,*S*)-3-hydroxy-4-ethoxybutyrate)] diblock copolymer was synthesized in two stages. The first poly[(*R*,*S*)-3-hydroxybutyrate) prepolymer was synthesized via anionic ring-opening polymerization of β-BL initiated with $AcNBu_4$. The polymerization was carried out in a solution of THF using round-bottom flasks equipped with magnetic stirring bars according to the previously reported procedure.[32,33] The progress of the reaction was measured by FTIR spectroscopy basing on the carbonyl group signals of BL and the poly(3-hydroxybutyrate) at 1815 and 1735 cm^{-1}, respectively. In order to characterize the obtained prepolymer, the samples were withdrawn from the reaction mixture before the addition of the second monomer for ^1H NMR and SEC analysis. The second EMPL monomer was dissolved in THF and then transferred to the reactor containing poly(*R*,*S*)-3-hydroxybutyrate) prepolymer. The progress of the copolymerization reactions was followed by FTIR spectroscopy, based on the measurements of the relative intensities of carbonyl group bands of EMPL lactone (at 1830 cm^{-1}) and the copolyesters formed (1740 cm^{-1}). After completion of the copolymerization reactions the resulting copolymers were precipitated in cold hexane and dried under vacuum.

Protonation of Copolyester Carboxylate End Groups. 10 wt % chloroform solution of copolymer samples were acidified with diluted HCl(aq), and the mixture was stirred dynamically for 10 min. After phase separation the organic layer was separated and acidified once more (similarly as described above). Then the polymer solution was washed 10 times with 10 mL of distilled water. Next the solvent was evaporated, and the copolymer was dried under vacuum at room temperature. Final products were characterized with ^1H NMR and GPC and ESI-MS techniques.

Measurements. *FT-IR Spectroscopy*. FT-IR spectra were recorded with FTS 40A Bio-Rad spectrometer.

Nuclear Magnetic Resonance (NMR). ^1H NMR spectra were recorded at 600 MHz with Bruker Avance II at room temperature. The $CDCl_3$ was used as a solvent, and tetramethylsilane (TMS) was applied as the internal standard. The spectra were recorded with 64 scans, a 2.65 s acquisition time, and a 11 μs pulse width.

Size Exclusion Chromatography (SEC). The number-average molecular weight (M_n) and molecular weight distribution index (M_w/M_n) were determined by SEC. The system was composed of a Spectra-Physics 8800 solvent delivery and a Shodex SE-61 refractive index detector. The analyses were carried out in THF at 35 °C at a flow rate of 1 mL/min, with the aid a set of two PLgel 5 μm MIXED-C high efficiency column (300 × 7.5 mm). A volume of 10 μL of sample solution in THF (concentration 1% w/v) was injected. PSSW GPC software was used to compute the average molar masses of the polyester samples by means of the calibration curve obtained using a set of polystyrene standards with narrow molecular weight distribution.

Electrospray Mass Spectrometry Analysis (ESI-MS). Electrospray mass spectrometry analyses (ESI-MS) were performed using a Finnigan LCQ ion trap mass spectrometer (Finnigan, San Jose, CA). The samples of the polyesters were dissolved in the chloroform/methanol system (2:1 v/v), and the solutions were introduced to the ESI source by continuous infusion using the instrument syringe pump at a rate of 3 μL/min. The LCQ ESI source was operated at 4.5 kV, and the capillary heater was set to 200 °C. Nitrogen was used as the nebulizing gas. For ESI-MS/MS experiments the ions of interest were isolated monoisotopically in the ion trap and collisionally activated. The helium damping gas present in the mass analyzer acts as a collision gas. The RF amplitude, which had a significant voltage range, was set to a value that caused the peak height of the parent ion to decrease by at least 50%. The analyses were performed in positive-ion mode.

Results and Discussion

The synthetic analogues of biodegradable natural poly(hydroxyalkanoates) (PHAs) can be obtained via anionic ring-opening polymerization (ROP) of simple β-lactones. Recently, it was also demonstrated that polymerization mechanism of

β-alkoxymethyl-substituted-β-lactones is similar to that proposed previously for anionic ROP of simple β-lactones such as β-propiolactone, β-butyrolactone, and α-methyl-β-pentyl-β-propiolactone carried out in the presence of the anionic initiators.[3]

Based on the previously reported results for purpose of this study, new model copolyesters were prepared via anionic simultaneous or stepwise ring-opening copolymerization of β-butyrolactone and β-ethoxymethyl-β-propiolactone, respectively (see Schemes 1 and 2). The resulting copolyesters were preliminary characterized by FT-IR, SEC, and ^{1}H NMR.

To obtain enhanced insight into the molecular structures of different types of copolymers complementary to NMR, the electrospray mass spectrometry technique (ESI-MS) and ESI-MS/MS method were applied.

Copolymer compositions and molar masses as determined by ^{1}H NMR and SEC are listed in Table 1. The molecular weight of the copolymers studied was tuned (by monomer-to-initiator ratio) to the M_n ca. 2000 due to the used in this study the ESI mass spectrometer with mass limit up to 2000 Da.

Some structural information about the synthesized copolyesters was obtained from NMR analysis. The ^{1}H NMR spectra of 3-HB/EMPL diblock and random copolyesters together with the signal assignments are displayed in Figures 1 and 2, respectively.

^{1}H NMR analysis revealed that the spectra are very similar, regardless of the studied copolyester, and according to the signal assignments, it is clearly seen that the both copolyesters obtained show signals characteristic for both 3-hydroxy-4-ethoxybutyrate (EMPL) and 3-hydroxybutyrate (3-HB) repeating units. Additionally, in both spectra the signal of acetate end group protons originating from the used initiator and the signals of protons of unsaturated (crotonate and 4-ethoxy-2-butenoate) end groups have been detected. In the case of diblock copolymer only one singlet at $\delta = 2.02$ ppm corresponding to protons of the acetate end group connected with 3-HB unit was observed, whereas in the case of random copolymer two signals (singlet at $\delta = 2.02$ ppm and at $\delta = 2.04$ ppm corresponding to the protons of acetate end group connected with 3-HB and EMPL units, respectively) were observed. Furthermore, a comparable amount of the both types of acetate end groups in the case of random copolyester studied indicates that the reactivities of β-ethoxymethyl-β-propiolactone and β-butyrolactone monomers in anionic ring copolymerization are similar, at least at the initiation stage.

In order to obtain some information about sequence distribution of the copolyesters studied, the ^{13}C NMR spectra were recorded. However, the determination of the sequence distribution with the aid of the ^{13}C NMR spectra of these oligocopolyesters was difficult. Because of the overlapping of the signals arising from comonomeric sequences on the signals corresponding to the fragments of chains with different tacticity, the obtained spectra turned out to be very complex. This is probably connected with the application of racemic β-(ethoxymethyl)-β-propiolactone (EMPL) and β-butyrolactone monomers for the synthesis. Moreover, there are no literature data available for correct assignments of the ^{13}C NMR signals of the two new copolyesters studied in this work. For these reasons the analysis of the ^{13}C NMR spectra was not performed, and instead the model copolyester samples were characterized by ESI-MS and ESI-MS/MS techniques.

Table 1. Copolyesters and Their Characteristics

sample	feed composition[a] [mol %]	composition[b] [mol %]	M_n[c] (M_w/M_n)[d]	3-HB prepolymer[e] M_n (M_w/M_n)
diblock copolyester	70/30	3-HB/EMPL 72/28	1600 (1.15)	1100 (1.11)
random copolyester	50/50	3-HB/EMPL 53/47	2300 (1.18)	

[a] Molar composition of the initial components mixture of (*R*,*S*)-β-butyrolactone (β-BL) and β-(ethoxymethyl)-β-propiolactone (EMPL). [b] Molar composition of obtained copolymers estimated from ^{1}H NMR. [c] Number-average molecular weight estimated by SEC. [d] Molecular weight distribution estimated by SEC. [e] Number-average molecular weight and molecular weight distribution of poly(*R*,*S*)-3-hydroxybutyrate prepolymer estimated by SEC.

ESI-MS Reveals the Molecular Level Structure of 3-HB/EMPL Random and Blocky Copolyester. Figures 3 and 4 display the positive ESI mass spectra together with spectral expansions in the mass range *m*/*z* 880−1120 respectively for 3-HB/EMPL diblock and random copolyesters.

Both ESI mass spectra recorded consist of a large number of singly charged ions and were rather complicated due to the various combinations of the two repeating units. The peaks corresponding to the individual copolyester chains with different size and different end groups tend to group themselves in clusters. Nevertheless, the high resolution achieved in the mass spectra allowed the different species to be distinguished.

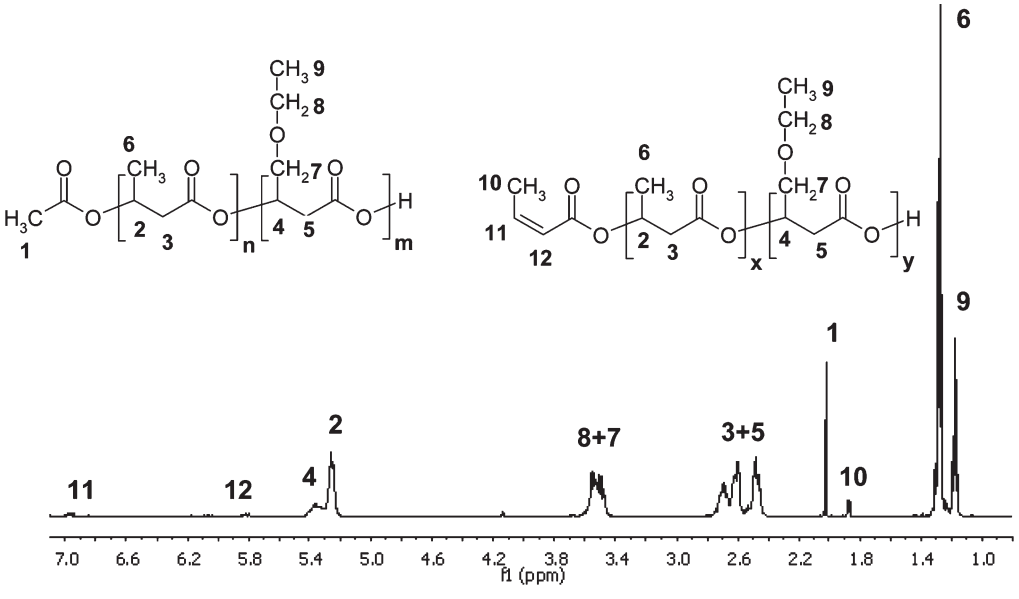


Figure 1. ^{1}H NMR spectrum of 3-HB/EMPL diblock copolyester sample.

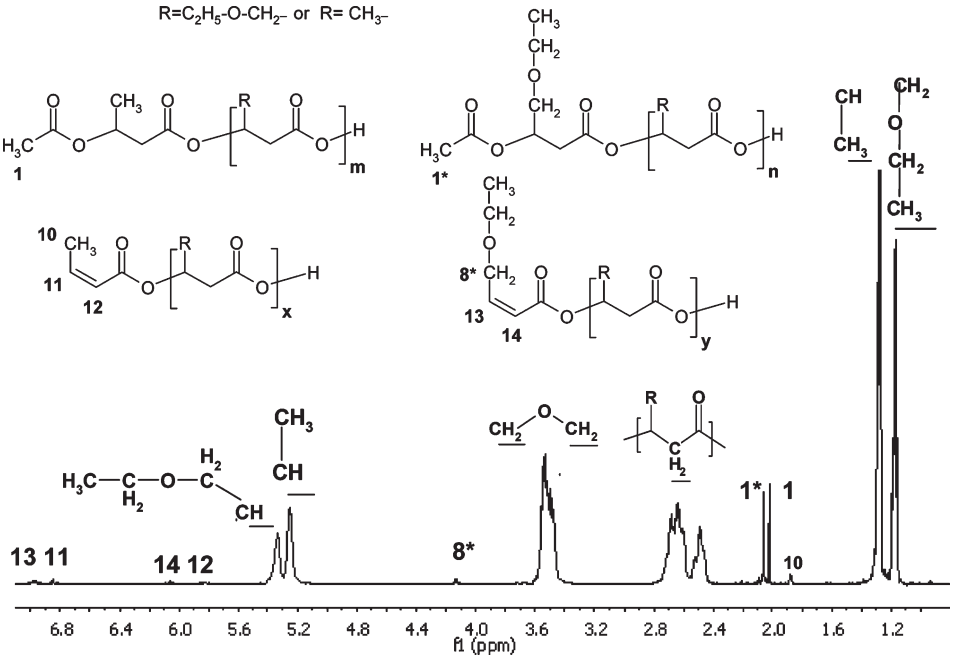


Figure 2. ^{1}H NMR spectrum of 3-HB/EMPL random copolyester sample.

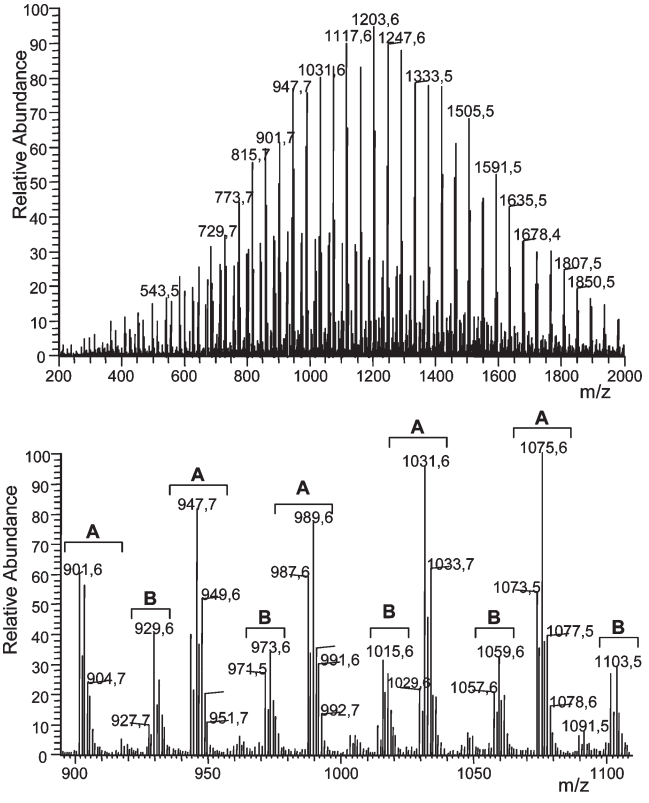


Figure 3. ESI mass spectrum of 3-HB/EMPL diblock copolyester together with spectral expansion in the mass range m/z 880−1120.

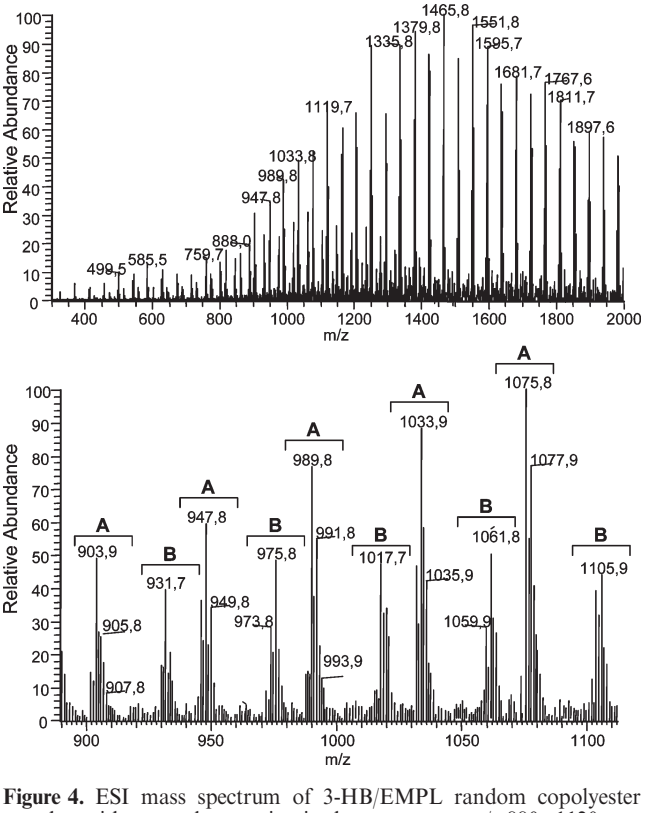


Figure 4. ESI mass spectrum of 3-HB/EMPL random copolyester together with spectral expansion in the mass range m/z 880−1120.

Two series of cluster ions, with a general chemical structure shown in the Scheme 3, are present in the mass spectra of the both copolyester samples studied. The major mass spacing between the respective signals belong to the neighboring clusters (series A or B) are equal to 44 Da, which corresponds to the difference between the molecular masses of individual 3-hydroxy-4-ethoxybutyrate (EMPL, 130 Da) and 3-hydroxybutyrate (3-HB, 86 Da) units. Regardless of the copolyester type, the main series of cluster peaks in both mass spectra (labeled as A, peaks apper at $m/z = 59 + n \times 86 + m \times 130 + 1 + 23$) correspond to sodium cationized linear 3-HB/EMPL copolyester chains terminated by acetate (derived from the initiator used) and carboxylic end groups. The second series of cluster peaks with significant low intensity and located especialy in the low mass range (labeled as B, peaks apper at $m/z = 85 + n \times 86 + m \times 130 + 1 + 23$ or $m/z = 129 + n \times 86 + m \times 130 + 1 + 23$) can be assigned to the sodium cationized linear 3-HB/EMPL copolyester oligomers terminated by

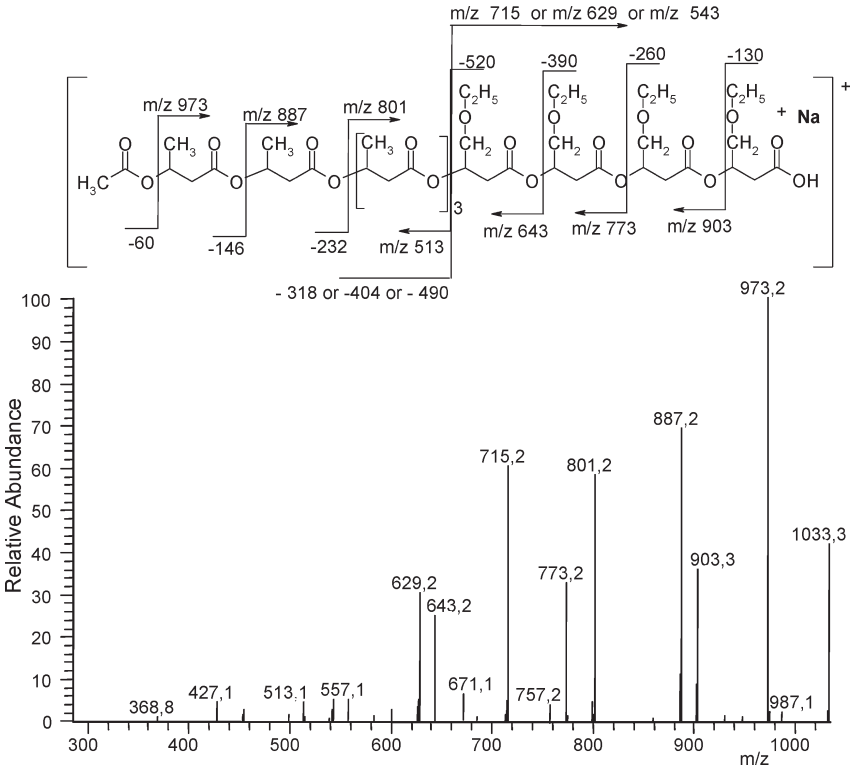
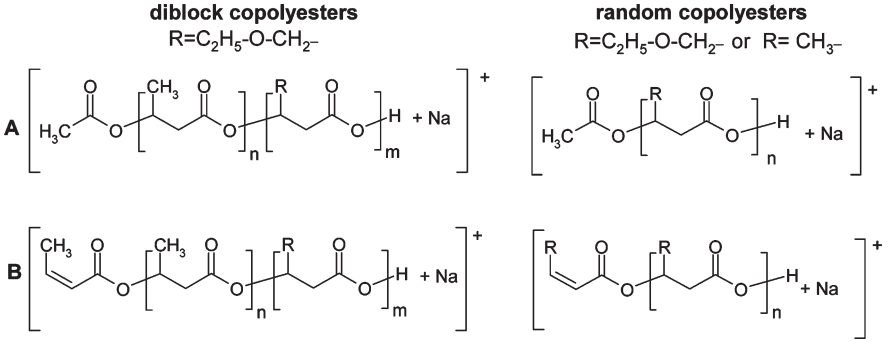


Figure 5. ESI-MS/MS spectrum of the sodiated precursor ion $[3\text{-}HB_5/EMPL_4 + Na]^+$, m/z 1033, selected from the simple ESI mass spectrum (series A) of diblock copolyester. Theoretical fragmentation pathway of the selected ion.

Scheme 3. General Chemical Structures of the Ions Appering in ESI Mass Spectra of 3-HB/EMPL Diblock and Random Copolyesters



unsaturated (crotonate or/and 4-ethoxy-2-butenoate) and carboxyl end groups. Furthermore, a small intensities peaks corresponding to the poly(3-hydroxybutyrate) and poly(3-hydroxy-4-ethoxybutyrate) homopolymers were detected in mass spectra of diblock and random copolyester samples, respectively.

Because of the large number of signals present in Figures 3 and 4, a full peak assignment was not performed. However, the peak assignments for the some crucial peaks shown in the respective spectral expansions in mass range m/z 880−1120 are presented in the table in the Suporting Information.

As can be seen from the above results, regardless of the type of studied copolyester (random or blocky) the respective mass spectra are very similar. The visible difference between these two spectra concerns only the mass range. Thus, the simple ESI-MS mass spectra provided information about the general structure of individual copolyester macromolcules including the chemical structure of their end groups. Hovewer, this analysis still has not provided enough information about arrangements of comonomer structural units along the individual copolyester chains. It may be due to the fact that in the case of copolymers several different oligomers with different sequences with particular chemical composition may contribute to the intensity of the ion of a given m/z value. Moreover, the number of theoretical sequences for an oligomer increases with degree of polymerization increase.

Structural Characterization of 3-HB/EMPL Random and Diblock Copolyester by Ion Trap ESI-MS/MS Analysis. Further insight into the structure of a individual copolyester chains can be achieved by multistage mass spectrometry (MS^n), where the molecular ion of interest is separated from all other ions formed during ionization and is induced to dissociate into fragments that can be used for seating arrangement of comonomer structural units along the copolyester chains.

In order to obtain more information about the specific sequence distributions and to clarify the structure of the two copolyesters studied, the ion trap ESI-MS/MS technique was applied. However, to decode sequencing data of the individual comacromolecules from their MS^n spectra, a basic knowledge of the fragmentation processes of copolyester studied is needed.

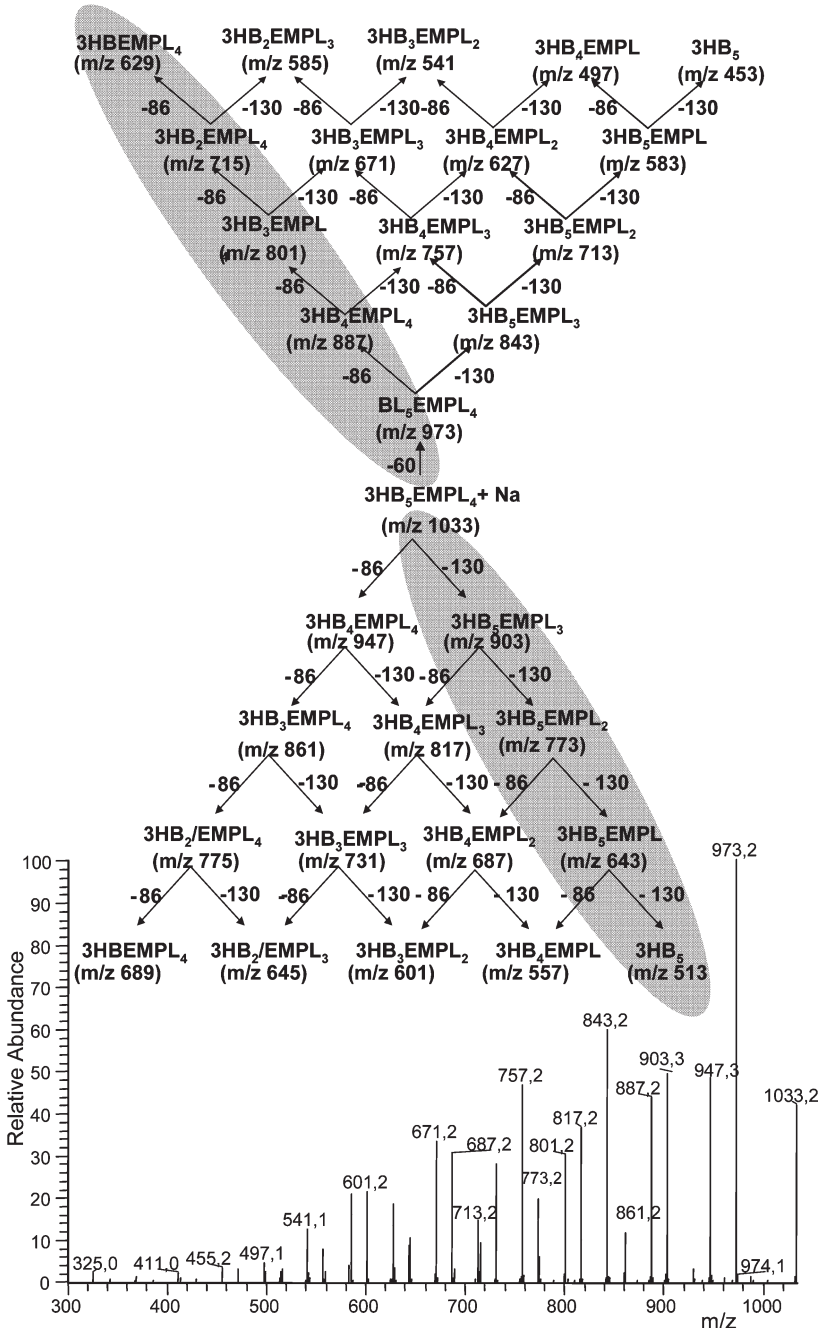


Figure 6. ESI-MS/MS spectrum of the sodiated precursor ion $[3\text{-}HB_5/EMPL_4 + Na]^+$, m/z 1033, selected from the simple ESI mass spectrum (series A) of random copolyester sample. Theoretical fragmentation pathway of the selected ion. The part of the scheme indicated in gray shows the product ions formed from diblock copolyester molecular ion at m/z 1033.

Previously performed fragmentation studies of individual macromolecular ions of poly(3-hydroxybutyrate)[14,15] and its synthetic copolymers poly[(*R*,*S*)-3-hydroxybutyrate-*co*-hydroxyhexanoate],[19,9] poly[(*R*,*S*)-3-hydroxybutyrate-*co*-L-lactide),[29] and poly(3-hydroxy-4-ethoxybutyrate)[3] revealed that the β-hydrogen rearrangement was the main mechanism that induced the fragmentation of such kinds of polyesters.

The structures of individual macromolecules of 3-HB/EMPL diblock and random copolyesters the ESI-MS/MS experiments were performed on sodiated precursor ions $[3\text{-}HB_5/EMPL_4 + Na]^+$, at m/z 1033, selected from the simple ESI mass spectra of both kinds of copolyesters, with the aim of differentiation at the molecular level. The selected ions consist of nine comonomer repeating units and contain acetate and carboxyl end groups (series A). The respective ESI-MS/MS spectra of these molecular ions together with theoretical fragmentation pathway are presented in Figures 5 and 6, respectively.

The obtained ESI-MS/MS spectra exposed differences between structure of the two selected molecular ions distinctly. A bigger diversity of the product ions formed during the fragmentation of sodiated molecular ion $[3\text{-}HB_5/EMPL_4 + Na]^+$, at m/z 1033, selected from mass spectrum of random and then from the diblock copolymer samples was observed.

On the basis of the fragmentation mechanism proposed previously for the poly(3-hydroxybutyrate) and poly(3-hydroxy-4-ethoxybutyrate) macromolecular ions the rearrangement of hydrogen originating in the $-CH_2-$ group leads to the selective ester bond cleavage between the

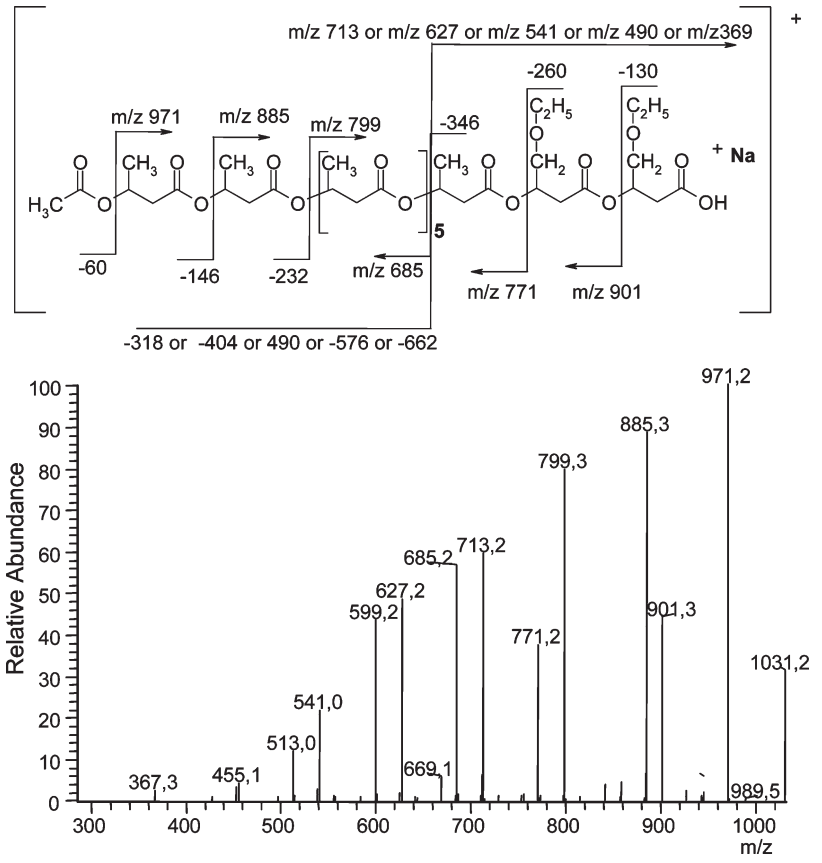
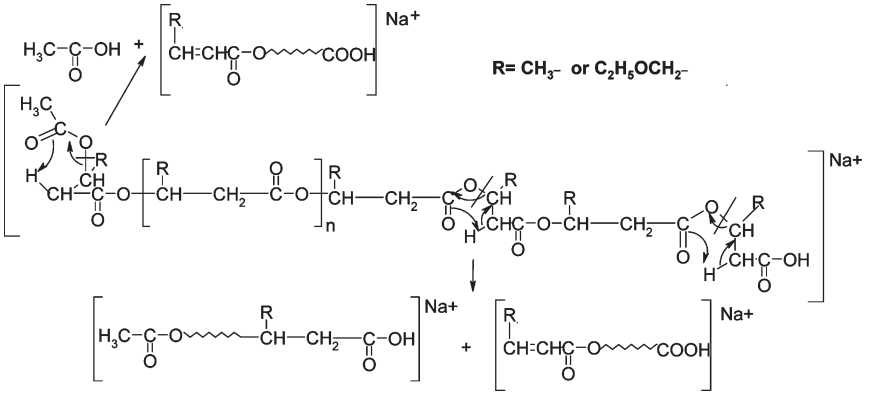


Figure 7. ESI-MS/MS spectrum of the sodiated precursor ion $[3\text{-}HB_8/EMPL_2 + Na]^+$ terminated by acetate and carboxyl end groups, at m/z 1031, selected from the simple ESI mass spectrum of diblock copolyester sample. Theoretical fragmentation pathway of the selected ion.

Scheme 4. Fragmentation of Sodiated Molecular Ions Terminated by Acetate and Carboxyl End Groups via β-Hydrogen Rearrangement Mechanism



two comonomer units in macromolecule with the formation of two kinds of product ions: one containing acetate and carboxyl end groups and the other with unsaturated (crotonic or 4-ethoxy-2-butenoic) and carboxyl end groups. The structures of the product ions formed during ESI-MS/MS experiments are presented in Scheme 4.

According to the structural assignment and based on the theoretical fragmentation pathway (see Figure 5), the product ions at m/z 903, 773, 643, 513, and 427 presented in the MS/MS spectrum of the sodiated molecular ion at m/z 1033 selected from the mass spectrum of diblock copolyester sample correspond to the 3-HB/EMPL copolyester oligomers terminated by acetate and carboxyl end groups, whereas the complementary product ions at m/z 973, 887, 801, 715, 629, 499, and 369 correspond to the 3-HB/EMPL copolyester chains terminated by crotonic and carboxyl end groups.

Similarly as in the case of diblock copolyester sample the fragmentation products of molecular ion at m/z 1033 selected from random copolyester sample composed of the macromolecules terminated by acetate and carboxyl end groups (see MS/MS spectrum and bottom part of theoretical fragmentation pathway, Figure 6) as well as macromolecules contained unsaturated (crotonic and 4-ethoxy-2-butenoic) and carboxyl end groups (see MS/MS spectrum and upper part of theoretical fragmentation pathway, Figure 6). However, the ESI-MS/MS spectrum of the sodiated molecular ion at m/z 1033 selected from the mass spectrum of random copolyester sample (Figure 6) in comparison with the spectrum shown in Figure 5 is more complex and contains additional product ions due to the presence in the case of random copolyester sample of a larger number of possible sequences.

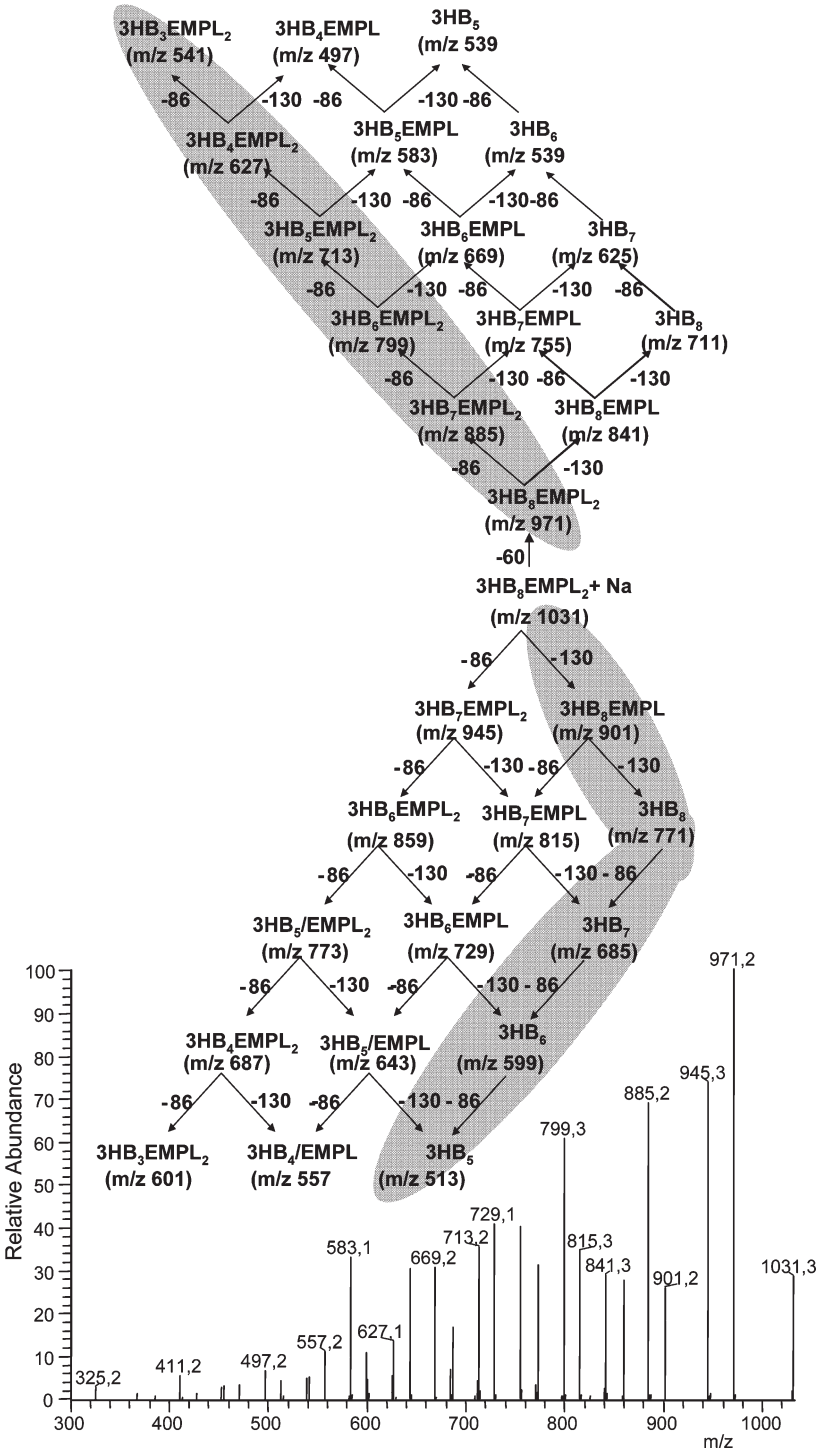


Figure 8. ESI-MS/MS spectrum of the sodiated precursor ion [3-HB_8/$EMPL_2$ + Na]$^+$ terminated by acetate and carboxyl end groups, at m/z 1031, selected from the simple ESI mass spectrum of random copolyester sample. Theoretical fragmentation pathway of the selected ion. The part of the scheme indicated in gray shows the product ions formed from diblock copolyester molecular ion at m/z 1031.

Differences between the product ions formed during fragmentation of the molecular ion at m/z 1033 selected from mass spectra of diblock and random copolyester samples are shown in Figure 6. A thorough examination of the scheme in Figure 6 revealed that all theoretically predicted structures of the product ions indeed appear in the MS/MS spectrum of the molecular ions at m/z 1033 selected from random copolyester sample (see MS/MS spectrum and scheme in Figure 6) whereas the product ions present in the MS/MS spectrum of the molecular ion at m/z 1033 selected from diblock sample constitute only a part of the ions in Figure 6 which are mainly located diagonally through the scheme (see MS/MS spectrum Figure 5 and part of scheme emphasized in gray in Figure 6).

Thus, the ion trap ESI-MS/MS experiments performed revealed that structure of the individual macromolecular ion fragmented has an influence on fragmentation product patterns.

In order to clarify this point and to verify if this hypothesis is reproducible, the ESI-MS/MS experiments were also performed on sodiated molecular ions [3-HB_8/$EMPL_2$ + Na]$^+$ at m/z 1031 selected from the mass spectra of diblock

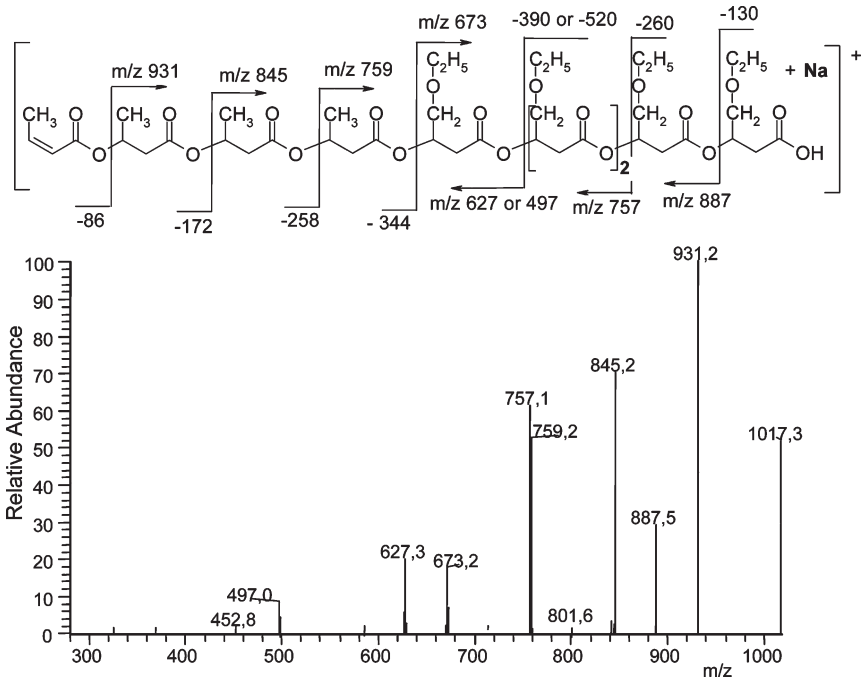


Figure 9. ESI-MS/MS spectrum of the sodiated precursor ion $[3\text{-}HB_4/EMPL_5 + Na]^+$ terminated by crotonate and carboxyl end groups, at m/z 1017, selected from the simple ESI mass spectrum of diblock copolyester sample. Theoretical fragmentation pathway of the selected ion.

and random copolyester samples. The respective ESI-MS/MS spectra of these ions are presented in Figures 7 and 8, respectively.

The insets in Figures 7 and 8 show the theoretical fragmentation pathways of the selected ions. Both ESI-MS/MS spectra of sodiated molecular ions $[3\text{-}HB_8/EMPL_2 + Na]^+$ also contain product ions composed of the macromolecules terminated by acetate and carboxyl end groups as well as macromolecules contained unsaturated (crotonic or/and 4-ethoxy-2-butenoic) and carboxyl end groups.

It is interesting to note that, again, a larger variety of the product ions was observed when the molecular ions at m/z 1031 was selected from mass spectrum of random then from diblock copolyester samples. The differences between these two fragmentation spectra are shown in Figure 8. Again, all theoretically predicted structures of the product ions are present in the MS/MS spectrum of molecular ion selected from random sample, whereas the fragmentation from both sides of the molecular ions selected from the diblock sample leads to the product ions which are present partly in Figure 8 (see ions emphasized in gray and mainly located diagonally through Figure 8).

As was already mentioned in this work, both types of copolyesters studied beside the 3-HB/EMPL copolyester macromolecules terminated by acetate end groups contained also some amount of low molecular weight copolyester chains terminated by unsaturated (crotonate or/and 4-ethoxy-2-butenoate) and carboxyl end groups. The formation of unsaturated (crotonate) end groups in β-butyrolactone polymerization as well as 4-ethoxy-2-butenoate end groups in polymerization of β-(ethoxymethyl)-β-propiolactone was already reported.[33,34,3] Thus, the formation of unsaturated end groups in the anionic ring-opening copolymerization of β-butyrolactone with β-(ethoxymethyl)- β-propiolactone initiated by activated acetate may be caused by chain transfer reaction to the monomer and/or by intermolecular carboxylate-induced α-deprotonation.

To verify the chemical structure of the end groups as well as sequence distribution in individual copolyester chains, the MS/MS experiments were performed for the molecular ions m/z 1017 selected from series B of diblock and random copolymers, respectively. The selected sodiated macromolecular ions $[3\text{-}HB_4/EMPL_5 + Na]^+$ contained comparable number of both comonomeric units and were terminated by unsaturated (crotonate or/and 4-ethoxy-2-butenoate) and carboxyl end groups. The results of fragmentation experiments and assignment of the chemical compositions for the formed product ions are shown in Figures 9 and 10, respectively.

The fragmentation of these macromolecular ions, which may occur from both sides of macromolecules, produces only one kind of product ion corresponding to the 3-HB/EMPL copolyester macromolecules terminated by unsaturated (crotonate or/and 4-ethoxy-2-butenoate) and carboxyl end groups (see Scheme 5 and fragmentation pathway in Figures 9 and 10).

The discernible differences were observed between the ESI-MS/MS mass spectra of selected ions m/z 1017 from the two copolymer samples studied indicated, similarly, on differences in the arrangement of comonomer units along their individual copolyester chains. The presence in MS/MS spectrum of all theoretically predicted structures of the product ions confirmed random distribution of comonomeric units along this copolyester chain (see MS/MS spectrum and fragmentation pathway in Figure 10), whereas the fragmentation at both sides of the molecular ion selected from the diblock sample leads to the smaller number of product ions (Figure 9). The difference between these two spectra is shown graphically in Figure 10. The product ions observed in the MS/MS spectrum (Figure 9) constitute only a part of the products formed in the case when fragmented ion was selected from the random sample (see part of scheme emphasized in gray in Figure 10). Moreover, the thorough examination of the composition of emphasized product ions clearly indicates the diblock structure of respective molecular ion fragmented.

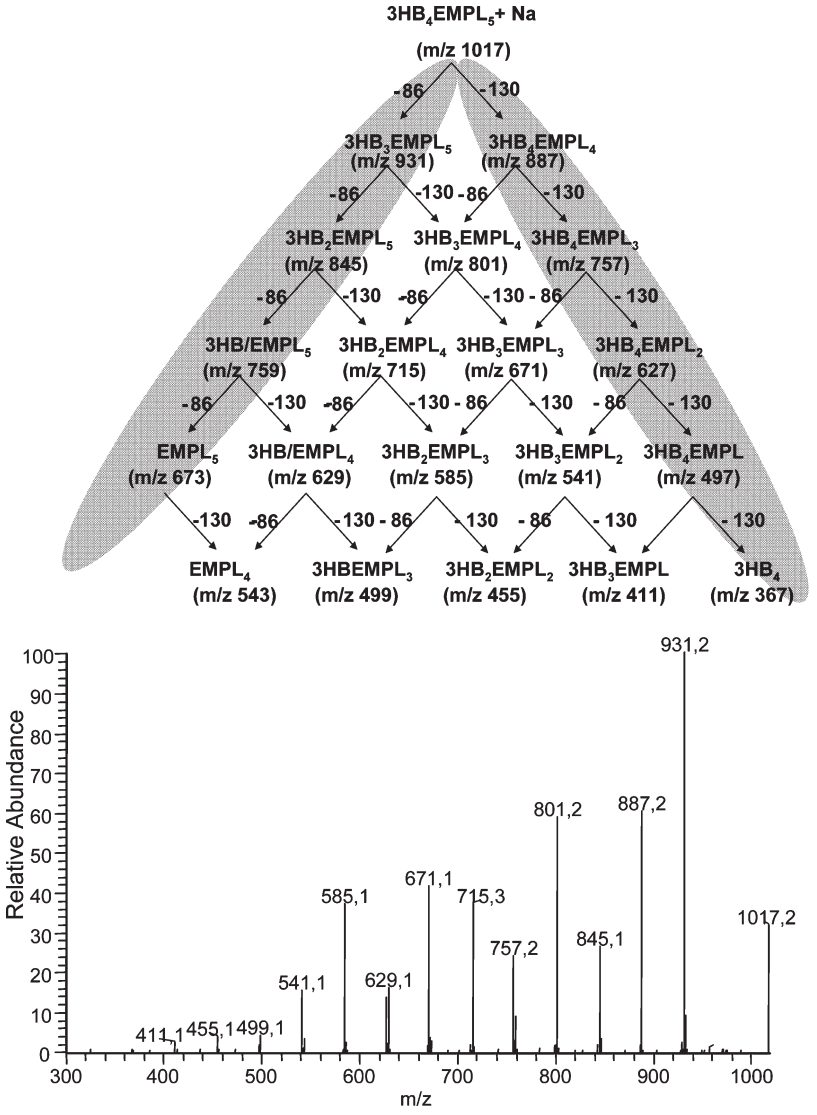
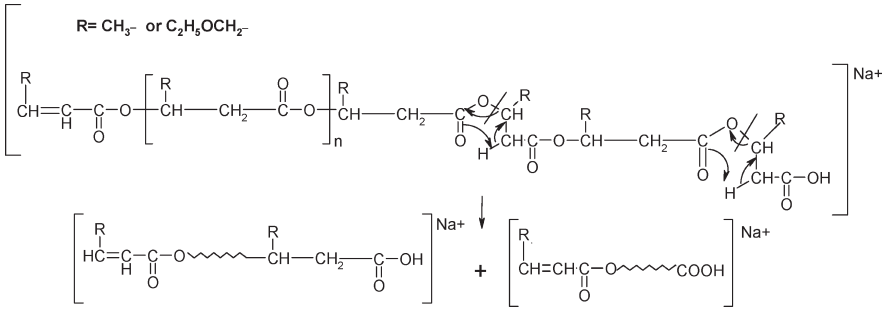


Figure 10. ESI-MS/MS spectrum of the sodiated precursor ion $[3\text{-}HB_4/EMPL_5 + Na]^+$ terminated by unsaturated (crotonate or 4-ethoxy-2-butenoate) and carboxyl end groups, at m/z 1017, selected from the simple ESI mass spectrum of random copolyester sample. Theoretical fragmentation pathway of the selected ion. The part of the scheme indicated in gray shows the product ions formed from diblock copolyester molecular ion at m/z 1017.

Scheme 5. Fragmentation of Sodiated Molecular Ions Terminated by Unsaturated (Crotonate or/and 4-Ethoxy-2-butenoate) and Carboxyl End Groups via β-Hydrogen Rearrangement Mechanism



Conclusions

The new copolyesters with different architecture (diblock and random) were synthesized via anionic ring-opening copolymerization of two racemic lactones, e.g., β-butyrolactone and β-ethoxymethyl-β-propiolactone initiated by tetrabutylammonium acetate. The ^{1}H NMR analysis supported by simple ESI-MS mass spectrometry provided information about the composition and general structure of copolyester macromolcules including the chemical structure of their end groups. Regardless of copolyester type or composition, resulting copolyesters contained predominantly linear macromolecular chains terminated by acetate end groups derived from the initiator used. Moreover, some amounts of copolyester chains terminated by unsaturated (crotonate and

4-ethoxy-2-butenoate) and carboxyl end groups were identified predominantly in the low mass range. The significant differences in molecular level structures of diblock and random copolyesters studied were discerned by application of ion trap ESI-MS/MS method. The performed ESI-MS/MS experiments revealed that sequence distribution presented in the fragmented individual copolyester macromolecular ions has an influence on their fragmentation product patterns. Thus, arrangements of comonomer structural units along the studied diblock and random copolyester chains was determined based on the investigation of their fragmentation product patterns.

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Supporting Information Available: Assignment of the chemical compositions for the sodium adducts appearing in the expanded regions at m/z 880−1120 of positive ESI mass spectra of 3-HB/EMPL blocky and random copolyesters (table). This material is available free of charge via the Internet at http://pubs.acs.org.

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