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Polyester-based Copolymers for Biomaterials Fabrication

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Summary: Results on synthesis of poly(3-hydroxybutyrate)s possessing one or two hydroxyl groups at one terminus of the chain and carboxylic group at the other chain end are reported. These polymers were further functionalised via transesterification with dimethyl H-phosphonate thus incorporating a reactive/biodegradable center in the polyester backbone. Block/star-like copolymers composed of hydrophilic PEG and hydrophobic poly(3-hydroxybutyrate) segments linked by phosphoester moiety were also obtained. Chemical structure and composition of the reaction products were analysed applying different spectroscopic techniques (^{1}H, and ^{31}P NMR, IR and ESI-MS) and size exclusion chromatography was applied to describe molecular weight averages and distribution.

Keywords: biodegradable; functionalization of polymers; phosphoesters; poly(3-hydroxybutyrate)

Introduction

Polyester derived biomaterials, i.e. PLA, PLGA, polyhydroxyalkanoates (PHA), have been extensively studied in tissue engineering or drug transport/release devices.[1–5] The advantages of these polymers are they tend to be biocompatible and can display varied degradation profiles as a function of (1) molecular weight characteristics, (2) crystallinity degree, and (3) comonomer and/or blend additive composition.

Polyhydroxyalkanoates (PHA) are a class of aliphatic thermloplastic polyesters that are biosynthesized by many bacteria and poly(3-hydroxybutyrate) (PHB) is the first identified and widely used member of this family.[6] Microbial PHB is highly isotactic, which leads to its degree of crystallinity in the range of 60–80%. The high crystallinity endows PHB with brittle properties and relatively poor hydrophilicity. In recent years, many efforts have been made to modify PHB, including physical and chemical methods.[7,8] An alternative approach to obtain amorphous material is the anionic polymerization of [R,S]-β-butyrolactone (β-BL) yielding atactic polymer.[9,10]

Another polymers possessing degradable linkages in the mainchain are the polyphosphoesters. They have been investigated as biomaterials for almost two decades.[11] The pentavalency of the phosphorus atom in the backbone of polyphosphoesters makes it possible to conjugate functional groups, including bioactive molecules, or segments in order to modulate the hydrophilic/hydrophobic balance. Convenient methods for conjugation of hydroxy- or amino- compounds have been reported based on the reactivity of H-phosphonate derivatives.[12–15]

The synthetic efforts were directed towards preparation of PHB-based copolymers targeted for tissue engineering

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purposes. H-Phosphonate group was incorporated in the polyester chain via transesterification of hydroxyl end groups of PHB with dimethyl H-phosphonate. Results on synthesis of block/star-like copolymers composed of hydrophilic PEG and hydrophobic PHB segments linked via phosphoester groups are also reported. The reaction composition and the chemical structure of the polymeric products were determined by NMR and IR spectroscopies, ESI-MS and SEC were applied to describe molecular weight averages and distribution.

Synthesis and Structure of Hydroxy-Functionalized PHBs

Ring-opening anionic polymerization of β-BL initiated with (R,S)-3-hydroxybutyric acid sodium salt in DMSO was applied in the synthesis of monohydroxy-terminated poly(3-hydroxybutyrate) (HO-PHB).[5,10] Polymerizations were carried out in THF solution when 2,2-bis(hydroxymethyl)butyric acid tetrabutyl ammonium salt was used as initiator for the preparation of PHBs bearing two hydroxymethyl groups at the chain terminus ($(HO)_2$- PHB)). In both cases the polymerization reaction proceeded quantitatively and PHBs having molecular weights close to calculated ones and expected chain configuration were obtained (Table 1). The structure of HO-PHB and $(HO)_2$-PHB and the assignment of the observed signals in the ^{1}H NMR spectra of the two products are given in Figure 1.

Synthesis of PHB-Based Block Copolymers Possessing H-Phosphonate Group in the Main Chain

The synthetic route for preparation PHB-based polymers possessing H-phosphonate groups is presented in Scheme 1. First step involved transesterification reaction between HO-PHB and dimethyl H-phosphonate. The process was carried out at two stages: (i) at gradually increasing temperatures from 90 °C up to 105 °C for 9 h under slow argon flow; (ii) under reduced pressure (2 mmHg) and at elevated temperatures (120 °C–140 °C) for 4 h. The starting reaction mixture contained dimethyl H-phosphonate with an excess to obtain at the first stage exclusively PHB with -OP(O)(H)OCH_3 end groups (product **2**). The ^{1}H NMR spectra presented in Figure 2 display the progress of the transesterification reaction. The two doublets at $\delta = 6.85$ ppm and $\delta = 6.84$ ppm with $^1J\{PH\} = 708$ Hz and $^1J\{PH\} = 706$ Hz indicate the formation of PHB bearing H-phosphonate end group. Additional confirmation is the shift of the signal of the methine H-atom at 4.22 ppm ($CH_3C(\underline{H})OH$) to 4.95 ppm due to the formation of the new phosphoester bond ($CH_3C(\underline{H})OP$-). Similarly, the resonance of the neighboring methyl group ($C\underline{H}_3C(H)OP$-) is shifted downfield by 0.14 ppm.

In the ^{31}P NMR spectrum (Figure 3A) the signals in the region 9.8–8.5 ppm can be assigned to the end phosphonate group

Table 1.
Results of anionic polymerization of [R,S]-β-BL[a] at room temperature.

Sample	Initiator	Solvent	$M_{n,th}$[d]	$M_{n,\ NMR}$	$M_{n,\ GPC}$	M_w/M_n
HO-PHB	HBA-Na[b]	DMSO	1500	1800	1900	1.10
$(HO)_2$-PHB-1	BHBA-TBA[c]	THF	1300	1300	1400	1.14
$(HO)_2$-PHB-2	BHBA-TBA[c]	THF	3000	2900	3100	1.13

[a] BL initial concentration varied between 1.5–2.0 mol/l; conversion in each experiment was equal to 100%;
[b] [R,S]-3-hydroxybutyric acid sodium salt (initial concentration was 0.14 mol/l);
[c] 2,2-bis(hydroxymethyl)butyric acid tetrabutyl ammonium salt (total BHBA concentration was 0.14 mol/l);
[d] Theoretical molecular weight calculated from the formula: $M_{n,th} = [M]_o/I_o \times 86 + M_i$, where $[M]_o$ and $[I]_o$ are the initial concentrations of the monomer and the initiator, respectively; 86 - molecular weight of BL monomer; M_i–molecular weight of the end group: 103 for HBA and 147 for BHBA.

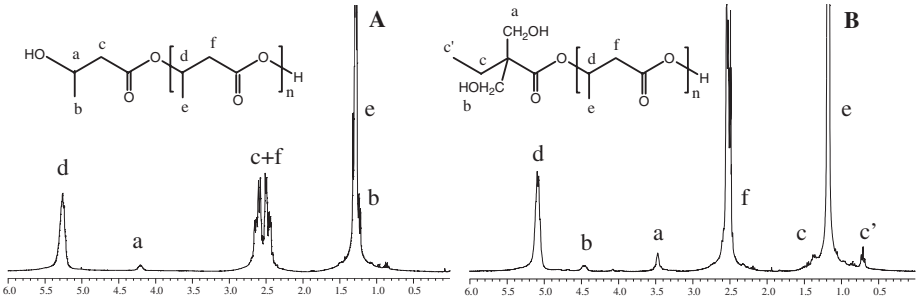
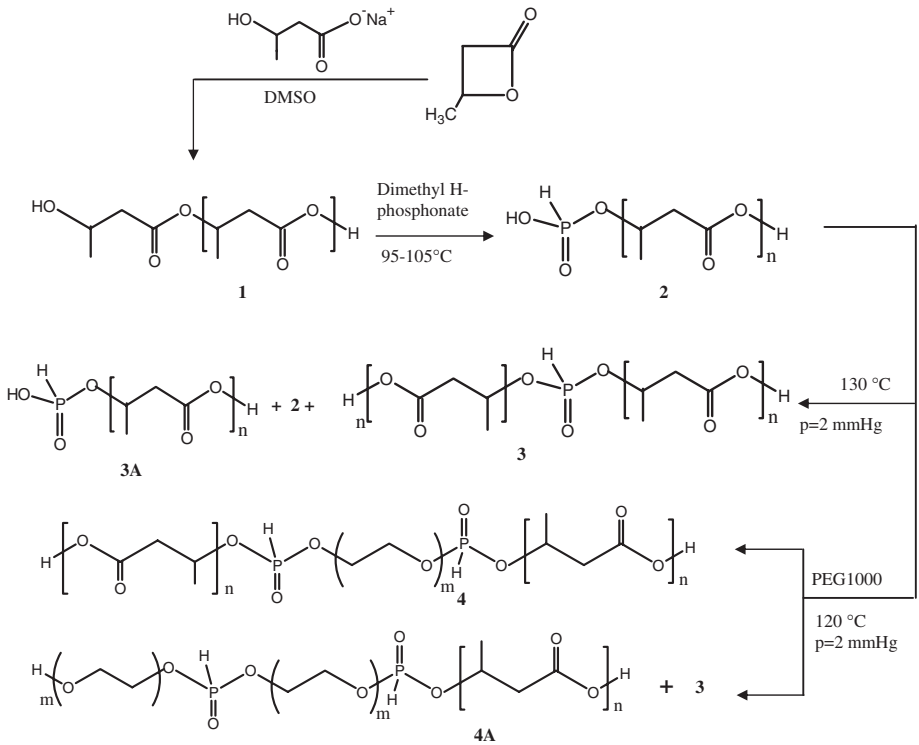


Figure 1.
^{1}H NMR spectra of (A) HO-PHB (in $CDCl_3$) and (B) $(HO)_2$-PHB (in DMSO-d_6).

of the PHB chain. The asymmetry of the phosphorus atom in the phosphoester group and the adjacent carbon atom results in splitting of the phosphorus resonance corresponding to the four possible configurations of the chain end (C(S)-P(S), C(S)-P(R), C(R)-P(R), C(R)-P(S)). The accurate determination of the value of the coupling constants ^{1}J{PH} from the ^{31}P{H} NMR spectrum is difficult because of the broad doublets as it is seen in Figure 3B. The approximate values obtained are in the range of 705–709 Hz that are close to those obtained from the ^{1}H NMR spectrum.

Next step targeted synthesis of higher molecular PHB via linking two PHB blocks through H-phosphonate group – a reactive center that can participate in



Scheme 1.
Synthetic route for preparation of PHB-based polymers possessing H-phosphonate groups in the main chain.

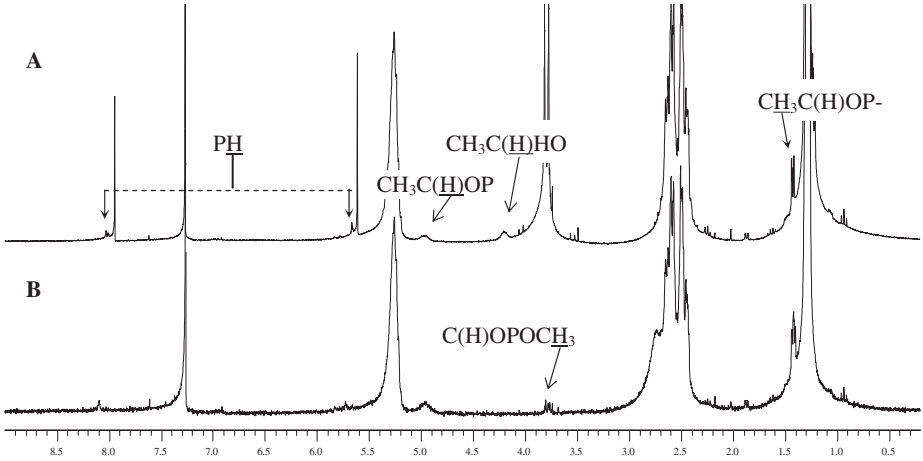


Figure 2.
^{1}H NMR analysis of the transesterification reaction of HO-PHB with dimethyl H-phosphonate: A) after 4 h reaction time at 90–95 °C and B) after 13 h reaction time at 90–140 °C (last 4 h under reduced pressure).

further reactions. The second stage of the transesterification reaction was carried out at elevated temperatures (120–140 °C) and under reduced pressure ($p \approx 2$ mmHg) - necessary conditions for accelerating the transesterification reaction of the second methyl ester group. No significant increase in the content of crotonate species (degradation products) was observed under the experimental conditions applied. The comparison of the two ^{1}H NMR spectra given in Figure 2 reveals the following: (i) all hydroxyl groups were converted into phosphoester groups (the signal at 4.22 ppm disappeared and a new signal at 4.95 ppm is observed); (ii) the unreacted dimethyl

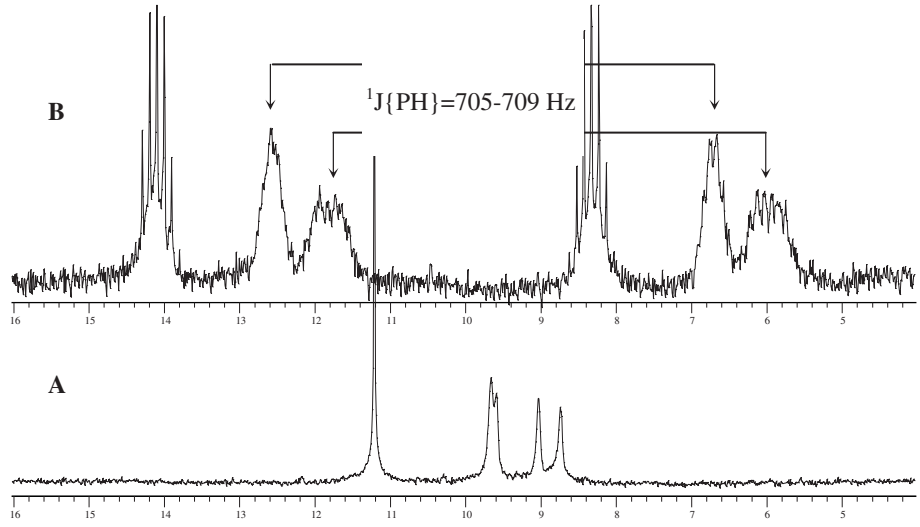


Figure 3.
^{31}P and ^{31}P{H} NMR spectra of the H-phosphonate functionalised PHB obtained at the first stage of the transesterification reaction between HO-PHB and dimethyl H-phosphonate (the spectrum was performed prior the excess of dimethyl H-phosphonate to be removed).

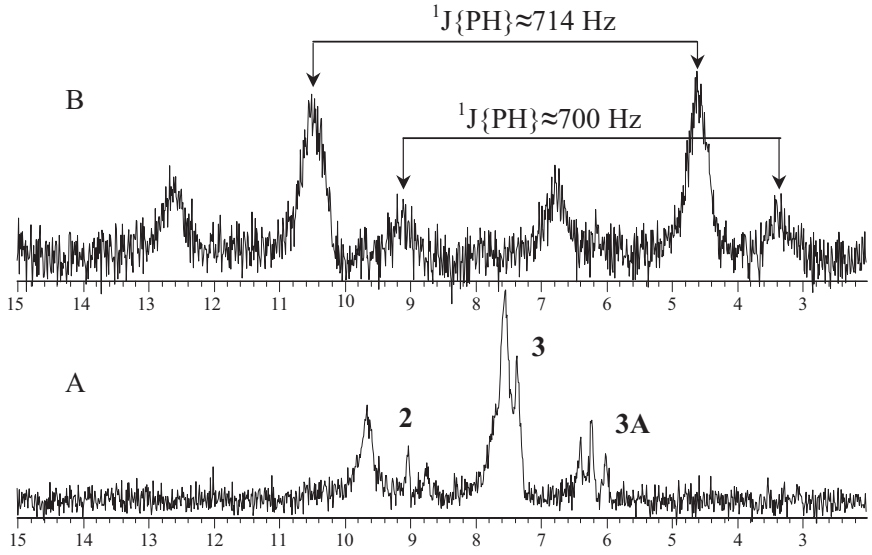


Figure 4.
^{31}P and ^{31}P{H} NMR spectra (A and B, respectively) of the transesterification products at the second stage of the transesterification process.

H-phosphonate was removed from the system – the characteristic doublet at 6.78 ppm with ^{1}J{PH} = 700 Hz is not seen in the spectrum B; (iii) the multiplet (overlapped doublets) between 3.82 ppm and 3.72 ppm is assigned to the methyl ester group in **2**, i.e. the reaction temperature and time were not sufficient for completion of the condensation reaction.

^{31}P and ^{31}P{H} NMR spectra of the reaction mixture indicates tree types of phosphonate structures. The signal (Figure 4A) in the region 8–7 ppm is assigned to the main product **3** of the reaction (yield 60 mol-%) – polyester composed of two PHB segments linked by H-phosphonate group. The corresponding doublet in the ^{31}P{H} NMR spectra (Figure 4B) displays ^{1}J{PH} with approximate value of 714 Hz. The comparison of the NMR spectra in Figure 3 and Figure 4 confirms that a portion (about 24 mol-%) of product **2** (signals in the region 10.0–8.7 ppm, Figure 4A) remained in the reaction mixture. The third group of signals in the region 6.4–6.0 ppm (Figure 4A) can be assigned to monoPHB ester of H-phosphonic acid (see Scheme 1, product **3A**) probably obtained by hydrolysis of polyester **2.** This assumption is supported by the lower value of the corresponding coupling constant ^{1}J{PH} ≈ 700 Hz.

The molecular weight characteristics obtained in the SEC measurements are listed in Table 2. The increase of the molecular weight and polydispersity of the polyester products with the progress of the transesterification process supports the analysis of the reaction composition and polymer structure based on the NMR data.

Product **2** was further reacted with PEG1000. ^{1}H and ^{31}P NMR spectra of the reaction product **4** are presented in Figure 5. The new multiplet in the region 4.33–4.15 ppm is a confirmation for PEG

Table 2.
SEC* data of the products obtained via transesterification of HO-PHB with dimethyl H-phosphonate.

Product	M_n	M_n/M_w
2	2 000	1.13
3	2 600	1.25
4	3 700	1.66

* SEC experiments were performed at 35 °C using a Spectra Physics 8800 instrument, polystyrene standards (PL-Lab) and $CHCl_3$ as an eluent at a flow rate of 1 ml/min.

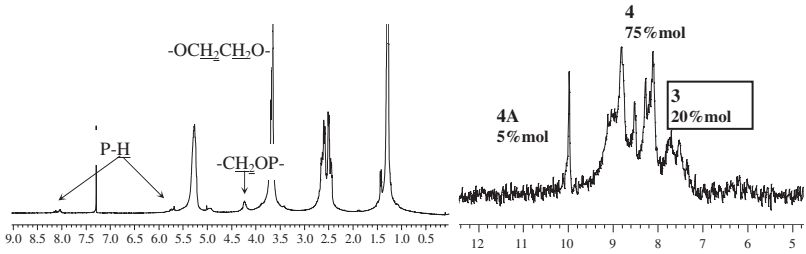


Figure 5.
^{1}H and ^{31}P NMR spectral analysis of the reaction product obtained via transesterification of PHB possessing methyl H-phosphonate end group with PEG1000.

participation in the transesterification reaction and formation of new phosphoester bond –$C\underline{H}_2$OP-. The formation of new H-phosphonate structures is also evidenced by the increased number of doublets in the region 6.95–6.85 ppm with $^{1}J\{PH\}$ values in the range 709–718 Hz.

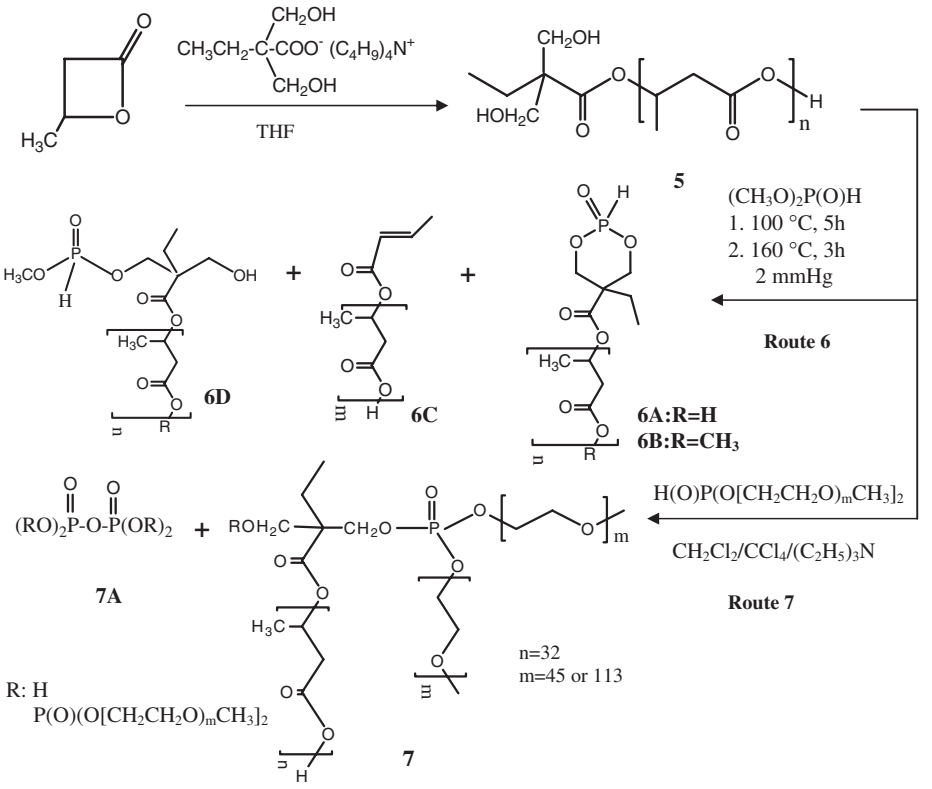
The signals observed in the ^{31}P NMR spectrum (Figure 5) of the reaction product can be attributed to three main structures. The assignment (shown in Figure 5) is made on the basis of published data about poly(oxyalkylene H-phosphonate)s[16] and those obtained in the present study.

Transesterification Reaction of $(HO)_2$-PHB with Dimethyl H-Phosphonate

The transesterification reaction of $(HO)_2$-PHB with dimethyl H-phosphonate yielded as main product PHB possessing six-member cyclic end group–2-hydro-2-oxo-1,3,2-dioxaphosphorinane ring (Scheme 2, route 6). The ^{1}H NMR spectrum (not shown) confirmed complete conversion of the hydroxy end groups in the starting $(HO)_2$-PHB (the multiplet in the region 4.0–3.6 ppm disappeared) and formation of new H-phosphonate structures (doublets at 6.96 ppm and at 6.1 ppm with $^{1}J\{PH\}$ values 681 Hz and 693 Hz, respectively). ^{31}P NMR spectrum displays a broad peak in the region 10.8–9.2 ppm and a second signal between 5.2 ppm and 4.0 ppm with ratio of peak integrals equal to 1:4, respectively. The value of the $^{1}J\{PH\}$ equal to 682 Hz for the doublets at 4.57 ppm was determined from the $^{31}P\{H\}$ NMR spectra. This signal was assigned to the P-atom in a dioxaphosphorinanyl residue at PHB chain terminus.

Both, the $^{1}J\{PH\}$ and chemical shift values are close to those published for 2-hydro-4-methyl-2-oxo-1,3,2-dioxaphosphorinane.[17] The broad signal between 10.8–9.2 ppm can be attributed to the presence of an open H-phosphonate structure at the chain terminus (**6D**). Data obtained from the SEC analysis revealed that no increase of the product molecular weight occurred during the reaction, on the contrary—a certain decrease in the molecular weight of the product in the course of the process was measured (from 1200 to 900 Da). This observation can be explained by acceleration of the polyester chain degradation at the elevated reaction temperature applied in the second stage of the process. A confirmation was the increased integral (almost twice) of the signal at 1.87 ppm (in the ^{1}H NMR spectrum) assigned to the increased content of crotonate groups.

The ESI-MS spectrum (Figure 6B) of the reaction product provides additional information about its composition and structure. Four sets of signals are observed and the most intensive signal is ascribed to molecular ion of the polymer chain with dioxaphosphorinanyl and carboxylic end groups (**6A,B**). Molecular ions of PHB oligomers with crotonate end groups (**6C**) are also detected, as well as chains with phosphonate and carboxylic acid methyl



Scheme 2.

Synthetic scheme of (HO)2-PHB modification: route 6 – transesterifiaction with dimethyl H-phosphonate; route 7: coupling with di(poly(oxyethylene)) H-phosphonates (Atherton-Todd reaction).

ester end groups (**6D**) (Scheme 2). However, their intensities were relatively low.

PHB based copolymers with H-phosphonate group incorporated in the main chain has the advantage of bearing a reactive center in the macromolecule. However, it should be mentioned the susceptibility of H-phosphonates to hydrolysis and the necessity these polymers to be stored or processed under dry conditions. The conversion of the P-H bond into a P-O bond, i.e. conversion of H-phosphonate group

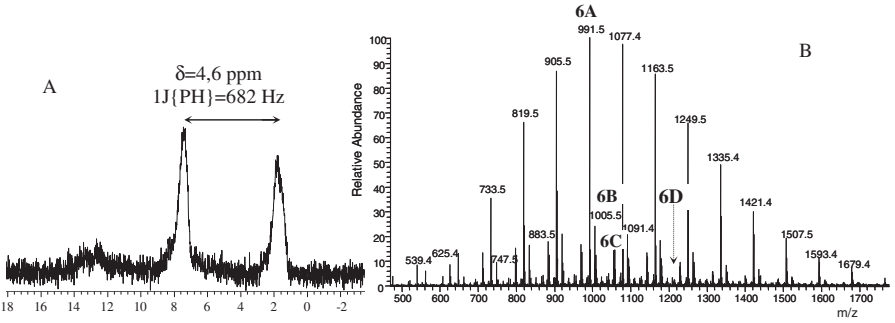


Figure 6.

$^{31}P\{H\}$ NMR (A) and ESI-MS (positive-ion mode) (B) spectra of the transesterification product between $(HO)_2$-PHB and dimethyl H-phosphonate.

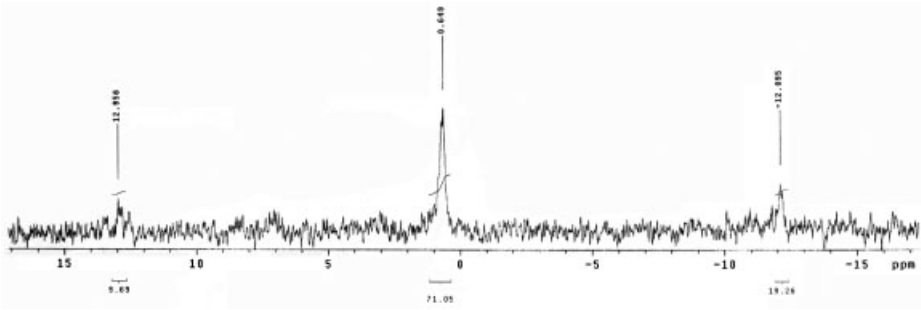


Figure 7.
$^{31}P\{H\}$ NMR spectrum of product 7 (refer to Scheme 2 2n = 32, m = 113).

into phosphate structure increases the hydrolytical stability of the polymer, and also affords possibility copolymers with different architecture to be synthesized.

Synthesis of Star-Like Copolymers of PHB and PEG

Atherton-Todd reaction[12,13] is a convenient method for coupling alcohols or amines to H-phosphonates. This reaction was used for the preparation of star-like copolymers bearing one PHB arm and two (or four) PEG arms. PHBs bearing hydroxyl end groups HO-PHB or $(HO)_2$-PHB) and previously synthesized diPEG esters of the H-phosphonic acid were used as starting compounds. The reaction proceeds at room temperature in the presence of CCl_4 as oxidizing agent and triethyl amine as catalyst. The yield of phosphate structures was about 70 mol-%. In all cases formation of pyrophosphate structures (between 10–20%) was also observed. ^{31}P NMR spectrum of one of the copolymers (**7**) obtained (Figure 7) illustrates the above-said.

Conclusions

PHB based polymers bearing H-phosphonate group in the main chain were synthesized and characterized. Some spectral characteristics of the P-H bond (spectral range of chemical shifts, coupling constants) in such structures were evaluated. Star-like copolymers composed of hydrophilic PEG and hydrophobic PHB segments linked by phosphoester moiety were also obtained. The synthesized polymers are targeted at biomaterial fabrication as degradability and hydrophilicity enhancers affording more friendly and compatible environment to the biological materials as well as delivery vehicles of bioactive agents incorporated and released by the biomaterial (i.e scaffold) thus assisting the successful tissue regeneration.

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