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Synthesis of fully bio-based poly (3-hydroxybutyrate)-oligo-2-ethyl oxazoline conjugates

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This work refers to the synthesis and characterization of poly (3-hydroxybutyrate)-b-oligo (2-ethyl oxazoline) (oligoEtOx). Cationic ring-opening polymerization of 2-ethyl oxazoline yielded poly (2-ethyl oxazoline) (oligoEtOx) with a hydroxyl end. Carboxylic acid-terminated PHB was reacted with oligoEtOx via dicyclohexylcarbodiimide chemistry to obtain PHB-b-oligoEtOx conjugates. The obtained PHB-b-oligoEtOx conjugates were successfully characterized by ^{1}H- and ^{13}C NMR, FTIR, DSC, and size exclusion chromatography. PHB-b-oligoEtOx conjugates can be promising biologic active materials.

KEYWORDS

bacterial polyester, PHB, oligo oxazoline, 2-ethyl oxazoline, cationic polymerization, two carboxylic acid-terminated PHB

Introduction

Poly (3-hydroxybutyrate) (PHB) is a microbial aliphatic biopolyester which is accumulated in bacterium cells from some carbon substrates (Ashby and Foglia, 1998; Kocer et al., 2003; Hazer and Steinbüchel, 2007; Chen, 2009; Ashby et al., 2019; Choi et al., 2020; Guzik et al., 2020; Bedade et al., 2021; Kacanski et al., 2023).

PHB is a crystalline polymer with melting transition (Tm) at approximately 170°C. It can also be synthesized by the anionic ring-opening polymerization of beta-butyrolactone (Hazer, 1996; Arkin et al., 2001).

The synthetic PHB is in R, S configuration, while bacterial PHB is only in R configuration (Caputo et al., 2022).

PHB modification reactions are important to prepare new PHB derivatives for some industrial and medical applications (Hazer, 2010; Hazer et al., 2012; Guennec et al., 2021). Some of them are halide derivatives (Arkin and Hazer, 2002; Yalcin et al., 2006; Erol et al., 2020), chitosan derivatives (Arslan et al., 2007), diethanol amine derivatives (Tuzen et al., 2016), trithiocarbonate derivatives (Hazer et al., 2020), methyl salicylate derivatives (Hazer et al., 2021), ricinoleic acid derivatives (Ullah et al., 2024), PEG derivatives (Hazer et al., 1999; Wadhwa et al., 2014), and caffeic acid derivatives (Abdelmalek et al., 2023).

Poly (2-ethyl-2-oxazoline) (oligoEtOx) is obtained by the cationic polymerization of 2-ethyl oxazoline (2-EtOx). OligoEtOx is a water-soluble polymer and is very popular in the field of biomedical and pharmaceutical applications (Vergaelen et al., 2023). Dual initiator techniques, including the carbocationic method and free radical polymerization, can be used to synthesize block copolymers (Hazer, 1991; Christova et al., 1997). In this manner, poly (2-ethyl-2-oxazoline) derivatives were successfully synthesized by polymer chemists

for medical applications (Miyamoto et al., 1989; Christova et al., 2002; Diab et al., 2004; Park et al., 2004; Hoogenboom et al., 2005; Becer et al., 2008; Li et al., 2021; Göppert et al., 2023).

Very recently, Becer et al. reported the synthesis of poly (2-ethyl oxazoline)-b-poly (acrylate) hybrid multiblock copolymers via a click reaction. They evaluate their self-assembly behavior into stomatocyte-like nanoparticles (Hayes et al., 2023). The multiamide structure of polyEtOx makes it a candidate to mimic peptides, and it shows an antibacterial effect against *Staphylococcus aureus* (Hoogenboom, 2009).

Poly (2-ethyl oxazoline) is a new class of functional peptide that mimics with potential in a variety of biological applications (Zhou et al., 2020). PolyEtOx is a thermosensitive polymer with a lower critical solution temperature (LCST), changing the aqueous solution temperature at approximately 62°C (Christova et al., 2003; Park and Kataoka, 2007; Obeid et al., 2009; Hoogenboom and Schlaad, 2011).

Winnik et al. reported the cloud point of aqueous methyl poly(I-propyl oxazoline) with Mn 10 K g/mol. Turbidity decreases with the increasing concentration from ~48°C to ~39°C.

Block copolymers containing hydrophilic and hydrophobic blocks gain the properties of both related blocks. These different polymer blocks can be arranged linearly or as brush-type copolymers (Minoda et al., 1990; Xu et al., 1991; Förster and Antonietti, 1998; Bronstein et al., 1999; Chen et al., 1999; Hu et al., 2008; Mai and Eisenberg, 2012; Kalayci et al., 2013; Glaive et al., 2024; Hosseini et al., 2024; Wang et al., 2024).

The insertion of the hydrophilic polymer in a block copolymer will improve the colloidal stability of the nanoparticles for biomedical applications (Balcı et al., 2010; Kalaycı et al., 2010; Karahaliloğlu et al., 2020; Wen et al., 2023; Kilicay et al., 2024).

PHB is a commercially available biodegradable natural aliphatic polyester for some biomedical applications, such as implant biomaterials, tissue engineering, and food packaging applications (Chen and Zhang, 2018; Mehrpouya et al., 2021; Abdelmalek et al., 2023). PHB derivatives can be used as novel biodegradable adsorbents for analytical applications (Wadhwa et al., 2014; Unsal et al., 2015; Tuzen et al., 2016; Altunay et al., 2020; Ullah et al., 2024; Ali et al., 2024) for drug delivery systems (Bayram et al., 2008; Kılıcay et al., 2011; Kilicay et al., 2024).

In this work, we report the synthesis of poly (3-hydroxybutyrate)-oligo-2-ethyl oxazoline, fully bio-based amphiphilic polymer conjugates. Two carboxyl-terminated PHB were synthesized by refluxing PHB with adipic acid in the presence of Stannous octoate. Then, the carboxyl-terminated PHB was reacted with the hydroxyl end of oligooxazoline, which was obtained by the ring-opening cationic polymerization of 2-ethyl oxazoline. The physicochemical characterization of the PHB-oligo-2-ethyl oxazoline conjugates was carried out in detail.

Experiment

Materials

2-Ethyl oxazoline (2-EtOx) was supplied from Sigma-Aldrich and was passed into the Al_2O_3 column before use. N, N′-Dicyclohexylcarbodiimide (DCC; 99%), dimethylaminopyridine (DMAP; 99%), stannous 2-ethylhexanoate (Sn-oct; ≥92.5%), methyl p-toluene sulfonate (MepTs), and all other chemicals were purchased from Sigma-Aldrich. Poly (3-hydroxybutyrate) (PHB) and microbial polyester (Mn 187,000 g/mol, Mw/Mn 2.5, Biomer Inc.) were supplied from Biomer (Germany) (Neugebauer et al., 2007).

Synthesis of oligo(2-ethyl oxazoline) (oligoEtOx)

2-Ethyl oxazoline was oligomerized by ring-opening cationic polymerization. A mixture of 2-ethyl oxazoline (2.01 g) and MepTs (0.20 g) as the catalyst was dissolved in acetonitrile (AcCN, 2.0 mL) in a reaction bottle. Argon was passed through the solution for 2 min. Polymerization was carried out at 100°C for 70 min. The polymer precipitated in excess diethyl ether. It was dried under vacuum at 40°C for 24 h (yield: 1.98 g, Mn 900 g/mol, and PDI: 1.57).

Synthesis of dicarboxylic acid-terminated PHB, PHB-COOH

A mixture of adipic acid (1.00 g), PHB (0.64 g), and Sn-oct (20 mg) was dissolved in $CHCl_3$ (20 mL). It was refluxed at 85°C for 4 h. After half of the solvent was evaporated, the product was precipitated from excess methanol and dried under vacuum at 40°C for 24 h. The yield was 0.86 g.

Characterization

^{1}H NMR spectra were taken with an Agilent NMR 600 MHz NMR (Agilent, Santa Clara, CA, United States) spectrometer equipped with a 3-mm broadband probe. FT-IR spectra of the substituted polymer samples were recorded using a Bruker Model, Tensor II instrument with the ATR technique in the transmissive mode and a scan rate of 4,000 to 450 cm^{-1}. A Viscotek GPCmax autosampler system, consisting of a pump, three ViscoGEL GPC columns (G2000H HR, G3000H HR, and G4000H HR), and a Viscotek differential refractive index (RI) detector, was used to determine the molecular weights of the polymer products. A calibration curve was generated with five polystyrene (PS) standards of molecular weight 2,960, 8,450, 50,400, 200,000, and 696,500 Da with low polydispersity. Data were analyzed using Viscotek OmniSEC Omni 01 software. Differential scanning calorimetry (DSC) was used in the thermal analysis of the obtained polymers. The DSC analysis was carried out under nitrogen using a TA Q2000 DSC instrument that was calibrated using indium (Tm = 156.6°C) and a Q600 Simultaneous DSC-TGA (SDT) series thermal analysis system. DSC measures the temperatures and heat flows associated with thermal transitions in the polymer samples obtained. The dried polymer samples were heated from −60°C to 220°C under a nitrogen atmosphere. All melting endotherms (Tm) were reported as peak temperatures, while all glass transition temperatures (Tg) were reported as midpoint temperatures. Thermogravimetric analysis (TGA) was

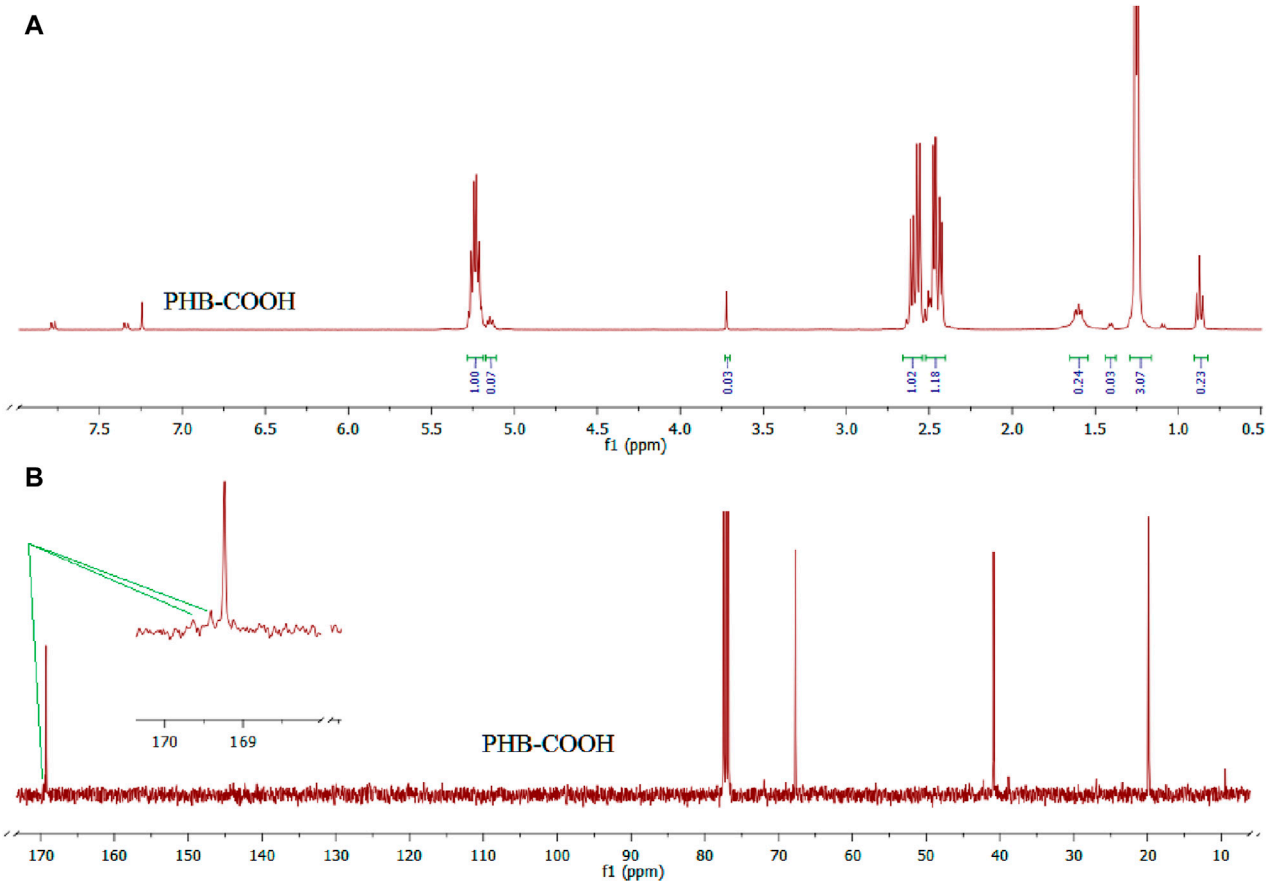

FIGURE 1
1H **(A)** and 13C **(B)** NMR spectra of the PHB-COOH sample.

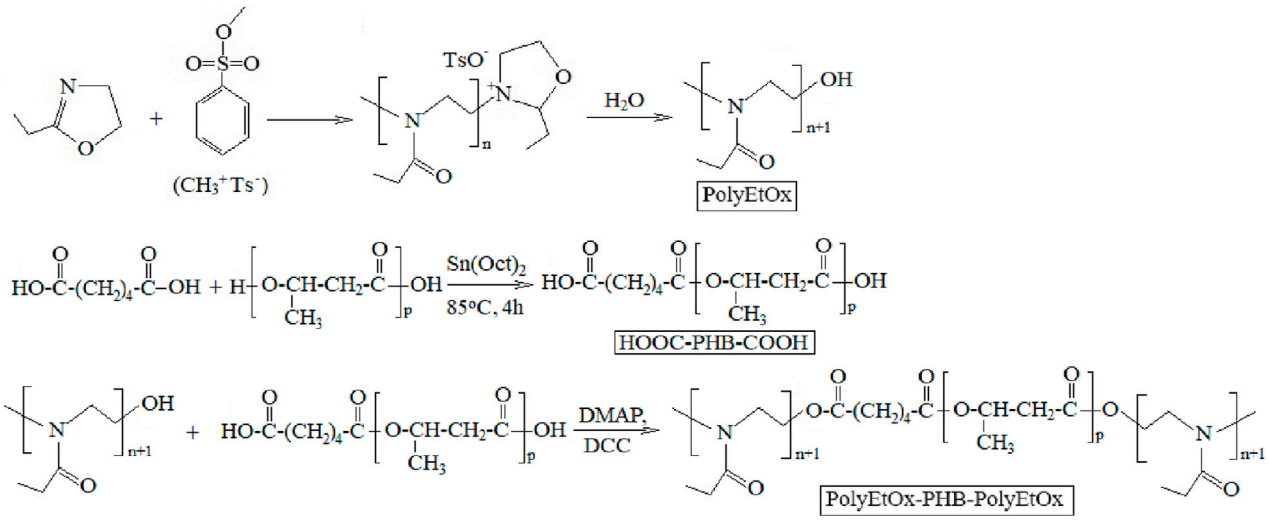

FIGURE 2
Reaction pathways of the synthesis of the oligoEtOx-b-PHB block copolymer.

used to determine the decomposition temperature (T_d) characteristics of the polymers by measuring the weight loss under a nitrogen atmosphere over time. In these analyses, the obtained polymers were heated from 20°C to 600°C at a rate of 10°C/min, and the results were determined based on the first derivative of each curve. Scanning electron microscopy (SEM)

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TABLE 1 Synthesis conditions and results of the PHB-b-oligoEtOx block copolymer at room temperature for 24 h.

Code	PHB(COOH)$_2$ (g)	PolyOx (g) (%)	DMAP (g)	DCC (g)	Yield (g) (%)
PHB-Ox-21	1.08	0.18 14	0.044	0.82	1.04 83
PHB-Ox-23	2.02	0.67 25	0.242	5.03	2.24 83
PHB-Ox-22	1.08	0.56 34	0.092	2.45	1.19 73
PHB-Ox-24	2.02	1.41 41	0.131	3.12	2.34 68

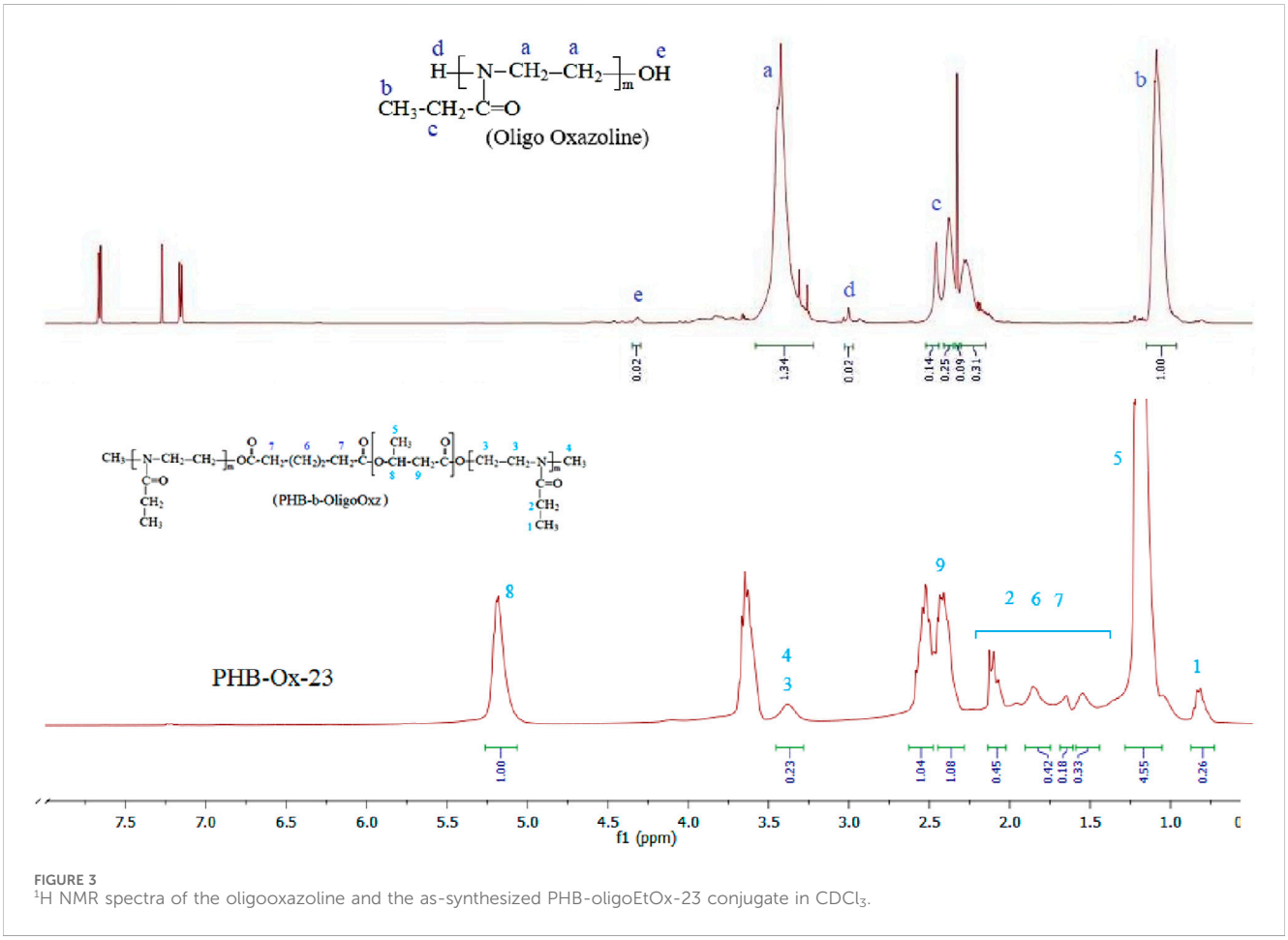

FIGURE 3
^{1}H NMR spectra of the oligooxazoline and the as-synthesized PHB-oligoEtOx-23 conjugate in $CDCl_3$.

imaging (Zeiss EVO lS10) was used for the characterization of the obtained polymers.

Results and discussion

Ring-opening cationic polymerization of 2-ethyl oxazoline, in the presence of MepTs, yielded oligo(2-ethyl oxazoline) (OligoOx). Oxazoline oligomers were obtained in several types, with molar masses changing from 700 to 900 g/mol. Characterization of OligoEtOx confirmed the polymer structure. The FTIR spectrum contained the characteristic signals at 3,462 cm^{-1}, 2,977–2,939 cm^{-1}, 1,624 cm^{-1}, and 1,187 cm^{-1} related to –OH, -C-H, amid carbonyl, and –N-CH_2- groups, respectively. Typical characteristic groups were also observed in the ^{1}H NMR spectrum at chemical shifts at 3.5 ppm (-N-C**H**$_2$-), 2.2–2.5 ppm (-C**H**$_2$-C(O)-), and 1.1 ppm (C**H**$_3$-CH_2-).

PHB with two carboxylic acid terminals was obtained by the reaction of an equimolar amount of adipic acid and PHB under reflux conditions at 85°C. The characteristic signals were observed in the 1H NMR and 13C NMR spectra of the as-synthesized PHB-COOH sample, which is seen in Figure 1, including 1H (a) and 13C (b) NMR spectra. 1H NMR, $\delta_{(ppm)}$: 1.3 ppm for –CH_3, 1.5 ppm for –CH_2-CH_2-, 2.4–2.6 ppm for –CH_2-COO-, 3.7 ppm for CH_2-OC(O)CH_2–, and 5.1–5.3 ppm for–CHO–. 13C NMR, $\delta_{(ppm)}$: 10 (–CH_2-CH_2-), 20 (CH_3-), 40 (-CH_2-C(O)-, 67 (-CH-O-), 169.1, and 169.2 carbonyls for PHB and adipic acid moieties, respectively.

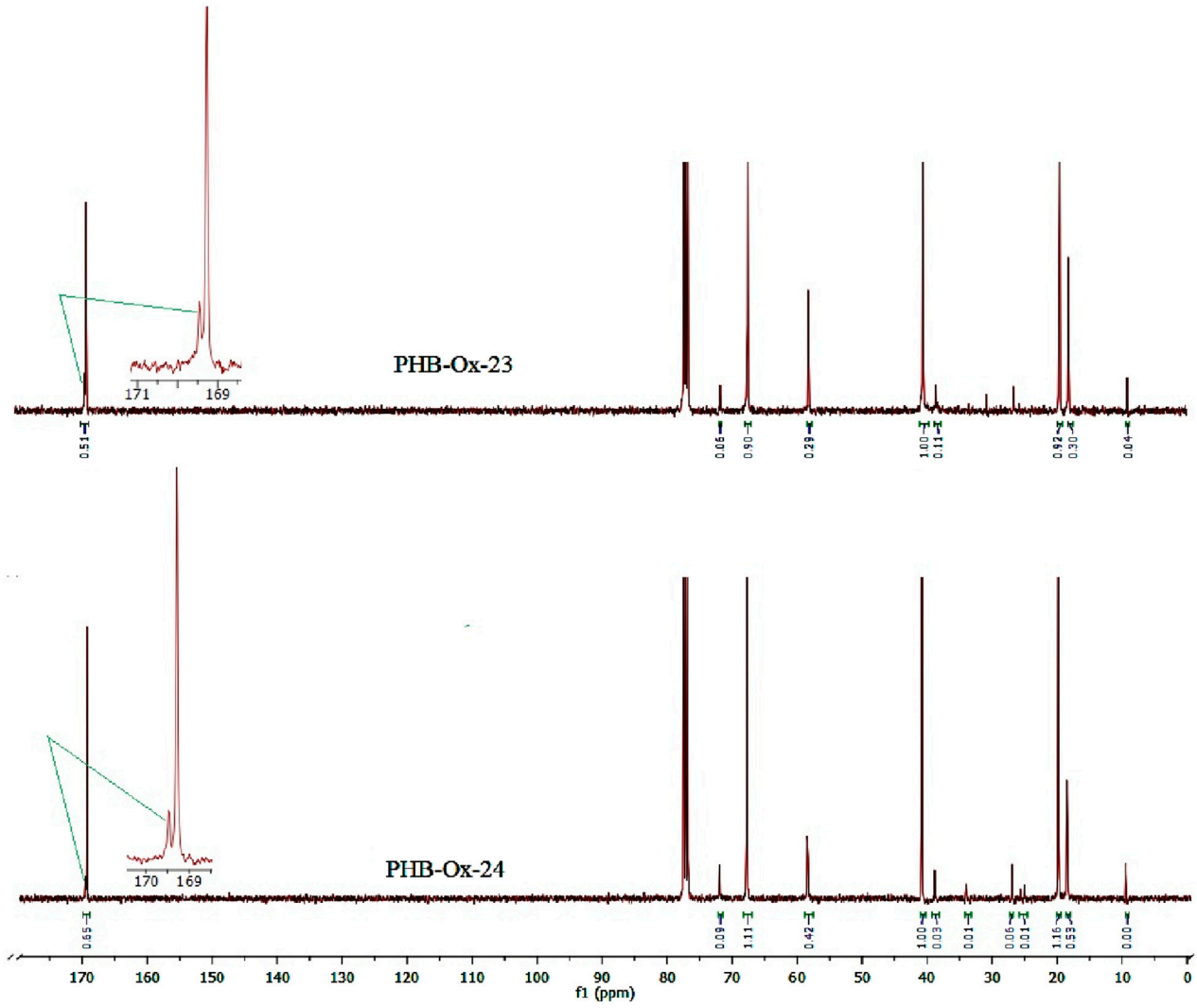

FIGURE 4
13C NMR spectra of the as-synthesized PHB-oligEtOx-23, -24 block copolymers in $CDCl_3$.

Synthesis of PHB-oligoEtOx polymer conjugates

OligoEtOx was capped with the carboxylic acid ends of PHB-COOH to produce the novel PHB-b-oligoEtOx block copolymer. The reaction pathways can be seen in Figure 2.

The reaction conditions and results are listed in Table 1. Changing the feed percentage of oligoEtOx from 14% to 41% against $PHB(COOH)_2$ was reacted at room temperature. The yield of the obtained block copolymer was gravimetrically determined. The polymer obtained was precipitated from the acidified diethyl ether and dried in vacuum. For further purification, it was soaked in distilled water for 24 h in order to remove the unreacted oligoEtOx residue.

Characterization of PHB-oligoEtOx conjugates was carried out by ^1H and ^{13}C NMR, FTIR, differential scanning calorimeter (DSC), and thermo-gravimetric analysis (TGA) techniques. PHB-Ox-21, −22, −23, and −24 samples contained the characteristic samples of oligoEtOx blocks at 3.4 and 3.6 ppm related to the $-C\mathbf{H}_2$-N- groups. Chemical shifts at 1.2 ppm ($C\mathbf{H}_2$-CH_2-) and 2.5 ppm (-$C\mathbf{H}_2$-C(O)-) were overlapped with those of PHB blocks. The amount of oligoOx blocks in the obtained PHB-oligoEtOx −21, −22, −23, and −24 conjugates was calculated while comparing them with integral values of the signals at 5.2 ppm (PHB) and 3.5 ppm (oligoOx) 19, 52, 17, and 14%, respectively. Figure 3 shows the ^1H NMR spectra of the comparison of oligoOx with the as-synthesized PHB-oligoOx-23 conjugate in $CDCl_3$.

^{13}C NMR spectra of the PHB-oligoEtOx-23, -24 block copolymers contained the characteristic signals of the PHB and oligoOx blocks. Chemical shifts: 19, 20 ppm (-$\mathbf{C}H_3$, PHB, oligoEtOx), 39, 40 ppm (-$\mathbf{C}H_2$-C(O)-, PHB, oligoEtOx), 58 ppm (-N-$\mathbf{C}H_2$-, oligoEtOx), 67 ppm (-$\mathbf{C}$H-O-, PHB), and 169.1 and 169.2 ppm (-$\mathbf{C}$=O, PHB and oligoOx). Figure 4 shows the ^{13}C NMR spectra of the as-synthesized PHB-oligoEtOx-23 and −24 conjugates in $CDCl_3$.

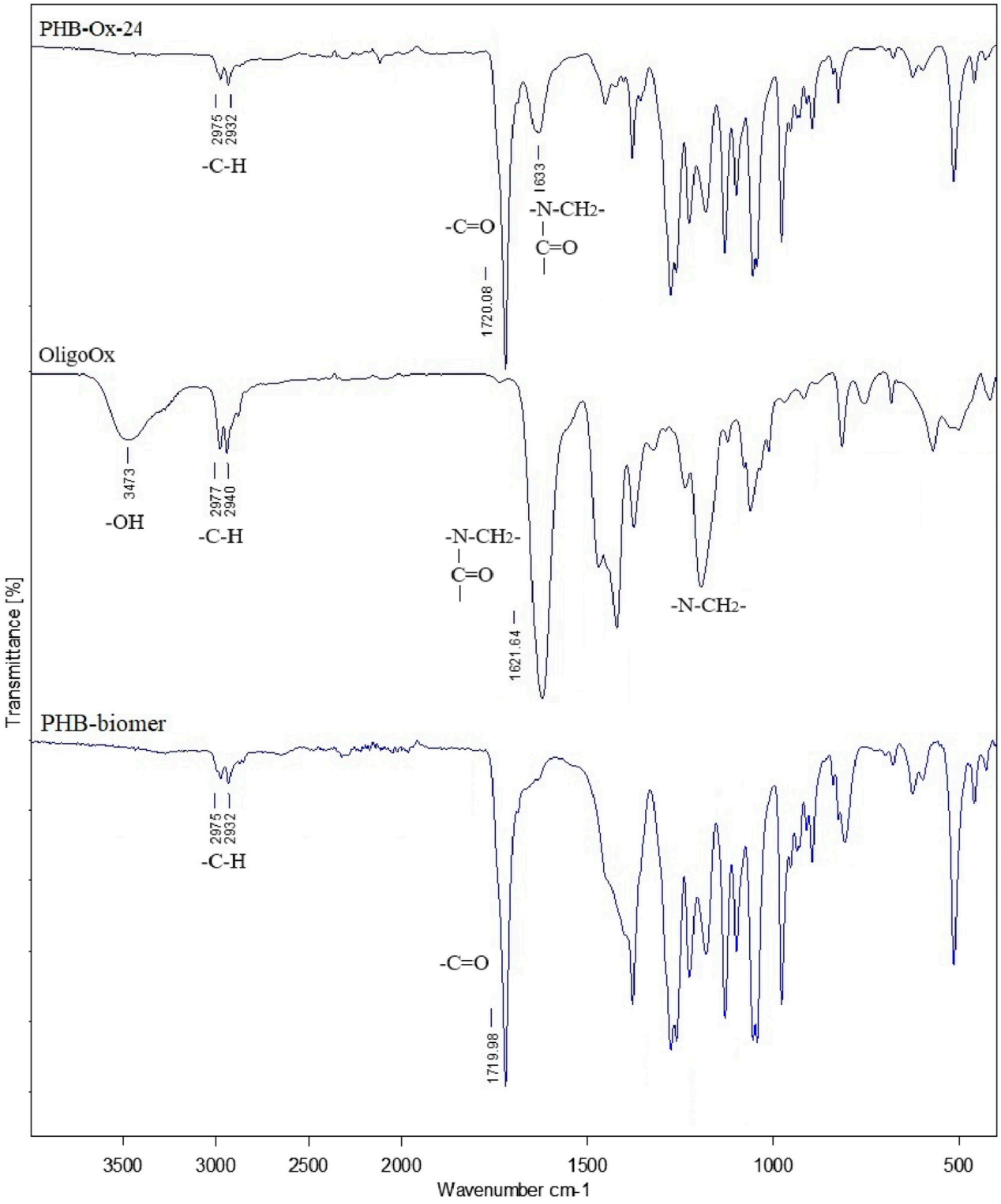

FIGURE 5
FTIR spectrum of PHB-oligEtOx-24 compared with the pristine oligEtOx.

Typical FTIR spectra of PHB-oligoOx-24, oligoOx, and pristine PHB compared with each other are shown in Figure 5. The typical characteristic signal of the oligoEtOx block was observed at 1,633 cm^{-1} related to the –N-C(O) group. The signals of the characteristic groups were marked on the related spectra.

Thermal properties of the block copolymers were measured using a differential scanning calorimeter (DSC). The oligoEtOx sample has a wide glass transition (Tm) between 10 and 76°C and the maximum at 64°C. In the PHB-oligoEtOx polymer conjugate, the same wide melting transition between 6 and

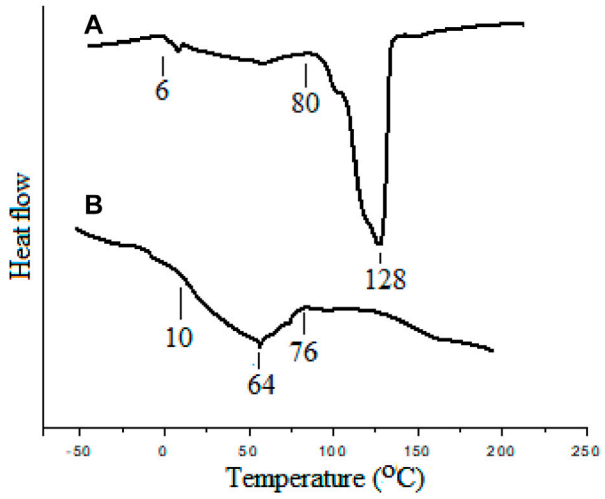

FIGURE 6
DSC curves of **(A)** PHB-oligoOx-21 and **(B)** oligoOx.

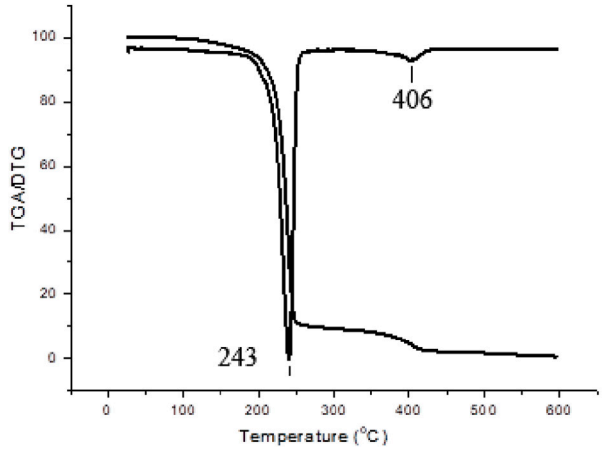

FIGURE 7
TGA/DTA curves of the PHB-oligoEtOx-22 conjugate.

80°C together with that of PHB at 128°C was observed. The PHB homopolymer has a melting transition at 170°C. The lower melting transition of the PHB block in the copolymer shows the plasticizing effect of oligoEtOx. Figure 6 shows the DSC curves of PHB-oligEtOx-21 and homo oligoOx. Homo oligoEtOx showed the glass transition temperature (Tg) at 10°C.

TGA analysis was done in the PHB-oligoEtOx conjugates. The all TGA/DTG curves contained two decomposition temperatures (Td)s: 243 and 406°C (for PHB-oligoOx-22), 249 and 381°C (for PHB-oligoOx-23), and 247 and 381°C (for PHB-oligoEtOx-24). The TGA/DTA curves of the PHB-oligoEtOx-22 conjugate are given in Figure 7. Decomposition of the PHB blocks changes between 243 and 249°C, while that of the oligo oxazoline blocks changes between 386 and 406°C (Bouten et al., 2015).

Conclusion

A fully biodegradable amphiphilic copolymer was obtained in this work. The hydroxyl end of oligoEtOx can easily be reacted with some other reagents to obtain polyoxazoline derivatives. Water-soluble hydrophilic oligoEtOx makes the hydrophobic polymers amphiphilic, which can be useful for medical applications. Combining natural and biodegradable hydrophobic properties of PHB with hydrophilic oligoEtOx yields a novel amphiphilic natural biopolymer.

Block copolymers containing hydrophilic and hydrophobic blocks gain the unique properties of both the related blocks. These different polymer blocks can be arranged linearly or as brush-type copolymers. The insertion of the hydrophilic polymer in a block copolymer can improve the colloidal stability of the biologic active nanoparticles for biomedical applications. Therefore, the PHB-b-oligoEtOx block copolymer can be a promising biopolymer for medical applications.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material; further inquiries can be directed to the corresponding author.

Author contributions

BH: Conceptualization, Methodology, Project administration, Writing–original draft, and Writing–review and editing. ÖA: Conceptualization, Formal analysis, Writing—review and editing. FK: Formal analysis, Methodology, and Writing–original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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