Notes

Novel Synthesis of Poly(3-hydroxybutyrate)

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1. Introduction

In the past decades polymer chemists have been actively involved in designing polymers for medical applications.[1−3] The natural polymer poly(3-hydroxybutyrate) produced by many microorganisms in bacteria cells contains small amounts of impurities such as proteins (ca. 2%) and lipids (ca. 0.5%).[4] These impurities cannot be removed easily from this bacterial polymer; therefore, we were looking for a method of preparation of pure poly(3-hydroxybutyrate).

It is possible to obtain synthetic poly(3-hydroxybutyrate) employing various methods and using various catalytic systems. Tin(II) 2-ethylhexanoate ($Sn(Oct)_2$) was proposed as one of the most frequently used catalysts for the polymerization of lactones and lactides. However, the mechanism of lactones polymerization with $Sn(Oct)_2$ has been a subject of controversy.[5,6]

On the other hand, Seebach and his group[7] developed a very elegant multistep condensation strategy starting from monomeric (*R*)-3-hydroxybutanoic acid using protection and deprotection of active groups at each condensation step. His method yielded well-defined linear low-molecular-weight polymers and cyclic (*R*)-3-hydroxyalkanoate oligomers, but it was very laborious and time-consuming.

In contrast to unsubstituted four-membered β-propiolactone, β-butyrolactone is not polymerized by common anionic initiators such as metal alkoxides and alkali metal carboxylate salts.[8] However, these initiators, when activated by the addition of a macrocyclic ligands such as crown ethers, are able to initiate polymerization of β-butyrolactone with the inversion of configuration from (*S*)-monomer to yield poly((*R*)-3-hydroxybutyrate) (PHB).[9]

We have previously shown[10] that the sodium salt of (*R*)-3-hydroxybutyric acid activated by a crown ether produces poly((*R*)-3-hydroxybutyrate) bearing only hydroxyl and carboxyl terminal groups from (*S*)-β-butyrolactone. The hydroxybutyrate anion of the initiator attacks the chiral carbon atom of the monomer (alkyl−oxygen bond scission) with inversion of configuration at the chiral atom. The polymer chain growth proceeds entirely via carboxylate anions. Polymers of desired molecular weights of up to 2×10^4 are synthesized. Moreover, the molecular weight distribution (PI) is relatively narrow ($M_w/M_n \sim 1.10$). Since the presence of the supramolecular complex crown ether used in this catalyst system influences the biological activity and induces toxicity of the produced polymers, we were looking for a pure catalytic system without crown ether or cryptands. Therefore, the novel method of oligo(3-hydroxybutyrate) synthesis presented in this paper is based on anionic ring-opening polymerization of β-butyrolactone in a highly polar aprotic solvent such as dimethyl sulfoxide (DMSO).

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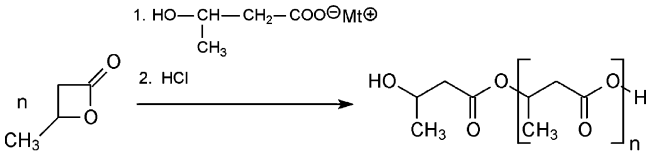
Scheme 1

Scheme 2



where: $Mt^{\oplus} = Na^{\oplus}$/ solvent = DMSO

Table 1. Results of the Anionic Polymerization of β-Butyrolactone[a] at Room Temperature

sample	$M_{n,th}$[b]	$M_{n,GPC}$	M_w/M_n[c]	time (h)
1	362	400	1.20	36
2	620	600	1.15	48
3	706	700	1.12	56
4	791	800	1.13	58
5	878	900	1.10	60
6	2082	2200	1.14	90
7	4000	4100	1.12	124

[a] Butyrolactone initial concentration was changed in the range from 1.0 to 2.0 mol/dm^3; conversion in each experiment was equal to 100%; initiator HBANa-(*R*,*S*)-3-hydroxybutyric acid sodium salt (initial concentration was changed in the range from 3.3×10^{-1} to 4.4×10^{-2} mol/dm^3). [b] $M_{n,th}$ is the theoretical molecular weight calculated from the formula $M_{n,th} = [M]_0/I_0 \times 86 + 104$, where $[M]_0$ and $[I]_0$ are initial concentrations of monomer and initiator, respectively; 86 molecular weight of butyrolactone monomer, 104 molecular weight of end group (3-hydroxybutyric acid). [c] Determined by gel permeation chromatography according to polystyrene standards with a low polydispersity.

2. Experimental Section

2.1. Polymerization of β-Butyrolactone. β-Butyrolactone (4-methyl-2-oxetanone) (Aldrich) was purified as described previously.[11] (*R*,*S*)-3-Hydroxybutyric acid sodium salt (Aldrich) was used as received. Dimethyl sulfoxide (DMSO, 99.8%, Aldrich) was dried over molecular sieves and used without additional purification. β-Butyrolactone was polymerized in DMSO solution under stirring in a previously flamed and argon-purged glass reactor, as described previously.[12] All polymerization experiments were performed at room temperature. The polymerization progress was measured by Fourier transform infrared spectroscopy (FT-IR) basing on the intensity of carbonyl group of monomer β-butyrolactone at 1820 cm^{-1}. When polymerization was completed, the solvent was stripped off and the residue was redissolved in $CHCl_3$. Next, the ethyl ether solution of HCl was added into the reactor, and the reaction mixture was washed six times with distilled water. Then the polymer was precipitated in hexane, dried under vacuum for

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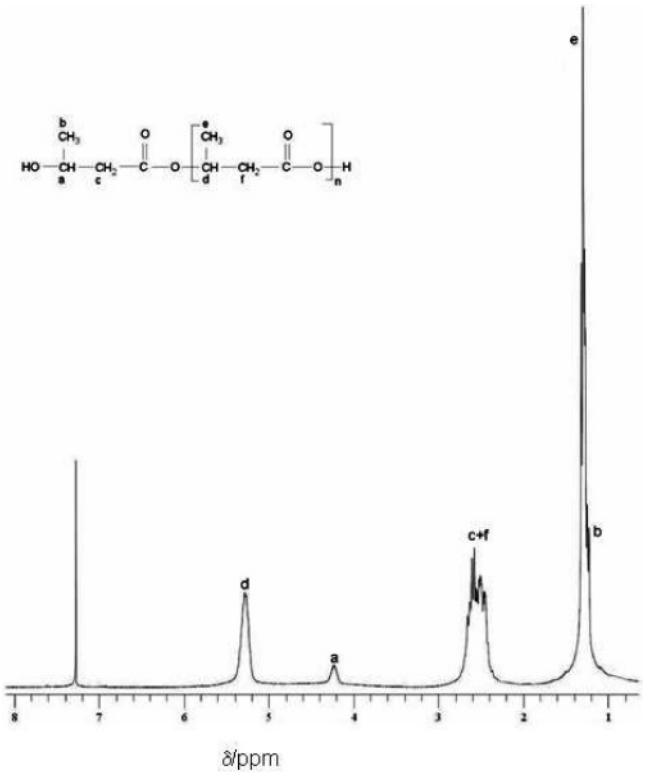

Figure 1. ^{1}H NMR spectrum of poly(3-hydroxybutyrate) ($M_{n,GPC}$ = 700).

48 h, and analyzed by ^{1}H NMR and ^{13}C NMR spectroscopy, ESI-MS spectrometry, and the GPC technique.

2.2. Chemical Analyses. The NMR spectra were recorded using a Varian VXR-300 multinuclear spectrometer. ^{1}H NMR and ^{13}C NMR spectra were run in $CDCl_3$ by using TMS as an internal standard. Gel permeation chromatography (GPC) was performed at 30 °C, using a Spectra Physics 8800 gel permeation chromatograph with two PL-gel packed columns (10^3 and 500 Å), THF was used as the eluent at a flow rate of 1 mL/min, and polystyrene standards with low polydispersity (PL-Lab.) were used to generate a calibration curve. Electrospray ionization mass spectrometry (ESI-MS) experiments were carried out using the Finningan LCQ ion trap mass spectrometer (Finningan, San Jose, CA). The samples were dissolved in methanol or in $CHCl_3$ at a concentration of 0.5 mg/mL, and such solutions were introduced into the ESI source by continuous infusion by means of instrument syringe pump at a flow rate of 3 μL/min. The ESI source was operated at 4.5 kV, with the capillary heater at 200 °C and sheath gas pressure 40 psi. Mass spectra in both positive and negative mode were acquired over the range of m/z = 50−2000. Infrared spectra were acquired on a BIO-RAD FTS-40A Fourier transform infrared spectrometer in the range 2000−1000 cm^{-1}.

3. Results and Discussion

The anionic polymerization depends strongly on interactions of anionic active species with the surrounding molecules and cation−anion association. Cation−anion associations and solvent−anion interactions are important factors determining anion reactivity. The influence of various solvents on anion reactivity has been studied in many nucleophilic reactions.[13] In our investigations, the novel method of ring-opening polymerization of β-butyrolactone is based on the activation of anions of polymerization species by highly polar aprotic solvents, which are responsible for this particular polymerization. The polymerization has been performed in the solution of active highly polar aprotic solvent such as DMSO. In such systems there exist equilibria of reactive ions as shown in the Scheme 1:

Dimethyl sulfoxide (DMSO) is a highly polar aprotic solvent with the dielectric constant ϵ_r = 46.70 and dipole moment μ = 3.90 D.[14] High-polarity and high donicity number (DN) DMSO (equal 29.8) activates the carboxylates species by separation of ion pairs. Solvent-separated ions pairs are very reactive in this system due to the activation of anions by the reduction of their interaction with cations.[10] The reactivity of the solvent separated ion pairs formed after the addition of a highly polar reactive solvent; e.g., DMSO increases, and the polymerization reaction proceeds fast. DMSO is a versatile and powerful solvent that dissolves a wide range of organic compounds and polymers.

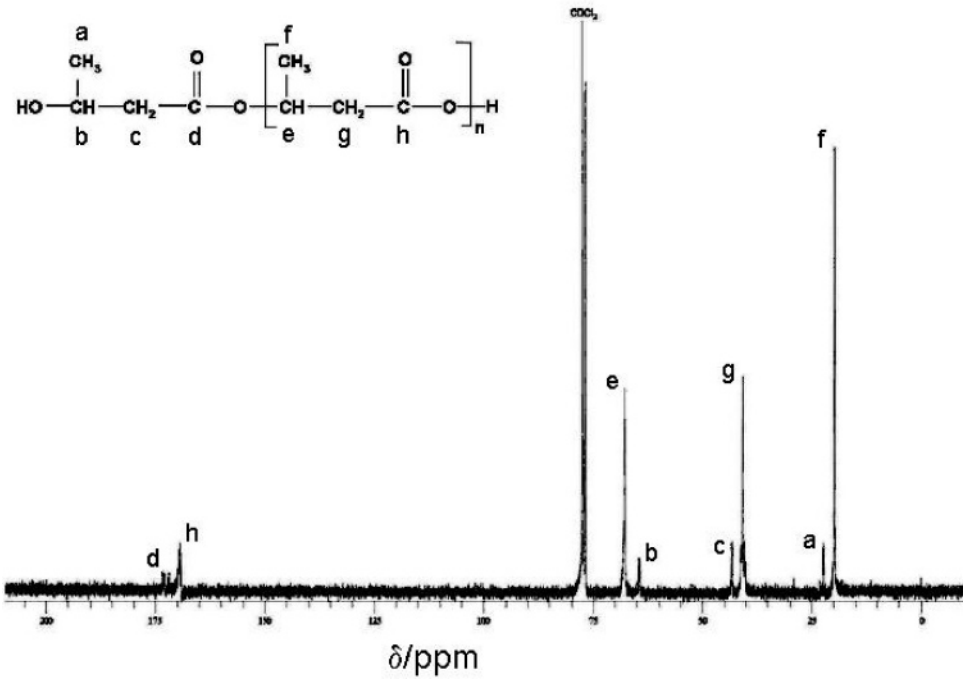

Figure 2. ^{13}C NMR spectrum of poly(3-hydroxybutyrate) ($M_{n,GPC}$ = 700).

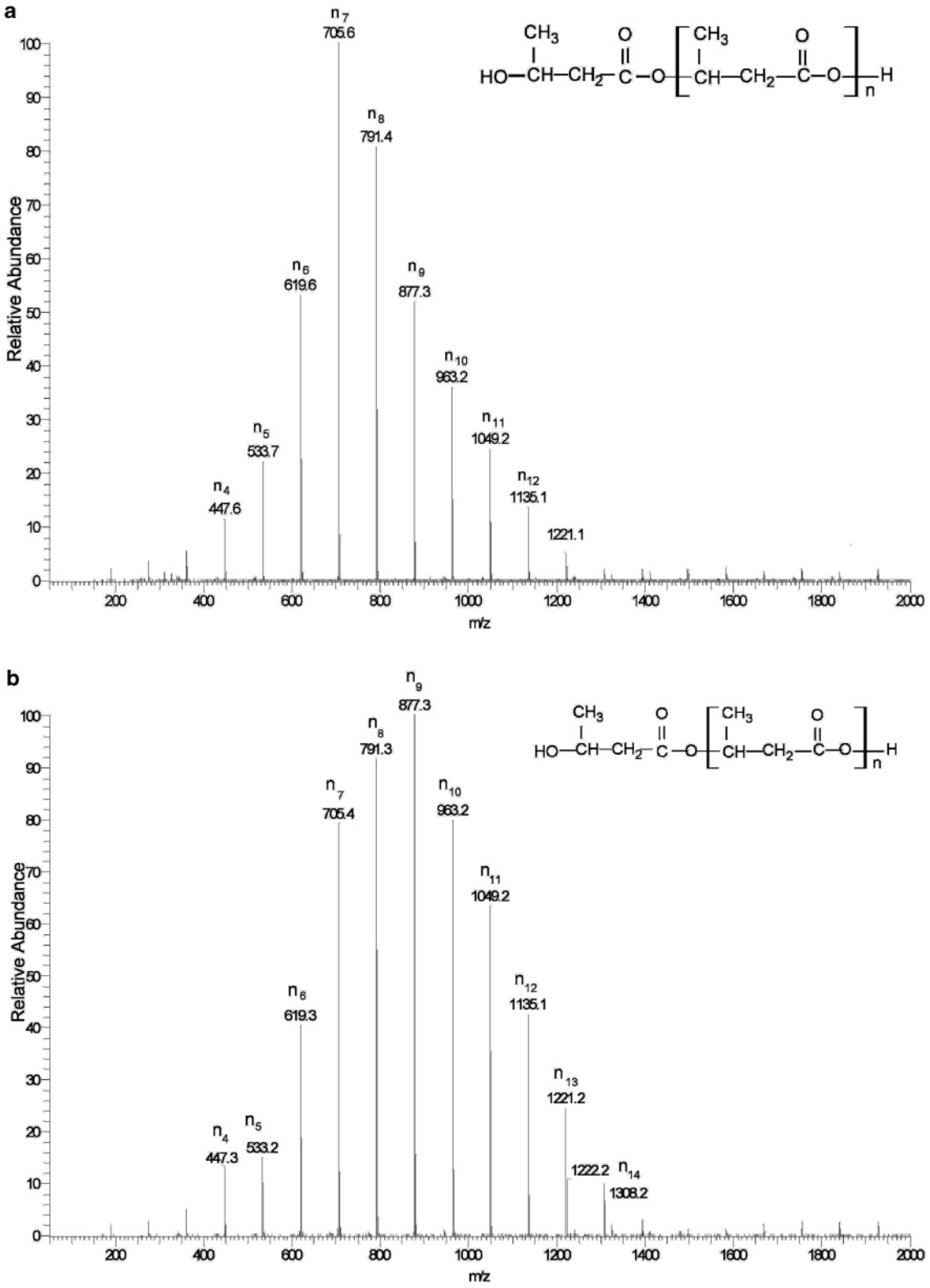

Figure 3. (a) ESI-MS (negative-ion mode) spectrum of poly(3-hydroxybutyrate) ($M_{n,GPC}$ = 700). (b) ESI-MS (negative-ion mode) spectrum of poly(3-hydroxybutyrate) ($M_{n,GPC}$ = 900).

Because of its low toxicity, DMSO is used in many unique applications, as neutral solvent in human and animal medical treatments.[15]

We present here the anionic polymerization of β-butyrolactone using highly polar dimethyl sulfoxide (DMSO) as a solvent activating the carboxylate growing species (Scheme 2).

In the reaction of four-membered β-butyrolactone, carboxylate anion of sodium 3-hydroxybutyrate activating polar solvent attacks the chiral carbon atom of the monomer implying alkyl−oxygen bond scission. The polymer chain growth proceeds via carboxylate anions, and polymers formed bear hydroxyl and carboxylic end groups. The molecular weight of the resulting linear polymers depends on the monomer-to-initiator molar ratio. The molecular weight distribution is relatively narrow (M_w/M_n = 1.1−1.2), which indicates the uniformity of polymers obtained.

CDV

Results of anionic polymerization of β-butyrolactone initiated with 3-hydroxybutyric acid sodium salt in highly polar solvent DMSO are presented in Table 1.

These results (Table 1) indicate that polymerization reaction of β-butyrolactone proceeds quantitatively in a relatively short time, and the resulting polymers possess predicted molecular weight and low molecular weights distribution, having molecular weights close to calculated. The proposed structure obtained of poly(3-hydroxybutyrate)s was confirmed by GPC chromatography, ^{1}H NMR and ^{13}C NMR spectroscopy, and ESI-MS spectrometry.

The analysis of ^{1}H NMR spectra of the obtained poly(3-hydroxybutyrate) (Figure 1) shows that, beside the signals characteristic for the polymer chain, there appears an additional signal at $\delta = 4.22$ ppm attributed to HO−C**H**(CH_3)− end group corresponding to the incorporated initiator.

The structure of the obtained poly(3-hydroxybutyrate) was analyzed also by means of ^{13}C NMR spectroscopy (Figure 2). The ^{13}C NMR spectrum of poly(3-hydroxybutyrate) shows characteristic signals of end groups, peak positioned at $\delta = 67.7$ ppm attributed to carbon with hydroxy group, and peak positioned at $\delta = 171.6$ ppm attributed to carbon of the carboxylic group.

In the ESI-MS spectrum (Figure 3a,b) of poly(3-hydroxybutyrate) the signals ascribed to molecular ions of the polymer chain with hydroxy and carboxylic end groups are present. Molecular ions with crotonate unsaturated end groups due to chain-to-monomer of transfer reaction are not visible in ESI-MS spectrum. The experimental results show that the polymerization of β-butyrolactone yields poly(3-hydroxybutyrate) having end groups typical for the natural PHB. Thus, linear monodisperse poly(3-hydroxybutyrate) is formed using highly polar solvent of 3-hydroxybutanoic acid sodium salt as the initiator.

The presented results indicate that anionic ring-opening polymerization of β-butyrolactone initiated by 3-hydroxybutyric acid sodium salt in highly polar aprotic low toxic solvent as dimethyl sulfoxide constitutes the convenient method of synthesis of well-defined, pure poly(3-hydroxybutyrate).

4. Conclusions

The novel system poly(3-hydroxybutyrate) synthesis using highly polar aprotic solvent as low-toxicity dimethyl sulfoxide (DMSO) is presented. Because of the strong ability of the solvation by the solvent used, the yields of poly(3-hydroxybutyrate) formed are high. The obtained poly(3-hydroxybutyrate) possess hydroxyl and carboxyl end groups, which are present in the structure of a natural poly(3-hydroxybutyrate). The result presented here shows great utility of highly polar solvent in the ring-opening reactions of β-butyrolactone. Thus, obtained poly(3-hydroxybutyrate) can find applications in medicine and pharmacology.

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