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Free radical crosslinking of unsaturated bacterial polyesters obtained from soybean oily acids

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Summary

Poly(-3-hydroxy alkanoate) containing unsaturated side chains, PHA-soybean, were produced by feeding *Pseudomonas oleovorans* with soybean oily acids obtained from soybean oil. Unsaturation of PHA-soybean were found to be 10 mol-% of unsaturated side chains.
Main saturated part of the biopolymer was Poly(3-hydroxy octanoate) with minor hexanoate and decanoate units. PHA films were crosslinked via free radical mechanism by means of thermally or under UV irradiation in the presence of benzoyl peroxide, benzophenon, and /or ethylene glycol dimethacrylate (EGDM). Crosslinking yield of the PHA films were found to be from 81 to 93 wt.-% from the sol-gel analysis. Swelling properties of the crosslinked PHA films in chloroform and toluene were also studied. Mc values of crosslinked PHAs were also calculated using Flory-Rehner equation. The crosslinked biopolyester obtained by thermally at 60 °C with benzoyl peroxide indicated the highest crosslinking density. Glass transition temperatures (Tg) of crosslinked biopolyester samples were changed from -33 to -45 °C while that of PHA-soybean was -60 °C .

Key words: Crosslinked PHAs, thermal crosslinking, UV irradiation, swelling measurements, Mc Value.

Introduction

Poly(3-hydroxy alkanoate)s, PHAs, are a class of reserve polyesters produced by a large number of bacteria when subjected metabolic stress. *Pseudomonas oleovorans* is a very versatile for PHA production because it can produce medium chain length polyesters
(MCL-PHA) from a wide variety of carbon substrates, including alkanes, alcohols, and alcanoic acids[1-4] . Biopolyesters have the properties of thermoplastic elastomers with melting transitions at 35-65°C related to the type of substrate. n-Octanoic acid, n-nonanoic acid and n-decanoic acid were used as substrate in order to obtain saturated PHAs [5-8] . PHAs with unsaturated side chains were produced from the substrates of 10-undecenoic acid and edible oily acids obtained from hazelnut, sesame, anchovy [9], and soybean [10-12] oils. Due to the biodegradable, biocompatible and natural properties, PHAs have been driving a considerable interests of biomedical and environmental applications. Despite these favourable properties, MCL-PHAs have low mechanical resistivity to be used directly as a polymer in the medical and industrial application. For this purpose, chemical modifications with peroxidic initiators of the saturated PHAs were reported [13,14] . PHAs with unsaturated side chains, obtained from soybean oily acids or 10-undecenoic acid using *Pseudomonas oleovorans*, were easily reacted from double bonds by means of grafting with polyazoesters[15], chlorination[10], epoxidation[16] and subsequently crosslinking[17]. An important objective for the use of chemical crosslinking in these bacterial elastomers is to improve their elastic response, but it is desirable to do so without the loss of biodegradability.
There are several fine works to obtain biodegradable polymers from pure soybean oil or copolymers with divinyl benzene[18]. However, cationic homopolymerization of pure soybean oil has proven relatively difficult[19] . This study refers to the simple, free radical crosslinking of bacterial polyesters obtained from soybean oily acids.

Experimental

Materials

Soybean oil was a commercial product obtained from soybean grown in Turkey. Soybean oil was hydrolysed in a 10% solution of KOH in ethanol, after which the solution was neutralised with a 10% solution of sulphuric acid in water to obtain the carboxylic acid substrates. Bacterial polyester containing olefinic groups in side chains was prepared by feeding *Pseudomonas oleovorans* with soybean oily acid, as described previously[9,10]. Mw, Mn and molecular weight distribution(MWD) of the PHA-soybean sample were 130000, 72000 and 1.8 , respectively.

Crosslinking Procedure

Crosslinked PHA film samples were obtained by the following procedures: 1.5 g of PHA- soybean was dissolved in 10 mL of chloroform. To this solution was added a given amount of benzoyl peroxide, benzophenon and/or EGDM (see Table 1). The chloroform solution was poured in an Al casting dish (Φ = 10 cm, depth = 0.3 cm). After evaporation of the solvent at room temperature in a hood, it was dried under vacuum for a day at room temperature and then it was processed under related reaction condition (Table 1). UV irradiation of the biopolyester films was carried out by a Spectroline E-series UV lamp with one longwave (λ=365 nm) tube. The distance between biopolyester sample and the lamp was 10 cm.

Sol-Gel Analysis and Swelling Experiment

Sol-gel analysis was performed to determine crosslinking degree. Typically , 0.05 g of rectangular PHA film (1x4cm) was placed in a 20 mL of chloroform. The sample was allowed to remain in the solvent for a day at room temperature. The swollen gel was taken from the solvent and dried in a vacuum oven at 20°C. The remaining solution was evaporated; the extracted polymer was dried in a vacuum oven at 20 °C and weighed. The sol fraction in the crosslinked crude sample was calculated [20].

$$\% \text{ sol fraction} = \frac{m_o - m_E}{m_o} \times 100 \qquad (1)$$

where m_E is the dry mass of the extracted sample and m_o is the mass of the dry sample.

Swelling experiments of the pure crosslinked PHA samples were carried out similarly in two solvents: chloroform and toluene. In that case the polymer films were allowed to remain in solvent for 24 h at room temperature and the swollen films were weighed as soon as they were taken out from the solvent. Swelling degrees of polymers at equilibrium[21] were determined using the equation (2) of swelling ratio, q_v

$$q_v = \frac{\text{volume of swollen polymer}(V_{\text{swollen polymer}})}{\text{Volume of dry polymer } (V_{\text{dry polymer}})} \qquad (2)$$

Polymer Characterization

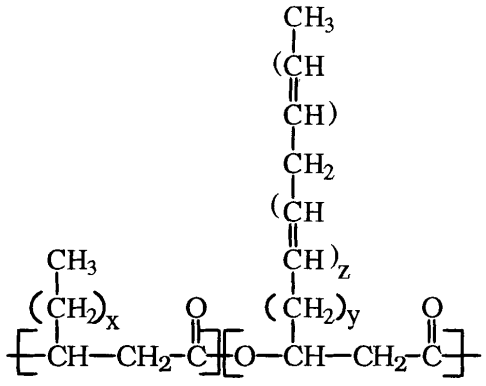
Molecular weights were determined by gel permeation chromatography , GPC. , with a Waters model solvent delivery system with a model 410 refractive index detector, and with 2 ultrastyragel linear columns (HRI and HT6E) in series. Tetrahydrofuran was used as the eluent at a flow rate of 1.0 mL/min. A calibration curve was generated with six polystyrene standards having molecular weights of $3x10^6$, 233 000, 22 000, 2150, 580 and 92 Daltons.

^{1}H-NMR spectra were recorded in $CDCl_3$ with TMS internal standard using Varian XL 200 ^{1}H-NMR. Thermal analysis was carried out 8-10 mg samples on a Du Pont 910 differential scanning colorimeter (DSC). The polymer samples were heated at a rate of 10°C/min from - 100 to 130°C, quickly cooled and then scanned a second time using the same heating rate and temperature range. Knauer type of vapour phase osmometer (VPO) was used in the determination of the electrical resistance ΔR of the PHA for calculation of χ parameter.

Results And Discussion

PHA containing unsaturation in side chains in a 10 L glass fermentation tank via feeding *Pseudomonas oleovorans* with soybean oily acids. Because of the negative affect of glycerine, hydrolysed oil was used in feeding bacterium. After 24 h fermentation time, 19 g of dry cell and 3.60 g of polyester were obtained. Mw, Mn and Molecular weight distribution(MWD) were found to be 130000, 72000 and 1.8, respectively. Typical double bond signals of PHA were observed at 2.0 and 5.4 ppm in NMR spectrum. Unsaturated units (-en, -dien and triens) in side chains were determined as 10 mol-% from GC-MS analysis. Saturated hexanoate, octanoate, decanoate units and the others were 3, 59, 18, and 10 mol-%, respectively (Scheme 1).

Scheme 1.



x
hexanoate : 2
octanoate : 4
decanoate : 6

z
-en :0
-dien :1
-trien :2

PHA-soybean

Crosslinking of PHA-soybean was carried out via free radical mechanism under UV irradiation with 365 nm wavelength, thermally at 60 °C or under atmospheric condition at room temperature. After each crosslinking procedure, originally viscose, sticky biopolyester became a smooth and less sticky elastomer film. Results and conditions of crosslinking of biopolyester were listed in Table 1. Six different crosslinking attempt led to the crosslinked biopolyester in high yield (81-93 wt.-%). PHAs obtained from anchovy (or sesame) oily acids were found to be crosslinked under laboratory atmospheric conditions for a quite longer time (e.g.2 months). Run no. 43 in Table 1 indicated the reproducibility for this kind of crosslinking reaction even with the PHA obtained from soybean. Molecular oxygen may cause crosslinking of the biopolyester via free radical mechanism as in case of crosslinking mechanism of the drying oils in the air . UV irradiation highly shortened the crosslinking time (2.4 day, run no.35 in Table 1). The affects of benzophenon as a photosensitizer, EGDM as an additional crosslinker (with or without benzoyl peroxide), were also studied. UV irradiation of the PHA-soybean films for run no: 35,38, 39 and 40 gave the similar crosslinked films for the time 1 to 2.4 day. Thermal crosslinking of the PHA was performed at 60 °C in the presence of benzoyl peroxide (run no. 41 in Table 1). Crosslinking time was very short in this case (2.4 hour).

Table 1. Crosslinking reaction condition of PHA

Run No	PHA	Benzo-phenon (g)	Benzoyl peroxide (g)	EGDM (g)	Time (day)	Reaction condition	Crosslinking yield (wt.-%)
35	PHA-soybean	-	-	-	2.4	UV	93
38	"	0,006	-	-	1.5	UV	81
39	"	-	-	0.010	1.3	UV	84
40	"	-	0.005	0.010	1.0	UV	89
41	"	-	0.050	-	0.1	Th,60 oC	87
43	"	-	-	-	120	L.a.c.	85

th.: thermal, L.a.c.:Laboratory atmosphric condition

Differential scanning calorimetry (DSC) thermograms showed that all crosslinked biopolyester samples obtained had glass transition temperatures (Tg) between from -33 to –45°C (Table 2). This was expected for the crosslinked PHA to have higher Tg than that of original PHA-soybean (-60 °C).

Swelling measurements were made in chloroform and toluen. Swelling degrees (qv) were calculated using Equation (2) and listed in Table 2. For this calculation, density of biopolyester was taken 1.02 g/cm^3 as that of poly(3-hydroxy octanoate)[22]. Swelling degrees of the crosslinked biopolyester samples UV irradiation and atmospheric condition were changed from 8 to 11 while only the one obtained thermally at 60 °C had swelling degree around 4 (run no.41 in Table 2).

Table 2. Swelling measurements, Mc values and thermal analysis results of the crosslinked biopolyesters.

Run No.	Tg(°C)	q_v		Mc ($gmol^{-1}$)
		in toluen	in chloroform	
35	-45	8.20	8.53	6391
38	-33	9.24	8.09	5772
39	-42	11.1	7.53	5024
40	-46	8.76	11.7	11583
41	-35	4.02	4.15	1485
43	-40	7.43	7.11	4491
PHA-soybean (original)	-60	-	-	-

Determination of Mc From Swelling Measurements

Mc of a network is a main characteristic parameters that serves as a reference in describing a network structure. Therefore as an initial attempt to characterise the network structure of PHA elastomers using the data obtained from the swelling measurements, the polymer-solvent interaction parameters of PHA- chloform systems (χ_1) were determined using the VPO (vapour pressure osmometer) method. Although VPO is not an absolute equilibrium method the determine the molar mass of the polymer , and consequently the thermodynamic data on polymer - solvent system, it has been reported that the obtain by the VPO are satisfactorily in agreement with the data obtained by the other absolute and equilibrium techniques such as membrane osmometer and Cahn electrobalance[23,24]. It has been established in the literature that the solvent activity of a polymer solution can be calculated using the following equation [25].

$$-\ln a_1 = \Delta R M_1 / 1000K \qquad (3)$$

Where a_1 is a solvent activity, ΔR is electrical resistance recorded by VPO, M_1 is the molecular weight of solvent, and K is the calibration constant of osmometer that was determined using benzyl (MW= 210) as the calibrating substance. According to the Flory-Huggins theory activity of solvent in a polymer solution can be written as,

$$\text{Ln } a_1 = \ln (1-V_2) + (1 - 1/x)\, V_2 + \chi_1 V_2^2 \qquad (4)$$

Where V_2 is volume fraction of polymer , x is the number of chain segments in a polymer molecule, assuming that the chain segments have approximately the same volume as the solvent molecules and χ_1 is the Flory-Huggins interaction parameters of the polymer-solvent system. The following equation can be derived from (3) and (4).

$$-\Delta R M_1 / 1000K = \ln (1-V_2) + (1 - 1/x)\, V_2 + \chi_1 V_2^2 \qquad (5)$$

The $\chi 1$ parameter of PHA-chloroform systems were calculated as 0.155 at 45 °C using the data from the VPO measurements in the eq. (5).

Mc values of crosslinked PHAs were also calculated using Flory-Rehner equation (6).

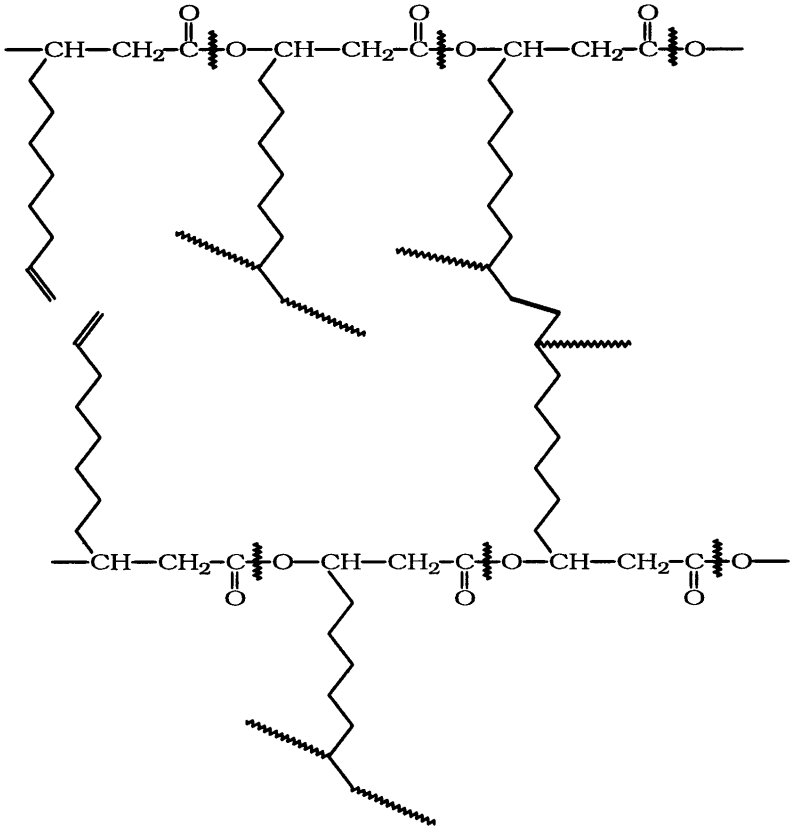
$$Mc = - V_1 * \rho \, (V_{2m}^{1/3} - 2 * V_{2m}/\Phi) \, / \, [\text{Ln}(1-V_{2m}) + V_{2m} + X_1 * V_{2m}^2] \qquad (6)$$

Where Mc is number-average molecular weight, V_1 is solvent molar volume (cm^3/mol), • is density of network

polymer, Φ is functionality of network polymer, V_{2m} is volume fraction of polymer in swolen gel at equilibrium and X_1 is 0.155.

Mc values of the crosslinked PHAs were listed in Table 2. Chain length between junction points (Mc) of the networks were found to be in range between from 4491 to 26323 when they irradiate or laboratory atmospheric conditions. This results also confirm that PHA-soybean has relatively low unsaturated side chains. Run no:40 shows that ethyleneglycol dimethacrylate, EGDM, as an additional crosslinker gets two times longer chain length between junction points. Thermal polymerization helps to get higher crosslinking density in case of run no: 41 with Mc of 1485 g/mol.

In order to understand the crosslinking structure, decrosslinking was performed. The crosslinked biopolyester (0.05 g) were hydrolysed in 20mL of aqueous 5 wt.-% of NaOH for a day at room temperature. Interestingly, wholly soluble units in aqueous solution were obtained after hydrolysis. So, we can say the crosslinking occurs in at least two polyester chain double bonds as shown in Scheme 2. Verification of the crosslinked bacterial polyester studies is under process.



Scheme 2.

Conclusion

Medical and industrial application of the biopolyesters can be verified by the modification reactions. Biopolyesters containing double bonds in side chains are useful for the chemical reactions. They can be easily obtained from renewable sources. Crosslinking reactions of the biopolyester containing double bonds was performed by thermally and UV irradiation with 365 nm wavelength. Sticky and soft polymer was transformed to smooth film which was easily handled. Crosslinking density can be increased by irradiation with shorter wavelength. It was also concluded that because the polymerization of soybean oil is difficult, even by the cationically, the biopolyester from soybean oily acids is more reactive than the relative oil to homopolymerize via free radical mechanism.

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