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Hydrophilic bacterial polyesters modified with pendant hydroxyl groups

M.Y. Lee[a], W.H. Park[a,*], R.W. Lenz[b]

[a]Department of Polymer Science and Engineering, Kumoh National University of Technology, Kumi, Kyungbuk 730-701, South Korea
[b]Department of Polymer Science and Engineering, University of Massachusetts, Amherst, MA 01003, USA

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Abstract

Poly-3-hydroxyalkanoates (PHAs) containing pendant diol groups were prepared by the chemical modification of unsaturated PHAs using potassium permanganate ($KMnO_4$) at 20°C, without a severe reduction in molecular weight. The introduction of hydroxyl groups in the products was verified from the IR, ^1H and ^{13}C NMR spectra. The degree of hydroxylation increased to approximately 60% after 3 h of reaction, but there was no further increase for the longer reaction times, and the degree of hydroxylation of products was almost constant at 50–60% after 3 h, irrespective of the unsaturated unit content of the original PHOUs or of the $KMnO_4$/unsaturated unit molar ratios, which varied from 0.7 to 2.0. The polymers which were 40–60% hydroxylated were completely soluble in polar solvents including an 80/20 acetone/water mixture, methanol and DMSO, indicating a considerably enhanced hydrophilicity of the modified PHAs. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Poly(3-hydroxyalkanoate); Hydroxylation; Hydrophilicity

1. Introduction

Poly(3-hydroxyalkanoate)s (PHAs) which are biosynthesized by a large variety of microorganisms, have potential for a wide range of biodegradable products. Of the microorganisms capable of producing PHAs, *Pseudomonas oleovorans* has been investigated extensively and is known to produce the PHAs with relatively long pendant side chains. The long pendant side chain in the PHAs can contain functional groups, such as olefin [1–4], halogen [5–7] and epoxide [8] groups, at the terminal position when the carbon source with such functional groups are fed, or cofed with a good carbon source, to the bacteria. The presence of functional groups in the PHAs provides sites for chemical modification, which can affect the physical properties of polymers, including their hydrophobicity and solubility. However, attempts to biosynthesize the PHAs with hydrophilic groups, especially carboxyl and amine groups or hydroxyl groups, on the side chain have been unsuccessful [9]. Instead, the investigation described in this report were directed at preparing the hydrophilic PHAs with hydroxyl groups by chemical modification of polymers with reactive functional groups in the side chain.

Poly(3-hydroxyoctanoate-*co*-3-hydroxyundec-10-enoate), PHOU, which is produced when *P. oleovorans* is fed a mixture of sodium octanoate and 10-undecenoic acid, has reactive unsaturated groups at the terminus of some of the side chains as shown in Scheme 1. PHOUs containing such unsaturated units can be modified to achieve unusual physical properties by converting those units into other functional groups, such as epoxide, carboxylic acid, and hydroxyl groups. In our previous studies [10–12] we developed a procedure for the quantitative conversion of the unsaturated groups to epoxide groups. In the present study, new PHAs with pendant hydroxyl groups were prepared by the chemical modification of PHOUs as shown in Scheme 1.

2. Experimental

2.1. PHOU biosynthesis

PHOU production was carried out using *P. oleovorans* as described in our previous report [10]. The PHOUs were isolated from lyophilized cells by extraction with hot chloroform in a Soxhlet extractor. The crude polymer was dissolved in chloroform, reprecipitated by addition of the solution to cooled methanol with rapid stirring, filtered, dried and stored in a sealed sample bottle at 4°C.

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[*] Corresponding author. Fax: +82-546-467-4050.
E-mail address: whpark@knut.kumoh.ac.kr (W.H. Park)

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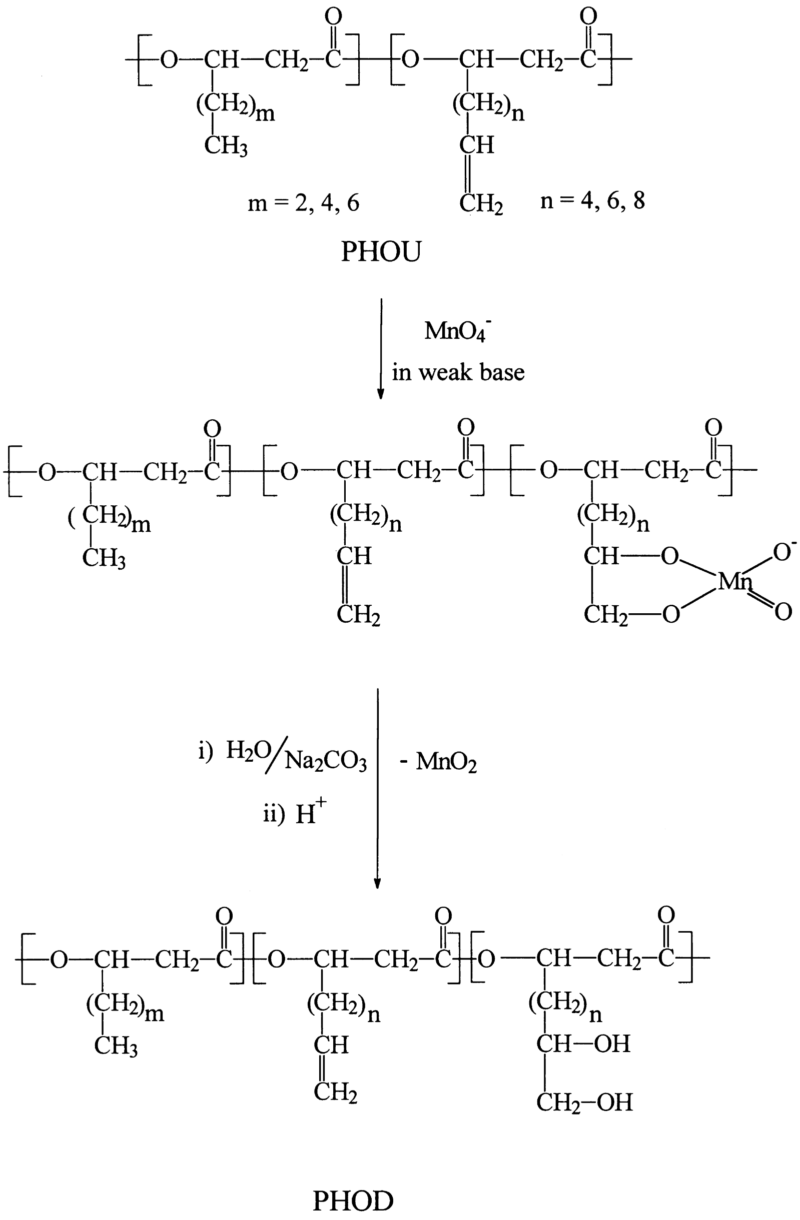

Scheme 1.

2.2. Hydroxylation of PHOU

PHOU (0.1 g) was dissolved in 100 ml of acetone. Two equivalents (based on unsaturated units in PHOU) of sodium bicarbonate ($NaHCO_3$) was added to the stirred solution followed by 0.7–2.0 equiv (based on unsaturated units in PHOU) of potassium permanganate ($KMnO_4$). The reaction solution became brown within several minutes as manganese(IV) oxide formed. The suspension was stirred for 1.5–24 h, and then poured into 50 ml of water containing 2.0 g of sodium carbonate, and suction-filtered. The yellow filtrate was slowly acidified with a concentrated sulfuric acid aqueous solution until a weak acidic pH (pH 5.5–6.5) was reached, which caused the precipitation of PHODs (hydroxylated products). The precipitate was isolated by centrifugation at 4000 rpm for 30 min. The pellets were water washed in the centrifuge tubes three times and the solid again recovered by centrifugation. The wet pellets were dissolved in acetone/water (80/20, v/v), and the solution was filtered to remove small quantities of insoluble materials. The products were isolated by drying in vacuum. The yield of the products was about 25–40%.

2.3. Product characterization

IR spectra were recorded on a Jasco 300E FT-IR Spectrometer. ^1H and ^{13}C NMR were recorded on a Bruker AC model at 200 MHz and chemical shift data were referenced to the TMS internal standard or deuterated solvent. The hydroxyl contents of products were determined from the

Table 1
Effect of unsaturated units content on the hydroxylation of PHOUs using potassium permaganate (reaction conditions: $KMnO_4$ (1.3 eqiv), reaction time (3 h))

Polymer	M_n ($\times 10^{-3}$)	M_w/M_n	Unsaturated groups (mol%)	Hydroxyl groups (mol%)	Conversion (%)
PHOU(55/45)	123	2.1	20	25	56
PHOU(18/82)	118	2.3	33	49	60
PHOU(7/93)	110	2.2	38	55	59

characteristic peaks of ^1H NMR spectra. Molecular weights of PHOUs and PHODs were determined by gel permeation chromatography (GPC) using polystyrene and poly(ethylene oxide) standards, respectively. Thermogravimetric analysis (TGA) was conducted using a TA Instrument 2950 (duPont Co.).

3. Results and discussion

PHOU samples with different unsaturated units were produced and characterized as previously described [10]. The molecular weights and unsaturated units content of the PHOU samples used in this study are given in Table 1. In the polymer code of Table 1, the first number in parentheses indicates the mol% of saturated O units, which are derived from the octanoic acid substrate, and the second number the mol% of unsaturated U units, which are derived from the 10-undecenoic acid substrate [10]. Fig. 1(a) shows a typical ^1H NMR spectrum of a PHOU copolymer with an U units content of 93 mol%, as determined from the ratio of the integration of peak HU7 to peak HO6. The ^{13}C NMR spectrum of this polymer is shown in Fig. 1(b). In this spectrum the carbon signals for the unsaturated units in PHOU occurred at 117.5 ppm for $-\underline{C}H{=}CH_2$ and 139.5 ppm for $-CH{=}\underline{C}H_2$.

All the PHOUs in Table 1 were readily soluble in non-polar solvents such as THF, chloroform, dichloromethane, acetone, and dioxane.

3.1. Hydroxylation of PHOU

Hydroxylation of alkenes is the most important method for the synthesis of 1,2-diols. Of the numerous oxidizing agents that bring about hydroxylation of alkenes, two of the most commonly used are potassium permanganate ($KMnO_4$), and peroxy acids, such as peroxyformic acid (HCO_2OH). For the hydroxylation of unsaturated, high molecular weight polymers, it is essential to use reactive mild reagents and conditions so that there are no side reactions, which can cause chain scission or undesired oxidation, especially further oxidation of the hydroxyl groups. For that reaction, potassium permanganate, $KMnO_4$, was chosen and the reactions were carried out in cold alkaline solution (20°C, pH 8–9). With these reaction conditions $KMnO_4$ is known to react with olefins in high yields and conversion with alkenes (60–70% conversions for alkenes with terminal vinyl group [13], 40–50% conversions for cyclic alkenes or non-vinyl type of alkenes [14]). PHOUs reacted with $KMnO_4$ to yield hydroxylated products (PHOD), as shown in Scheme 1. In this reaction, permanganate ion (MnO_4^-) adds to the double bond to form an intermediate cyclic ester, and the mild hydrolysis of the cyclic ester results in the formation of diol groups.

3.2. Effect of reaction time on hydroxylation

Fig. 2 shows ^1H NMR(a) and ^{13}C NMR(b) spectra of hydroxylated product (PHOD-1) obtained after 1.5 h. The peaks due to $C\underline{H}$–OH and/or $C\underline{H}_2$–OH appeared at 3.3–3.7 ppm with decrease in the peaks at 5.70 and 4.88 ppm of the olefin groups. The ^1H NMR spectrum showed that the products contained approximately 60% of unreacted olefin groups in the side chains of PHOD. The data in Table 2 show the effect of reaction time on the hydroxylation of PHOU(7/93) under the reaction condition with constant $KMnO_4$ (1.3 equiv). Conversion increased to 59% from 0 to 3 h, and no further increase occurred from 3 to 24 h, indicating that the degree of hydroxylation may be limited by the amount of cyclic ester formed in the reaction. Hydroxylation reactions of olefins with $KMnO_4$ usually gives a conversion of 60–70% for alkenes with terminal vinyl group. The lower conversion (41–59%) of unsaturated units in PHOU(7/93) could be explained by steric hindrance to the hydroxylation reaction of the bulky, cyclic manganese ester. The molecular weight data for the PHODs in Table 2 showed that there was no significant molecular weight reduction in products after the hydroxylation. IR spectra of PHOU and PHOD-1 revealed that the absorption peak of hydroxyl groups were broad in the region of 2500–3500 cm^{-1}, and the appearance of this peak was associated with decrease in the absorption peak at ~1650 cm^{-1} of the unsaturated group (carbon–carbon double bond) (Fig. 3).

3.3. Effect of unsaturated unit content in PHOU on hydroxylation

The hydroxylation of PHOUs was also carried out on a series of polymers with different unsaturated unit, U, contents for 3 h. The data in Table 1 show the effect of U content on the hydroxylation of PHOUs under the reaction condition with an equivalent amount of $KMnO_4$ (1.3 equiv). The degree of hydroxylation of its products was almost constant after 3 h, in the range of 55–60% for the three PHOUs studied, irrespective of the unsaturated unit content of the initial PHOU samples, indicating again that the bulky intermediate cyclic ester may limit its extent of the reaction.

Table 3 shows the effect of $KMnO_4$/unsaturated units

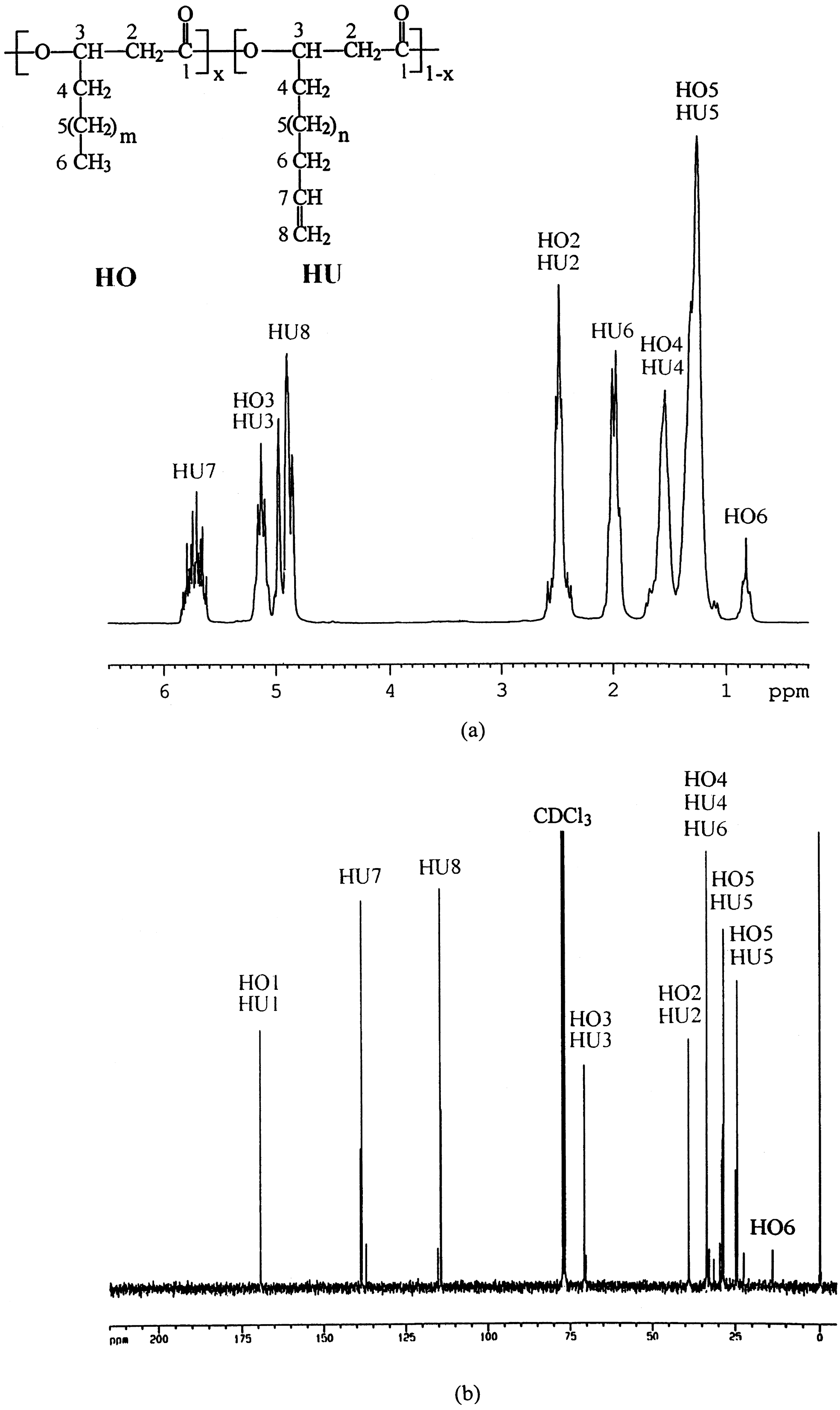

Fig. 1. (a) ^{1}H and (b) ^{13}C NMR spectra of PHOU (7/93).

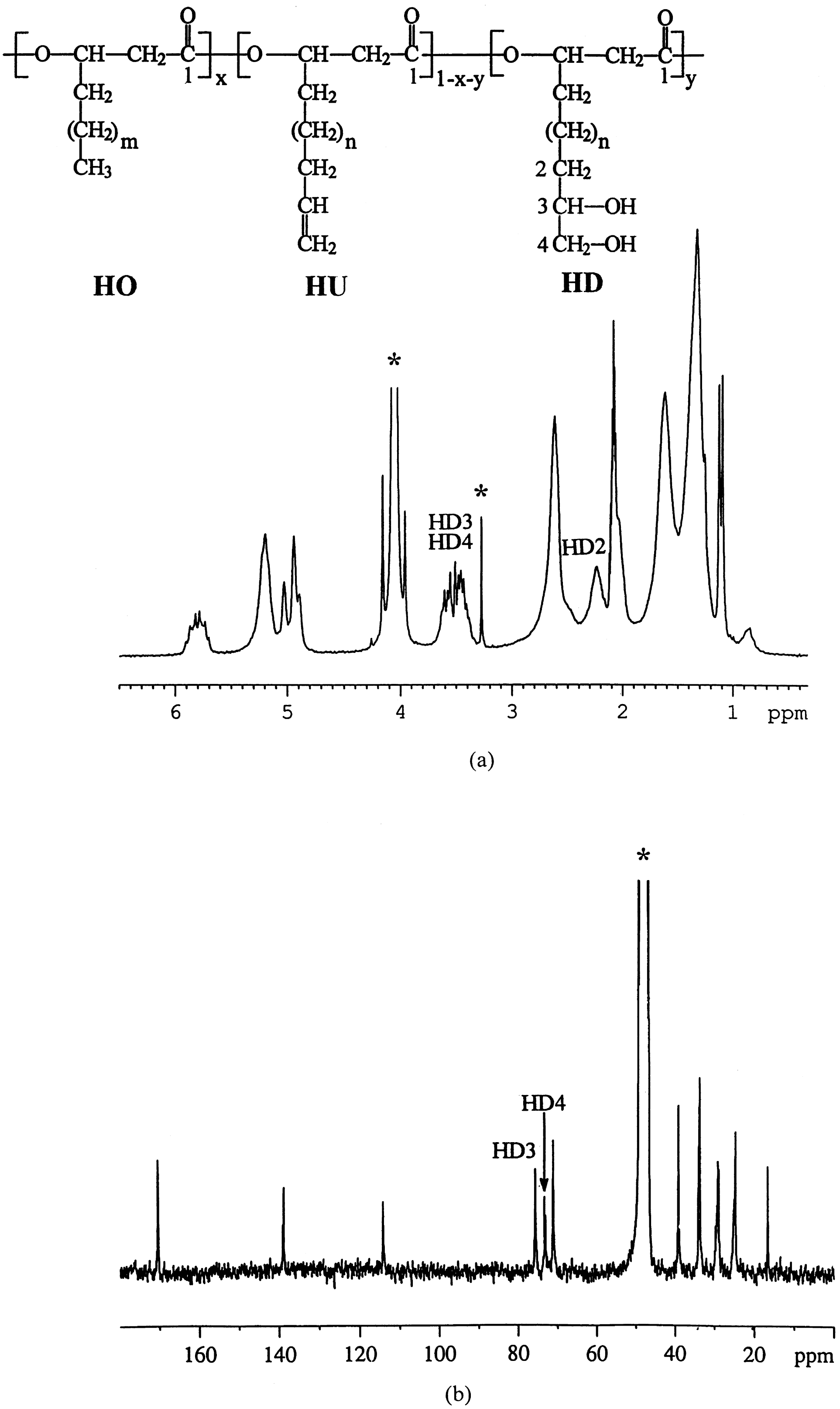

Fig. 2. (a) ^{1}H and (b) ^{13}H NMR spectra of PHOD-1. * indicates the peaks by solvent (acetone/water).

Table 2
Effect of reaction time on the hydroxylation of PHOU (7/93) by potassium permaganate ($KMnO_4$: 1.3 eqiv)

Polymer	Reaction time (h)	Unsaturated groups (mol%)	Hydroxyl groups (mol%)	Conversion (%)	M_n ($\times 10^{-3}$)	M_w/M_n
PHOU (7/93)	–	93	–	–	110[a]	2.2[a]
PHOD-1	1.5	55	38	41	81[b]	2.0[b]
PHOD-2	3	38	55	59	86[b]	2.1[b]
PHOD-3	6	39	54	58	84[b]	2.1[b]
PHOD-4	12	42	51	55	–	–
PHOD-5	24	40	53	57	–	–

[a] Determined by polystyrene standard.
[b] Determined by poly(ethylene oxide) standard.

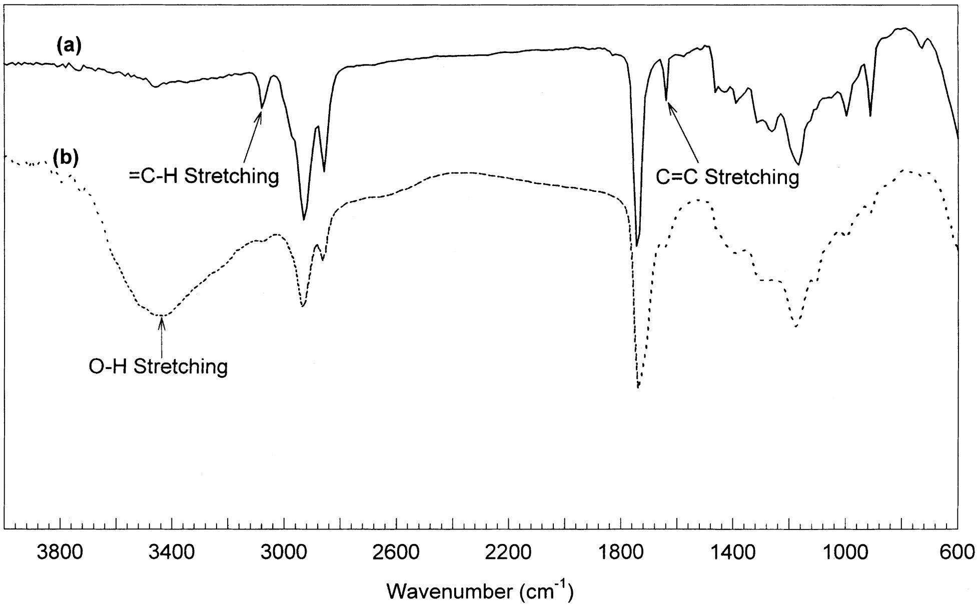

Fig. 3. IR spectra of (a) PHOU (7/93) and (b) PHOD-1.

molar ratio on the hydroxylation of PHOUs for 3 h. The conversion was in the range of 52–59% and was unaffected by the amount of $KMnO_4$ from 0.7 to 2.0 equiv based on unsaturated units in PHOU.

3.4. PHOD properties

The formation of PHOD was also confirmed by their change in solubility. While PHOU was insoluble in a polar solvent, PHOD was soluble in methanol, acetone/water (80/20, v/v) and DMSO, even with 40–60% of double bonds unconverted, but it was insoluble in non-polar solvents such as chloroform, THF, acetone.

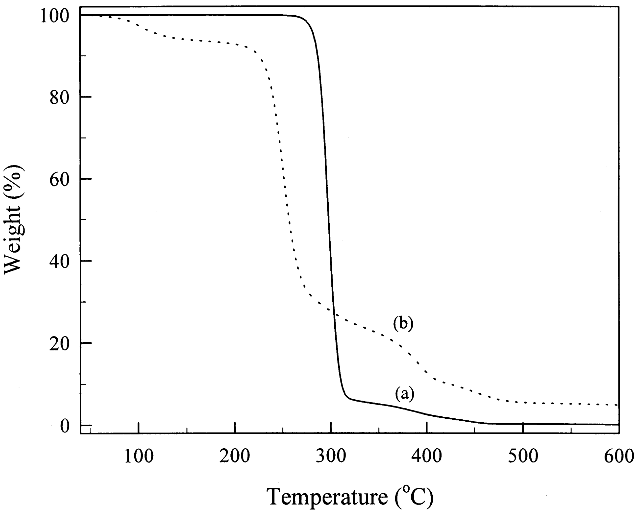

Fig. 4. TGA thermograms of (a) PHOU (7/93) and (b) PHOD-1.

The thermal stabilities of PHOU and PHOD-1 were compared by TGA with the results shown in Fig. 4. The maximum decomposition temperatures for PHOU and PHOD-1 were 296 and 251°C, respectively. For PHOD-1, the slight weight loss detected above 100°C was caused by vaporization of water, associated with the hydrophilic polymer. This reduced thermal stability of PHOD may be associated with the hydroxyl group in the side chain of PHOD,

Table 3
Effect of potassium permanganate content on the hydroxylation of PHOU(7/93) (reaction time: 3 h)

Reaction	$KMnO_4$ (eqiv)	Unsaturated groups (mol%)	Hydroxyl groups (mol%)	Conversion (%)
1	0.7	40	53	57
2	1.0	42	51	55
3	1.3	38	55	59
4	2.0	45	48	52

because the hydroxyl groups could react with the ester linkages in the main chain to accelerate the molecular weight loss and volatilization.

Acknowledgements

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