Hydroxylation of Pendant Vinyl Groups of Poly(3-hydroxy Undec-10-enoate) in High Yield

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ABSTRACT: Poly(3-hydroxy undec-10-enoate) (PHU) was synthesized by feeding *Pseudomonas oleovarans* with 10-undecenoic acid and nearly 100% hydroxylation of double bonds of PHU was achieved by hydroboration–oxidation reaction using 9-borobicyclononane. The disappearance of vinyl signals of PHU in proton and ^{13}C NMR spectra was observed. Acetylation of hydroxylated PHU was conducted for molecular weight measurements. Molecular weight and polydispersity of hydroxylated PHU were found to be 10,000 and 1.23, respectively, while that of the originals was 32,000 and 2.42. Decomposition temperatures of original and hydroxylated PHU were 280 and 200°C, respectively. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 97: 2132–2139, 2005

Key words: PHU; PHAs; biopolyesters

INTRODUCTION

Poly(3-hydroxyalkanoate)s (PHAs) are a group of storage polymers produced by many bacterias in response to growth restriction by a nutrient other than the carbon source.[1–3] One of the known microorganisms capable of producing PHAs, *Pseudomonas oleovorans*, has been investigated extensively and results were reported.[4–11] This microorganism produces PHAs with relatively long pendant side chains. This long side chain can contain some functionalities such as halogen,[4] methyl branching,[5] phenyl,[6] and olefin.[7–10] The presence of functionalities, especially olefins, in PHAs provides sites for chemical modifications, which can affect the physical properties of the polymer and, due to their biodegradability, assure their use in various areas such as medical applications and the packing industry. In this manner, chlorination,[8] epoxidation,[9] and crosslinking[10,11] of the olefinic groups in the PHA have been reported in detail. Edible oily acids such as soybean, hazelnut, sesame, fish, and linseed oils are suitable substrates to obtain PHAs containing olefinic groups on the pendant side chains.[7] They can also be biosynthesized using a mixture of octanoic and/or only 10-undecenoic acid as a substrate.[9,10] Poly(3-hydroxy undec-10-enoate) (PHU) has reactive unsaturated groups at the terminus, as shown in Scheme 1. Hydroxylation of the bacterial polyesters containing unsaturated pendant side chains is important for their medical applications. However, attempts to biosynthesize the PHAs with hydrophilic groups such as hydroxyls on the side chain have been unsuccessful.[12] On the other hand, partial hydroxylation of unsaturated side chain in the PHU using potassium permanganate has recently been reported.[13] In this work, we report complete hydroxylation of the unsaturated side chains in the PHU, using a different reactive system.

EXPERIMENTAL

Materials and characterization

Tetrahydrofurane (THF, Aldrich, Germany) used as solvent was refluxed over sodium and benzophenone until a purple color was obtained; it was then distilled under argon atmosphere. Chloroform (Aldrich) used as a cosolvent (THF/chloroform, v/v, 6/1) was dried and distilled two times under argon atmosphere. 9-Borobicyclononane (9-BBN; Aldrich sure seal, 0.5*M* solution in THF) was used as received. Dimethyl formamide (DMF), for extraction of hydroxylated PHU, was supplied from Aldrich and used as received. For oxidation reaction, a 30% solution of hydrogen peroxide (H_2O_2) in water was used, supplied from Aldrich.

Instrumentation

The ^1H, HETCOR, and attached proton test (ATP) NMR spectra of samples were measured using a

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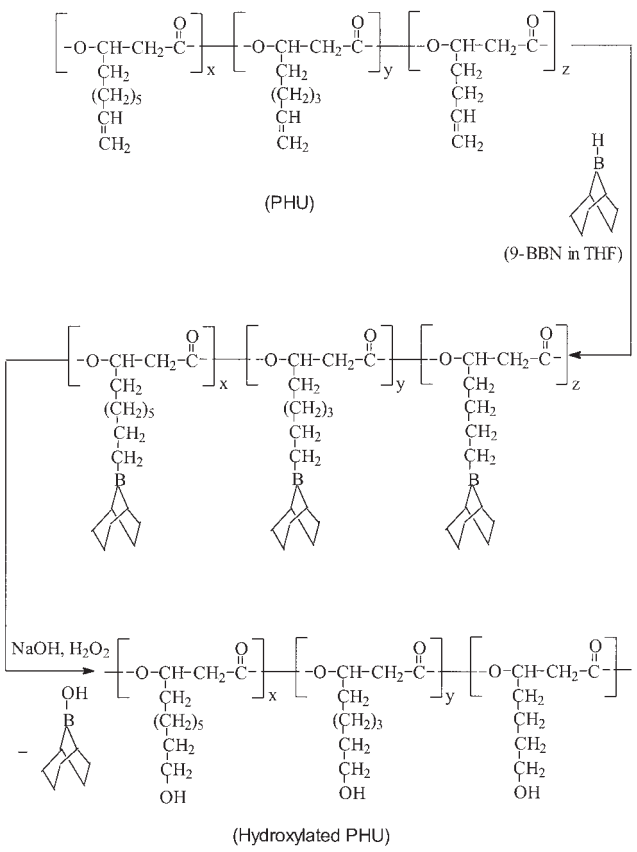

Scheme 1

Bruker-AC 200L, 200-MHz NMR spectrometer, using tetramethylsilane as an internal reference. A Perkin-Elmer Spectrum One model spectrophotometer was used in obtaining the FTIR spectra of samples. The molecular weights of the samples were determined with gel permeation chromatography (GPC) using a Waters 510 HPLC pump with a Waters 410 refractometer and a Waters Stryragel HR1+HT6E column system. A primary calibration method was used for chromatograms in which polystyrene as a standard and THF as an eluting solvent were applied. The thermal gravimetry (TG), derivative thermogravimetry (DTG), and differential scanning calorimetry (DSC) curves of the PHU and hydroxylated PHU samples were recorded on a Perkin-Elmer Pyris 1 Thermal analysis system under nitrogen atmosphere at a 10°C/min heating rate. Both TGA and DSC systems were calibrated before running.

Biosynthesis of PHU

Biosynthesis of PHU was carried out by growing *P. oleovorans* in the presence of undecanoic acid as described in the literature.[1] Polymer was extracted from the lyophilized cells using chloroform and precipitated with methanol. The number of average molecular weight $\bar{M}_n$ and polydispersity of the PHU was 32,000 g/mol and 2.42, respectively, as determined by GPC.

Hydroxylation of PHU

Due to the insufficient solubility of PHU in THF, the hydroboration reaction was carried out in chloroform–THF mixture. A total of 0.5 g of PHU (3.49 mmol vinyl groups) was weighed into a 100-mL round-bottom flask (RB) equipped with a Teflon-coated magnetic stirring rod and a rubber septa. After being sealed, the RB flask was purged with dry argon for 0.5 h and 5 mL chloroform was transferred under argon atmosphere, which resulted in a viscous solution. A total of 30 mL of THF was transferred into the RB flask under argon atmosphere and the mixture was purged with argon for an additional 0.5 h; then 8.4 mL 9-BBN (4.2 mmol, 20% excess) was added with special care under an argon atmosphere. After the reaction was allowed to proceed with stirring for 12 h at room temperature, it was cooled to −25°C and 1 mL anhydrous methanol was injected into the solution to destroy unreacted 9-BBN. After 10 min of stirring, 1.5 mL of 3*N* NaOH (4.5 mmol) was injected; after another 15 min of stirring 1.5 mL of H_2O_2 was added (30% H_2O_2 in water, 13 mmol) and the solution became opaque. After the oxidation reaction was allowed to proceed for 1 h at −25°C, the RB flask was slowly warmed up to 40°C within additional 1 h and cooled down to room temperature with vigorous stirring. The solution was filtered and 1*N* HCl was added dropwise until the basic solution was neutralized.[15]

Solvent was evaporated under reduced pressure until approximately 5 mL remained, and hydroxylated PHU was extracted with DMF and filtered and the solvent was removed under vacuum at 60°C to obtain 0.45 g of hydroxylated polymer, yield 90%, fully soluble in methanol and mostly soluble in pure water.

RESULTS AND DISCUSSION

PHU was synthesized by feeding *P. oleovorans* with 25 m*M* 10-undecenoic acid as seldom carbon-source in a 10-L fermentor, yielding 18 wt % of polymer as a dry cell weight. The molecular weight and polydispersity of PHU were found to be 32,000 and 2.42, respectively. The ^{13}C and ^{1}H NMR spectra of the PHU confirm the structure given in the literature.[9,13] In good agreement with the reported assignments, HETCOR and ATP analyses of the original PHU indicated that the ^{1}H NMR signals at 5.67–5.89 (m), 5.11–5.34 (m), and 3.97–5.05 (m) are from olefin CH, CH next to oxygen, and olefin CH_2, respectively[13], and the ^{1}H NMR spectrum of the polymer obtained from 10-undeconoic acid itself showed that 99 mol % of the repeating units contain alkene substituents, as reported in the literature.[9,13]

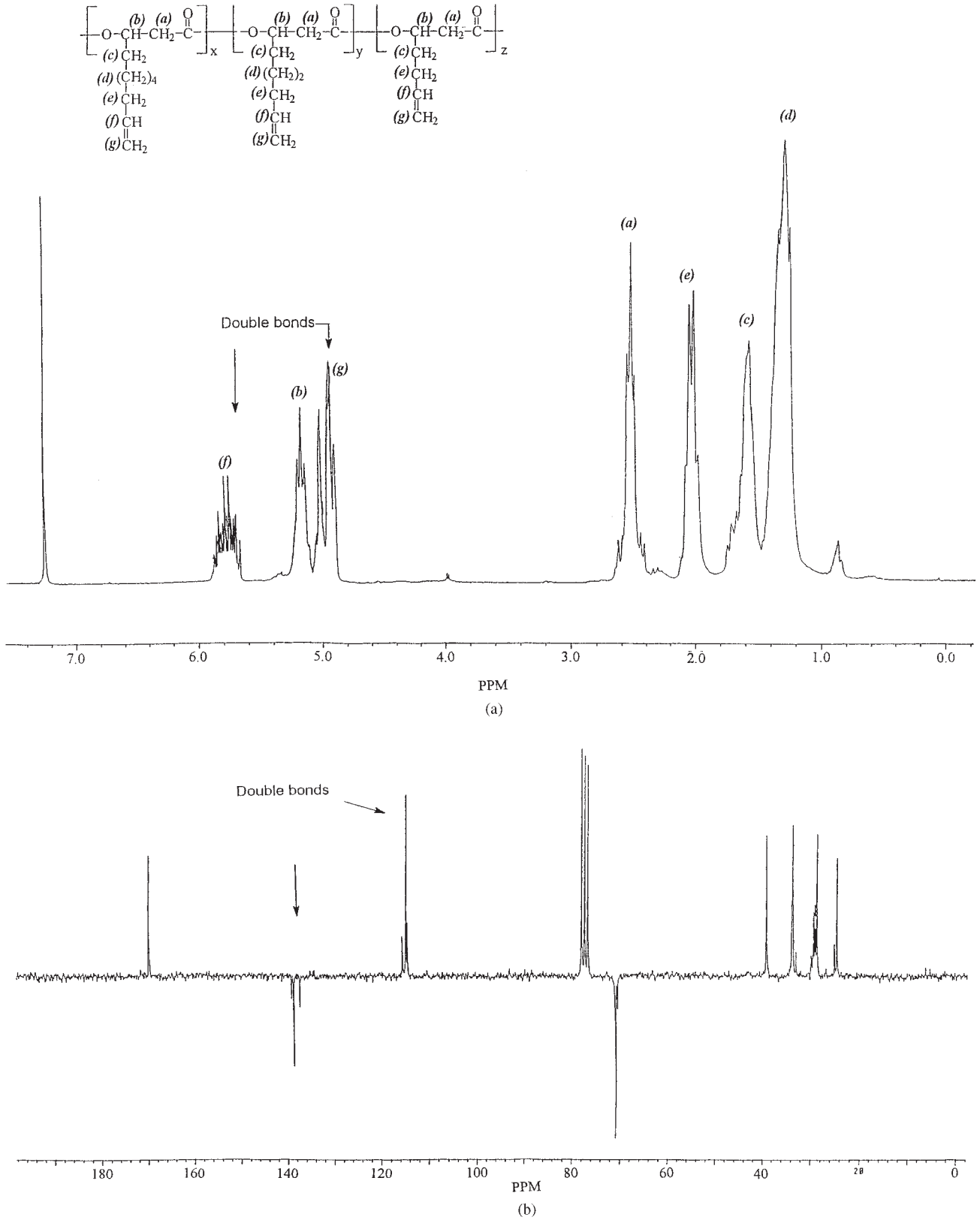

Figure 1 (a) ^{1}H NMR spectrum of original PHU. (b) ^{13}C NMR spectrum of original PHU.

The hydroxylation of PHU was performed using 9-borobicyclononane, which attaches only to the vinyl ends. This was achieved in quantitative conversion. The methanol-soluble portion of the modified polymer constituted the major fraction, while a small amount was soluble in water, which was not subjected

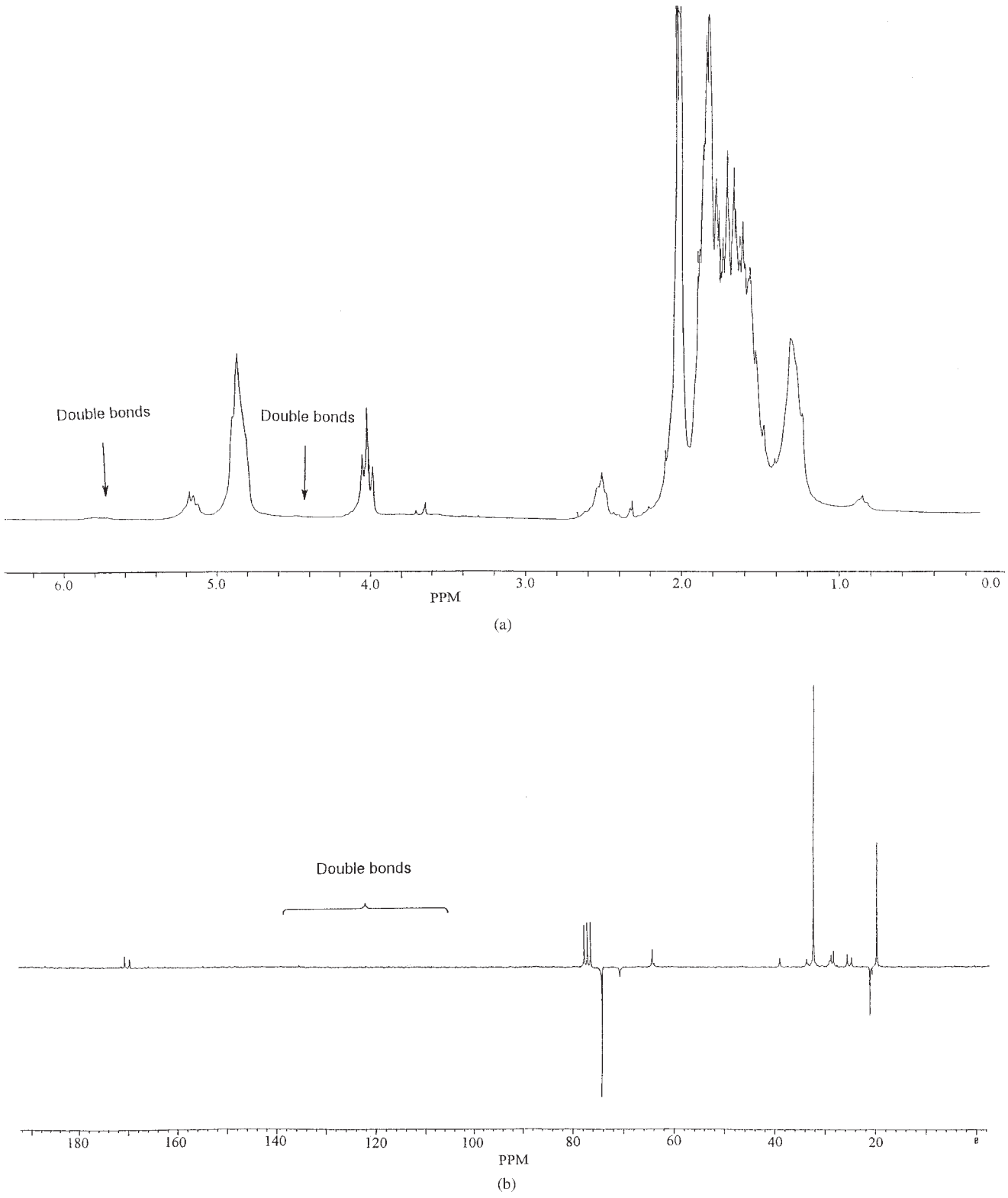

Figure 2 (a) ^{1}H NMR spectrum of hydroxylated PHU. (b) ^{13}C NMR spectrum of hydroxylated PHU.

further analyses. Considering that use of permanganate leads to the formation of a partial glycol derivative of PHU and the hydroxylation can only be achieved in 50–60% at vinyl ends,[13] quantitative hydroxylation of PHU with borobicyclononane demonstrates important progress.

Comparative spectra of proton and ^{13}C NMR of original and hydroxylated PHU are given in Figures 1

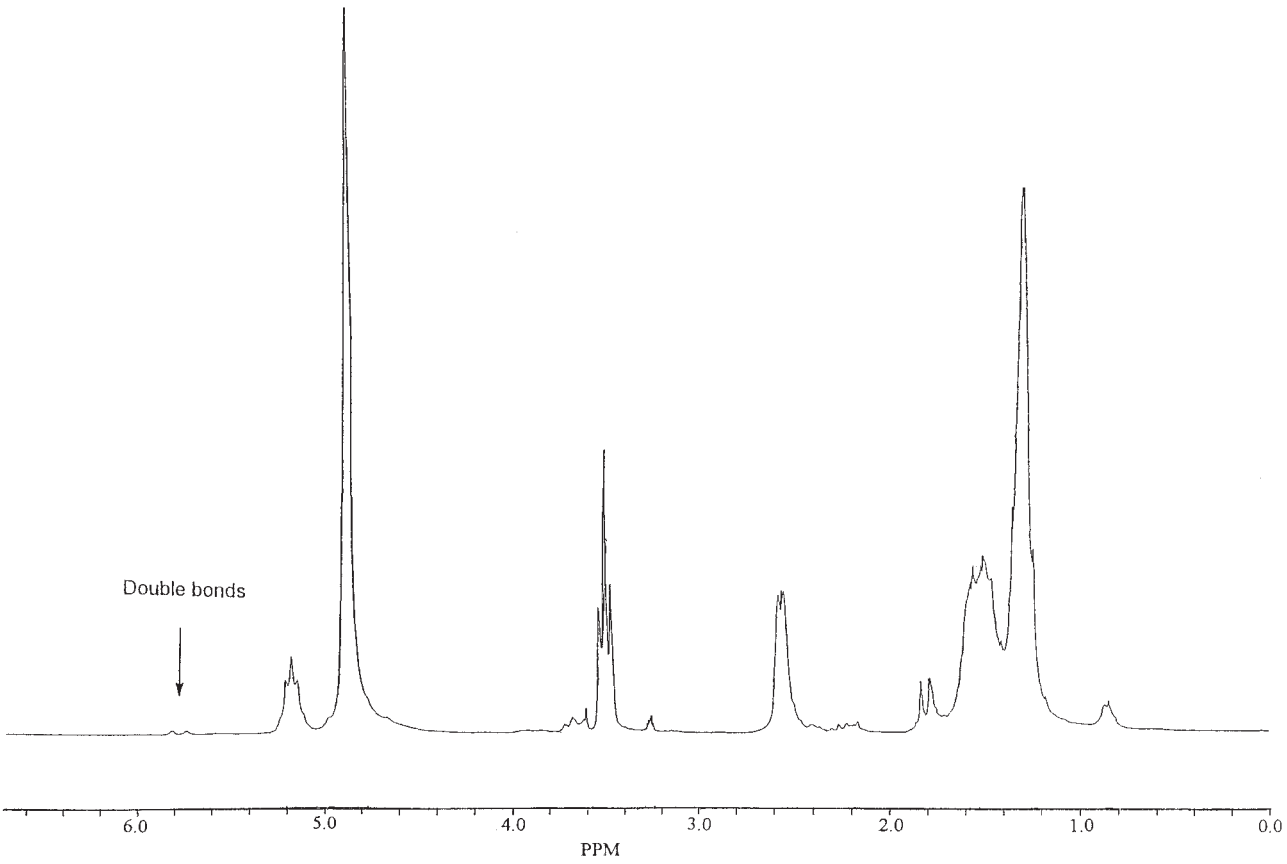

Figure 3 ^{1}H NMR spectrum of acetylated PHU.

and 2, respectively. After the hydroxylation process, olefin signals at 5.0 and 5.8 ppm in proton NMR of original PHU (Fig. 1a) had almost completely disappeared (Fig. 2a). In the same manner, the olefin signals in the ^{13}C NMR spectrum of the original sample at 115 and 138–140 ppm (Fig. 1b) had completely disappeared after hydroxylation (Fig. 2b).

To improve the solubility in chloroform and THF, hydroxylated PHU was acetylated with acetic anhydride via its hydroxyl groups and proton NMR spectrum was recorded in $CDCl_3$. Peaks at 5.72–5.88 ppm of proton NMR spectrum also showed the presence of only a trace of double bonds (Fig. 3). After the acetylation, a broad hydroxyl peak of hydroxylated PHU in the FTIR spectrum disappeared, which indicates complete acetylation of the hydroxyl groups. The molecular weight of the acetylated PHU, which is soluble in THF, was M_w = 8100, and polydispersity was 1.105, which indicates a considerable decrease in molecular weight.

In the TG and DTG thermograms of original PHU (Fig. 4), two characteristic weight loss steps are observed. This can be better seen from the DTG curve. The first weight loss step is probably due to the release of low-molecular-weight decomposition products of polyester linkage, and the second step is due to the decomposition of thermally crosslinked residue from the first step. The pendant vinyl bonds can generate crosslinks at relatively high temperatures and this phenomenon results in insoluble and more thermally stable polymer products.[16] However, hydroxylated PHU decomposes in a single step and at lower temperature, which is a result of nearly complete conversion of unsaturated bonds(Fig. 5). This reduced thermal stability can mainly be attributed to the lower molecular weight of the hydroxylated PHU. Although lower decomposition temperature could be the result of the lower molecular weight, it is difficult to speculate on the effects of the hydroxyl groups on physical properties, since both the original and the hydroxylated polymers do not have similar molecular weights. In the DSC thermogram of PHU (Fig. 6a), before endothermic decomposition, an exotherm was obtained nearly at 300°C. This exotherm can be attributed to the thermal crosslinking of unsaturated double bonds present on the terminus of the pendant side chain of PHU. However, in the DSC thermogram of hydroxylated PHU (Fig. 6b) no exotherm was observed. This is further evidence of the complete hydroxylation of unsaturated terminus present on the pendant side chains of PHU.

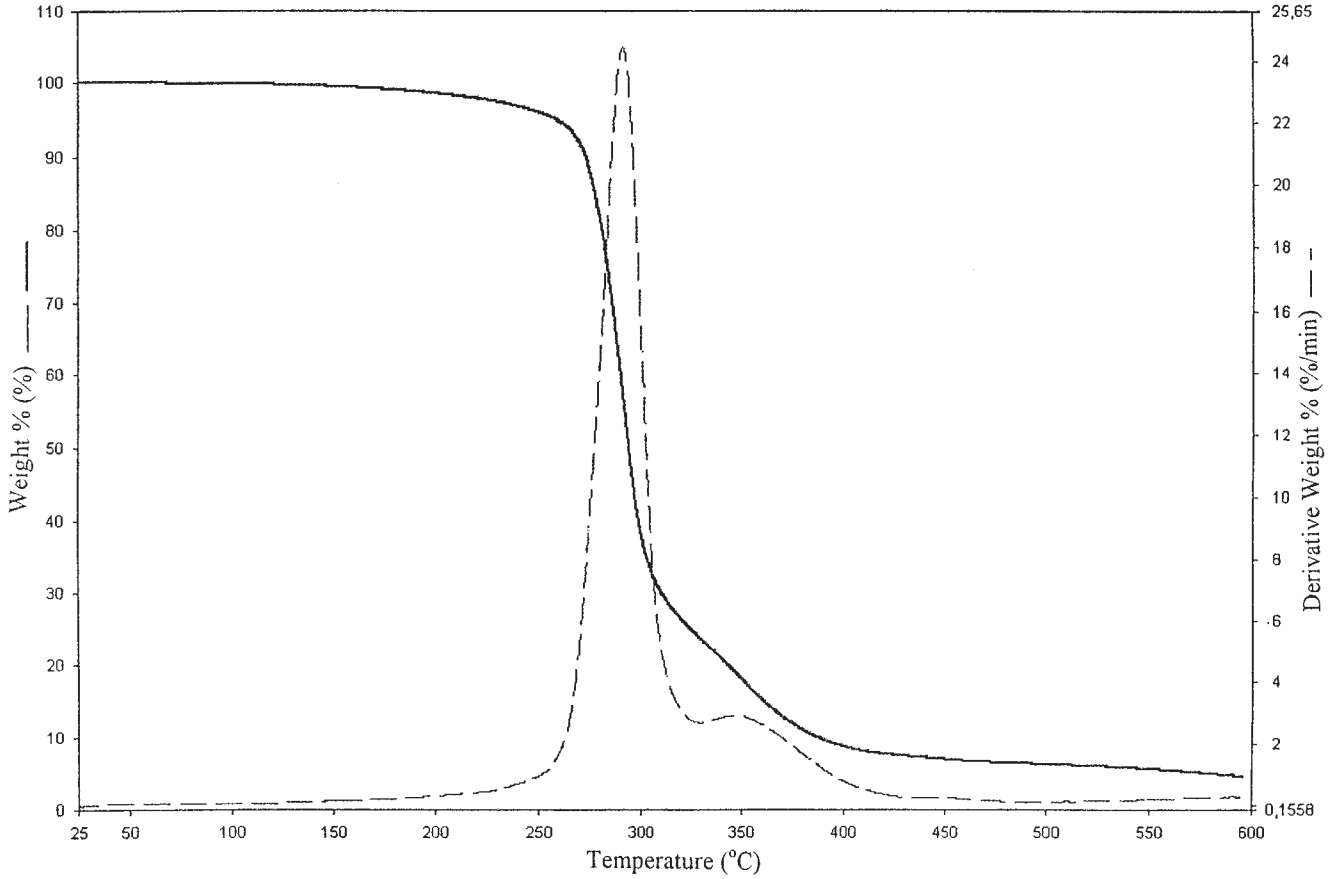

Figure 4 TG and DTG thermograms of original PHU.

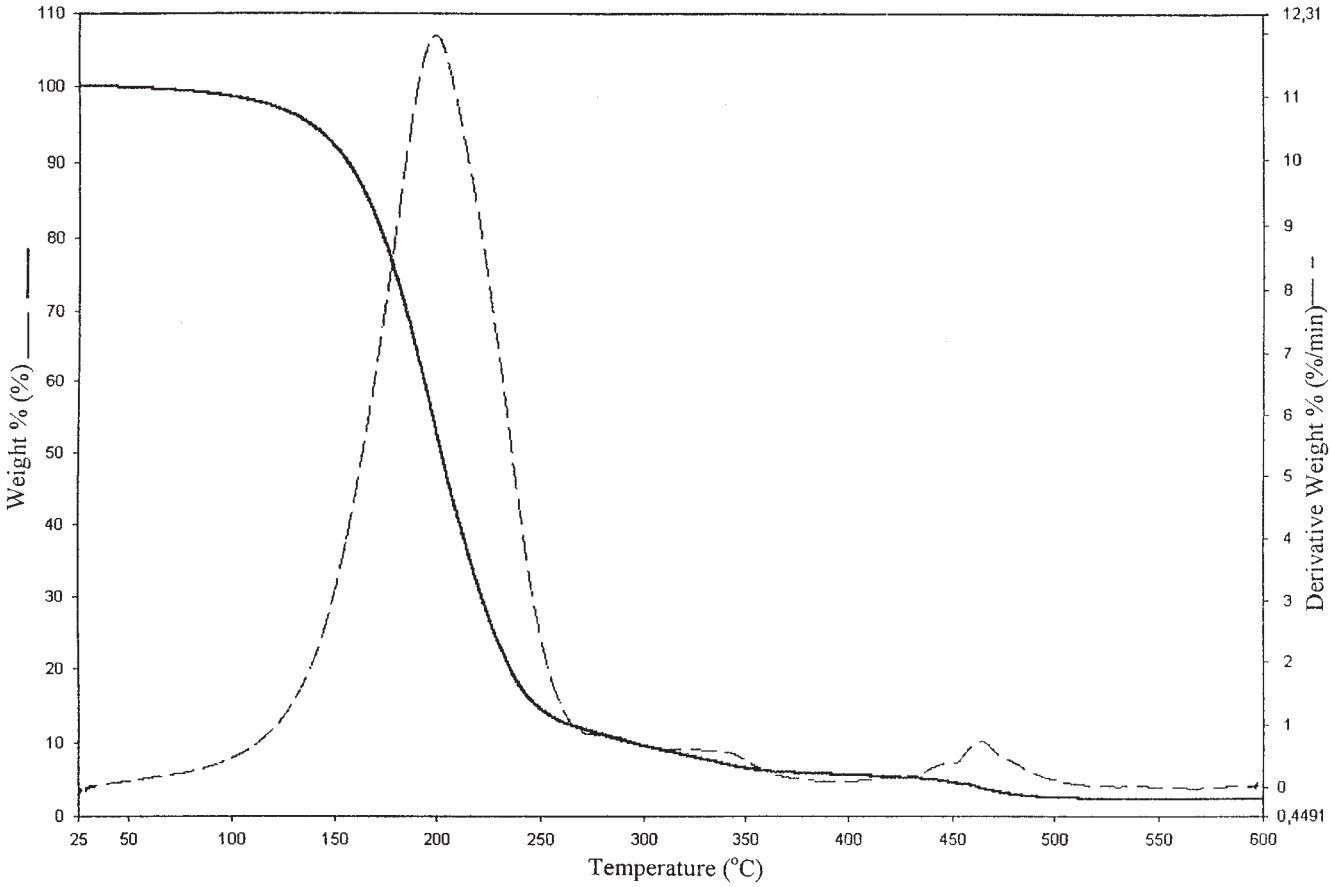

Figure 5 TG and DTG thermograms of hydroxylated PHU.

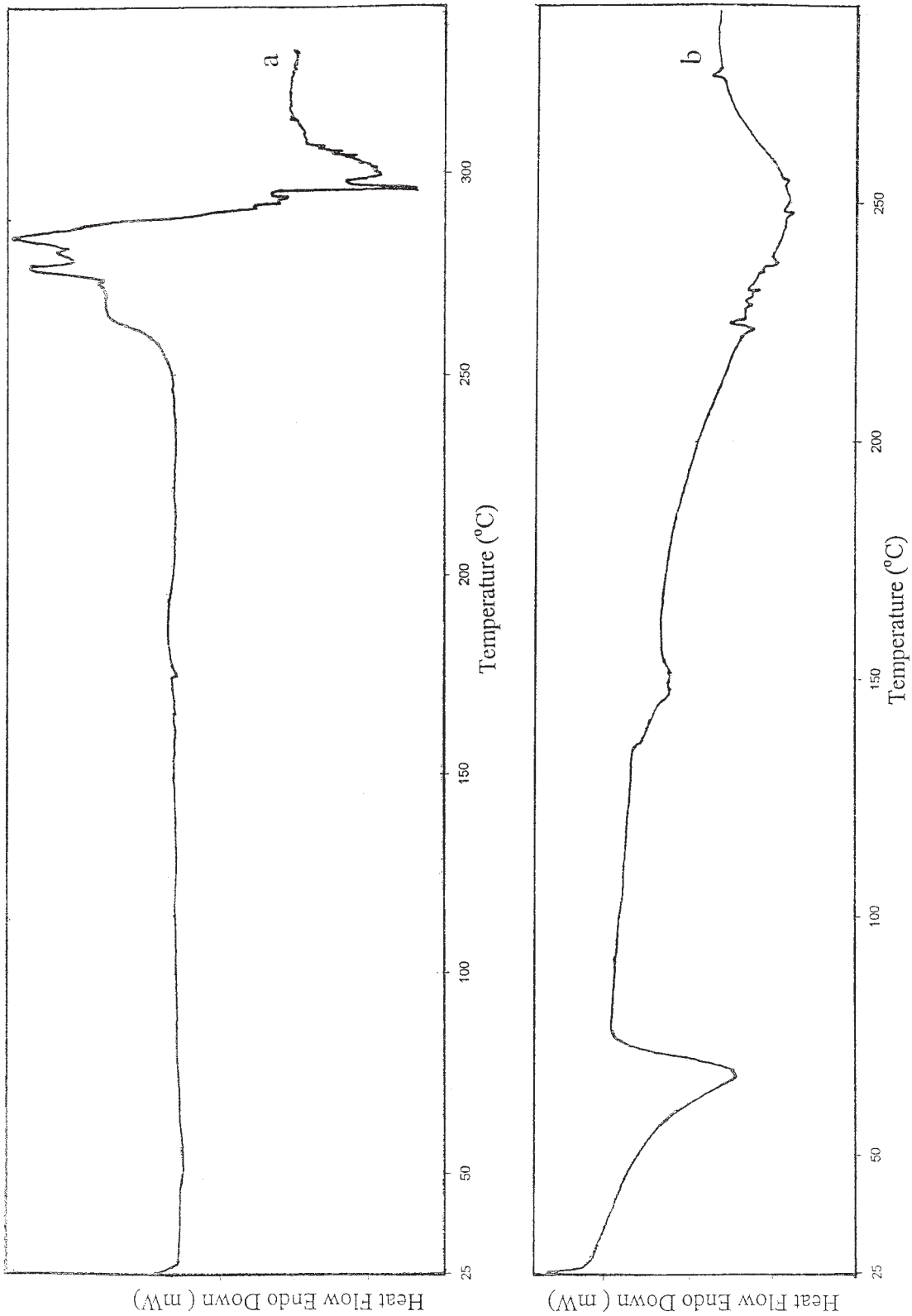

Figure 6 DSC thermogram of (a) PHU and (b) hydroxylated PHU.

CONCLUSIONS

The hydroxylation of pendant vinyl groups of PHU is achieved in high yield (nearly 100%). After hydroxylation, the thermal stability and the molecular weight of the hydroxylated PHU showed small decreases; however, full solubility in methanol and almost full solubility in water are achieved. The increase in percentage of hydroxyl groups of PHU and its improved water solubility are important for preparing drug delivery systems, artificial organs, and tissue engineering applications.

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