Preparation and Structural Characterization of Water-Soluble *O*-Hydroxypropyl Chitin Derivatives

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ABSTRACT: Water-soluble hydroxypropyl chitin (HPC) derivatives were prepared by the reaction of chitin with propylene oxide in homogeneous conditions using a dimethylacetamide/5% lithium chloride solvent system. The reaction conditions for a proper substitution of the hydroxypropyl group were an 80°C reaction temperature, 24-h reaction time, and 48-h aging time. ^{1}H-NMR and ^{13}C-cross-polarization/magic angle spinning NMR spectroscopy were used for determining the substitution value and reaction site of substitution. It was found that the substitution reaction occurred mainly at the C_6—OH group in the chitin molecules and water-soluble chitin derivatives could be obtained at a substitution value higher than 0.35, which is known as a critical substitution value. X-ray diffraction analysis and FTIR spectroscopy showed that the structural characteristics of HPC derivatives were dependent on the substitution value. As the substitution value of the HPC derivative increased the α-chitin crystal structure changed to the water-soluble β-chitin form in higher substitution values. Differences in the interchenic hydrogen bonding, interplanar spacing of the crystal lattice plane, and crystallinity can explain the structural changes upon substitution. The thermal decomposition temperature was also related to the characteristics of the crystalline structure, depending on a critical substitution value. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 80: 2624–2632, 2001

Key words: water-soluble hydroxypropyl chitin; dimethylacetamide/lithium chloride solvent system; critical substitution value; β chitin; crystalline structure

INTRODUCTION

Chitin has the same chemical structure as cellulose, except that the acetamide group is substituted instead of the secondary hydroxyl group of the α carbon in cellulose molecules. Because of this similar structure, any cellulose derivatization techniques can be easily applied to chitin. However, chitin can be digested by lysozymes whereas cellulose is nondegradable, although both of them are polysaccharides with rigid structures.[1]

Because of its structural characteristics of high crystallinity and strong hydrogen bonding, chitin is insoluble in most organic solvents and has a limited application field. Also, the reactivity of chitin is generally low under heterogeneous conditions and chitin derivatives are therefore very difficult to form. A strong acid can dissolve chitin easily, but a considerable reduction of the molecular weight occurs.

The dimethylacetamide/lithium chloride (DMAc/LiCl) solvent system is known to be useful for the preparation of a homogeneous solution of natural polysaccharide such as chitin, dextran, amylose, amylopectin, or cellulose without chemical modification or the reduction of molecular weight. As a

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result, the derivatives of natural polysaccharides can be synthesized in a solution state.[2] In the case of cellulose, the polar aprotic nature of the DMAc/LiCl solvent allows a wide range of organic reactions, including typical alcohol modification reactions such as esterification, carbamate formation, and tosylation.[3] Similar to the modifications of cellulose, approaches for versatile application of chitin were attempted through chlorination, bromination, and acylation in the DMAc/LiCl solvent.[4–6]

There are several reports about water-soluble chitosan derivatives prepared by the hydroxylation reaction of chitosan.[7,8] The hydroxylation can enhance the hydrophilicity of chitosan, and the material has a high water-absorbing property. In the hydroxylation reaction of chitin, Sannan and Sobue[9] reported that a 6-O-hydroxypropyl chitin (HPC) derivative could be obtained from chitin powder dispersed in water with propylene oxide (PO). Recently, Kim et al.[10] also reported the thermal characteristics of HPC prepared heterogeneously using alkali chitin and PO. However, the products of the heterogeneous reactions could not be predicted because they were varied, in spite of the same experimental conditions.

Indeed, most of investigations concentrated on the chitosan derivatives and few studies were done for the preparation of water-soluble chitin derivatives. This was due to the low reactivity and heterogeneous nature of the reaction, as well as the lack of a dissolution system of chitin. In this study a homogeneous chitin solution was prepared using the DMAc/LiCl solvent system, and water-soluble chitin derivatives with various substitution values were synthesized by the reaction of chitin with PO. Several analytical methods were used for the structural characterization of HPC derivatives.

EXPERIMENTAL

Hydroxypropylation of Chitin

Chitin (Sigma, coarse flake, practical grade from crab shell) was purified by treating it with 1N NaOH for 3 h at 80°C and then with 1N HCl for 12 h at room temperature in order to extract the calcium carbonate and protein remaining. The dry weights of the chitin samples were measured using a moisture analyzer (Mark II HP, Denver Instrument Co.).

A viscous chitin solution was obtained by dissolving chitin in the DMAc/5% LiCl solvent system with stirring for 24 h at room temperature (liquor ratio = 1 : 150). It is reported[11–14] that chitin and cellulose can be dissolved with the DMAc/LiCl solvent system according to the dissolution mechanism of complex formation. The weight ratio of LiCl and DMAc is an important factor for the dissolution performance, and 5–11% (w/w) LiCl/DMAc is recommended as a proper mixing condition. In this study the LiCl/DMAc weight ratio was fixed at 5% because of the reextraction of LiCl salt for a ratio of above 7% LiCl. The insoluble fraction was removed from the chitin solution using nonwoven Miracloth (Calbiochem Co.).

The PO (99+%, Aldrich) was slowly added to the chitin solution with vigorous shaking at room temperature for a homogeneous mixing of the viscous solution. After the temperature reached 80°C, the reaction was continued for 24 h with stirring and aged for another 48 h at room temperature. The HPC was then dialyzed under distilled water for 3 days using a Spectra/Por⊕ (MWCO = 12,000–14,000, Spectrum) to remove the DMAc/LiCl and water-soluble poly(PO) (PPO) homopolymer of low molecular weight that was present in the chitin solution. During a dialysis procedure, the low substituted chitin derivatives changed to a gel (water-insoluble fraction, IF),
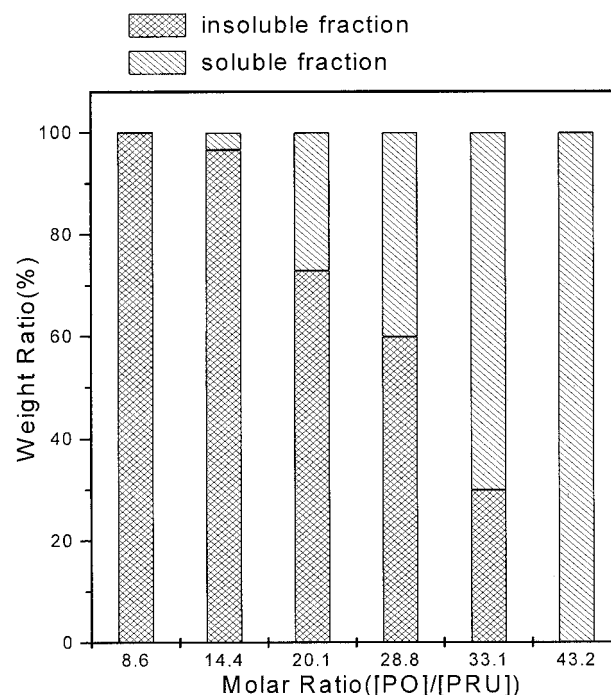
Figure 1 The weight ratio of the soluble and insoluble fraction of hydroxypropyl chitin derivatives.
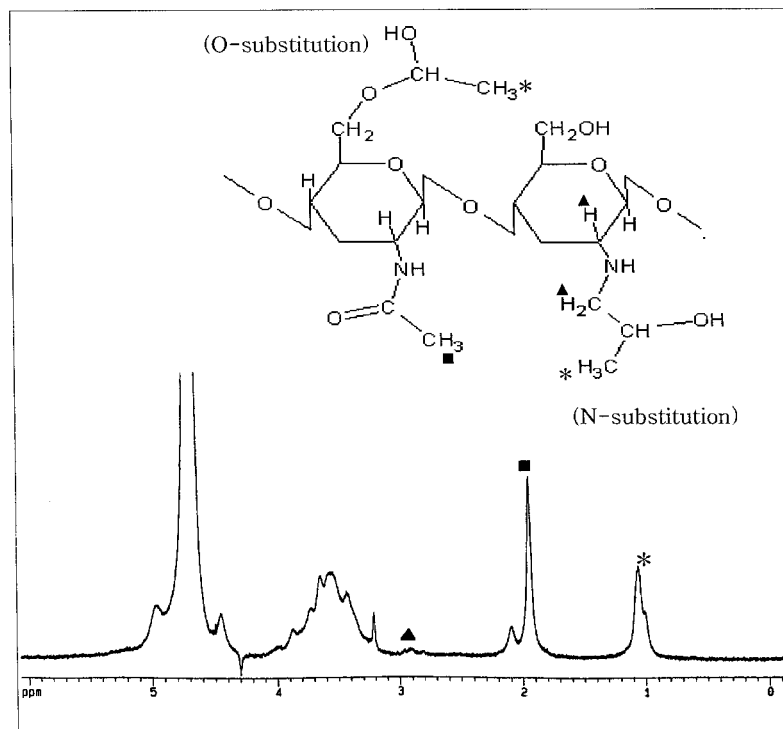
Figure 2   A typical ^{1}H-NMR spectrum of the hydroxypropyl chitin derivative.

which could be separated from the water-soluble fraction (SF) of high substituted chitin derivatives using Miracloth. Finally, both of the fractions were treated with methanol to remove the PPO homopolymer of high molecular weight.

Analytical Methods

An FTIR spectrometer (M series, Midac Co.) was used for the determination of the degree of acetylation, as well as the structural transition. The thickness of the thin film was controlled as about 10 μm for proper measurements. The degree of acetylation of the chitins was measured with the Baxter et al. method[15] in which the absorbance ratio of the amide I band (1655 cm^{-1}) and OH band (3450 cm^{-1}) was used. The degree of acetylation of the original chitin was calculated as 80%.

^{1}H-NMR spectra of chitin derivatives were recorded on a Jeol JNM-LA400 spectrometer at 300 K to determine the substitution value and site of the HPC. The water IF was hydrolyzed by DCl in D$_2$O (37% w/w) at 80°C for 8 h prior to the NMR measurements while the water SF was prepared in D$_2$O without any hydrolysis step. Also, the substitution site of HPC was determined using ^{13}C-cross-polarization/magic angle spinning (CP/MAS)-NMR spectroscopy. ^{13}C-NMR spectra were

obtained with a 400-MHz Bruker Avance 400WB DSX-400 spectrometer.

The transition of the crystal structure and crystallinitiy of HPC was examined in the form of a thin film using an X-ray diffractometer (D/MAX-3C, Rigaku). The maximum thermal decomposition temperature was also measured in order to figure out the structural changes with substitution values of the HPC indirectly. A Rheometric Scientific TGA1000 was used for thermogravimetric analysis (TGA) at a heating rate of 20°C/min.

RESULTS AND DISCUSSION

Factors such as the reaction temperature, reaction time, and aging time significantly affect the substitution value of the chitin derivative. In a previous study[16] the optimum reaction conditions were reported for the preparation of HPC derivatives using the DMAc/5% LiCl solvent system. The 80°C reaction temperature, 24-h reaction time, and 48-h aging time were fixed throughout the experiments to obtain the substitution value (SV) of 80–85%.

Figure 1 shows the weight ratio percent of the water IF and SF of the HPC derivatives prepared
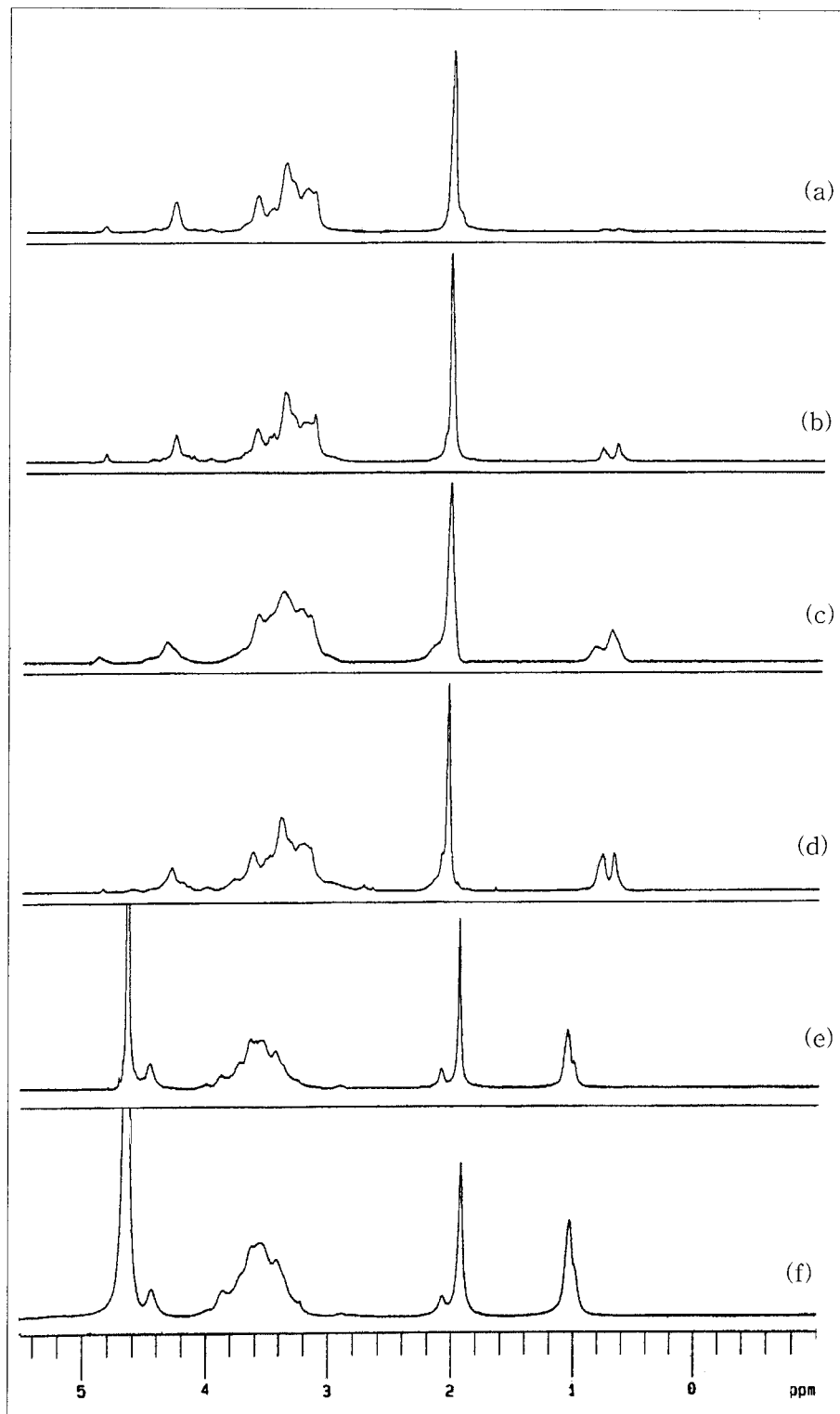
Figure 3 ^{1}H-NMR spectra of chitin and hydroxypropyl chitin derivatives with substitution values of 0 (chitin, spectrum a), 0.15 (spectrum b), 0.30 (spectrum c), 0.38 (spectrum d), 0.44 (spectrum e), and 0.91 (spectrum f).

with various PO/chitin molar ratios. Here the concentration of chitin is expressed as a molar concentration of the polymer repeating unit ([PRU]) of chitin. As PO/PRU molar concentration ratio increased, the weight ratio of the SF markedly increased and a completely water-soluble chitin derivative was obtained at the molar ratio of above 43.2. The water SF was not obtained in the case of a low molar ratio of PO/PRU, which was less than about 10%.

Figure 2 shows a typical ^{1}H-NMR spectrum of the HPC derivative prepared in this study. The chemical shifts associated with PO homopolymers were absent in the spectrum, indicating that the homopolymers were completely removed by the methanol treatment in the preparation of HPC derivatives. The SV and site of chitin were determined by the method suggested by Maresch et al.[17] using ^{1}H-NMR spectra. The SV was calculated by comparing the peak area of the 1.0- and 2.8-ppm chemical shifts with that of the 2.0-ppm peak attributed to the original acetamide group). Here the peak at 1.0 ppm appeared because of the substitution reaction of the hydroxyl group (O-substitution), and the peak at 2.8 ppm was due to the substitution of the amine group (N-substitution).

The degree of deacetylation of chitin was 80% (calculated by IR spectrum), and therefore the value of the acetamide group was converted to 100% for the calculation of the SV. Also, the peak area at 1.0 ppm was corrected properly because this peak was overlapped for the substitution of the hydroxyl and amine groups.

^{1}H-NMR spectra of HPC derivatives with various SVs are shown in Figure 3. The low substituted derivatives [Fig. 3(a–d)] were dissolved in 37% DCl-D$_2$O because of their insoluble nature while soluble derivatives [Fig. 3(e,f)] were dissolved in D$_2$O for ^{1}H-NMR measurements. There was no significant indication for the substitution reaction of the amine group even at high SV (0.91) because of the peak absent at the 2.8-ppm chemical shift. Therefore, it can be said that the substitution reaction takes place mostly at the hydroxyl groups in chitin. It may be assumed that the substitution occurs at C$_6$—OH rather than C$_3$—OH because of the higher reactivity, the lack of intramolecular hydrogen bonds with neighboring repeating units, and the lack of steric hindrance against neighboring acetamide groups.[18–20] However, a true substitution reaction becomes more complex be-
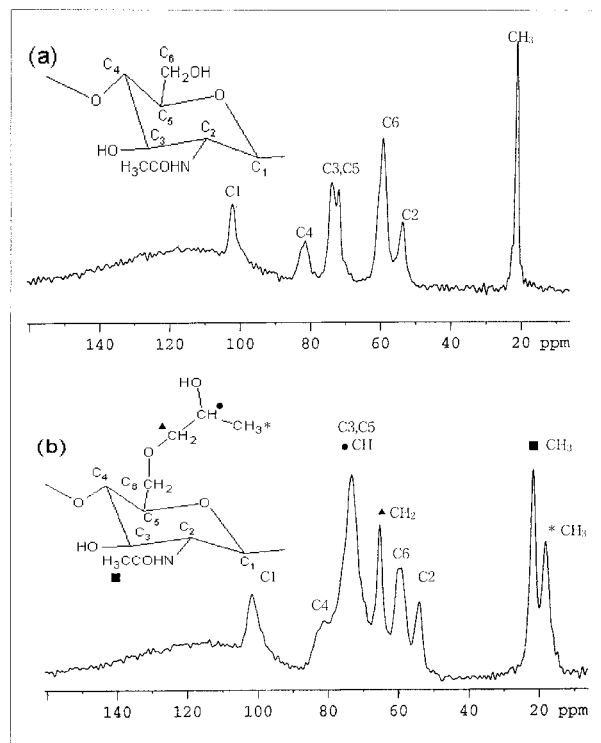

Figure 4 ^{13}C-CP/MAS spectra of (a) chitin and (b) the HPC (SV = 0.62) derivative.

cause of successive PO ring-opening polymerization on the reaction site.

In order to find the detail of the reaction site of PO substitution, the ^{13}C-NMR spectroscopy was examined. Figure 4 shows ^{13}C-CP/MAS-NMR spectra of the chitin and HPC derivative. Compared with the NMR spectrum of α-chitin [Fig. 4(a)], it was confirmed that the hydroxypropyl group could be introduced into the chitin molecule because of the appearance of new peaks at the 18- and 65-ppm chemical shifts. It was also found that a monomeric unit of PO (not several PO sequences produced by a graft copolymerization) might be substituted with the hydroxyl group in chitin because only two CH$_3$ peaks appeared at 21 (attributed to CH$_3$ in the acetamide group) and 18 ppm (attributed to CH$_3$ in the 2-hydroxypropyl group).

It is clearly evident that the substitution reaction of PO occurs mainly at the C$_6$—OH group. The single peak at 60 ppm, attributed to the C$_6$ position of chitin, splits into a doublet with different environmental conditions according to the hydroxypropyl group introduced. Also, the peak intensity is decreased on the basis of the C$_2$ intensity. Although the peaks at around 75 ppm,
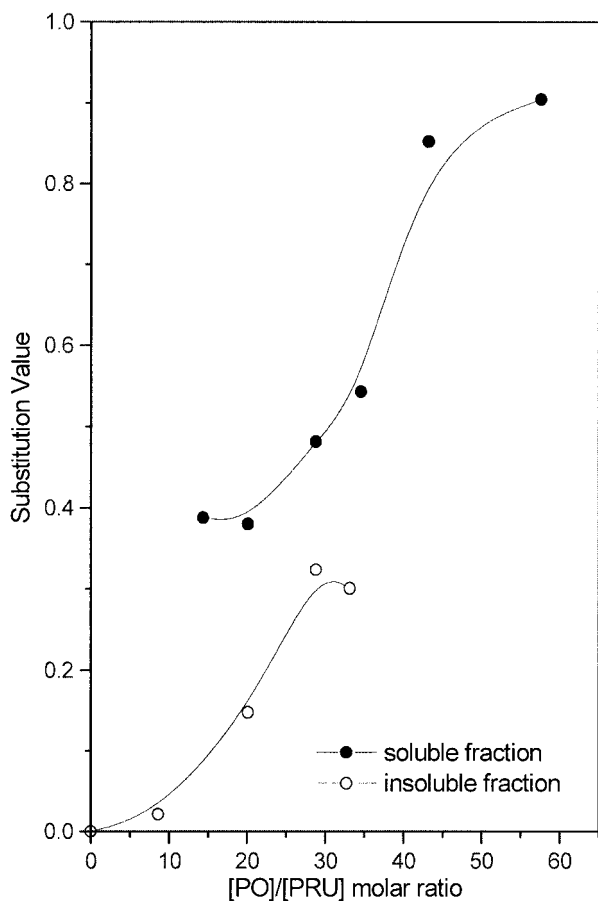
Figure 5 The substitution values of the soluble and insoluble fraction of hydroxypropyl chitin derivatives.

attributed to C_3, C_5, and CH in the hydroxypropyl group, are not fully resolved because of their similar chemical shifts, the C_3—OH group seems to be hardly involved in the substitution reaction in consideration of the relative peak intensity, as well as the area, of C_3 and C_5.

It was already mentioned in Figure 1 that the SF and IF existed in HPC derivatives, depending on the PO/PRU molar ratio. The SVs of the SF and IF were calculated independently from the ¹H-NMR spectra and are plotted against PO/PRU molar ratios in Figure 5. As a the PO/PRU concentration ratio increases, the SV increases moderately and reaches a maximum value of 0.9. A very high PO/PRU concentration ratio (>60) could not be used experimentally for the preparation of the HPC derivative because of the extremely high viscosity of the reaction product. Therefore, it was practically impossible to prepare the HPC derivative with an SV higher than 1.0. The approaching maximum SV of 1.0 is one

piece of evidence that the primary hydroxyl group (C_6—OH) in chitin is the main site of the substitution reaction with PO. As shown in Figure 5, the HPC derivative may be water soluble when the SV is higher than about 0.4 whereas it may be water insoluble when the SV is 0.35 or less. Therefore, the critical SV at which a chitin derivative becomes soluble in water may exist in the range of 3.5–4.0.

Chitin has a highly ordered crystalline structure and has been classified into three polymorphic forms of α-, β-, and γ-chitin, which differ in the arrangement and hydrogen bonding of molecular chains within the crystalline regions.[21,22] X-ray diffraction analysis was carried out in order to find the changes of the crystalline structure upon the substitution reaction.

Figure 6 shows X-ray diffractograms of chitin and HPC derivatives. As expected, the original chitin (SV = 0) has a typical α-chitin structure. As the SV increases to 0.33, the characteristic peaks of α-chitin, corresponding to [220] and [110] planes, diminish gradually and then disappear completely. In other words, the crystalline structure of α-chitin transforms to β-chitin in the vicinity of a critical SV known as a water-soluble or
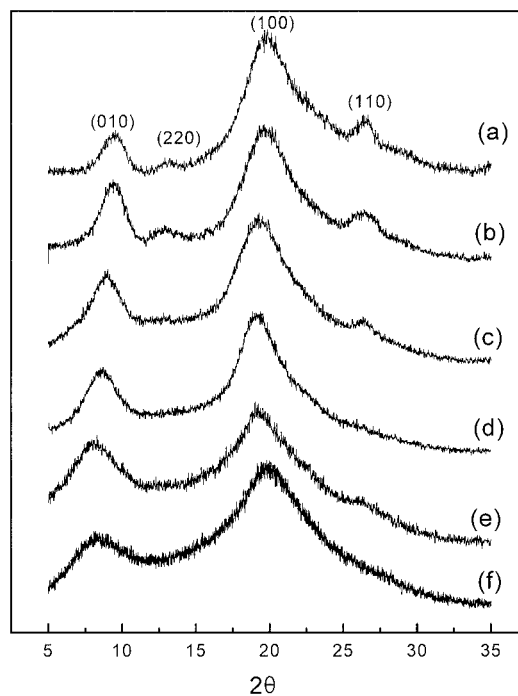
Figure 6 X-ray diffractograms of HPC derivatives with different substitution values of 0 (chitin, spectrum a), 0.02 (spectrum b), 0.19 (spectrum c), 0.33 (spectrum d), 0.48 (spectrum e), and 0.91 (spectrum f).

Table I Degree of Crystallinity of HPC Derivatives with Different Substitution Values

| Chitin Derivatives	Substitution Value	Crystallinity (%)
Chitin	0	84.8
Water-insoluble fraction of HPC	0.02	72.1
	0.19	61.6
Water-soluble fraction of HPC	0.33	56.5
	0.48	53.9
	0.91	48.2

water-insoluble boundary. Therefore, the difference in the crystalline structure can explain the water solubility of HPC derivatives affected by the SV.

Maresch et al. reported that α-chitin consisted of strong hydrogen bonds of C_3—OH—O(5) and C_6—OH—O=C (intramolecular H bond) and of NH—O=C (intrasheet H bond) and C_6—OH—OH—C_6 (intersheet H bond) along the molecular axis while intersheet H bonds are absent in β-chitin.[17]

It was previously mentioned in the NMR analysis that the substitution reaction of the hydroxypropyl group occurred mainly at the C_6—OH group of chitin in which strong intramolecular and intersheet hydrogen bonds were involved. An increase of the SV induces the breakage of hydrogen bonds, especially C_6—OH—OH—C_6 intersheet bonds, and the loosening of the interchenic spacing of chitin molecules in crystalline regions. Considering the [010] plane in the X-ray diffractograms, the interplanar spacing of this plane (d_{010}) was changed from 9.0 to 11.3 Å
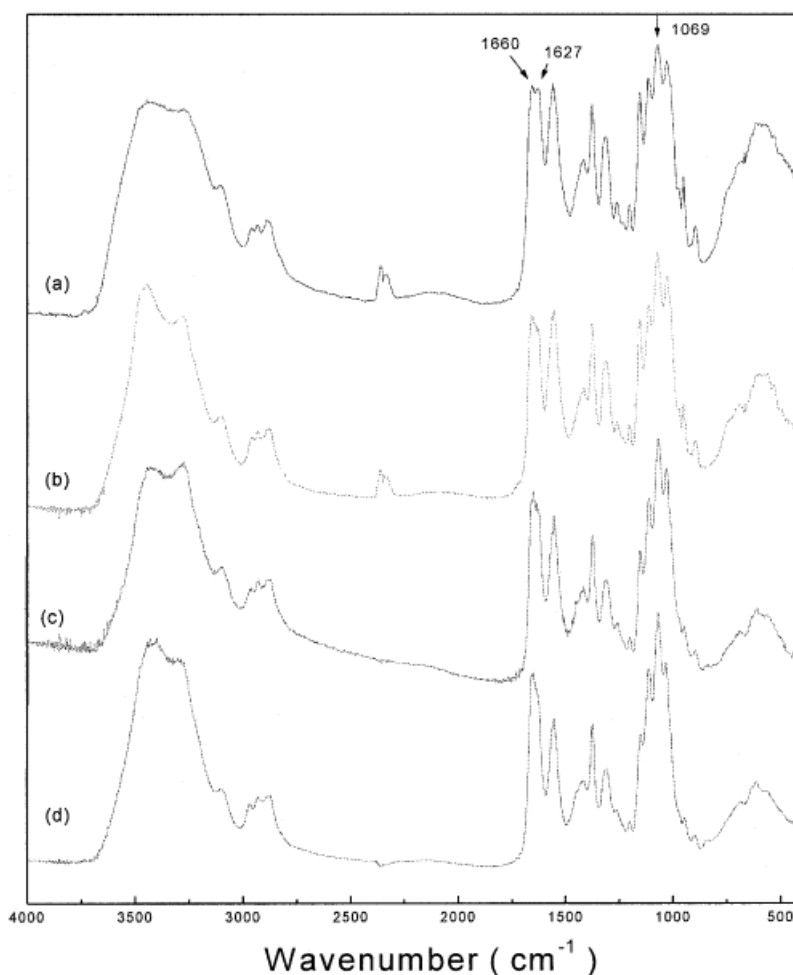
Figure 7 FTIR spectra of HPC derivatives with different substitution values of 0 (spectrum a), 0.02 (spectrum b), 0.39 (spectrum c), and 0.62 (spectrum d).
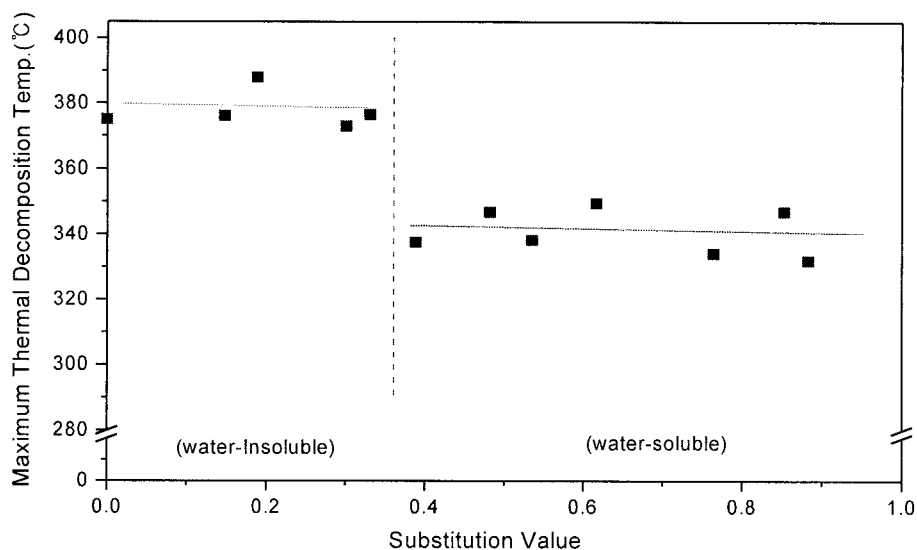
Figure 8 The maximum thermal decomposition temperature of HPC derivatives with different substitution values.

(corresponding to a Bragg angle shift of the [010] plane from $2\theta = 9.8–7.8°$) as the SV increased. Therefore, the antiparallel α form (SV < ca. 0.33) was converted to the water-soluble parallel β form (SV > 0.3).

It is expected that the substitution can lead to the reduction of the crystallinity of HPC derivatives because of the changes of the crystalline morphology. The degree of crystallinity of the HPC derivatives was calculated from the X-ray diffractograms and is shown in Table I. As the SV increases, the degree of crystallinity decreases in general. In the case of lower SV, corresponding to the water IF, the crystallinity changes markedly up to a certain critical SV. On the other hand, there is no significant change of crystallinity in the water SF, which exhibits a high SV.

The structural transition due to the changes of hydrogen bonding can be confirmed by IR spectroscopy. As shown in Figure 7, the absorption band of C=O stretching at 1627 cm^{-1}, attributed to hydrogen bonding between the acetamide carbonyl group and C$_6$—OH group, disappears with the increase of the SV. On the other hand, the amide I band at 1660 cm^{-1} is intact, indicating that the hydrogen bonding of amide groups is not changed with substitution. This result also supports the fact that the $\alpha \rightarrow \beta$ form conversion occurs upon substitution, which is in good agreement with the X-ray diffraction results. According to the IR spectra, the relative intensity of the C—O stretching band at 1067 cm^{-1} is increasing

while that of the absorption band at 1030 cm^{-1}, which is related to primary alcohol group, is decreasing. This may explain that the substitution reaction takes place at the C$_6$—OH group.

The solubility of HPC derivatives is strongly dependent on a critical SV of the hydroxypropyl group because of their crystalline nature. If so, thermal decomposition can be affected by the SV. Figure 8 shows the maximum thermal decomposition temperatures calculated from the first derivatives of the TGA curves. On a basis of the critical SV (ca. 0.38), the maximum temperature was observed at around 380°C for the water IF (SV < 0.38) whereas it was observed at around 340°C for the water SF (SV > 0.38). This can be explained by the differences in the crystalline structure. The higher temperature (380°C) corresponds to the characteristic decomposition of the α-chitin form and the lower temperature (340°C) is that of the β-chitin form.

CONCLUSIONS

Water-soluble HPC derivatives were prepared with PO using a DMAc/LiCl solvent system. As a result of the ^{1}H-NMR and ^{13}C-CP/MAS-NMR spectra, the reaction site of hydroxypropyl substitution was identified as predominantly occurring at the C$_6$—OH in the chitin molecules.

The solubility of the HPC derivatives was strongly dependent on an SV of the hydroxypro-

pyl group and the critical SV, determining water solubility or insolubility, was found to be around 0.35. X-ray diffraction analysis and IR spectroscopy confirmed that a β-chitin crystal structure was transformed from α-chitin upon substitution for the water-soluble HPC derivative. This result can be explained by the crystalline nature relating to the intersheet hydrogen bonding, the interplanar spacing of the crystal lattice plane, and the crystallinity. The thermal decomposition behavior also depends on the structural characteristics affected by an SV.

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