

Synthesis and Characterization of Amphiphatic Carboxymethyl-hexanoyl Chitosan Hydrogel: Water-Retention Ability and Drug Encapsulation

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Carboxymethyl-hexanoyl chitosan (NOCHC) amphiphatic hydrogel with excellent water-absorption and water-retention abilities under neutral conditions was successfully synthesized for the first time and then employed as a carrier for delivering amphiphatic agents. NOCHC is a water-soluble chitosan derivative bearing the carboxymethyl (hydrophilic) group and the hexanoyl (hydrophobic) group, which was synthesized using *N,O*-carboxymethyl chitosan (NOCC) as the starting precursor. Water-absorption ability (W_c), water-retention ability, and drug encapsulation efficiency of the NOCHC hydrogel were investigated in terms of the degree of carboxymethyl and hexanoyl substitution. It was found that the amount of moisture uptake was dependent on the relative humidity as well as degree and nature of such substitution. The hexanoyl substitution affected significantly the water-absorption ability by altering the number of water-binding sites and the state of water under low humidity and the fully swollen state, respectively. In addition, the presence of hydrophobic hexanoyl substitution significantly retards water mobility during deswelling, causing better water-retention ability. Moreover, compared with that of pristine chitosan and NOCC, the encapsulation efficiency of ibuprofen (partially hydrophobic agent) was significantly enhanced with the incorporation of the hexanoyl group. These results demonstrate that the newly developed NOCHC amphiphatic hydrogel showed enhanced water-absorption ability, water-retention ability, and amphiphatic drug encapsulation efficiency compared with NOCC and chitosan.

Introduction

In recent years, much attention has been focused on chitosan (CS) hydrogels for wound dressing, cartilage tissue engineering, and drug-loaded implants owing to its glycosaminoglycan-like structure and outstanding characters such as biodegradability and low price.^{1–3} However, insufficient swelling ability under neutral physiological conditions, poor solubility in organic solvents, and lack of amphiphatic nature have limited its uses. Therefore, a number of chitosan derivatives have been developed over the years in order to improve desirable properties, thus enhancing its bioapplicability.^{4–6}

For the occlusive films used in wound healing, it has been reported that healing under a wet environment is faster than that under a dry environment.^{7,8} In addition, hydrogel-based scaffold in a highly swollen state has been used for delivering cell with bioactive agents.⁹ Therefore, it is beneficial to employ chitosan derivatives with excellent water-absorption ability and water-

retention ability in neutral physiological condition for both burn-dressing and cell encapsulation application.¹⁰ On the other hand, an increase in hydrophobicity of drug-loaded hydrogel is required to improve drug transport across the buccal mucosa and to enhance the encapsulation efficiency for many amphiphatic bioactive agents such as vancomycin and ibuprofen.⁴ Therefore, it is necessary to develop a chitosan-based hydrogel with high encapsulation efficiency of bioactive agents with a wide range of hydrophilic/hydrophobic nature, to replace currently employed hydrophilic or hydrophobic polymeric matrixes.^{11–14} On this base, amphiphatic carboxymethyl-hexanoyl chitosan (NOCHC) bearing hydrophilic and hydrophobic substitutions was synthesized for the first time and expected to possess excellent water-absorption ability and water-retention ability in neutral condition, as well as high encapsulation efficiency for the therapeutic agent with partial hydrophobicity.

In general, the water-absorption ability and water-retention ability (deswelling behavior) are determined by the intermolecular interactions in water solution such as hydrogen bonding and hydrophobic and electrostatic interactions, which depend on the macromolecular structure and the state of water.^{15,16} It is known that the state of water in hydrophilic polymers can be classified into three types: (i) nonfreezable bound water with tight water–polymer interaction, (ii) freezable bound water with loose water–polymer interaction, (iii) free (freezable) water without water–

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(1) Ishihara, M.; Obara, K.; Ishizuka, T.; Fujita, M.; Sato, M.; Masuoka, K.; Saito, Y.; Yura, H.; Matsui, T.; Hattori, H.; Kikuchi, M.; Kurita, A. *J. Biomed. Mater. Res.* **2003**, *64A*, 551–559.

(2) Kim, S. E.; Park, J. H.; Cho, Y. W.; Chung, H.; Jeong, S. Y.; Lee, E. B.; Kwon, I. C. *J. Controlled Release* **2003**, *91*, 365–374.

(3) Drury, J. L.; Mooney, D. J. *Biomaterials* **2003**, *24*, 4337–4351.

(4) Martin, L.; Wilson, C. G.; Koosha, F.; Uchegbu, I. F. *Eur. J. Pharm. Biopharm.* **2003**, *55*, 35–45.

(5) Kurita, K.; Ikeda, H.; Yoshida, Y.; Shimohjoh, M.; Harata, M. *Biomacromolecules* **2002**, *3*, 1–4.

(6) Sashiwa, H.; Kawasaki, N.; Nakayama, A.; Muraki, E.; Yamamoto, N.; Aiba, S.-i. *Biomacromolecules* **2002**, *3*, 1126–1128.

(7) de Queiroz, A. A. A.; Ferraz, H. G.; Abraham, G. A.; Fernandez, M. D.; Bravo, A. L.; Roman, J. S. *J. Biomed. Mater. Res.* **2003**, *64A*, 147–154.

(8) Koyano, T.; Minoura, N.; Nagura, M.; Kobayashi, K. *J. Biomed. Mater. Res.* **1998**, *39*, 486–490.

(9) Temenoff, J. S.; Park, H.; Jabbari, E.; Sheffield, T. L.; LeBaron, R. G.; Ambrose, C. G.; Mikos, A. G. *J. Biomed. Mater. Res.* **2004**, *70A*, 235–244.

(10) Chen, L. Y.; Du, Y. M.; Zeng, X. Q. *Carbohydr. Res.* **2003**, *338*, 333–340.

(11) Missirlis, D.; Tirelli, N.; Hubbell, J. A. *Langmuir* **2005**, *21*, 2605–2613.

(12) Cleary, J.; Bromberg, L. E.; Magner, E. *Langmuir* **2003**, *19*, 9162–9172.

(13) Can, Z.; Ping, Q. N.; Zhang, H. J.; Jian, S. *Carbohydr. Polym.* **2003**, *54*, 137–141.

(14) Le Tien, C.; Lacroix, M.; Ispas-Szabo, P.; Mateescu, M. A. *J. Controlled Release* **2003**, *1*, 1–13.

(15) Guan, Y. L.; Shao, L.; Yao, K. D. *J. Appl. Polym. Sci.* **1996**, *61*, 2325–2335.

(16) Vashuk, E. V.; Vorobieva, E. V.; Basalyga, I. I.; Krutko, N. P. *Mater. Res. Innov.* **2001**, *4*, 350–352.

polymer interaction.¹⁵ Various approaches have been proposed to explore the relationship between water–polymer interaction and water mobility in hydrogels.^{17,18} However, it remains unclear if the hydrophobic hexanoyl group influences the water state, water-absorption, and deswelling behaviors of amphiphatic hydrogel.

The cytotoxicity of NOCHC was investigated in our previous work indicating that the NOCHC hydrogel did not show any cytotoxicity in vitro.¹⁹ This present study systematically investigates the influence of the substitution of the carboxymethyl and hexanoyl groups on water-absorption ability and deswelling kinetics of the NOCHC. In addition, ibuprofen (IBU) was employed as a partially hydrophobic model drug to further understand its loading efficiency in the NOCHC hydrogel since the therapeutic effect (modulation of tissue perfusion) of IBU on burns has been reported.²⁰ This study is expected to provide fundamental understanding on the amphiphatic NOCHC hydrogel used for biomedical, drug delivery, and cosmetic application requiring super-swelling or super water-retention properties.

Materials and Methods

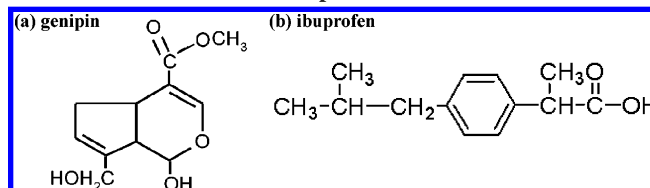
Materials. Chitosan ($M_w = 215\,000\text{ g}\cdot\text{mol}^{-1}$, deacetylation degree = 85–90%) was supplied from Aldrich-Sigma. Ibuprofen was purchased from Aldrich-Sigma (I4883).

Synthesis of NOCC and NOCHC. NOCHC was synthesized via selecting *N,O*-carboxymethyl chitosan (NOCC) as a starting precursor because its skeletal structure favored the formation of hyaluronic acid-like structure.¹⁰ The synthesis of NOCC with various degrees of substitution has been reported in many studies.²¹ In brief, 5 g of chitosan was suspended in 2-propanol (50 mL) at room temperature while being stirred for 30 min. The resulting suspension was gently mixed with 12.5 mL of NaOH solution at different concentrations for controlling the degree of substitution. The mixture containing NaOH of 10M was mixed with 7 g of chloroacetic acid to prepare NOCC sample with a low degree of carboxymethyl substitution. The mixtures containing NaOH of 13.3M were mixed with 7 g and 25 g of chloroacetic to prepare the NOCC sample with medium and high carboxymethyl substitutions, respectively. These three resulting suspensions were stirred for 30 min and heated to 60 °C for 4 h, followed by filtration, washing by methanol solution, and drying. The obtained chitosan derivatives with low, medium, and high degree of carboxymethyl substitutions were named as NOCC-1, NOCC-2, and NOCC-3, respectively. NOCC-2 and NOCC-3 were selected as starting precursors for the synthesis of NOCHC because it is hard to dissolve NOCC-1 in neutral water to achieve a homogeneous synthesis. Each of the NOCC samples (2 g) was dissolved in distilled water (50 mL) while being stirred for 24 h. These resulting solutions were mixed with methanol (50 mL), followed by the addition of hexanoyl anhydride at concentration of 0.1 M, 0.3 M, and 0.5 M for the NOCHC samples with low, medium, and high hexanoyl substitution, respectively. After the reaction time of 12 h, the resulting solutions were collected by dialysis membrane after dialysis with ethanol solution (25% v/v) for 24 h. The obtained ethanol/water (volume ratio = 3:2)-soluble chitosan derivatives were named as NOCHC. NOCHC samples with various carboxymethyl and hexanoyl substitution degrees were named and shown in Table 1. For subsequent material characterization, chitosan-derived solutions of 1.3% (w/v) were prepared by dissolving obtained derivatives in DI water. These solutions were then cross-linked with various amounts of genipin (molecular structure is shown in Scheme 1(a))

Table 1. Sample Name and Corresponding Estimation of Substitution Degree by ¹H-NMR

	degree of substitution	
	carboxymethyl group	hexanoyl group
chitosan	0	0
NOCC-1	0.06	0
NOCC-2	0.32	0
NOCHC-2A	0.32	0.259
NOCHC-2B	0.32	0.461
NOCC-3	0.50	0
NOCHC-3A	0.50	0.264
NOCHC-3B	0.50	0.483

Scheme 1. Molecular Structures of (a) Genipin and (b) Ibuprofen



solution (1% w/v) to prepare hydrogels with same degree of cross-linking (constant amino residue/genipin ratio).

Material Characterization. Proton nuclear magnetic resonance spectroscopy (¹H NMR) spectra were used to confirm the sites and degrees of substitution recorded by NMR spectrometer (Varian unitynova 500) at 270 MHz. Attenuated total reflectance Fourier transformed infrared spectroscopy (ATR-FTIR) spectra were recorded on a spectrometer (Bomem DA8.3, Canada) using a film type sample (4 cm × 0.5 cm). The ATR-FTIR spectra were taken with a resolution of 2 cm⁻¹ in the range of 4000–400 cm⁻¹. The state of water was characterized by a differential scanning calorimeter (DSC, Perkin-Elmer instrument).²² Each dried sample was weighed in an aluminum pan, followed by the addition of various amounts of water into the sample pan. Samples were quenched from room temperature to 213 K and conditioned at 213 K for 10 min prior to the DSC test. DSC curves were then obtained by reheating the sample to 300 K at a scanning rate of 10 K min⁻¹. The maximum content of nonfreezable bound water ($W_{nf,max}$) could be determined by detecting the endothermic peak assigned to the first-order phase transformation of water in the samples with various contents of water. The endothermic peak of freezable bound water is not detected until a critical amount of water is added to the sample. The critical amount of water is defined as $W_{nf,max}$, which is correlated with the number of tight water binding sites.

Moisture Absorption and Retention Test. The characterization of water-absorption ability was measured following the report of Chen et al.¹⁰ Briefly, the samples were dried in a vacuum chamber with P₂O₅ for 24 h prior to the moisture absorption test which was carried out by putting the sample in an incubator with relative humidity of 40% and 70% at 20 °C for 48 h. Water-absorption ability (W_c) was determined by the percentage of weight change of the dry sample:

$$W_c (\%) = 100\% \times (W_w - W_d)/W_d \quad (1)$$

where W_w and W_d are the weights of the sample after and before the absorption test. Saturated water-absorption ability ($W_{c,sat}$, equilibrium swelling ratio) was also determined by eq 1 and measured after putting the samples in DI water (pH = 7) at 20 °C for 48 h.

The water-retention ability was evaluated by measuring water mobility during dynamic deswelling test.²³ Fully swollen hydrogels were placed in desiccators containing silica gel at 37 °C and removed from desiccators at regular intervals for weighing. The weights of fully swollen hydrogels (W_{wet}), hydrogels at each juncture (W_i), and dry sample (W_{dry}) were recorded. The deswelling profile could be

(17) Vazquez, B.; Roman, J. S.; Peniche, C.; Cohen, M. E. *Macromolecules* **1997**, *30*, 8440–8446.

(18) Morillon, V.; Debeaufort, F.; Capelle, M.; Blond, G.; Voilley, A. *J. Agric. Food Chem.* **2000**, *48*, 11–16.

(19) Liu, T. Y.; Liu, T. Y.; Chen, S. Y.; Chen, S. C.; Liu, D. M. *J. Nanosci. Nanotechnol.*, in press.

(20) Barrow, R. E.; Ramirez, R. J.; Zhang, X. J. *Burns* **2000**, *26*, 341–346.

(21) Sugimoto, M.; Morimoto, M.; Sashiwa, H.; Saimoto, H.; Shigemasa, Y. *Carbohydr. Polym.* **1998**, *36*, 49–59.

(22) Agrawal, A. M.; Manek R. V.; Kolling W. M.; Neau S. H. *J. Pharm. Sci.* **2004**, *93*, 1766–1779.

(23) Sen, M.; Guven, O. *Eur. Polym. J.* **2002**, *38*, 751–757.

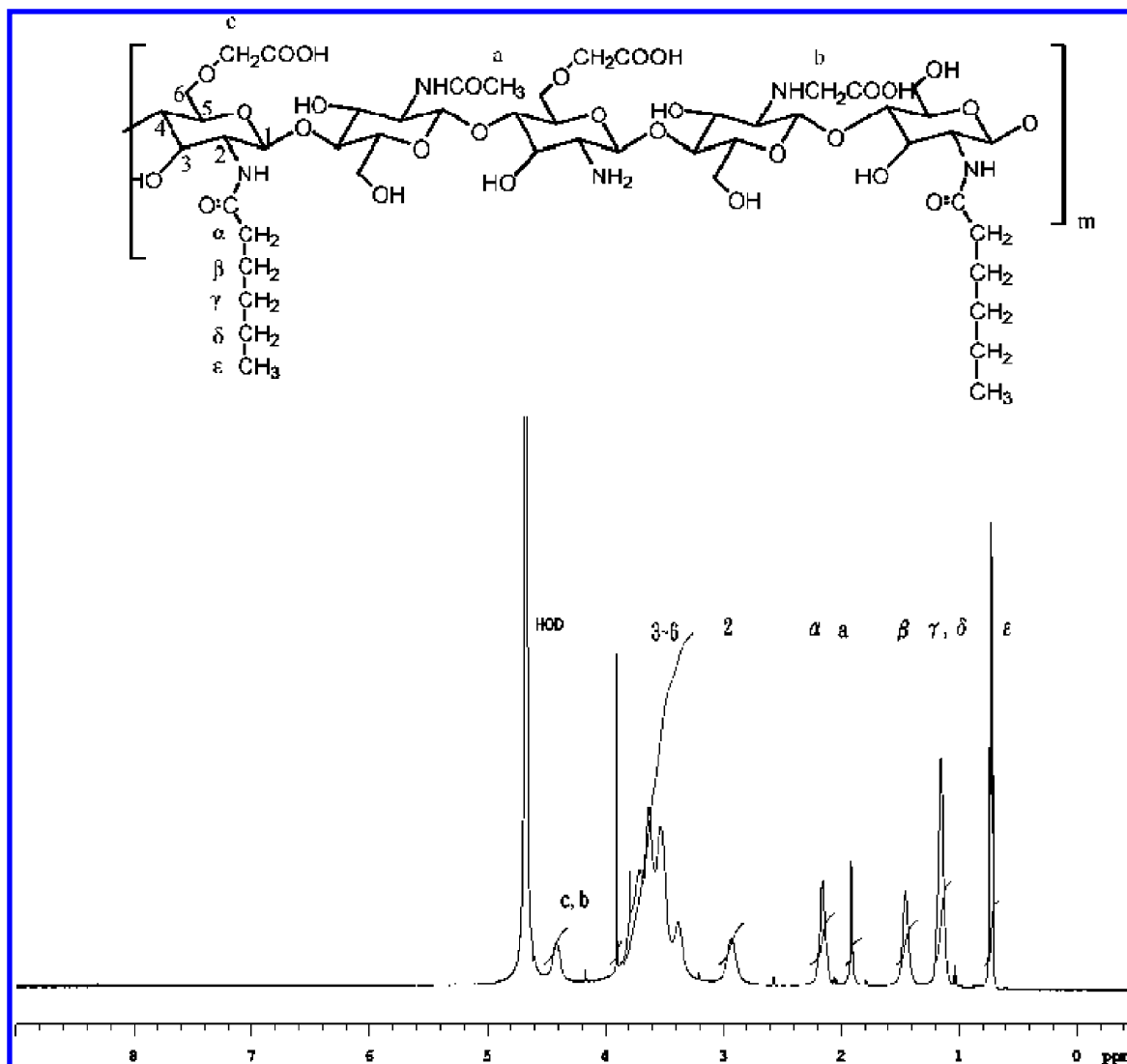


Figure 1. ^1H NMR spectrum of sample NOCHC-2B.

presented by plotting (M_t/M_0) -time curves, where M_0 is the initial amount of water in polymer ($M_0 = W_{\text{wet}} - W_{\text{dry}}$), and M_t is the amount of water remaining in the polymer at any given time ($M_t = W_t - W_{\text{dry}}$).

Drug Encapsulation Efficiency. Ibuprofen was employed as a partially hydrophobic model drug (molecular structure is shown in Scheme 1b). Ibuprofen-loaded monolithic films (matrix films) prepared by chitosan derivatives were obtained using the film-casting method. In brief, chitosan/acetic acid solution, NOCC/DI water solutions, and NOCHC/ethanol/DI water solutions with pH values of 7.0 were adjusted by NaOH solution (1 M), followed by mixing these polymeric solutions with saturated IBU/DI solution and genipin solution to form IBU-loaded polymeric solutions (IBU concentration: 1.2 mg/mL). Subsequently, those drug-loaded polymeric solutions were poured into Petri dish to form drug-loaded matrix films after cross-linking and drying at 50 °C for 2 days. To calculate the real encapsulation efficiency, these genipin-cross-linked matrix films were rinsed with ethanol solution for 30 s followed by measuring the amount of IBU rinsed out to ethanol solution (L_2) determined by UV-visible spectroscopy (Agilent 8453) at 264.4 nm through the use of predetermined standard concentration-intensity curve. The IBU encapsulation efficiencies (E) could be determined by eq 2

$$E = (L_1 - L_2)/L_1 \times 100\% \quad (2)$$

where L_1 is the initial loading amount of IBU incorporated.

Results and Discussion

Synthesis of NOCHC. Carboxymethyl-hexanoyl chitosan was synthesized using NOCC as the starting precursor. Upon substi-

tution reaction, a hydrogen atom on the amino group or hydroxyl group can be replaced by the hexanoyl group. Figure 1 shows the molecular structure and the ^1H NMR spectrum of NOCHC-2B. The chemical shift at 3.2 and 3.9 ppm was probably assigned to the proton of the segment with low molecular weight due to the acidic process with mild heating. The chemical shift at 1.9 and 2.9 ppm was assigned to the protons on the acetyl group and C2 position (H-2), respectively. In addition, the ring protons (H-3 to H-6) are considered to be resonated at 3.6–4.0 ppm. Moreover, the chemical shifts at 4.2 and 4.4 ppm were designated to the protons of NCH_2CO (N-position) and OCH_2CO (O-position) of NOCHC, respectively. On the other hand, the chemical shifts at 0.7 ppm (CH_3), 1.1 ppm ($\text{C}_\delta\text{H}_2$), 1.2 ppm ($\text{C}_\gamma\text{H}_2$), 1.5 ppm (C_βH_2), and 2.2 ppm ($\text{COC}_\alpha\text{H}_2$) were assigned to the protons on the hexanoyl group. This demonstrates hexanoyl substitution replaced some of the amino and hydroxyl sites of the *N,O*-carboxymethyl chitosan. The method for determining the degree of carboxymethyl substitution has been described in the literature, and the result is shown in Table 1.²¹ The degree of hexanoyl substitution was determined by eq 3 and shown in Table 1.

$$\text{DH} = \left(\frac{\text{integrated area at 0.75 ppm, CH}_3/}{\text{integrated area at 2.9 ppm, } ^1\text{H}} \right) \times 1/3 \quad (3)$$

where DH is the substitution degree of the hexanoyl group at amino sites.

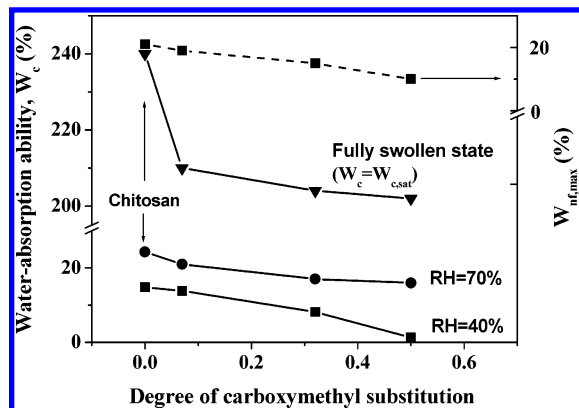


Figure 2. Dependence of carboxymethyl substitution on water-absorption ability (W_c , solid line) and maximal nonfreezable water amount ($W_{nf,max}$, dash line).

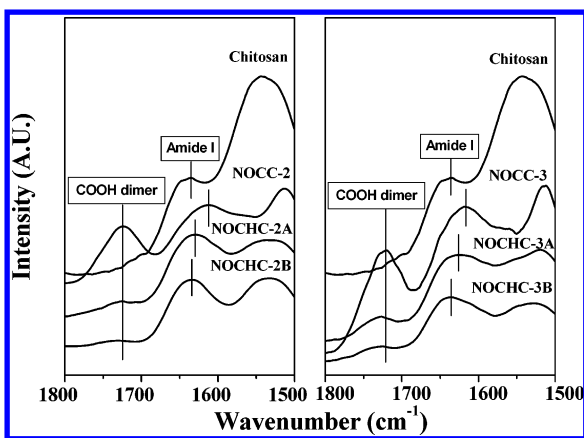


Figure 3. ATR-FTIR spectra of chitosan derivatives.

Moisture Absorption Test. The influence of carboxymethyl substitution on W_c (moisture absorption ability) and $W_{nf,max}$ (maximal nonfreezable water amount) values under the environment with various degrees of relative humidity (RH) is shown in Figure 2. Under low humidity (RH = 40%), the value of W_c was decreased with increasing degree of carboxymethyl substitution. This phenomena was also observed for $W_{nf,max}$ which is related to the number of tight water-binding sites, suggesting that W_c is closely correlated with the number of tight water-binding sites. In other words, water-binding site is the dominant factor for W_c under low humidity condition and decreased with increasing degree of carboxymethyl substitution. This is due to the fact that the samples with high carboxymethyl substitution favored the formation of intermolecular hydrogen bonding (polymer–polymer interaction), resulting in a structure with fewer tight water-binding sites (water–polymer interactions). This is further evidenced by the ATR-FTIR spectra shown in Figure 3. The characteristic peaks at 1730 cm^{-1} for the samples NOCC-2 and NOCC-3 were assigned to the carboxymethyl dimer ($\text{O}=\text{COH}\cdots\text{O}=\text{COH}$), suggesting an intermolecular interaction. Moreover, as compared with unmodified chitosan, it was found that the amide I characteristic peaks (1635 cm^{-1}) of samples NOCC-2 and NOCC-3 shifted significantly to lower wavenumber (red shifts $\delta = 20\text{ cm}^{-1}$), which was due to the formation of intermolecular hydrogen bonding between the amide group and carboxylic group ($\text{O}=\text{CNH}_2\cdots\text{O}=\text{COH}$) in the modified chitosan. This hydrogen bonding is stronger than that in unmodified chitosan (amide resonance H-bonding: $\text{O}=\text{CNH}_2\cdots\text{O}=\text{CNH}_2$) because the dipole moment of an N–H bond is smaller than that

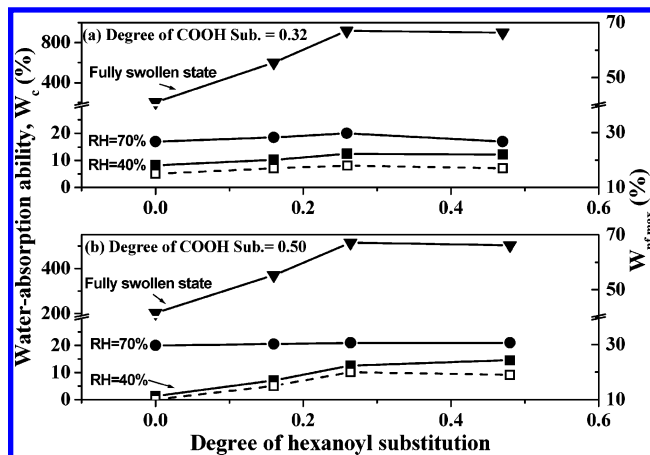


Figure 4. Dependence of hexanoyl group on water-absorption ability (W_c , solid line) and maximal nonfreezable water amount ($W_{nf,max}$, dash line).

of an O–H bond.²⁴ These strong intermolecular bonds reduced the number of tight water-binding sites (water–polymer interaction), resulting in the lowest water-absorption ability detected for the sample with the highest degree of carboxymethyl substitution (NOCC-3) under low humidity. However, the W_c of sample NOCC-3 was significantly enhanced when hexanoyl substitution was introduced (NOCHC-3A), as shown in Figure 4. This agrees with the finding that $W_{nf,max}$ of sample NOCHC-3A was significantly higher than that of sample NOCC-3. This implies that the number of tight water-binding sites increased with the incorporation of hexanoyl groups because it could inhibit the formation of intermolecular hydrogen bonding (i.e., $\text{O}=\text{CNH}_2\cdots\text{O}=\text{COH}$, $\text{O}=\text{COH}\cdots\text{O}=\text{COH}$) in NOCC, which is further demonstrated by the ATR-FTIR spectra shown in Figure 3. The red shift for the amide I characteristic peak observed for samples NOCHC-2A and NOCHC-3A was of a lesser extent than that for samples NOCC-2 and NOCC-3, respectively. Additionally, characteristic peaks at 1723 cm^{-1} assigned to carboxymethyl dimer were broadened and shifted to a higher wavenumber region for samples NOCHC-2A and NOCHC-3A. These two findings in the ATR-FTIR spectra suggest that the intermolecular hydrogen bonding in sample NOCC was inhibited to a certain extent (H-bonding inhibition effect) as the hexanoyl group was introduced, resulting in extensive water–polymer interactions and higher water-absorption ability under condition of low relative humidity.

For the fully swollen condition, the $W_{c,sat}$ value (i.e., equilibrium swelling ratio) was significantly decreased with increasing carboxymethyl substitution as shown in Figure 2. This can be ascribed to the fact that the intermolecular volume for free water was drastically reduced by the intermolecular electrostatic attraction (between COO^- and NH_3^+) and the above-mentioned intermolecular hydrogen bonding. Therefore, a higher $W_{c,sat}$ value was obtained for the unmodified chitosan compared with those samples with high carboxymethyl substitution (NOCC-2 and NOCC-3). However, as shown in Figure 4, the $W_{c,sat}$ values of samples NOCC-2 and NOCC-3 were significantly enhanced as the hexanoyl group was introduced (NOCHC-2A and NOCHC-3A). This is attributed to the fact that the formation of electrostatic attraction and the intermolecular hydrogen bond in NOCC was inhibited by the hexanoyl groups (NOCHC), which significantly enlarge the intermolecular volume for free water as further demonstrated by DSC test, and it will be discussed later. Conse-

(24) Smith, B. C. *Infrared spectral interpretation: a systematic approach*; CRC Press: Boca Raton, 1999; Chapter 4.

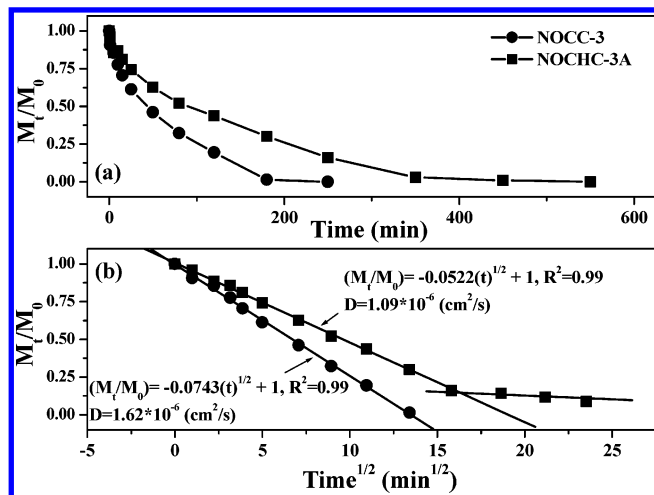


Figure 5. Deswelling profiles of samples NOCC-3 and NOCHC-3A.

quently, $W_{c,\text{sat}}$ values were significantly enhanced for NOCHC compared with NOCC. However, as the hexanoyl substitution was further increased, the above-mentioned H-bonding inhibition effect is compensated by hydrophobic interaction which acts as an intermolecular interaction and reduces the intermolecular volume for free water.²⁵ Therefore, the $W_{c,\text{sat}}$ value was slightly decreased with increasing hexanoyl substitution.

Under medium relative humidity (RH = 70%), as shown in Figure 2 and Figure 4, it was found that W_c values were not significantly altered with increasing carboxymethyl and hexanoyl substitutions which could be explained as follows. For all samples, W_c values were larger than the corresponding $W_{\text{nf,max}}$ but much lower than $W_{c,\text{sat}}$. Under this condition, water-absorption ability was mainly determined by the hydration volume (i.e., the amount of freezable bound water occupying the hydration layer) of the hydrophilic group. In other words, water-absorption ability was not altered significantly with the tight water-binding sites for nonfreezable bound water and the intermolecular volume for free water. Therefore, the W_c value was less sensitive to the degrees of carboxymethyl and hexanoyl groups under medium relative humidity.

Moisture Retention Characterization. The deswelling behavior of fully swollen hydrogel can be illustrated by plotting (M_t/M_0) versus time (t) as shown in Figure 5(a). The deswelling kinetics of hydrogels investigated in this study could be characterized by further plotting (M_t/M_0) $-t^{1/2}$ curves (Figure 5b) using eq 4 derived simply from Higuchi's model:²⁶

$$M_t/M_0 = -kt^{1/2} + 1 \quad (4)$$

where M_0 is the initial amount of water in polymer, M_t is the amount of water remaining in the polymer at any given time, and k is a constant.

Figure 5b shows that a linear (M_t/M_0) $-t^{1/2}$ curve of the sample NOCC-3A exhibited a one-stage profile which can be fitted to Higuchi's model, suggesting that the water release followed a diffusion control mechanism. To further understand the deswelling mechanism, the state of water in hydrogel was characterized by the DSC test. It is known that the state of water, and its corresponding DSC curve can be classified into three types: (i) nonfreezable bound water (where the DSC endothermic peak of water cannot be detected), (ii) freezable bound water (endothermic peak of water is much lower than 4.8 °C), (iii) free (freezable)

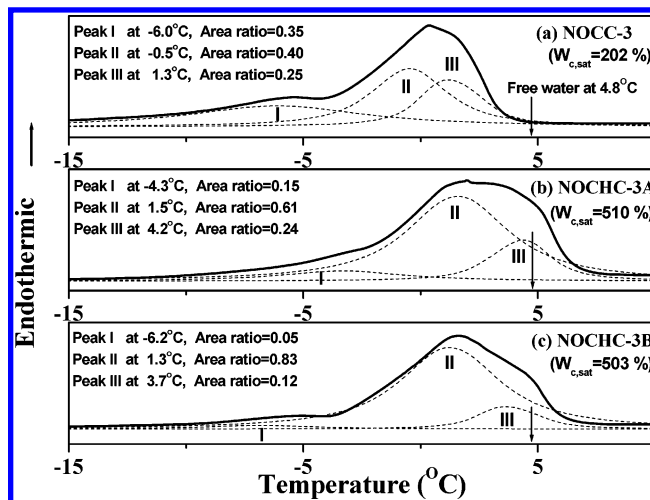


Figure 6. DSC curves of chitosan derivatives (carboxymethyl substitution degree = 0.5) measured at $W_c = 200\%$. Dash lines represent the curve fitting by Lorentzian curve-fitting procedure.

water (endothermic peak of water is close to 4.8 °C).²² DSC curves of the samples with high carboxymethyl substitution (0.5) measured at $W_c = 200\%$ are shown in Figure 6. A heavily overlapped band (solid line) was observed and could be divided into three peaks (dash lines) using the Lorentzian curve-fitting procedure. In Figure 6a, the endothermic peak of free water was not observed for the NOCC-3 hydrogel even under fully swollen state. Instead, the endothermic peaks at 1.3 °C and below assigned to freezable bound water were observed. Hence, it is reasonable to believe that the single release (deswelling) stage of sample NOCC-3 is mainly due to dehydrated-evaporation of freezable bound water. By contrast, Figure 5b shows that sample NOCHC-3A exhibited a two-stage profile, suggesting the state of water in sample NOCHC-3A was significantly different from that in sample NOCC-3 as previously described. This assumption is further supported by DSC curves shown in Figure 6b, the peak III of sample NOCHC-3A was very close to 4.8 °C, revealing that the NOCHC-3A hydrogel contained free water at and above $W_c = 200\%$. On the other hand, the peaks II and III were assigned to freezable bound water. Hence, it is reasonable to believe that the first and second release stages of sample NOCHC-3A are mainly due to dehydrated-evaporation of free water and freezable bound water, respectively. This demonstrates that the hexanoyl group played an important role in affecting free water. However, as the hexanoyl substitution was further increased (NOCHC-3B), the peak III shifts toward low temperature (Figure 6c). It implies that the intermolecular volume for free water was decreased with further increasing hexanoyl substitution.

An interesting finding can be seen in Figure 5b that the diffusion coefficient of free water released from sample NOCHC-3A was lower than that of freezable bound water released from sample NOCC-3. This means that the “bound” water has a faster diffusion rate compared with that of the “free” water through a given medium. This unusual result is probably attributed to a so-called “hydrophobic effect” under which water becomes more structured and less mobile in the vicinity of the hydrophobic group.²⁷ Hence, the diffusion coefficients of water molecules from sample NOCC-3 was higher than that from sample NOCHC-3A upon deswelling. This also implies that the water-retention ability of NOCHC is much better than that of NOCC.

Drug Encapsulation Efficiency. The influence of both carboxymethyl and hexanoyl substitutions on the encapsulation

(25) Bajpai, S. K. *J. Appl. Polym. Sci.* **2001**, *80*, 2782–2789.

(26) Siepmann, J.; Peppas, N. A. *Adv. Drug Delivery Rev.* **2001**, *48*, 139–157.

(27) Tamai, Y.; Tanaka, H.; Nakanishi, K. *Macromolecules* **1996**, *29*, 6750–6760.

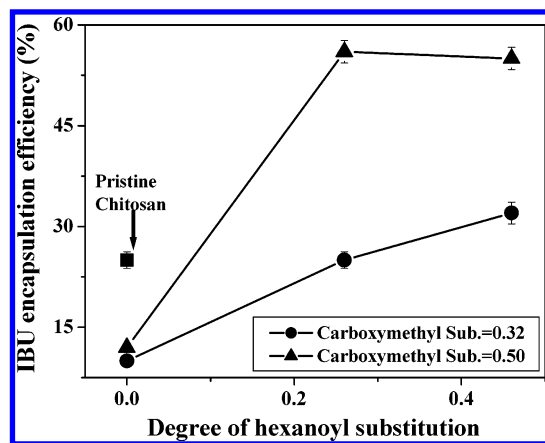


Figure 7. Dependences of degrees of carboxymethyl and hexanoyl substitution on ibuprofen encapsulation efficiency.

efficiencies of IBU within the samples are displayed in Figure 7. As can be seen, the encapsulation efficiency of NOCC was lower than that of pristine chitosan. It was probably due to the electrostatic attraction forces between COO^- (from IBU) and NH_3^+ (from chitosan), and this became more pronounced for the latter NH_3^+ groups that are richer in pristine chitosan than in its modified version, i.e., NOCC. In addition, IBU encapsulation efficiency was significantly enhanced when some of the amino groups in NOCC were substituted by the hexanoyl groups. It was ascribed to an increasing hydrophobicity of the polymer with the substitution of the hexanoyl group. On this base, it was believed that the critical micelle concentration (CMC) of IBU in polymeric solution was increased. Thus, IBU tended to be dissolved in the NOCHC polymeric solution in the form of molecules rather than micelles.²⁸ On the contrary, it was observed that IBU has a tendency to form micelles segregating on the surface of the NOCC polymer matrixes, thus lowering the real encapsulation efficiency because IBU segregation was easily rinsed out before release test. In addition, IBU in molecular form after encapsulation could increase the opportunity of drug–polymer electrostatic attraction such as $\text{IBU-COO}^- \cdots \text{NH}_3^+$ -polymer together with drug–polymer hydrogen bonding including $\text{IBU-COOH} \cdots \text{O}=\text{COH}$ -polymer and $\text{IBU-COOH} \cdots \text{O}=\text{CNH}_2$ -polymer, and this caused a higher miscibility of IBU in NOCHC matrixes. Consequently, encapsulation efficiencies of IBU in NOCHC-2A and NOCHC-3A were significantly higher than those in NOCC-2 and NOCC-3, respectively. Moreover, as shown in Figure 7, for the NOCHC samples with medium degree of carboxymethyl substitution (NOCHC-2A and NOCHC-2B), the encapsulation efficiency was increased with increasing hexanoyl substitution because the hydrophobicity of sample NOCHC-2B is higher than that of sample NOCHC-2A. However, for the samples with a high degree of carboxymethyl substitution (NOCHC-3A and NOCHC-3B), the hexanoyl group did not affect drug encapsulation efficiency

in a considerable manner because the amino group was proportionally decreased for the sample with a high degree of carboxymethyl and hexanoyl substitution (NOCHC-3B). Thus, the binding sites between IBU and polymer were mainly contributed by carboxymethyl substitution ($\text{IBU-COOH} \cdots \text{O}=\text{COH}$ -polymer) rather than the amino group alone. Therefore, although hydrophobicity of NOCHC-3B was higher than that of NOCHC-3A, the difference in encapsulation efficiency between NOCHC-3A and NOCHC-3B was not pronounced.

It is known that the Hildebrand solubility parameter is the most common value to indicate the miscibility of polymers.²⁹ However, the Hildebrand solubility parameter has its limitation to illustrate the drug encapsulation efficiency in some cases because a high encapsulation efficiency could be achieved via uniformly dispersing drug micelles in the polymer matrix despite poor miscibility. In other words, under certain conditions, the solubility parameter must be considered together with the structure (i.e., size and dispersal) of the drug micelles in the polymer matrix to explain the real encapsulation efficiency and release kinetics, which is not fully conclusive and awaits more detailed study. On the other hand, only the NOCC derivatives substituted with a hexanoyl group (alkyl length is 6 carbons) were investigated in the present study. However, our recent experiments for the NOCC derivatives with various alkyl lengths (6–16 carbons) seem to demonstrate differing cell adhesion behaviors, drug release profiles, and pH-sensitivity, which are being investigated and will be reported separately.

Conclusion

Amphiphatic hydrogel derived from chemically modified chitosan was successfully synthesized bearing both carboxymethyl and hexanoyl substitutions. Water-absorption ability decreased with increasing substitution with the carboxymethyl group but was significantly increased with the introduction of the hexanoyl group because it altered the number of water-binding sites and the state of water under low humidity and the fully swollen condition, respectively. Moreover, compared with NOCC, NOCHC also demonstrated enhanced water-retention ability. On the other hand, improved encapsulation efficiency for model drug ibuprofen was achieved for the NOCHC sample with high hexanoyl and carboxymethyl substitution. It is feasible to employ NOCHC hydrogel as a novel and potential amphiphatic carrier to effectively encapsulate and deliver numerous amphiphatic drugs, such as vancomycin and naproxen, for cell encapsulation or wound dressing material with excellent water-retention ability and predesigned drug release function for enhanced therapeutic efficacy.

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(28) Rodriguez, R.; Alvarez-Lorenzo, C.; Concheiro, A. *Eur. J. Pharm. Sci.* **2003**, *20*, 429–438.

(29) Sperling, L. H. *Introduction to physical polymer science*, 3rd ed.; Wiley-Interscience: New York, 2001; Chapter 4.