

Preparation of *O*-Hydroxypropyl-*N*-butyl Chitosan under Microwave Irradiation

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ABSTRACT: *O*-Hydroxypropyl-*N*-butyl chitosan (C_4 -HPCS) was prepared by microwave irradiation and phase-transfer catalysis; this consisted of two steps: (1) the synthesis of *O*-hydroxypropyl chitosan (HPCS) with chitosan and propylene oxide and (2) the synthesis of C_4 -HPCS with HPCS and 1-butyl bromide. The results of the experiment are as follows: Fourier transform infrared spectroscopy and $^1\text{H-NMR}$ displayed the characteristic peaks of C_4 -HPCS, thermogravimetric analysis showed that C_4 -HPCS was stable until 240°C , the critical micelle concentration was 0.025 wt %, the surface tension was equal to 65.70 ± 0.09 mN/m, the hydrophile-lipophile balance number value was 13.55, and the emulsifying power, foaming expansion, and foaming volume stability were 73.10, 45, and 94 wt %, respectively. This indicated that C_4 -HPCS had superior surface performance and more excellent hydrophilicity. In addition, the microwave irradiation and phase-transfer catalysis used in the experiment were considered to be more environmentally friendly and time-saving methods. © 2014 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* **2015**, *132*, 41527.

KEYWORDS: applications; biopolymers and renewable polymers; foams; surfactants; surfaces and interfaces

Received 9 July 2014; accepted 14 September 2014

DOI: 10.1002/app.41527

INTRODUCTION

Chitosan (CS), the unique alkaline cationic polysaccharide containing free amino groups in nature, is a linear semicrystalline copolymer of β -(1,4)-2-acetamido-2-deoxy-D-glucose and β -(1,4)-2-amino-2-deoxy-D-glucose units.¹ CS has been proven to have significant biological activities, including antifungal, antibacterial, antitumor, and antioxidant activities,^{2–6} and its advantages, including biocompatibility, biodegradability, nontoxicity, immune-enhancing effects, blood-coagulating effects, and wound-healing activities, have also been reported.^{7–11} It has been broadly applied in the fields of agriculture, medicine, functional food, biopolymer catalyst, and so on.^{12–15} Nevertheless, extended applications of CS are often restrained by its inferior processability and water solubility.^{16,17} Recently, many researchers have investigated the preparation of water-soluble CS derivatives via a variety of chemical modifications, including quaternization, *O*-acylation, phosphorylated, crosslink copolymers, grafting copolymerization, and so on.^{18–20} For example, Tharun et al.²¹ investigated the preparation of water-soluble CS via quaternization and microwave irradiation, and Feng and Xia²² reported the preparation of a water-soluble derivative of CS by acylation with fumaric acid. Elsabee et al.²³ reviewed the surface-active properties of CS and its derivatives. However, these articles mainly focused on the resolution of the water solubility problem through chemical modifications to

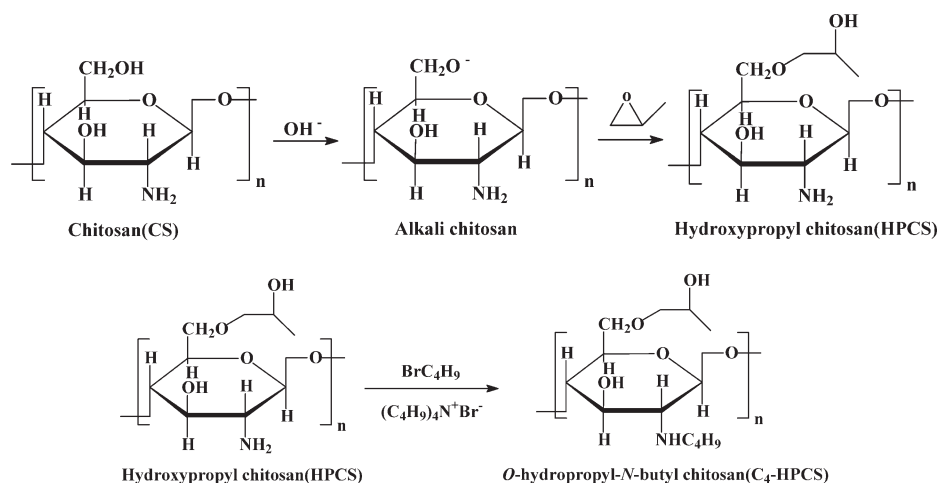
enlarge CS applications or only studied their surface-active properties. The preparation of water-soluble CS with a superior surface activity under more efficient and energy-saving conditions have recently attracted our attention. The surface activity properties, biocompatibility, and high preparative performance render CS with a wide range of applications in both biological and industrial fields. This field has been seldom reported.

In this study, hydroxypropyl and butyl groups were introduced on the $C_6\text{-OH}$ of CS and -NH_2 , respectively, to prepare excellent surface-active and water-soluble CS derivatives. Simultaneously, microwave irradiation and phase-transfer catalysis were innovatively integrated to boost the reaction efficiency.²⁴ Fourier transform infrared (FTIR) spectroscopy, $^1\text{H-NMR}$, thermogravimetry, and elemental analysis techniques were used to characterize the chemical structure and physical properties, and the surface performances of *O*-hydroxypropyl-*N*-butyl chitosan (C_4 -HPCS) was evaluated by thermal stability, critical micelle concentration (cmc), surface tension (γ), the value of hydrophile lipophile balance number (HLB), and the foaming and emulsifying performances.

EXPERIMENTAL

Materials and Equipment

CS from shrimp shells was acquired from Zhejiang Golden-Shell Biochemical Co., Ltd. (Taizhou, China). The degree of



Scheme 1. Synthesis routes, chemical structures, and abbreviations.

deacetylation was 92.2% (determined by elemental analysis), and its molecular weight was 590,000 (as determined by gel permeation chromatography). All of the chemicals, including isopropyl alcohol, tetramethyl ammonium hydroxide, propylene oxide, *N*-butane bromide, tetrabutyl ammonium bromide, and *N,N*-dimethylformamide, were analytical reagents and were used without further purification. MAS-II microwave oven was produced by Sineo Microwave Chemistry Technology Co., Ltd. (Shanghai, China).

Preparation of *O*-Hydroxypropyl Chitosan (HPCS) and *C*₄-HPCS

CS (2.0 g) and isopropyl alcohol (2 mL) were added to 3 g of a 40 wt % NaOH solution after 30 min of stirring to avert the aggregation of CS; this was then swelled for 2 h at room temperature and frozen for 20 h in the refrigerator. After thawing, isopropyl alcohol (20 mL), 1 mL of 10 wt % tetramethyl ammonium hydroxide and propylene oxide (35 mL) were added to the previous mixture before stirring for 1 h at room temperature. The CS mixture was transferred into a round-bottomed flask, and a magnetic stirrer was put into it. Then, the reaction flask was placed into a microwave oven, and the temperature was adjusted to a series of different temperatures. The mixture was heated for 40 min under microwave irradiation. After cooling and filtering, the filter cake was washed with isopropyl alcohol, distilled water, and acetone several times successively. Finally, the pale yellow powder HPCS was obtained after drying and grinding.

The obtained HPCS was dissolved in distilled water before it was stirred for 30 min at a temperature of 40°C; *n*-butyl bromide (7 mL) and tetrabutyl ammonium bromide (0.05 g) were added to the mixture and then reacted for 80 min at a temperature of 75°C. After cooling, dilute hydrochloric acid was added to the mixture until it was neutralized to the correct pH of 7. The reaction system was added to a large amount of ethanol; then, we precipitated, filtered, and washed the filter cake with methanol/water (85:15 v/v) and pure ethanol. Ultimately, the

yellow powder product *C*₄-HPCS was obtained after drying and grinding.

Measurement Methods

The molecular structures of the target products were characterized by a Shimadzu IR spectrometer (IRAFFINITY-1, Japan) with KBr pellets with a 400–4000 cm⁻¹ wave-number ranges and 4-cm⁻¹ resolutions. NMR spectral analysis (VNMRS 400 MHz) was also used to characterize *C*₄-HPCS.

The C/N proportional analysis of the target products was characterized by a Vario EL CUBE elemental analyzer (Elementar Co., Germany). The degree of substitution (DS) was calculated by the changes in the C/N ratio according to the following equation:²⁵

$$\frac{C}{N} = \frac{12(6+3DS)}{14} \quad (1)$$

Thermogravimetric analysis (TGA) was performed with a Sync Analyzer (Netzsch, Germany) under a nitrogen atmosphere of 0.15 MPa from 25 to 600°C at a heating rate of 10°C/min. The cmc value was obtained by the measurement of the γ s of the *C*₄-HPCS aqueous solutions at different concentrations with the Wilhelmy type at 30 ± 0.1°C with automatic γ equipment (BZY-1, Shanghai Hengping Instrument Factory, China).

The foaming performances were evaluated by the following methods. Volume (V_{10} ; mL) of the 0.1 wt % surfactant solution was added exactly into the scale tube and shaken vigorously 25 times. Then, we recorded the volume (V_{10} ; mL) of foam and liquid. After 10 min, the volumes (V_t 's; mL) of foam and liquid were recorded again. Furthermore, the foaming expansions (FEs) and foaming volume stabilities (FVSs) were calculated by the following equations, respectively:²⁶

$$FE = \frac{(V_{10} - V_{10})}{V_{10}} \times 100\% \quad (2)$$

$$FVS = \frac{V_{tt}}{V_{10}} \times 100\% \quad (3)$$

The emulsifying performances were measured by the following methods. The same volume of liquid paraffin was emulsified by

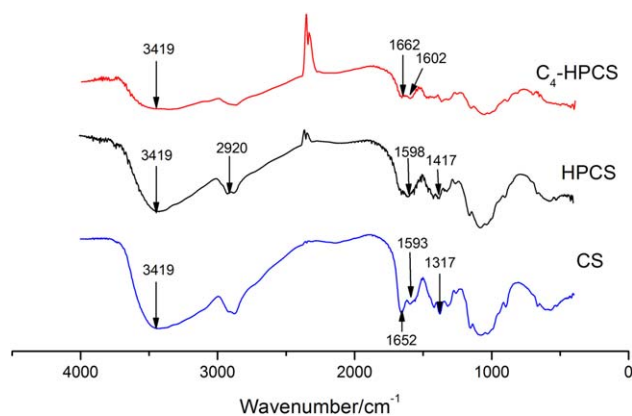


Figure 1. FTIR spectra of CS, HPCS, and C_4 -HPCS. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

6 mL of a 0.1 wt % surfactant solution. The volumes of the oil and water layers were recorded once every 30 min, and the emulsifying power was demonstrated by the volume fraction of the emulsion layers in the entire liquid.²⁷

The HLB value was measured by the water number method.²⁸ The calorimetric tube was added to a mixture of 25 mL of *N,N*-dimethylformamide/benzene (20:1 v/v) and the produced target (0.5 g). Water bath heating was provided to dissolve the previous mixture. After the mixture was cooled to $25 \pm 1^\circ\text{C}$, distilled water was slowly added dropwise with stirring. When we could see the printed III Times New Roman characters blurring on the other side of the paper from the front view of the curvature, it signified the end. Then, the volume of distilled water (amount of water used) was recorded, and the HLB value was obtained according to the standard curve.

RESULTS AND DISCUSSION

Synthetic Routes and Principles

Because CS has a lot of reaction groups, including C_2 - NH_2 , C_3 - OH , and C_6 - OH , the reaction occurred to the amino

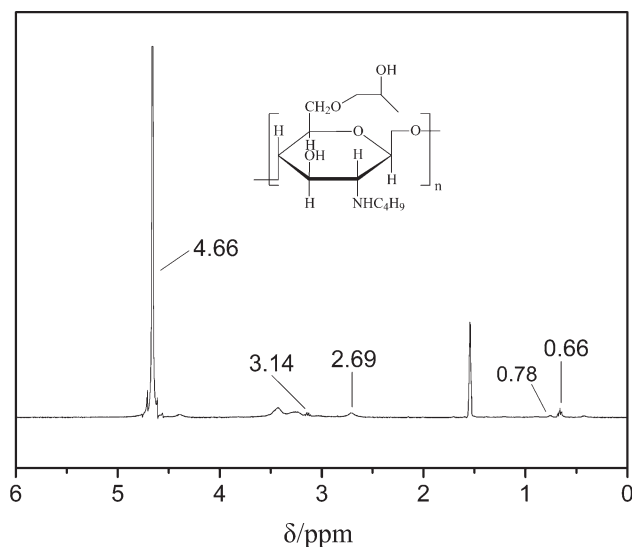


Figure 2. ^1H -NMR spectra of C_4 -HPCS.

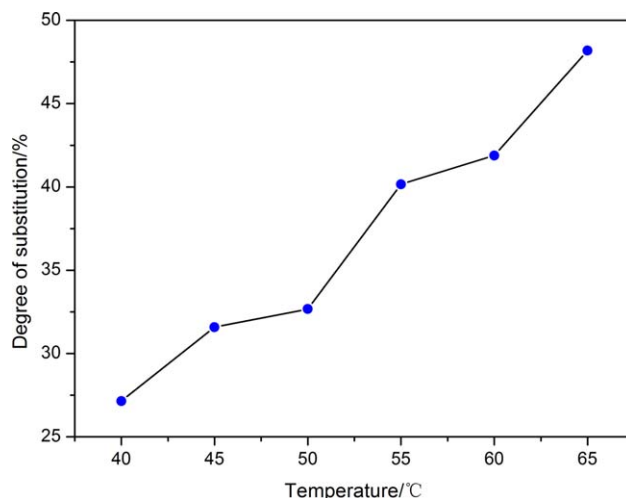


Figure 3. Curve on the DS of HPCS with different reaction temperatures. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

groups and hydroxyl groups. The reaction capacity of the amino group was much stronger under acidic or neutral conditions. In this experiment, C_6 - OH was substituted by the hydroxypropyl under alkaline conditions; this was attributed to nucleophilic ring-opening addition reaction.

The butylate reaction of HPCS was nucleophilic substitution. On the basis of the HPCS solution as the aqueous phase and *n*-butyl bromide as the organic phase, phase-transfer catalyst tetrabutyl ammonium bromide was added to transfer the aqueous phase HPCS to the organic phase; this drove the reaction to proceed. The synthesis routes, chemical structures, and abbreviations are presented in Scheme 1.

FTIR and NMR Spectral Analysis

The FTIR spectra of CS, HPCS, and C_4 -HPCS samples obtained under microwave irradiation and phase-transfer catalysis are shown in Figure 1.

According to the spectra, HPCS retained characteristic intensity peaks of the original CS at 3419 cm^{-1} ; this was the superimposition of the $-\text{OH}$ stretching vibration absorption peaks and $-\text{NH}$ stretching vibration absorption peaks.²⁹ After the hydroxypropyl was introduced onto CS, the $\text{C}-\text{H}$ stretching vibration absorption peaks at 2920 cm^{-1} were strengthened; this indicated that the product structure contained methyl and methylene, and the alkyls were connected to CS. The decrease in the absorption peak at 1652 cm^{-1} present in CS illustrated the reaction between the hydroxypropyl and a small amount of amino. The $\text{C}-\text{O}-\text{C}$ stretching vibration absorption peaks at 1000 – 1200 cm^{-1} explained that hydroxypropylation occurred mainly on C_6 - OH . In the meantime, the CS peaks at 1593 and 1652 cm^{-1} were fitted to form the absorption peaks of amino deformation vibrations at 1598 cm^{-1} because of the deacetylation of residual acetyl on CS. Moreover, according to the C_4 -HPCS IR spectrum, we discovered that the mixing band of HPCS at 1500 – 1640 cm^{-1} was separated after the reaction. The amino deformation vibration absorption peak at 1598 cm^{-1} disappeared and was obviously divided into two peaks at 1602

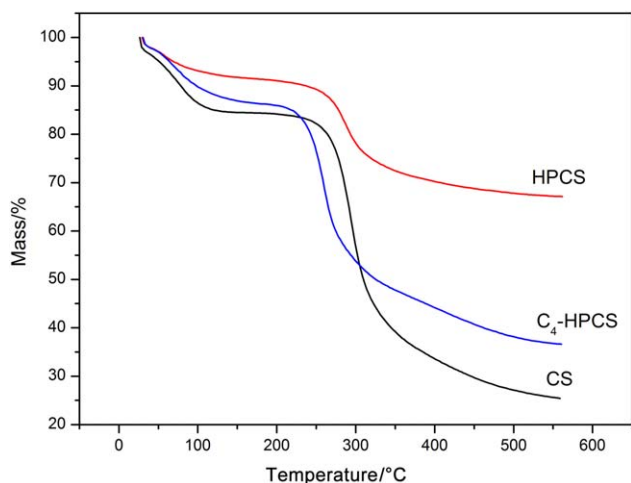


Figure 4. TGA of CS, HPCS, and C_4 -HPCS. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

and 1662 cm^{-1} , respectively; this indicated that the substitution reaction occurred to some amino groups; namely, these alkyl groups had been grafted onto the CS groups.

Figure 2 exhibits the $^1\text{H-NMR}$ spectra of C_4 -HPCS. As shown, the most intensive signal at a chemical shift value (δ) = 4.66 ppm corresponded to the solvent water. The peaks at δ values of 0.78 and 0.66 ppm were attributed to the methyl and methylene protons of the quaternized CSs. In addition, the chemical shifts at δ values of 2.69 and 3.14 ppm belonged to the hydrogen atom on the carbon atom connected to the $-\text{NH}-$ group. All of the previous proved that the target product C_4 -HPCS was acquired.

DS of HPCS

The C/N elemental proportion of HPCS was analyzed by an elemental analyzer, and a control variable method was used to evaluate the effects of the reaction temperature on the DS. The variation of the DS of HPCS with the reaction temperature is reported in Figure 3.

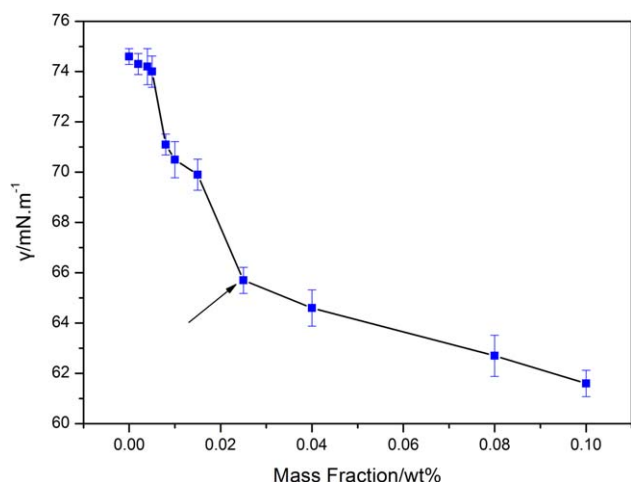


Figure 5. Curve of the concentration impact affecting γ of C_4 -HPCS. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table I. Foaming Performance of C_4 -HPCS

	V_{10} (mL)	V_{1t} (mL)	V_{t0} (mL)	V_{tt} (mL)	FE (%)	FVS (%)
C_4 -HPCS	7.40	8.39	13.40	12.62	45.00	94.00

V_{1t} , volume of liquid no including the volume of the foams.

As shown in Figure 3, it was not complicated to determine that the DS of HPCS increased with increasing reaction temperature, and the best DS products were obtained at 65°C . The reason for this was that the increase in the reaction temperature was beneficial for the penetration between the reactants, whereas the contact between propylene oxide and the active groups of the reactants accelerated the rate of the hydroxypropylated reaction. Meanwhile, side reactions also occurred, such as the degradation of the molecular chains. What is more, the propylene oxide boiling point was about 34°C ; this indicated that it was volatile. When the temperature was too high, the loss of propylene oxide was considerable during the reaction, so generally, a temperature of 60°C was optimal.

Performances of C_4 -HPCS

TGA. Figure 4 displays TGA thermograms of CS, HPCS, and C_4 -HPCS.

Apparently, the curves of CS, HPCS, and C_4 -HPCS were similar in the heating process; this demonstrated two discrete weight losses corresponding to the loss of water and the degradation of the polymer chains: From room temperature to 140°C , the weight loss rates were 14.52, 8.66, and 13.74%; these losses were triggered mainly by the loss of adsorbed water and crystal water. The loss from 240 to 450°C was greater. The weight loss rates were 54.68, 22.52, and 45.40% because the diminutive molecules of CS, HPCS, and C_4 -HPCS, respectively, such as acetyl groups, hydroxypropyl groups, and alkyl groups, were taken off because of the decomposition reaction.³⁰ Moreover, this indicated that C_4 -HPCS began to decompose at about 240°C . Meanwhile, CS and HPCS began to decompose at about 260°C . This supported the fact that the thermal stability of C_4 -HPCS was slightly worse than those of CS and HPCS.

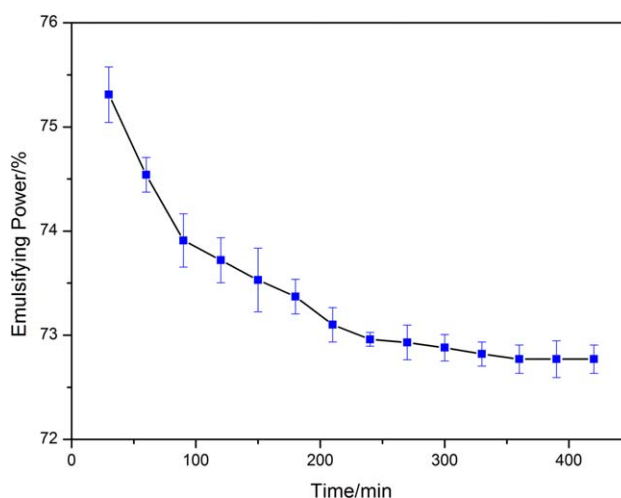


Figure 6. Emulsifying power of C_4 -HPCS. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table II. HLB Values and Corresponding V_{wc} Values

	Sample												
	1	2	3	4	5	6	7	8	9	10	11	12	13
ϕ (Tween 80; %)	100	95	90	85	80	70	60	50	40	30	20	10	0
ϕ (Span 85; %)	0	5	10	15	20	30	40	50	60	70	80	90	100
HLB value	15.0	14.3	13.7	13.0	12.4	11.0	9.7	8.4	7.1	5.8	4.4	3.1	1.8
V_{wc} (mL)	3.20	2.56	2.10	1.80	1.60	1.25	1.00	0.74	0.62	0.53	0.45	0.40	0.33

ϕ , concentrations of surfactants.

γ and cmc Values. Different concentrations of C_4 -HPCS aqueous were prepared, and their γ s were measured. The γ curve plotted with the different concentrations is shown in Figure 5.

According to the research,³¹ it has been proven that CS cannot reduce the γ of water. However, after modification, C_4 -HPCS had a significant surface activity. Figure 5 shows that as the concentration increased, γ decreased, and when the concentration reached a certain value, the γ tended to be smooth. This was because C_4 -HPCS adsorbed on the surface and generated hydrophilic skeletons in the water by hydrophobic group outward orientation; this made γ decrease in speed. When the amount reached a certain level, micelles were formed by the molecules in the solution thanks to hydrophobic association, and the γ of the solution was almost unchanged; this reflected the specific characteristics of the surfactant. As shown, its cmc was pointed out by an arrow. In this study, the cmc of C_4 -HPCS was 0.025 wt %, and the corresponding γ was equal to 65.70 ± 0.09 mN/m.

Foaming Performance. The measurement and evaluation of the foaming performances of C_4 -HPCS are shown in Table I. The foaming performance of surfactant included its foaming power and foaming stability.

As shown in Table I, with the FE value became larger, the foaming power was better and the FVS was higher; this indicated that the foam was more persistent. In this experiment, we knew that the C_4 -HPCS foaming power was 45 wt %, and its FVS was 94 wt %.

Emulsifying Performance. The test and evaluation of the emulsifying performance of C_4 -HPCS are shown in Figure 6.

The emulsifying power of C_4 -HPCS was observed to change little over time. The data shown in Figure 6 demonstrated that the emulsifying power value was 73.10 wt % or greater. This illustrated that C_4 -HPCS showed excellent emulsifying performances for the melted paraffin. From the perspective of molecular structures, C_4 -HPCS molecules with hydrophobic groups tended to adsorb at the interface film to form a more compact arrangement; this made it easier to associate at the same time. What is more, C_4 -HPCS, as a polymer, had a definite viscosity, which

played a crucial role in the emulsifying stability. From what we have discussed in this part, we safely drew the conclusion that the emulsifying power of C_4 -HPCS was remarkable.

Values of HLB. The HLB values and the corresponding volumes of water consumed (V_{wc} 's) of the surface activity are shown in Table II. The data in Table I could be used to draw a smooth curve about the HLB value and the corresponding V_{wc} which is the HLB standard curve. With the water number by this standard curve, we could measure the V_{wc} values of the samples, which we could use to get the HLB values of samples.

As shown in Table III, the V_{wc} of C_4 -HPCS measured by the water number method number was 2.00 mL; this illustrated that the HLB value of C_4 -HPCS was 13.55. As we all know, the greater the HLB value is, the stronger the hydrophilicity will be; namely, the greater the solubility of water will be. Therefore, C_4 -HPCS had a strong hydrophilicity. Whether the C_4 -HPCS surfactant can be used as an emulsifier and detergent will be judged preliminarily by its HLB values.

CONCLUSIONS

In this article, C_4 -HPCS was successfully prepared under microwave irradiation and phase-transfer catalysis; this greatly reduced the reaction time from the conventional 16 h to 120 min. In the C_6 -OH hydroxypropyl substitution reaction, 60°C was the most advisable. Moreover, thermal analysis showed that C_4 -HPCS was stable until 240°C, the cmc was 0.025 wt %, and the γ was equal to 65.70 ± 0.09 mN/m. FE and FVS of the 0.1 wt % C_4 -HPCS solutions were 45 and 94 wt %, respectively. In addition, an emulsifying power of 73.10 wt % or higher was observed, and an HLB value of 13.55 was obtained. As a whole, water-soluble C_4 -HPCS with a distinguished surface activity could be prepared under more efficient and energy-saving conditions.

ACKNOWLEDGMENTS

This work was funded by the Shenzhen University Challenge Cup University Student Extracurricular Academic Innovative Research Project (contract grant number FM017). The authors thank Bo Liu and Zhongkuang Luo for enlightening discussions and the reviewers for their constructive criticism and critical input.

REFERENCES

- Luo, J. W.; Wang, X. Y.; Xia, B.; Wu, J. J. *Macromol. Sci. Pure Appl. Chem.* **2010**, *47*, 952.

Table III. HLB Values of C_4 -HPCS

	V_{wc} (mL)	HLB value
C_4 -HPCS	2.00	13.55

2. Guo, Z.; Ren, J.; Dong, F.; Wang, G.; Li, P. *J. Appl. Polym. Sci.* **2013**, *127*, 2553.
3. Geng, X.; Yang, R.; Huang, J.; Zhang, X.; Wang, X. *J. Food Sci.* **2013**, *78*, M90.
4. Hussein, M. H. M.; El-Hady, M. F.; Shehata, H. A. H.; Hegazy, M. A.; Hefni, H. H. H. *J. Surfactants Detergents* **2013**, *16*, 233.
5. Philippi, C.; Loretz, B.; Schaefer, U. F.; Lehr, C. M. Chitosan-Based Systems for Biopharmaceuticals: Delivery, Targeting and Polymer Therapeutics; John Wiley & Sons: New York, **2012**; p 381.
6. Woranuch, S.; Yoksan, R. *Carbohydr. Polym.* **2013**, *96*, 495.
7. Arai, K.; Kinumaki, T.; Fujita, T. *Bull. Tokai. Region Fish. Res. Lab.* **1968**, *89*, 165.
8. Chen, W. R. *Clin. Dev. Immunol.* **2013**, *2013*, 1740.
9. Okamoto, Y.; Yano, R.; Miyatake, K.; Tomohiro, I.; Shigemasa, Y.; Minami, S. *Carbohydr. Polym.* **2003**, *53*, 337.
10. Chang, J.; Liu, W.; Han, B.; Peng, S.; He, B.; Gu, Z. *Wound Repair Regen.* **2013**, *21*, 113.
11. Singh Dhillon, G.; Kaur, S.; Jyoti Sarma, S.; Kaur Brar, S.; Verma, M.; Yadagiri Surampalli, R. *Current Tissue Eng.* **2013**, *2*, 20.
12. Sachdev, D. P.; Cameotra, S. S. *Appl. Microbiol. Biotechnol.* **2013**, *97*, 1005.
13. Azadi, A.; Hamidi, M.; Rouini, M.-R. *Int. J. Biol. Macromol.* **2013**, *62*, 523.
14. Qin, Y.-Y.; Yang, J.-Y.; Lu, H.-B.; Wang, S.-S.; Yang, J.; Yang, X.-C.; Chai, M.; Li, L.; Cao, J.-X. *Int. J. Biol. Macromol.* **2013**, *61*, 312.
15. Baig, R. B. N.; Varma, R. S. *Green Chem.* **2013**, *15*, 1839.
16. Luo, B.; Yang, J.; Zhao, J.; Hsu, C.; Li, J.; Zhou, C. *J. Appl. Polym. Sci.* **2012**, *125*, E125.
17. Alves, N.; Mano, J. *Int. J. Biol. Macromol.* **2008**, *43*, 401.
18. Jayakumar, R.; Selvamurugan, N.; Nair, S.; Tokura, S.; Tamura, H. *Int. J. Biol. Macromol.* **2008**, *43*, 221.
19. Domard, A. *Carbohydr. Polym.* **2011**, *84*, 696.
20. Romero, M.-A.; Domard, A.; Petit, D. *Polymer* **1993**, *34*, 3004.
21. Tharun, J.; Kim, D. W.; Roshan, R.; Hwang, Y.; Park, D.-W. *Catal. Commun.* **2013**, *31*, 62.
22. Feng, Y.; Xia, W. *Carbohydr. Polym.* **2011**, *83*, 1169.
23. Elsabee, M. Z.; Morsi, R. E.; Al-Sabagh, A. *Colloids Surf. B* **2009**, *74*, 1.
24. Liu, M.; Hu, W. X. *Adv. Mater. Res.* **2013**, *616*, 1711.
25. Domard, A.; Rinaudo, M.; Terrassin, C. *Int. J. Biol. Macromol.* **1986**, *8*, 105.
26. Dutkiewicz, J.; Tuora, M. Advances in Chitin and Chitosan, In Advances in Chitin and Chitosan; Elsevier: New York; **1992**, p. 496.
27. Pei-Kun, M. Industrial Analysis of the Synthetical Detergents; Light Industry: Beijing, 1988.
28. Yumin, T. Synthetic Detergent and Its Application; China Textile: Beijing, 2006.
29. Sahu, A.; Goswami, P.; Bora, U. *J. Mater. Sci. Mater. Med.* **2009**, *20*, 171.
30. Wang, X.; Du, Y.; Yang, J.; Wang, X.; Shi, X.; Hu, Y. *Polymer* **2006**, *47*, 6738.
31. Pepić, I.; Filipović-Grčić, J.; Jalšenjak, I. *Colloids Surf. A* **2008**, *327*, 95.