Synthesis of O-Hydroxypropyl-*N*-Alkylated Chitosans and Characterization of Their Surface Features

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A class of novel amphiphilic chitosan derivates, the polymeric surfactant O-hydroxypropyl-*N*-alkyl chitosans (Cₙ-HPCS), was synthesized by first modifying chitosan into the more hydrophilic O-hydroxypropyl chitosan and then alkylating the amino groups with different long carbon chains. The structures were characterized using ^{1}H NMR and FT-IR and several surface activity parameters, including surface tension, critical micelle concentration (CMC), hydrophile-lipophile balance (HLB), foam characteristics and emulsibility, were measured. The critical micelle concentration values ranged from 0.016 g/L to 0.05 g/L and the hydrophile-lipophile balance values were between 5.33 and 13.89. The structures displayed good foam characteristics and the emulsibility was better than that of Tween 60. Because of these good surface activity properties, the Cₙ-HPCS have many potential surfactant applications.

Key Words: Chitosan modification, Amphiphilicity, Polymeric surfactant.

INTRODUCTION

Surface active agents or surfactants are amphiphilic molecules, with both hydrophilic and hydrophobic features. The hydrophobic part of these molecules is usually a long hydrocarbon chain that may be branched. The hydrophilic part may be anionic, cationic or simply a polar group (*e.g.*, -OH). This dual functionality permits amphiphiles to accumulate at interfaces such as those between a solution and air, a solution and a solid (*e.g.*, at the walls of a container) or most practically interesting, between two liquids, such as water and oil. Polymers are high molar mass substances composed of a large number of repeating units. Naturally occurring polymers include proteins, starches, cellulose and latex. Combining surfactants and polymers often improves the desired properties of the product. Surfactants are usually added to control the dispersion, flocculation and wetting properties of suspensions, whereas polymers are usually added to meet rheological requirements. Polymer surfactants have a few key advantages over other surfactants: (i) they do not have a critical micelle concentration (CMC) and may be used at concentrations below the normal critical micelle concentration of the monomer, (ii) they are stable in the presence of a high content of organic solvents and (iii) they can be used in conjunction with inclusion molecules (*e.g.*, cyclodextrins) without significant interference from the formation of host-guest complexes with the surfactant[1]. Various industries, including the cosmetic industry, the oil suspension and removal engineering industry and the detergent products manufacturing industry are increasingly pursuing natural polymeric surfactants due to health concerns and the practical advantages of these materials.

Chitosan is a polymer of β(1→4) linked 2-amino-2-deoxy-β-D-glucosamine that is manufactured using alkaline deacetylation of chitin that is extracted primarily from arthropods, insects and mushrooms. Compared with synthetic materials, chitosan is nontoxic, non-irritant, non-allergenic and non-mutagenic and exhibits greater biocompatibility and biodegradability[2]. However, applications of chitosan are limited because of water insolubility and low acid-concentration solubility properties. The utility of chitosan can be greatly expanded by using surface modifications such as quaternization[3], hydroxyalkylation[4], carboxyalkylation[5] and other methods[6] to introduce functional groups that improve aqueous solubility.[7] The surface activity and the aggregation properties of chitosan in water have been described previously[8]. The surface tension of chitosan in water at 25 ºC and pH 5.06 was 71.45 mN/m, a value that is close to the surface tension of pure water, 71.99 mN/m at 25 ºC. This result indicates that native chitosan molecules are excluded from the air/solution interface and do not exhibit surface activity.

This paper describes a simple method to produce chitosan-derived surfactants. The hydrophilic O-hydroxypropyl chitosan backbone is prepared first, then long, hydrophobic carbon chains (C₄, C₈, C₁₂, C₁₆) are grafted onto it to form a polymeric surfactant with a hydrophilic backbone and hydrophobic side

chains, as shown in **Scheme-I**[9]. The full synthesis of O-hydroxypropyl-*N*-alkyl chitosan is detailed in **Scheme-II**.
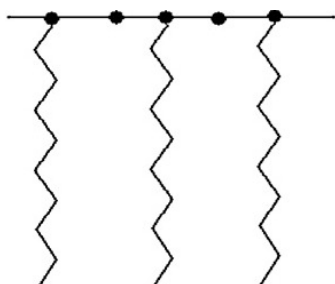
Scheme-I Schematic diagram of polymeric surfactants with a hydrophilic backbone and hydrophobic side chains

$$R = C_nH_{2n+2} \ (n = 4,8,12,16)$$

when n = 4,8, phase transfer catalyst is tetrabutyl ammonium bromide; when n = 12,16, phase transfer catalyst (PTC) is cetyltrimethyl ammonium bromide.
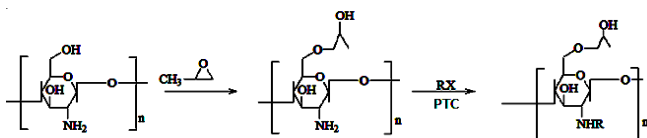
Scheme-II Synthesis of O-hydroxypropyl-*N*-alkyl chitosan

Because of the special behaviour of amphiphilic chitosan derivatives, the possible applications of a variety of these compounds have been intensively studied[10]. These studies of amphiphilic chitosan derivatives, however, did not include detailed examinations of fundamental surfactant activity properties, such as surface tension, critical micelle concentration, hydrophile-lipophile balance (HLB), foam characteristics and emulsibility. In this paper, the structures of a novel class of amphiphilic chitosan derivatives were characterized with ^{1}H NMR and FT-IR and the surfactant activity properties of surface tension, critical micelle concentration, hydrophile-lipophile balance, foam characteristics and emulsibility were evaluated. These data provide valuable reference information for future practical applications of these amphiphilic chitosan materials.

EXPERIMENTAL

Chitosan (shrimp shell, powders, MW 50 kDa, DD > 90 %) was supplied by Zhejiang Golden-shell Biochemical Co. Ltd., (China) and used without further treatment. Isopropyl alcohol and propylene oxide were purchased from Sinopharm Chemical Reagent Co. Ltd., (China). All other chemicals were obtained from Beijing Chemical Works (China). All reagents were of analytical grade and were used without further purification.

Synthesis of O-hydroxypropyl chitosan (O-HPCS): The synthesis of O-HPCS was performed according to an improved version of the method of Xie *et al.*[11]. A 2 g sample of chitosan powder was soaked in 5 mL of 50 % (w/w) aqueous NaOH for several hours, then lyophilized. The alkalized, dried chitosan was mixed with 20 mL of isopropanol in a three-mouth flask and stirred for 0.5 h at room temperature followed by dropping 1 mL catalyst-tetramethyl ammonium hydroxide and 20 mL propylene oxide, stirring for 1 h at room temperature.

The solution was then heated at 60 ºC for 8 h. After cooling to room temperature, the isopropanol was removed, 20 mL of distilled water was added and the mixture was stirred until the precipitate was completely dissolved. The resulting solution was centrifuged at 4000 rpm for 10 min and the supernatant was evaporated at 80 ºC to remove the remaining isopropanol and propylene oxide to yield a pure alkaline solution of O-HPCS.

Alkylation of water-soluble chitosan: The alkaline solution of O-HPCS was stirred at 40 ºC for 0.5 h, then 5 mL alkyl halide was added and the mixture was heated to 80 ºC. After adding 0.06 g of the phase transfer catalyst (PTC), the mixture was stirred for 8 h. Then, the mixture was cooled to room temperature and the pH was adjusted to 7 with dilute hydrochloric acid. The crude product was precipitated with acetone, collected by vacuum filtration, washed with methanol and anhydrous alcohol and vacuum dried at 60 ºC to yield a pale white solid.

Characterization and identification: ^{1}H NMR spectra were collected on a 600 MHz Bruker AV spectrometer. Chitosan and an O-hydroxypropyl-*N*-alkyl chitosan (*e.g.*, C16-HPCS) were dissolved in 5 % (v/v) CD_3COOD/D_2O. FT-IR spectra were recorded on FTIR-8900 infrared spectrometer (Shimadzu Kyoto, Japan) in KBr discs. The intrinsic viscosity [η] and molecular weight (M) of the chitosan derivates were measured with a 140 mm ± 0.5 mm ubbelohde viscometer and calculated according to the Mark-Houwink equation[12].

Measurement of liquid surface tension and critical micelle concentration: A concentration series of aqueous solutions of the C_n-HPCS was prepared and the solutions were allowed to stand for 1 day to allow the solids to completely dissolve. The surface tensions were measured with a QBZY-2 automatic surface tension meter (Fangrui Shanghai, China) at 25 ± 0.1 ºC. The surface tension-concentration plots were used to determine the critical micelle concentrations[13,14].

Measurement of hydrophile-lipophile balance: The hydrophile-lipophile balance values were measured using the water number method[15,16]. Briefly, 0.5 g of surfactant was suspended in 25 mL of a *N,N*-dimethyl formamide-benzene mixture (100 + 5) in a colourimeter tube and heated at 70-80 ºC until the surfactants were fully dispersed. The mixture was then titrated with distilled water with continuous magnetic stirring until it became turbid.

Evaluation of foaming properties and emulsifying power: A test tube containing 10 mL of aqueous surfactant solution was shaken vigorously 25 times. The heights of the foam and liquid regions were recorded and the tube was set aside. The heights of the foam and the liquid were measured and recorded again after 10 min and 30 min. The foaming properties were evaluated according to the following formulae[17]:

$$\text{Foam expansion (FE)} = \frac{V_{t0} - V_{10}}{V_{10}} \times 100\%$$

$$\text{Foam liquid stability (FLS)} = \frac{V_{10} - V_{1t}}{V_{10}} \times 100\%$$

$$\text{Foam volume stability (FVS)} = \frac{V_{tt}}{V_{t0}} \times 100\%$$

where, V_{10} is the starting liquid volume, V_{1t} is the liquid volume at a given time after shaking, V_{t0} is the total volume of foam and liquid immediately after shaking and V_{tt} is the total volume of foam and liquid at a given time after shaking.

To evaluate the emulsifying power of the C_n-HPCS surfactants, a 10 g/L solution of each compound was prepared and 10 mL aliquots of these solutions were mixed with 10 mL of liquid paraffin oil. The surfactant/paraffin mixtures were shaken vigorously 25 times and the separation of the emulsion was determined visually by pouring the emulsion into a 25 mL graduated cylinder and monitoring the appearance of the oil layer at the top of the solution[18]. The volumes of oil and water were recorded every 0.5 h. The ratio between the volume of the emulsion layer and the total liquid volume indicates the emulsifying power of the surfactant[19].

RESULTS AND DISCUSSION

Synthesis and characterization of chitosan derivatives: The synthesis of O-hydroxypropyl-*N*-alkyl chitosan is briefly summarized in **Scheme-I**. The structural changes in the chitosan derivatives were confirmed using FT-IR (Fig. 1) and ^{1}H NMR (Fig. 2). The infrared spectra of the four derivatives are very similar (Fig. 1). The stronger absorptions around 2890 cm^{-1} and 2915 cm^{-1} can be attributed to C-H stretching vibrations arising from the introduction of methyl and methylene groups. The splitting of the chitosan amide peak at 1640-1500 cm^{-1} indicates that substitution reactions occurred at -NH_2. The strong absorptions of C-O-C at 1200-1000 cm^{-1} and -OH at 3440 cm^{-1} can be assigned to the replacement of the C_6-OH groups of native chitosan by a large number of hydroxypropyl groups. These features of the IR spectra indicate that chitosan was successfully conjugated with both hydroxypropyl groups and alkyl groups.
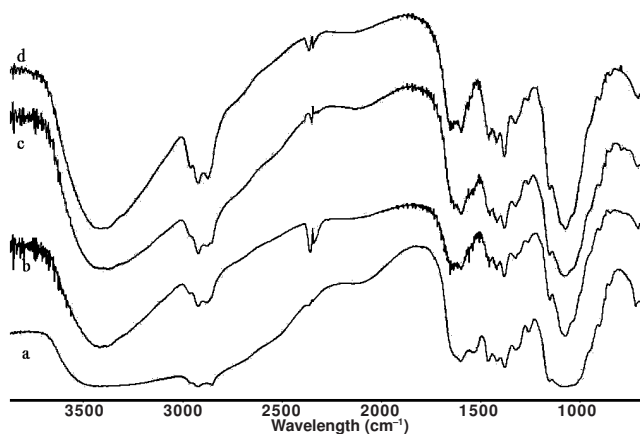

Fig. 1.   FT-IR spectra of a) C4-HPCS, b) C8-HPCS, c) C12-HPCS, d) C16-HPCS

The ^{1}H NMR spectra of native and modified chitosan are shown in Fig. 2. Compared with native chitosan, the ^{1}H NMR spectrum of O-HPCS revealed new signals at δ 1.0-1.2 ppm and δ 4.2-4.5 ppm that could be assigned to the methyl and methenyl hydrogens of the hydroxypropyl groups (-$CH_2CHOHCH_3$), enhanced signals at δ 3.4-3.6 ppm that could be attributed to the added methylene hydrogens around the newly formed ether linkage and no significant changes

elsewhere, consistent with the conversion of the C6-OH of chitosan to a hydroxypropyl ether. Compared with O-HPCS, the ^{1}H NMR spectrum of C_n-HPCS (n = 16) revealed new signals at δ 0.86 and δ 1.09 that could be assigned to the methyl and methene hydrogens of the alkyl groups (-C_nH_{2n+1}) and greatly increased signal strength at δ 1.09, also consistent with the introduction of alkyl groups. The signals at δ 2.69 can be assigned to the hydrogen with the carbon connected to the amino groups and the enhancing of which indicated that the alkylation reaction was occurred at the amino position of chitosan.
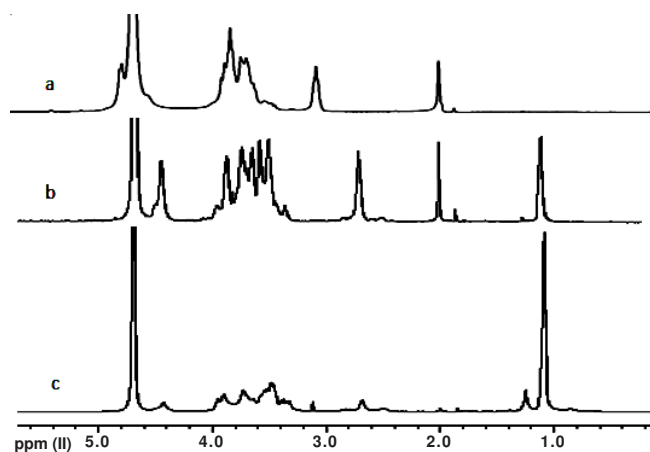

Fig. 2.   ^{1}H NMR spectra of a) native chitosan, b) O-HPCS and c) C_n-HPCS (n = 16). Resonances at d 4.6-4.8 arise from the solvent.

Measurement of liquid surface tension and critical micelle concentration: The reference value for the average surface tension of pure water at 23 ºC obtained in this study, 72.54 ± 0.74 mN/m (Fig. 3), was consistent with previously reported results[13]. The surface activity and the aggregation properties of native chitosan in water have been previously described[20]. The surface tension value was very close to that of pure water, an indication that native chitosan molecules are excluded from the air/solution interface and do not exhibit surface activity. In contrast, the surface tension of aqueous solutions of the four C_n-HPCS chitosan derivatives decreased steeply at very low concentrations (Fig. 3). At higher concentrations of the chitosan derivatives, the surface tension of the solutions plateaued. This surface tension trend is characteristic of most surfactants[14]. When the concentration of the surfactant reaches a certain level, the liquid surface becomes saturated with adsorbed surfactant molecules and the surface tension of the solution can no longer change. This point, when micelles just begin to form, is known as the critical micelle concentration (CMC). As shown in Fig. 4, the critical micelle concentration values of the four C_n-HPCS chitosan derivatives decrease from 0.05 g/L to 0.016 g/L as the length of the carbon chains increases. This correlation between critical micelle concentration and carbon chain length is consistent with the results of Negm *et al.*[21]. The minimum surface tension produced by the surfactant also decreases as the length of the carbon chains increases. The lowest surface tension value in this study, 24 mN/m, was observed with the C16-HPCS solution.

Measurement of hydrophile-lipophile balance: An hydrophile-lipophile balance value calibration curve was prepared according to the water number method[22]. The

composition of the standard solutions is given in Table-1 and the resulting calibration curve is shown in Fig. 4. As shown in Table -2, the hydrophile-lipophile balance values of the chitosan derivatives decreased as the length of the alkyl carbon chain increased. The most hydrophilic chitosan derivative was C4-HPCS, with an hydrophile-lipophile balance of 13.89, whereas the most lipophilic chitosan derivative was C16-HPCS, with an hydrophile-lipophile balance of 5.33. A high hydrophile-lipophile balance value indicates that the surfactant is readily soluble in water. A low hydrophile-lipophile balance value indicates that the surfactant is difficult to dissolve in water and effectively decreases the surface tension by setting the molecules at the water surface in motion. The hydrophile-lipophile balance data suggest that this class of novel surfactants may be useful as emulsifiers or detergents[23].
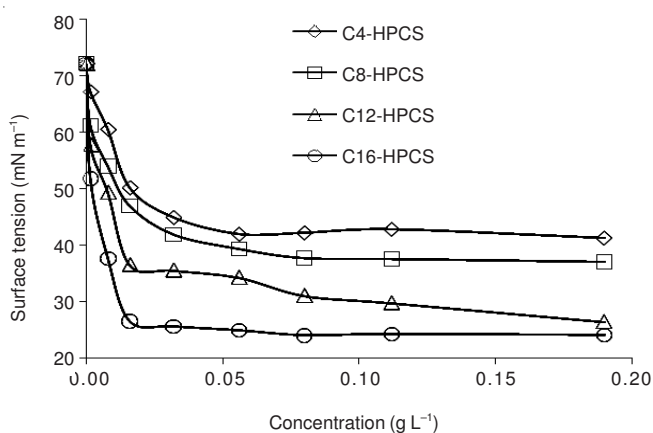

Fig. 3. Effect of Cn-HPCS surfactants on aqueous surface tension

TABLE-1
HYDROPHILE-LIPOPHILE BALANCE (HLB)
VALUE CALIBRATION GRADIENT

Formulation (F)	Tween 80 (%)	Span 85 (%)	HLB value	Water number (mL)
F1	100	0	15.0	3.20
F2	95	5	14.3	2.56
F3	90	10	13.7	2.10
F4	85	15	13.0	1.80
F5	80	20	12.4	1.60
F6	70	30	11.0	1.25
F7	60	40	9.7	1.00
F8	50	50	8.4	0.74
F9	40	60	7.1	0.62
F10	30	70	5.8	0.53
F11	20	80	4.4	0.45
F12	10	90	3.1	0.40
F13	0	100	1.8	0.33
F14	(Tween 40)100	0	15.6	4.20

Evaluation of foaming properties and emulsifying power: The foam expansion and stability of the C$_n$-HPCS derivatives are presented in Table-3. The long carbon chain of C16-HPCS allows it to foam easily and it exhibited the best foam expansion, with an foam expansion value of 85 %. All four surfactants exhibited foam liquid stability value of 5 %, which indicates that a small amount of the liquid was held in the foam as thick liquid films. The foam volume stability values ranged from 84.1 % to 90.8 %, but did not vary significantly.

These high values indicate that all of the C$_n$-HPCS surfactants created stable, long-lasting foams.
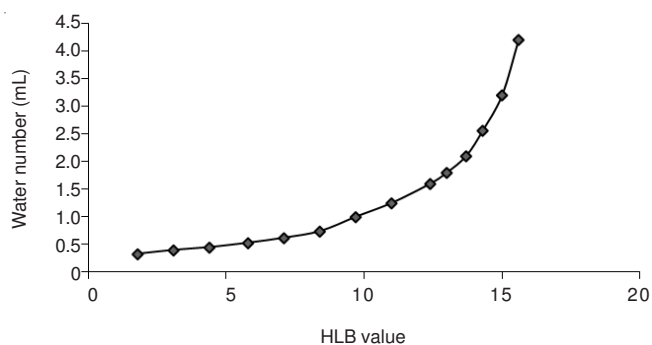

Fig. 4. Water number versus hydrophile-lipophile balance value calibration curve

TABLE-2
HYDROPHILE-LIPOPHILE BALANCE (HLB)
VALUE OF C$_n$-HPCS

CTS derivate	Water number	HLB
C4-HPCS	2.19	13.89
C8-HPCS	2.00	13.53
C12-HPCS	1.65	12.51
C16-HPCS	0.50	5.33

TABLE-3
FOAM PROPERTIES OF C$_n$-HPCS (10 g/L, t = 10 MIN)

	Foam expansion (%)	Foam liquid stability (%)	Foam volume stability (%)
C4-HPCS	30	5.0	89.2
C8-HPCS	80	5.0	90.8
C12-HPCS	82	5.0	84.1
C16-HPCS	85	5.0	88.3

All of the C$_n$-HPCS derivatives exhibited greater than 50 % emulsibility and performed better than the reference compound Tween 60 (Fig. 5). Although the emulsibility was initially quite high at or above 80 %, it dropped rapidly to a stable plateau between 60 % and 70 %. These results suggest that this series of compounds effectively dispersed at the interface between water and oil, reducing interfacial tension and allowing the water to infiltrate the oil phase and form an emulsion. The most effective emulsifier among the chitosan derivatives was C8-HPCS.
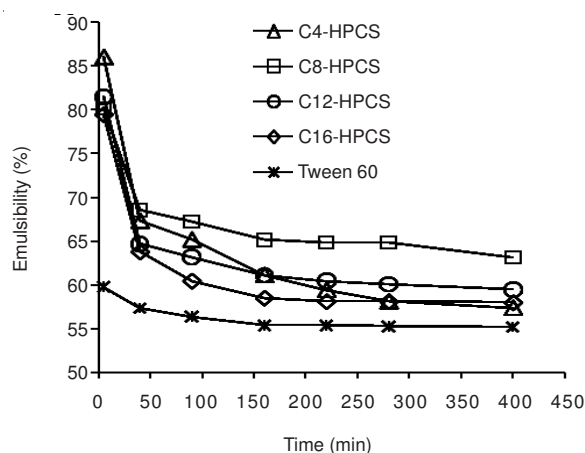

Fig. 5. Emulsifying power of C$_n$-HPCS

Conclusion

A novel class of surface-active O-hydroxypropyl-*N*-alkyl chitosan derivates, the C_n-HPCS, was synthesized successfully and efficiently. The intermediate product required no neutralization or drying steps, allowing the final product to be prepared directly from chitosan without introducing unnecessary impurities. The chemical and physical properties of the C_n-HPCS were characterized by ^{1}H NMR and FT-IR. The surface activity was correlated with the length of the carbon chains. A small amount of C_n-HPCS greatly decreased the surface tension of distilled water and the critical micelle concentration values decreased from 0.16 g/L to 0.5 g/L as the length of the carbon chains increased. The hydrophile-lipophile balance values varied similarly with the length of the carbon chains. C16-HPCS produced the greatest foam expansion. All of the C_n-HPCS exhibited low foam liquid stability, high foam volume stability and better emulsibility than Tween 60. Overall, the O-hydroxypropyl-*N*-alkyl chitosans displayed excellent surfactant potential and may be useful in many surface activity related applications such as cleaning and emulsifying.

REFERENCES

1. C. Akbay, N.L. Gill, R.A. Agbaria and I.M. Warner, *Electrophoresis*, **24**, 4209 (2003).
2. X.G. Chen, C.S. Liu, C.G. Liu, X.H. Meng, C.M. Lee and H.J. Park, *Biochem. Engg. J.*, **27**, 269 (2006).
3. D. Britto and O.B.G. Assis, *Carbohydr. Polym.*, **69**, 305 (2007).
4. Y.J. Xie, X.F. Liu and Q. Chen, *Carbohydr. Polym.*, **69**, 142 (2007).
5. H.T. Pang, X.G. Chen, H.J. Park, D.S. Cha and J.F. Kennedy, *Carbohydr. Polym.*, **69**, 419 (2007).
6. W. Sajomsang, *Carbohydr. Polym.*, **80**, 631 (2010).
7. M. Recillas, L.L. Silva, C. Peniche, F.M. Goycoolea, M. Rinaudo and W.M. Argüelles-Monal, *Biomacromolecules*, **10**, 1633 (2009).
8. I. Pepic, J.F.-Grcic and I. Jalšenjak, *Colloids Surf. A: Physicochem. Eng. Aspects*, **327**, 95 (2008).
9. M.Z. Elsabee, R.E. Morsi and A.M. Al-Sabagh, *Colloid. Surf. B: Biointerf.*, **74**, 1 (2009).
10. S.-B. Lao, Z.-X. Zhang, H.-H. Xu and G.-B. Jiang, *Carbohydr. Polym.*, **82**, 1136 (2010).
11. Y. Xie, X.-X. Shang, F.-X. Jiang and J.-G. Hu, *J. Clin. Rehabilitative Tissue Engg. Res.*, **12**, 4546 (2008).
12. H. Ortega-Ortiz, B. Gutiérrez-Rodríguez, G. Cadenas-Pliego and L. Ibarra Jimenez, *Braz. Arch. Biol. Technol.*, **53**, 623 (2010).
13. G. Dehghan-Noudeh, M.Housaindokht and B.S.F. Bazzaz, *J. Microbiol.*, **43**, 272 (2005).
14. S.M. Zhao, Surfactant-Principle, edn. 1, p. 136 (2005).
15. H.L. Greenwald, G.L. Brown and M.N. Finemann, *Anal. Chem.*, **28**, 1693 (1958).
16. X.B. Xu, G.W. Zhang and Huayuping, *J. Chin. Cereal. Oils Assoc.*, **8**, 26 (1993).
17. X. Chen, Y.H. Yuan, L.P. Zhang and X. Hong, *Chin J. Synth. Chem.*, **12**, 85 (2004).
18. K.M. Dollo and B.K. Sharma, *J. Surfact. Deterg.*, **14**, 131 (2011).
19. W.P. Sui, X.L. Yang, Q. Yang and G.H. Chen, *Chin. J. Appl. Chem.*, **9**, 890 (2002).
20. J. Pepic, Filipovic-Grcic and I. Jalšenjak, *Coll. Surf. A: Physicochem. Eng. Aspects*, **327**, 95 (2008).
21. N.A. Negm and I.A. Aiad, *J. Surfact. Deterg.*, **10**, 87 (2007).
22. Y.M. Tang, Synthetic Detergent and it's Application. China Textile Press, p. 391 (2006).
23. S.K. Hait and S.P. Moulik, *J. Surfact, Deterg.*, **4**, 3 (2001).