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Carbohydrate Research 339 (2004) 1113–1118

Carbohydrate
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Surface and aggregate properties of an amphiphilic derivative of carboxymethylchitosan

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Received 1 October 2003; accepted 3 February 2004

Abstract—A new kind of amphiphilic derivative of carboxymethylchitosan, a group of (2-hydroxyl-3-butoxyl)propylcarboxymethylchitosans (HBP-CMCHS), has been synthesized, and the surface and aggregate properties have been studied by means of surface tension, surface pressure and fluorescence measurements. HBP-CMCHS can adsorb on the surface to decrease the surface tension of the solution. The adsorption film was quite stable, which can make the relative compressed pressure increase dramatically with the decrease of the surface area. In solution, hydrophobic aggregations were identified by the decrease in the ratio of the fluorescence emission intensity of the first and third pyrene vibronic peaks (I_1/I_3). Results showed that the aggregation began to form at a concentration similar to that of the polymer transfer to the air–water interface. Aggregate formation of the polymers is a gradually compact process with hydrophobic associations. Increase of DS and addition of NaCl to the HBP-CMCHS solution can make the surface tension decrease, make the aggregation occur at lower concentration, and make the aggregation more hydrophobic.

Keywords: Amphiphilic derivative of carboxymethylchitosan; (2-Hydroxyl-3-butoxyl)propylcarboxymethylchitosans (HBP-CMCHS); Surface tension; Surface pressure; Aggregation

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1. Introduction

Polymeric surfactants have attracted the attention of various industrial and academic research groups for a number of years because their possessing properties are frequently superior to those of conventional surfactants and polymers.[1–3] These compounds include various synthetic amphiphilic polymers and natural po1ymers that have been used as hydrophilic backbones to prepare amphiphilic polymers by hydrophobic substitution. Such amphiphilic polymers are of growing interest with respect to biological importance and pharmaceutical or biotechnological applications.[4–6]

As a naturally occurring polysaccharide, chitin and its deacetylated derivative, chitosan, show many unique properties, such as biocompatibility, biodegradation, biological activity and low toxicity. Many derivatives have been prepared to meet different needs.[7–9] Some of them are amphiphilic polymers and have characteristics of polymeric surfactants.[10–14]

Carboxymethylchitosan (CMCHS) is one of the water-soluble derivatives of chitosan. In the present work, CMCHS was hydrophobically modified by butyl glycidol ether (BGE), to yield a group of amphiphilic chitosan derivatives, (2-hydroxyl-3-butoxyl)propylcarboxymethylchitosans (HBP-CMCHSs). Surface activity properties were studied by means of surface tension and surface pressure measurements. Fluorescence spectroscopy was combined to study the aggregation in solution.

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doi:10.1016/j.carres.2004.02.002

2. Experimental section

2.1. Materials

Carboxymethylchitosan of biomedical grade purity ($M_W = 8.0 \times 10^4$; degree of carboxymethyl substitution is 91% and degree of deacetylation is 84%) was supplied from Qingdao Biochemical Pharmacy Co., China. Pyrene as a fluorescence probe was purchased from Sigma Chemical Co. and used without further purification. Butyl glycidol ether (BGE) was supplied from Shanghai Resin Co., China. The other chemicals were all AR grade. The water was purified by distillation.

2.2. Synthesis of CMCHS derivatives

Some finely powdered KOH was added to the proper amount of 2-propanol and stirred to dissolve. Then while stirring consistently, CMCHS was added in, and the mixture was further stirred for 1 h. A known BGE content was then added dropwise over a period of time at 50 °C, and the mixture was kept at 50 °C for several hours while stirring. The products were filtered and washed with acetone, and then dried in a vacuum at 50 °C. For water-soluble products, the precipitates were redissolved in deionized water, dialyzed against deionized water and freeze-dried. The degree of the substitution (DS) is defined as the number of (2-hydroxyl-3-butoxyl)propyl groups per 100 anhydroglucose units of chitosan and was determined by elemental analysis (taken as the average of three measurements). The detailed synthetic procedures and the structural analysis of HBP-CMCHS by IR, ^{1}H NMR, ^{13}C NMR, and X-ray diffraction spectra and elemental analysis has been submitted to another journal,[15] and the structure of CMCHS and HBP-CMCHS has been studied by a quantum chemical method.[16]

2.3. Measurement of surface tension

The surface tension measurements of CMCHS and HBP-CMCHS solutions at various concentrations were carried out separately on a Krüss K12 Processor Tensionmeter by the plate method of Wilbelmy at 30 ± 0.1 °C. The surface tension data were taken as the average of three measurements. All solutions were kept at room temperature for 1 day before surface tension measurements.

2.4. Measurement of surface pressure

The surface pressure measurements were carried out on a film balance apparatus (type HBM Face LB, Japan) at 20 °C. The solution of HBP-CMCHS was put in the trough of a film balance, and the surface pressure was observed until it did not increase, indicating that the surface adsorption to the surface had reached equilibrium. After setting the surface pressure to zero, the surface film was compressed at a speed of 5 mm min^{-1}. The relative compressed surface pressure was record at each different film area.

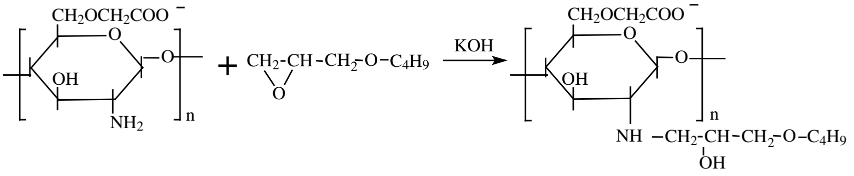
2.5. Fluorescence measurements

Steady-state fluorescence measurements were carried out on a RF-540 Spectrofluorimeter (Japan) at 30 ± 0.1 °C. A known volume of pyrene in MeOH was put into a test tube and evaporated under a nitrogen purge to remove the solvent. Then, an aqueous solution of HBP-CMCHS was added into the test tube. The final concentration of pyrene was 1×10^{-6} mol dm^{-3}. The solutions were sonicated for 15 min in an ultrasonic bath and then kept at room temperature for 2 h before spectroscopic analysis. The excitation wavelength of pyrene was 335 nm. The hydrophobic index, I_1/I_3, was calculated as the ratio of the intensities at the first (374 nm) and the third (385 nm) vibrational peaks of monomeric pyrene in the pyrene emission spectra.

3. Results and discussion

3.1. Surface activity properties of HBP-CMCHS

Figure 1 shows the surface tension–concentration plots of CMCHS and HBP-CMCHS with different degrees of substitution (DS). It can be seen that CMCHS has little surface activity properties for there is no hydrophobic group in the polymer. After being modified with the (2-hydroxyl-3-butoxyl)propyl group, the resulting derivatives, HBP-CMCHSs are converted to amphiphilic polymers with these hydrophilic backbones substituted by hydrophobic groups. They adsorb on the surface with the hydrophilic backbone in the solution, while the hydrophobic groups point up towards the air to reduce



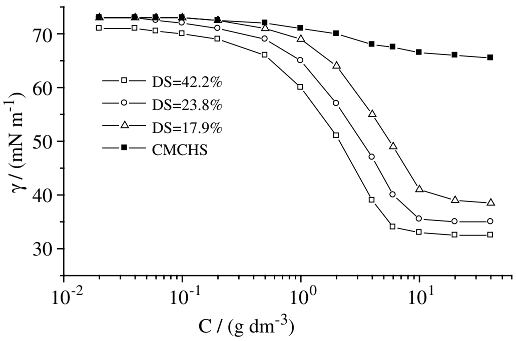


Figure 1. Surface tension–concentration plots of CMCHS and HBP-CMCHS at different degree of substitution.

the surface tension of water. When the concentration is big enough, the surface adsorption is complete and the surface tension no longer decreases.

From the figure it can also be seen that with the increase of degree of substitution (DS) of the hydrophobic group, the surface tension decreases at the same concentration of the derivatives, for there are more hydrophobic groups on the surface. The concentration of adsorption decreases for the same reason.

For the derivative of DS is 42.2%, surface tension was measured in the presence of different amounts of NaCl. From Figure 2 we can see that addition of NaCl to the solution of the HBP-CMCHS made the surface tension lower and gave two transitions. This kind of interesting phenomena was also observed in the studies of another derivative of CMCHS, (2-hydroxyl-3-*p*-nonylphenoxyl)propylcarboxymethylchitosan (HNP-CMCHS) in the presence of NaCl,[17] but for the derivatives of long hydrophobic group and low DS, such as (2-hydroxyl-3-dodecanoxyl)propylcarboxymethylchitosans (HDP-CMCHS), two transitions were not observed even in the presence of NaCl.[18] The phenomena of two transitions have also been reported for other polymeric surfactants.[19–21] It was considered as the formation of an intramolecular micelle.

The electrolyte can compress the electric double layer of the anionic carboxymethyl groups on the hydrophilic backbones of the HBP-CMCHS and make the polymers more flexible. So it is easy for the polymers to concentrate on the surface of the solution and reduce surface tension, and it is easy to form aggregates by hydrophobic group association in solution. For the derivatives of high enough DS, the hydrophobic chain could even self-associate with each other in the same macromolecule to form intramolecular aggregates at low concentrations, and make the surface tension get to a lowest point. With an increase in concentration, hydrophobic chains of different macromolecules could associate to form intermolecular micelles in solution. In my opinion, the minimum of surface tension may be the ‘intra’ start to take place, and the maximum corresponds to the maximum of ‘intra’. After this maximum, aggregates start to form, and the surface tension decreases. The structural transformation of the association of hydrophobic chain on the surface and solution made the surface tension decrease abnormally. The aggregations that can form before the surface tension levels off will be confirmed by fluorescence measurements made afterward. This procedure has been found in another report of lipophile-grafted styrene–maleic anhydride copolymer.[22]

3.2. Surface film of HBP-CMCHS

Amphiphilic molecules are molecules that can adsorb to the air–water interface and form a film. Molecules are spontaneously inserted into the film only up to a certain surface pressure, known as the equilibrium spreading pressure. Surface pressure can be thought of as the repulsive force resulting from molecules in a film being in close proximity with each other. If the surface area of the film is compressed, surface pressure may increase. For the low-molecular-weight water-soluble amphiphiles, when the surface is compressed, some of the molecules in the film can submerge into the subphase and make the compressed pressure increase slightly.[23] But for molecules of polymeric amphiphile, HBP-CMCHS, the situation is different (Fig. 3).

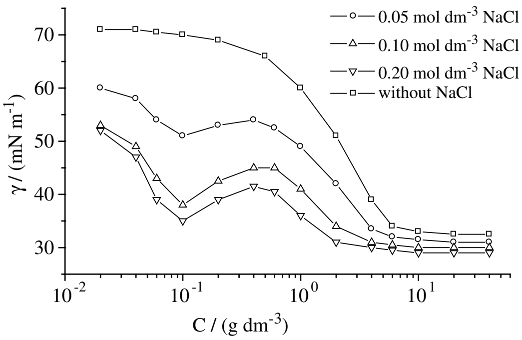


Figure 2. Surface tension–concentration plots of HBP-CMCHS (DS 42.2%) in the presence and absence of NaCl.

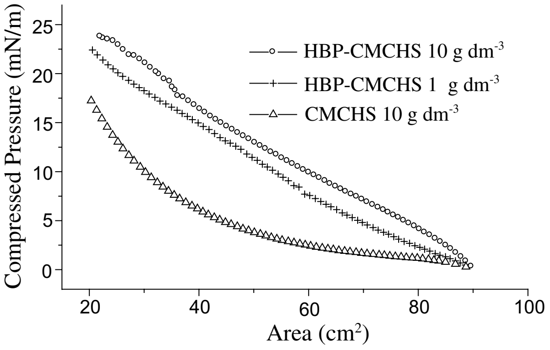


Figure 3. Compressed curve of the adsorption film of CMCHS and HBP-CMCHS (DS 42.2%).

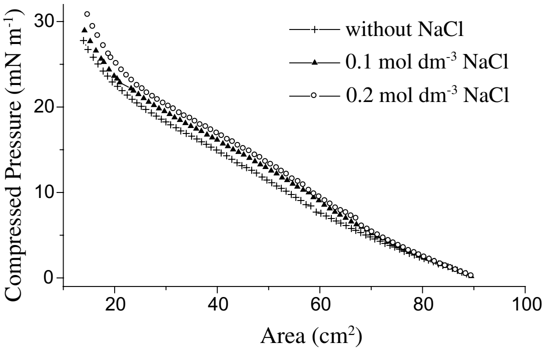


Figure 4. Compressed curve of the adsorption film of HBP-CMCHS (1 g dm^{-3}, DS 42.2%) in the presence of NaCl.

The HBP-CMCHS solution is placed in the trough of a film balance, and the surface pressure is observed until it reaches equilibrium. After the surface pressure has been set to zero, the surface film is compressed and the relative compressed surface pressure is recorded at each different film area. The result is shown in Figure 3. It is shown that with the decrease of the surface area, the relative compressed pressure increases slowly for CMCHS because there is little CMCHS concentrated on the surface. Only after the surface is compressed to about half of the area, the relative compressed pressure gradually increases. But for HBP-CMCHS, the relative compressed pressure increases dramatically with the decrease of the surface area. Even for the solution of 1 g dm^{-3} whose surface adsorption has not reached saturation, the relative compressed pressure can also increases linear with the decrease of the surface area. It means that the amphiphilic derivative of carboxymethylchitosan has a good ability to form a surface film. It can arrange in a more compact structure or even may form multiple layers on the surface layer instead of coming into the solution like small molecules.

Additions of NaCl made the relative compressed pressure increase only a little (see Fig. 4). There are two sides of the effects for NaCl. On the one hand, the electrolyte can compress the electric double layer of the anionic carboxymethyl on the hydrophilic backbone and inhibit the carboxyl from ionization, make the polymers more flexible, so the repulsion between different groups of the molecules decrease, resulting in the decrease of the surface pressure. On the other hand, there are more molecules adsorbed on the surface, which make the surface pressure increase. As a result, the relative compressed pressure increases little as the surface is being compressed.

3.3. Aggregate formation of HBP-CMCHS

In order to study the formation of aggregates in a solution of HBP-CMCHS, fluorescence measurements were used to detect the emission spectra of pyrene in the solution. There are five peaks in the emission spectra of pyrene, and the emission intensity of the first peak (374 nm) and of the third peak (385 nm) is sensitive to the microenvironment. So the intensity ratio of the emission at 374 and 385 nm (I_1/I_3) has been used to monitor the solution behavior of surfactants and/or polymers. A bigger I_1/I_3 value means a greater polarity of the solution around pyrene. Therefore, the formation of the aggregates with a hydrophobic inner core can be detected by means of plotting I_1/I_3 versus polymer concentration.[24]

Figure 5 shows the I_1/I_3 value of pyrene in solution as a function of HBP-CMCHS concentration. When the concentration is below 10^{-1} g dm^{-3}, the I_1/I_3 values are close to that of pure water (1.7) for there are few aggregates formed. Above 10^{-1} g dm^{-3}, the I_1/I_3 value gradually decreases, indicating that the microenvironment around pyrene is getting more hydrophobic. It can be interpreted as the formation of aggregates with the hydrophobic core getting more compact. The I_1/I_3 value can even get to 0.8 at the concentration of 40 g dm^{-3}, which is similar to that of the normal ionic surfactants such as SDS.

In Figure 5, the surface tension (γ) of HBP-CMCHS solution is also plotted as a function of the polymer concentration. It can be found that the I_1/I_3 began to decrease at the concentration similar to that of the polymer transfer to the air–water interface. After the surface adsorption is completed and the surface tension levels off, the I_1/I_3 value still decreases slightly. This indicates that the polymers begin to form aggregates by their hydrophobic chains in solution while some of them transfer to the air–water interface to decrease the surface tension. After the surface adsorption reaches equilibrium, the micropolarity in the polymer aggregates still gets less polar due to the hydrophobic core getting more compact. The aggregate formation of the polymers is a process that gradually becomes more compact with hydrophobic association.

These phenomena are quite different from the surfactants with low molecular weight such as $C_{12}BE$.[25] For

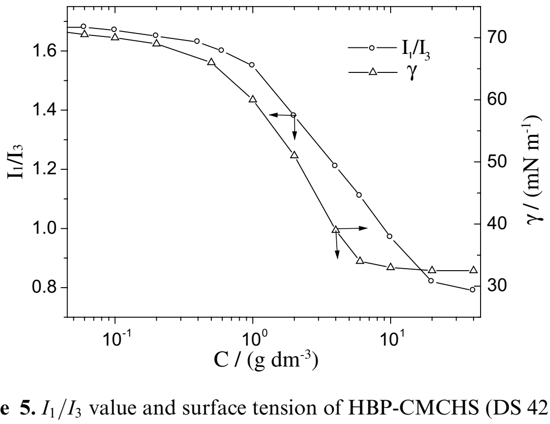


Figure 5. I_1/I_3 value and surface tension of HBP-CMCHS (DS 42.2%) as a function of polymer concentration.

those surfactants, only after the surface is saturated, that is, the surface tension reaches an equilibrium value, and aggregates (micelle) can form in solution, leading to the dramatic decrease of I_1/I_3, which can be used to determine the critical micelle concentration (CMC). After the normal micelles form, I_1/I_3 reaches an equilibrium value. But for the amphiphilic polymer HBP-CMCHS, aggregates can form at very low concentration while the surface tension decreases. As the surface tension reaches an equilibrium value, the additional excess polymer can only make the inner core of the aggregates a little more compact, so the decreasing rate of I_1/I_3 slows down compared with that of the aggregates that begin to form.

The plots of I_1/I_3 values versus polymer concentrations for CMCHS and HBP-CMCHS composed of different degrees of DS are shown in Figure 6. It can be found that in CMCHS solution the I_1/I_3 values are close to that of pure water (1.7), for there is no hydrophobic microdomination formed in the anionic polymer. Only at high concentration can the polymer render the aqueous solution less polar than water. So the I_1/I_3 values come down slightly. For HBP-CMCHS, at very low polymer concentration, I_1/I_3 values are close to that of pure water indicating that few aggregates form in solution at low concentration. With the increase of concentration, the I_1/I_3 value decreases, and the HBP-CMCHS of the higher DS decreases first, which indicates that increasing of DS can make it easy for aggregates to form for there are more hydrophobic chains to associate. At the same concentration, the higher the DS, the lower the I_1/I_3 value. This means that the hydrophobicity increases with increases in the DS. This is consistent with the results of the surface tension studies.

I_1/I_3 was also measured in the presence of different amounts of NaCl for the derivative of DS = 42.2%. From Figure 7 it can be seen that addition of NaCl to the solution of HBP-CMCHS made I_1/I_3 decrease. This demonstrates that the electrolyte can compress the electric double layer of the anionic carboxymethyl groups and make the polymer more flexible. So it is easier for the hydrophobic groups to associate, and the aggregates can be more compact and hydrophobic. But different from the surface tension curve, there are no transitions in the I_1/I_3 curves. This may suggest that at low concentration, the 'intramolecular' aggregates are quite loose. There is water in the inner core, and that makes it quite hydrophilic. More work should be done to study the conformation of the polymer aggregates.

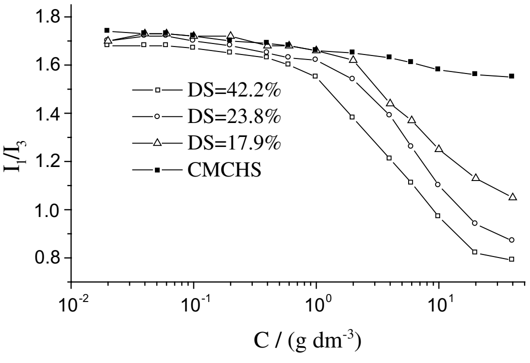


Figure 6. I_1/I_3–concentration plots of CMCHS and HBP-CMCHS at different degree of substitution.

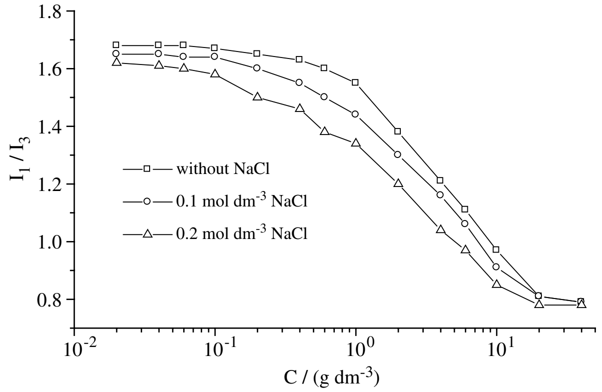


Figure 7. I_1/I_3–concentration plots of HBP-CMCHS (DS 42.2%) in the presence and absence of NaCl.

4. Conclusions

The hydrophobically modified carboxymethylchitosan (CMCHS) derivatives, the (2-hydroxyl-3-butoxyl)propylcarboxymethylchitosans (HBP-CMCHSs), have good surface activities and can form aggregates in solution. They can adsorb on the surface to decrease the surface tension of the solution and form a fairly stable film that can make the compressed pressure increase with the surface being compressed. In solution, HBP-CMCHSs can aggregate by the hydrophobic chain at concentrations similar to those of the polymer transfer to the air–water interface, and make the I_1/I_3 (the intensity ratio of pyrene emission at 374 and 385 nm) decrease. The formation of aggregates is a process of the molecules becoming gradually compact with increasing hydrophobic association. Increase of DS and addition of NaCl to the HBP-CMCHS solution can make the surface tension decrease, can make the aggregation occur at lower concentration, and can make the aggregation more hydrophobic.

Acknowledgements

This work is supported by the Science Research Awards Foundation for Yøung Scientists of Shandong Province, China (02BS108).

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