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Comparison of curdlan and its carboxymethylated derivative by means of Rheology, DSC, and AFM

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Abstract—Curdlan was carboxymethylated in an aqueous alkaline medium using monochloroacetic acid as the etherifying agent. The structure of carboxymethylated curdlan (CMc) was analyzed by FT-IR and NMR spectroscopy, which revealed that the carboxymethyl group was introduced mainly at the C-6 position as well as at the C-2 and C-4 positions. Furthermore, CMc was compared with the native curdlan by using rheology and DSC methods. It was found that in water, both polysaccharides behaved as pseudoplastic fluids and fit the power law and Herschel–Bulkley rheological models well. Both the storage shear modulus G' and the loss shear modulus G'' of CMc aqueous solutions decreased and became more frequency dependent with decreasing concentration in comparison with the curdlan aqueous suspensions. The modulus–temperature curve also suggested that the gel characteristic of curdlan has been lost after chemical modification, which is consistent with the DSC results. AFM images revealed differences in the conformation of native and carboxymethylated curdlan, which changed from the aggregation of macromolecules to triple helices. All the experimental results suggest that the hydrogen bonds that bind curdlan with interstitial water to form the micelles have been destroyed completely and that the hydrophobic interactions related to the methylene groups at C-6 formed above 55 °C disappeared due to the introduction of the hydrophilic carboxymethyl group.

Keywords: Curdlan; Carboxymethylation; Physico-chemical property; Gelation; Hydrophobicity; Hydrogen bond

1. Introduction

Curdlan is a bacterial polysaccharide formed by fermentation of *Alcaligenes faecalis* with a linear structure composed entirely of β-(1→3)-D-glucopyranosidic linkages.[1] It has utility as a food additive due to its ability to form an elastic gel[2] and shows strong bioactivities;[3] these unusual properties of curdlan have not been observed in other synthetic or natural compounds. In the past 40 years since the discovery of curdlan in 1964 by Harada, our understanding of curdlan has undergone a dramatic evolution. More recently, physical characteristics determined by rheological and thermal measurements and conformational/morphological analysis[4–13] as well as physiological functions such as anti-tumor and anti-HIV activities have been reported.[14]

Curdlan is insoluble in water, which limits its biological applications. Water insolubility is generally attributed to the existence of extensive intra/intermolecular hydrogen bonds. Sulfation is a main method to realize or confer biological activity to polysaccharides, and inhibitory effects against HIV in vitro as well as structural studies on curdlan sulfate have been investigated extensively.[15–17] Carboxymethyl substitution is considered as another method to improve the functional properties for many polysaccharides.[18–20] In the case of curdlan, its carboxymethylated derivative has good water solubility as well as good bioactivity, and it has been added successfully to cosmetics due to its immunocompetence.[21] However, the physico-chemical properties of carboxymethylated curdlan have not been examined yet, as far as the authors are aware. This

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paper reports the comparison of curdlan and its carboxymethylated derivative by means of rheology, DSC, and AFM, and gives a possible interpretation for the change of the macromolecular structure after carboxymethylation.

2. Experimental

2.1. Materials

Curdlan was a gift from Takeda-Kirin Foods Corporation (Tokyo, Japan) and was used without further purification. All chemical reagents used were analytical grade and were obtained from commercial resources in China.

2.2. Carboxymethylation

The procedure is briefly as follows: A suspension of 3.0 g of curdlan in 100 mL 2-propanol was stirred for 30 min at room temperature. Then, 8.0 mL of a 30 wt% sodium hydroxide solution was added dropwise to the suspension and vigorous stirring was continued at room temperature for 90 min. Next, 3.0 g of monochloroacetic acid (MCA) was added over three separate intervals of 10 min each and the mixture was stirred at 55 °C for 2 h. The suspended product was recovered by filtration and washed successively with MeOH–water, MeOH, and acetone. The precipitate was dissolved in water and dialyzed against distilled water at 4 °C for 4 days. The retentate was frozen and lyophilized to give the final product. The degree of substitution (DS) of the CMc sample was determined by conductometric back titration in accordance with a previous report.[22] The molecular characteristics of carboxymethylated curdlan are given in Table 1. The obtained CMc sample displays good water solubility, even with its high molecular weight, and because of the remarkable improvement in hydrophilicity, the chemical modified curdlan cannot be dissolved in Me_2SO, which is a good solvent for the native curdlan.

2.3. Preparation of curdlan suspension

Despite the lack of the water solubility, curdlan is saturable in water; a homogenizer can make a well-dispersed suspension of curdlan.[10] To obtain reproducible measurement results, it is essential that the curdlan does not separate when left standing. Curdlan was first soaked in water and homogenized at room temperature, and paste-like suspensions were obtained. Before each measurement, the suspension of curdlan was stirred by using a magnetic stirrer at room temperature.

Table 1. Molecular characteristics of carboxymethylated curdlan

Sample	DS	$[\eta]$ (dL/g)	$M_w \times 10^{-6}$	M_w/M_n
CMc	0.83	1.62	4.78	2.99

Note: The M_w of native curdlan is 1.92×10^6 obtained from the Mark–Houwink equation in 0.3 M NaOH proposed by Nakata et al.[5]

2.4. Structural analysis

IR spectra were recorded on a Paragon 1000 FT-IR spectrophotometer (Perkin Elmer, Inc., USA) in the wavenumber range of 500–4000 cm^{-1}. Samples were prepared by the KBr-disk method and measured at room temperature. The ^{13}C NMR spectrum was recorded on a Mercuryplus 400 MHz spectrometer (Varian, Inc., USA) at 27 °C. The polysaccharides were dissolved in D_2O or NaOD aqueous solutions and the samples were measured with Me_4Si as the internal standard. The intrinsic viscosity of CMc, $[\eta]$, was measured using an Ubbelohde-type viscometer in 0.1 M NaCl aqueous solution at 37 °C. The molecular weight and polydispersity index of the CMc were measured in a 0.1 M NaCl aqueous solution with a laser light scattering instrument (DAWN DSP, Wyatt Technology Co., USA) at 37 °C.

2.5. Rheological measurements

A stress-controlled fluids spectrometer RS1 (Thermo Haake, Inc., Germany) was used for the steady shear and dynamic viscoelasticity measurements. The rheometer was equipped with a transducer with a sensitivity limit of 0.0005 g cm in torque. Parallel plate geometry was used with 60 mm diameter and 1.0 mm gap. Dispersions and solutions were poured directly onto the plate and the free surfaces were covered with a layer of silicon oil to avoid dehydration during the measurement.

For steady shear measurement, the shear rate was changed from 0.1 to 100 s^{-1}. The rheological data for dispersions and solutions were fitted to the Herschel–Bulkley (HB) (Eq. 1) and power law (Eq. 2) models, respectively, as follows:

$$\tau = \tau_y + K\dot{\gamma}^n \quad (1)$$

$$\tau = K\dot{\gamma}^n \quad (2)$$

where τ is the shear stress; τ_y is the yield stress; $\dot{\gamma}$ is the shear rate; K is the consistency index (Pa S^n); and n is the flow behavior index. The value of n and K were obtained by regression of the double logarithmic plot of shear stress versus shear rate.

For the dynamic viscoelastic measurements, a stress sweep test was performed for each sample to determine the range of linear viscoelasticity. The storage shear modulus G' and the loss shear modulus G'' were observed as a function of frequency from 0.1 rad/s to 100 rad/s at room temperature and at a strain of 1%,

which is within the region of linear viscoelastic response, and as a function of temperature from room temperature to 70 °C at a constant frequency of 1 Hz in the linear viscoelastic regime. The heating and cooling rates were fixed at 1 °C/min.

2.6. Differential scanning calorimetry (DSC)

DSC measurements were performed with a Setaram micro DSC-III calorimeter (Caluire, France). An approximately 800 mg sample was hermetically sealed into the DSC pan, and an appropriate quantity of distilled water was used as a reference material to obtain a flat baseline. The test temperature was in the range from 20 to 80 °C, and the scan rate was 1 °C/min.

2.7. Atomic force microscopy (AFM)

Solid curdlan was dissolved in 5 mM NaOH to make a 1 mg/mL stock solution after stirring for a few days. Samples of native curdlan in solution were made by diluting 1 mg/mL stock solution to the required concentration with 5 mM NaOH solution. Diluted samples were stirred and equilibrated for 24 h prior to AFM sample preparation. A similar procedure was used for making the samples of CMc in water. AFM samples were prepared using the drop deposition method. A 5 μL aliquot of the solution was pipetted directly onto a freshly cleaved mica surface, and the surface was allowed to dry in air (about 10 min) in an enclosed Petri dish, and then the samples were dried for more than 24 h in a desiccator.

Scanning probe microscopy images were obtained using a NanoScope III atomic force microscope (Digital Instruments, Santa Barbara, CA) and collected by using tapping mode atomic force microscopy (TmAFM). All measurements were performed in air at ambient pressure and humidity (ca. 50% RH).

3. Results and discussion

3.1. FT-IR spectroscopy analysis

Figure 1 shows the FT-IR spectra of curdlan and CMc. The spectra are dominated by a broad band at about 3370 cm^{-1}, which is assigned to the stretching vibration modes of OH groups. This band tends to shift to a higher wavenumber upon chemical modification. The shift in wavenumber is attributed to the weakening of hydrogen bonds. The peak at ca. 1644 cm^{-1} in the spectrum of native curdlan is attributed to the existence of water, which seems to be unable to be completely eliminated from the sample. A similar phenomena can be seen for some other polysaccharides such as konjac glucomannan.[23,24] In the CMc spectrum, the absorption band at

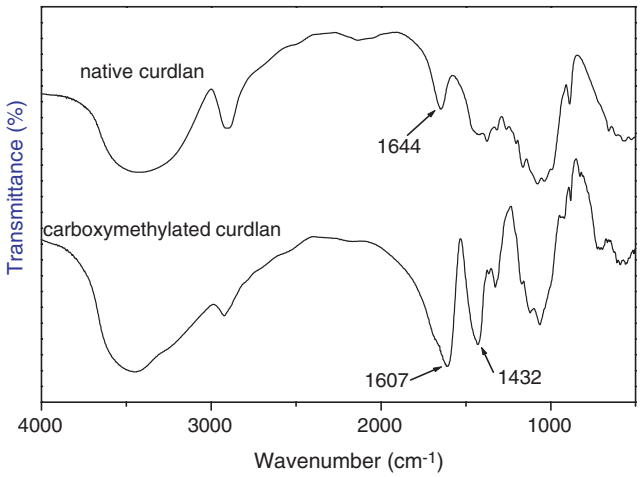

Figure 1. FT-IR spectra of native and carboxymethylated curdlan.

Table 2. Infrared absorption regions and assignments for curdlan and CMc

Wavenumber (cm^{-1})	Fragment
3370	OH
2917	$CHCH_2$
1644	H–O–H
1373	CH
1317	CH_2
1261	C–OH
1234	C–O
1160	C_1–O–C_3
1080	C–O
1607*	C=O
1432*	C=O

Symbol * refers to the characteristic peaks of the CMc molecule.

1607 cm^{-1} is due to the asymmetrical COO^- stretching vibration, whereas the band at 1432 cm^{-1} is due to the symmetrical COO^- stretching vibration. A detailed band assignment is not presented in this work, but the main absorption peaks and their tentative assignments are shown in Table 2.

3.2. ^{13}C nuclear magnetic resonance (NMR)

^{13}C NMR spectra of curdlan and CMc samples in solutions are shown in Figure 2, in which the curdlan spectrum is similar to that obtained by Saito et al.[25] When the concentration of NaOD is below 0.20 M, the signals for the chain will not be present because of restricted mobility, and only a small signal from C-6 appears clearly because of its low steric hindrance (Fig. 2A). At a NaOD concentrations between 0.19 and 0.24 M, a conformation transition occurred because NaOD would break up hydrogen bonds, stabilizing the single and multiple helical conformations. This transition might be considered as the helix–coil transition.[26] Moreover, when the concentration of NaOD exceeds 0.24 M, there seem no obvious changes in the peak intensities.

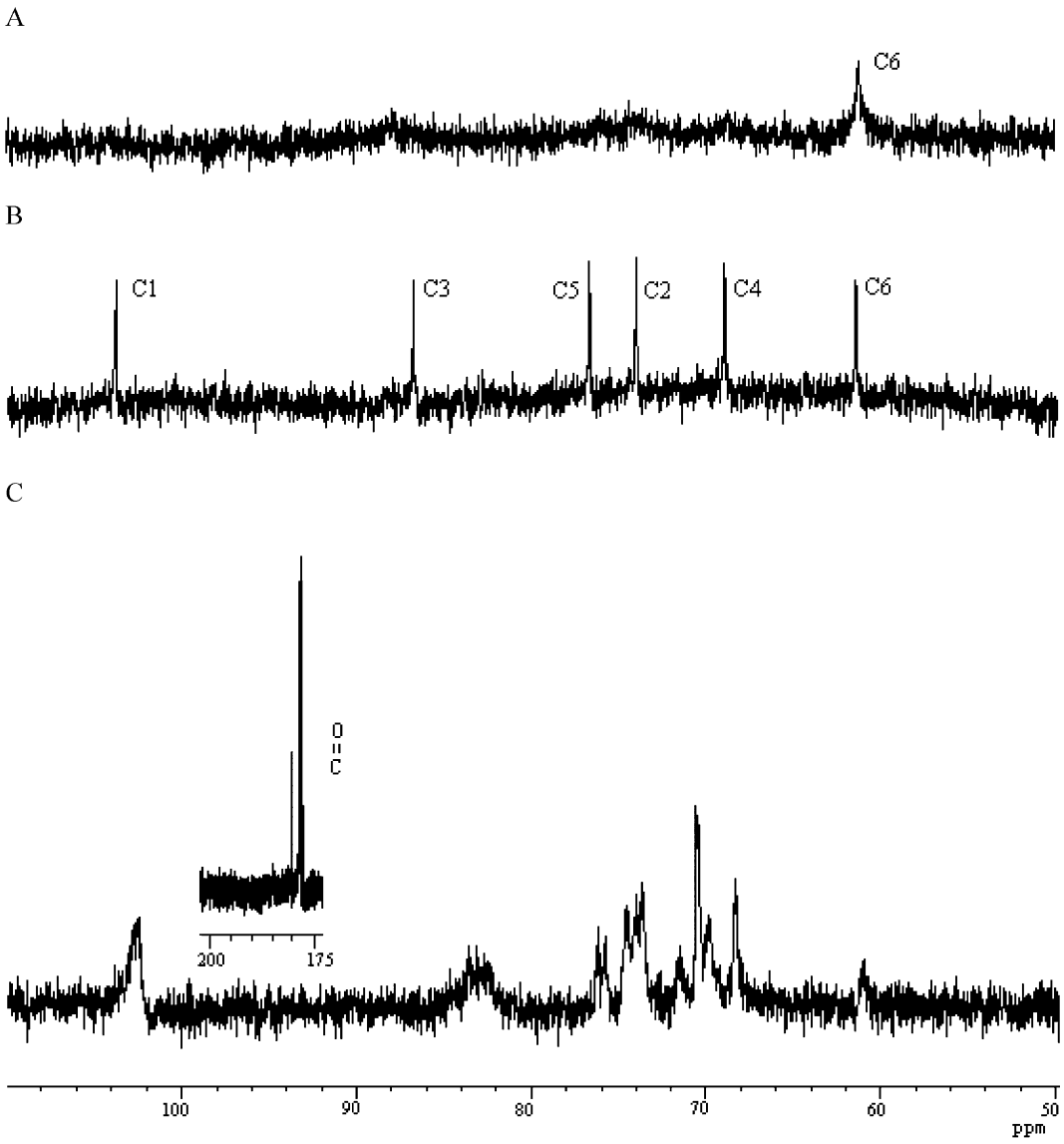

Figure 2. ^{13}C NMR spectra of native and carboxymethylated samples: (A) curdlan in 0.15 M NaOD; (B) curdlan in 0.4 M NaOD; (C) CMc in water.

According to a comparison with β-(1→3)-D-glucan,[27] the peaks at 103.7 (C-1), 73.9 (C-2), 86.7 (C-3), 68.6 (C-4), 76.6 (C-5), 61.3 (C-6) ppm are attributed to the signals of the backbone chain, as shown in Figure 2B.

After carboxymethylation, good solubility is achieved due to the introduced hydrophilic carboxymethyl groups, and the signals of aqueous CMc solution in the NMR spectra are legible, as shown in Figure 2C. Those signals of the backbone chain mentioned above are still detectable, indicating that the structure of the original polysaccharide remained. The spectrum of CMc shows a new peak due to the carbonyl group at about 178 ppm, as evidence of carboxymethyl substitution in agreement with the abovementioned FT-IR results. The distinct increase in peak intensities around 70 ppm is attributed to the shift of primary carbon (C-6) from the region around 60 ppm after substitution of the $-CH_2COOH$ group on the primary carbon. All these results are in line with the NMR experimental results for a branched (1→3)-β-D-glucan structure,[25] and the introduction of a carboxymethyl group in the C-6 position resulted in a weakening of the signals at 61.3 ppm and subsequent appearance of the signals of the side chain C-6′ at ca. 70 ppm.

Yoshida et al.[15] found that sulfate groups were introduced to the C-6, C-4, and C-2 positions in the ratio of 100%, 5%, and 40%, respectively, for curdlan sulfation, and believed that the first sulfation of the glucose units of curdlan occurred at the C-6 position because it is a primary alcohol and has a relatively lower steric hindrance than those of C-2 and C-4. In the present work, the peak of C-6 shifted from $\delta = 61.3$ ppm to around 70.0 ppm and the peak of C-5 from $\delta = 76.6$ to 74.6 ppm, suggesting that the carboxymethylation of

curdlan also occurred mainly in C-6 position. In addition, several signals appeared at ca. 178 ppm and between 70 and 80 ppm in Figure 2C, indicating the variation of the substitution position. The substituted C-2 and C-4 signals are presumed to be assigned to the peaks at ca. 74.0 and 75.8 ppm, respectively. From the areas of these peaks, which correspond to the amount of substitution, the degree of introduction of the carboxymethyl group is in the order of C-6 > C-2 > C-4.

3.3. Steady shear properties of native and chemically modified curdlan in water

Figure 3a shows the steady shear viscosity, η, of curdlan suspensions as a function of shear rate. The viscosity decreases remarkably with increasing shear rate, that is, curdlan suspensions exhibit a shear-thinning behavior and no Newtonian region can be detected, whereas the 2% and 3% CMc solutions (Fig. 3b) exhibit only a slight shear thinning. The steady shear viscosity of 6% and 10% CMc solutions show a gradual decrease in the low shear rate region, followed by a fall at high shear rates, and the onset of shear-thinning behavior shifts to lower shear rates with increasing polymer concentration, as has been observed for many other polymer solutions.[28]

Figure 4 shows that the rheological experimental data are well described by the Herschel–Bulkley and power law rheological models for the two samples at various concentrations at 30 °C, respectively. Table 3 shows the magnitudes of all parameters (τ_y, K, n) obtained from the two models. For curdlan suspensions, there is an increase in the value of yield stress with the increase of concentration while no yield stress is observed for CMc solutions. The flow behavior index n of CMc solutions ranges from 0.86 (for 2%) to 0.75 (for 6%), suggesting that CMc solutions with higher concentrations are more shear thinning than those with low concentrations.

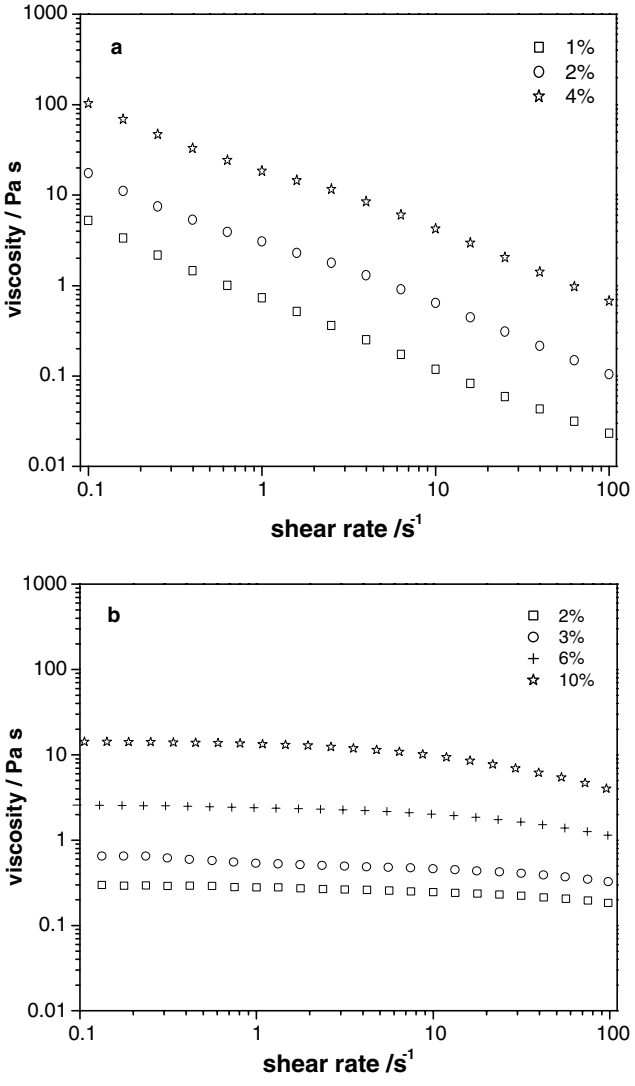

Figure 3. Steady shear viscosity as a function of shear rate for curdlan suspensions (a) and CMc solutions (b) at 30 °C.

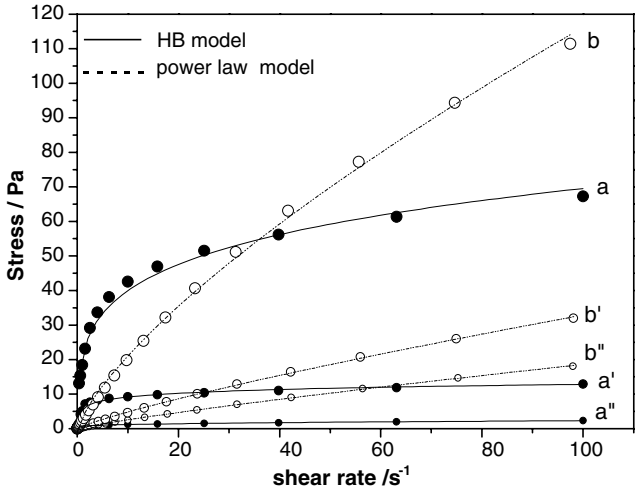

Figure 4. Effect of concentration on the flow behavior for curdlan aqueous suspensions (a, a′, a″) and CMc solutions (b, b′, b″) at 30 °C: (a) 4%; (a′) 2%; (a″) 1%; (b) 6%; (b′) 3%; (b″) 2%.

Table 3. Yield stress τ_y, consistency index K, and exponent n for curdlan suspensions (a, a′, and a″) and for aqueous CMc solutions (b, b′, and b″) at various concentrations at 30 °C

Sample	τ_y (Pa)	K (Pa S^n)	n
Curdlan			
a″: 1%	0.4	0.32	0.39
a′: 2%	3.8	3.20	0.22
a: 4%	14.0	14.0	0.29
CMc			
b″: 2%	—	0.35	0.86
b′: 3%	—	0.70	0.83
b: 6%	—	3.73	0.75

On the other hand, the consistency coefficients K for both of the samples increase with increasing concentration of the polysaccharide.

3.4. Dynamic viscoelasticity measurements of native and modified curdlan in water

The mechanical spectra of native and modified curdlan in water are shown in Figure 5. Solid-like behavior of curdlan suspensions is observed at 30 °C as shown in Figure 5a. The common characteristic feature of the curves is that G' predominates over G'' through the accessible frequency range as has been reported previously.[6,10] The variation of $\log G'$ with $\log \omega$ remains linear over three decades with a common slope of ~0.04, showing only a slight frequency dependence, and the appearance of a minimum or a plateau region in G'' is related to entanglements among polymeric chains. Similar behavior was observed for suspension of microparticles of cellulose[7] and many polymer gels such as amylose, agar, bovine serum albumin, and β-conglycinin gels.[29]

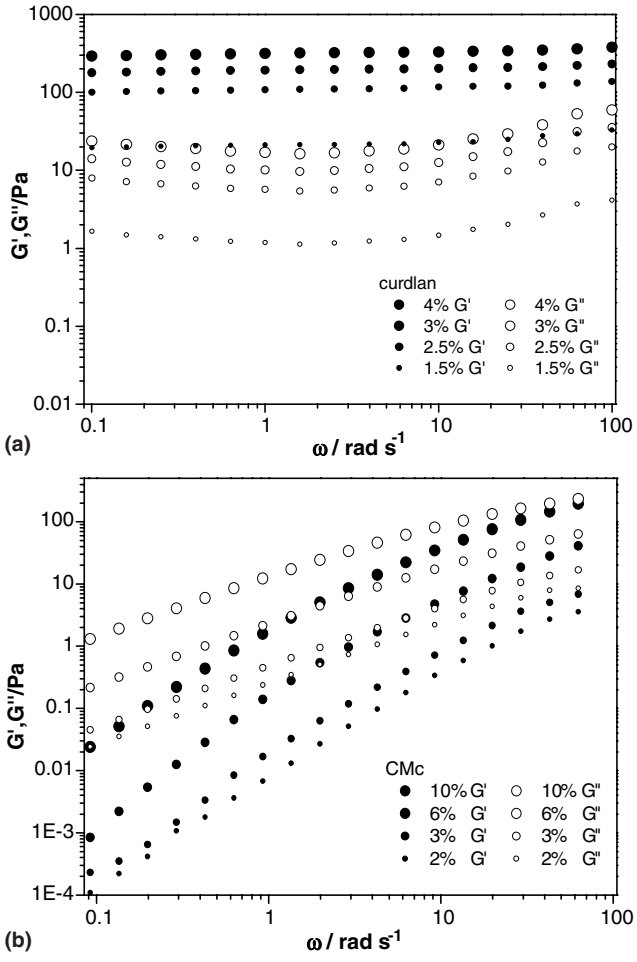

Figure 5. Frequency dependence of G' and G'' of curdlan suspensions (1.5–4%) and CMc solutions (2–10%) at 30 °C.

The mechanical spectra for CMc aqueous solutions of various concentrations at 30 °C are shown in Figure 5b. Liquid-like behavior is observed with $G'' > G'$, G' increasing more rapidly with frequency than G'' over the accessible frequency range, as expected for a liquid system. Both moduli were strongly frequency dependent. For 2% solution $G' \propto \omega^2$, $G'' \propto \omega$, and the exponent deviated from 2 and 1 with increasing concentration of polymer solution. This is in good agreement for common polymer solutions.[28,30]

3.5. Modulus–temperature dependence

The temperature dependence of G' and G'' for a curdlan aqueous suspension and a CMc aqueous solution is shown in Figure 6. For the former, G' decreases at temperature range from 40 to 50 °C, which is related to the swelling of curdlan because of the breakup of hydrogen bonds.[10] Upon further heating, both moduli increase remarkably at about 55 °C and then increased gradually with increasing temperature, suggesting the formation of the thermo-irreversible gels due to hydrophobic interactions. The fact that the heating and cooling curves always deviated from each other implies that the temperature dependence of modulus must reflect a structure change. Upon cooling, G' and G'' began to increase drastically at about 40 °C, which may be due to enhancement of network structures by the development of additional cross-links via hydrogen bonds involving water, as revealed by DSC.[31]

For the carboxymethylated samples, obviously liquid-like character is observed with $G'' > G'$, and the G' is too small to be detected at high temperature due to the remarkable decrease of the elastic components and the instrumental limitation. It was found that upon heating that the modulus decreases linearly, and the curves of

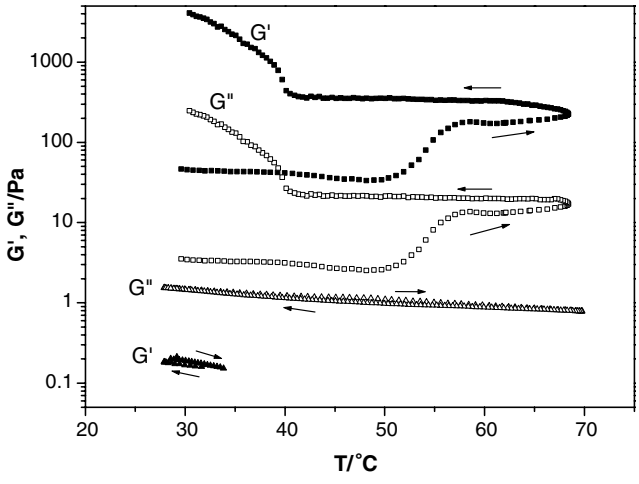

Figure 6. Temperature dependence of G' and G'' for 2% curdlan suspension (■, □) and CMc solution (▲, △) at a constant frequency of 1 Hz.

heating and cooling superposed well, indicating that the change of modulus with temperature is reversible for the CMc solution, there being no hydrogen bonds involved during the processes of heating and cooling. On the other hand, the fact that the modulus did not increase markedly above 55 °C and that no gel formation occurred indicates that the intrinsic hydrophobic structure for the native curdlan had been influenced by the chemical modification.

3.6. DSC measurements of native and carboxymethylated curdlan in water

The DSC heating and cooling curves for native curdlan and CMc are shown in Figure 7. For curdlan, the endothermic peak on heating can be attributed to the disordering of the structure or swelling of curdlan particles accompanying the breakup of hydrogen bonds.[10,31] In contrast, the appearance of exothermic peaks upon cooling is considered as the formation of hydrogen bonds involving water as shown in Figure 7a and b.

The DSC curve for 2% CMc solution is entirely different from that of native curdlan. No clear endothermic or exothermic peaks appear during heating and cooling as seen in Figure 7c and d, suggesting that both processes do not involve any change in hydrogen bonds, which is in good agreement with the above rheological experiments shown in Figure 6. Thus, it is evident that the hydrogen bonds that maintain the primary molecular structure have been destroyed by the carboxymethylation, and subsequently curdlan lost the ability of gel formation.

3.7. AFM images of native curdlan and CMc in solution

Curdlan is not soluble in water at room temperature but can be dissolved in an alkaline aqueous solution, cadoxen aqueous solution, and Me_2SO. The conformation of curdlan in a sodium hydroxide aqueous solution varied from a triple helix to random coil depending on the concentration of NaOH used.[26] Fulton and Atkins[23] investigated the molecular structure and gelling mechanism of curdlan using X-ray diffraction and infrared spectroscopy, and they proposed that triple helices are dominant for most curdlan molecular chains. The triple strand molecules are bound by hydrogen bonds to the interstitial water of crystallization to form a micellar domain. In other words, interstitial water forms the hydrogen-bonded network with the helices, binding them into a micellar structure. The diameter of the micelles was estimated to be about 8 nm.[32] Bo et al.[33] and Tabata et al.[34] reported that dissociation began to occur in 0.07 M NaOH (pH 12.8) and complete dissociation observed at 0.2 M NaOH (pH 13.3) for scleroglucan, a branched version of curdlan. Furthermore, Stipanovic and Giammatteo[35] also found that the dissociation occurred in 0.05 M NaOH by measuring the steady shear viscosity of curdlan solution at different NaOH concentrations, and the results were supported by their ^{13}C NMR experiments. The destruction of hydrogen bonds becomes less remarkable with decreasing concentration of NaOH. So with the 5 mM NaOH that was used in the present work, the micellar structure of curdlan cannot be dissociated, and triple helices still exist as in the solid state.

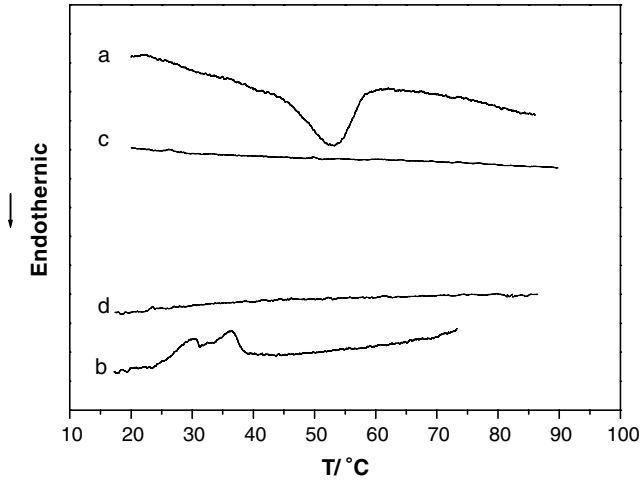

Figure 7. DSC heating and cooling curves of 2% aqueous dispersion of curdlan (a, b) and CMc solution (c, d) during heating and cooling at a rate of 1 °C/min.

From the AFM images, the main feature that can be observed is that multiple curdlan molecules form thick rope-like structures. We believe that the rope is the form of micelles, which are aggregated molecules of curdlan. The height measured for this micelle is between 6.13 and 7.42 nm as shown in Figure 8. Because the AFM observation is performed at normal humidity, there will be a layer of water present on the mica surface. Despite dehydration in air, Figure 8 should represent the micelles within a hydrated film, resulting in the decrease of the height measured. Therefore, we consider that the size of the micelles is about 8 nm, which is consistent with the conclusion by Fulton and Atkins.[23] In addition, a few triple helices, which are dissociated from the micelles are also observed clearly.

Figure 9 shows a TmAFM image of CMc dissolved in water on the mica surface. The image is quite different from that shown in Figure 8. No aggregation can be observed and the molecule appears as an extended chain. The measured vertical thickness is approximately 1.75 nm. We consider that its conformation is in the form of a triple helix comparing with the single chain dimension of curdlan in Me_2SO in which the height is about 0.65 ± 0.05 nm. In addition, when the difference in experimental methods is taken into account, this result is consistent with the triple strand thickness expected from X-ray fiber diffraction, in which the diameter of the curdlan triple helices is reported to be

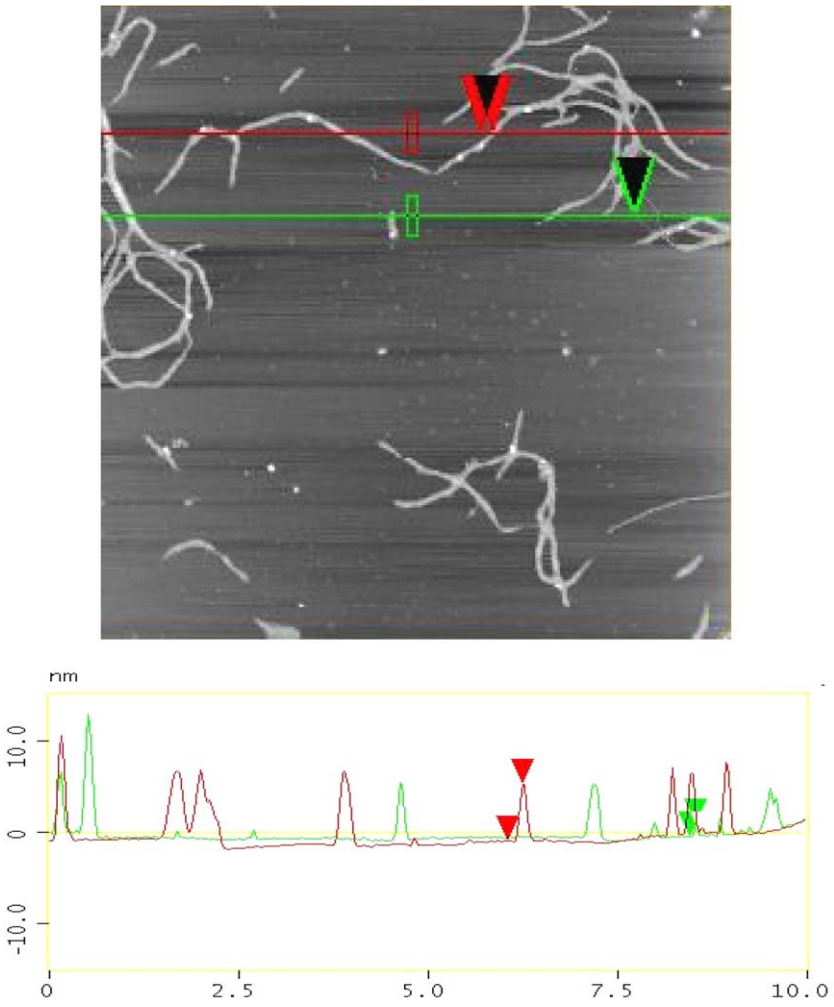

Figure 8. TmAFM image of curdlan (10 mg/L, dissolved in 5 mM NaOH) deposited onto mica surface. Image size: 10.0 μm × 10.0 μm.

1.56 nm.[36] Furthermore, according to the values of M_w in Table 1, taking into account the effect of the degradation of curdlan in a concentrated alkaline solution, the M_w of the curdlan derivatives is approximate three times that of the native curdlan in 0.3 M NaOH, in which the molecules of curdlan take a random coil conformation. It is reasonable to say that the chain of the curdlan derivatives in water is in the form of a triple strand, similar to that of schizophyllan in water.[37] Also, a few single chains (ca. 0.65 nm) that dissociated from the triple helical chains can be detected due to the substitution on C-2 position, as shown in Figure 9.

The AFM image might provide a good basis to explain the potentially enhanced bioactivity of the curdlan after carboxymethylation. The relatively stiff chain conformation of carboxymethylated derivative is beneficial for enhancing the bioactivity, as many common anti-tumor polysaccharides, such as lentinan and schizophyllan that have a triple-helix conformation with extreme stiffness.[38,39]

3.8. The effect of the carboxymethyl group on the molecular structure of curdlan

Research on physico-chemical properties of the native and modified curdlan may help us better understand the aggregation mechanism of curdlan. In aqueous suspensions, curdlan essentially exists in the form of triple helices, which are maintained mainly by hydrogen bonds. The single helix chain is formed by intramolecular hydrogen bonds between OH(4) and the adjacent hemiacetal oxygen atom of the D-glucopyranosyl residue, and the triple helical structure is linked by the intermolecular hydrogen bonds between OH(2) of every backbone glucopyranose, while intermolecular hydrogen bonds between OH(6) and the interstitial water will form the micelles. As for the carboxymethylated derivative prepared in the present work, the groups introduced are mainly in the C-6 position, which may destroy the capacity to form the micelles of curdlan molecules, but exert little effect on the hydrogen bonds

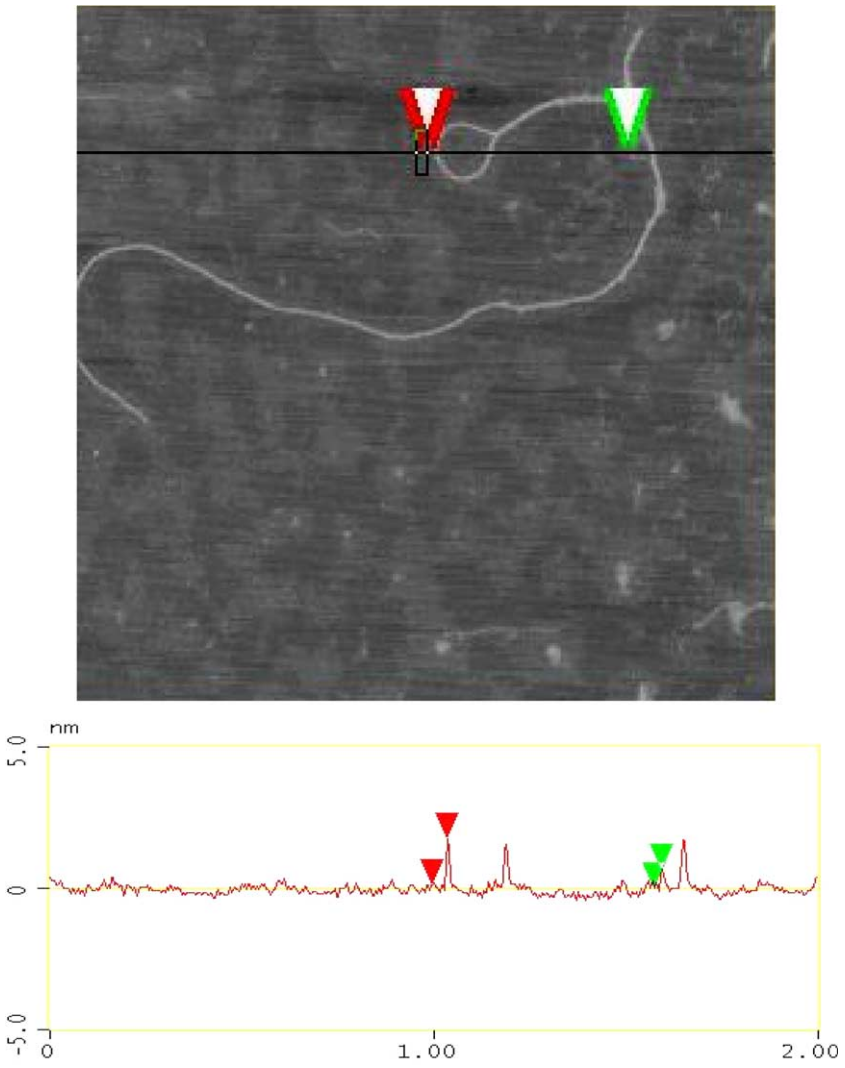

Figure 9. TmAFM image of CMc (20 mg/L, dissolved in pure water) deposited onto mica surface. Image size: 2.0 μm × 2.0 μm.

at C-2, C-4 positions, and the derivative can maintain its original triple strand helical structure.

The hydrophilic groups introduced can affect the hydrophobicity of curdlan as well. On the backbone of a curdlan molecule, bulky groups of hydroxyls and hydroxymethyls are in sterically favorable equatorial positions while all the small hydrogen atoms occupy the less sterically favorable axial positions. The three axial hydrogen atoms at C-1, C-3, and C-5 of D-glucopyranosyl residues form a non-polar, relatively hydrophobic face while the equatorial side groups form a more polar, hydrophilic face. The hydroxymethyl group can be rotated easily to some stable position to add its hydroxyl group to the hydrophilic face and add its methylene to the hydrophobic face,[40] thereby contributing to the hydrophobicity. Tako and Hanashiro[11] considered that the formation of an irreversible gel at high temperature is possibly caused by the hydrophobic interaction relative to the methylene at C-6. Because the introduced groups are mainly in the C-6 position, the hypothesis that the main intrinsic hydrophobic position for the native curdlan is in the C-6 position might be supported. The weakening of the hydrophobicity of curdlan after carboxymethylation could deprive curdlan of its gelling ability.

4. Conclusions

Curdlan displays good water solubility after carboxymethylation, but the gelling ability is entirely lost due to the weakening of the hydrophobic property at the C-6 position. For the carboxymethylated derivative of curdlan, there are no molecular aggregates in solution and the conformation mainly exists in the form of triple strand helices. Therefore, because the group introduced is more hydrophilic, the hydrophobic effect of curdlan itself is greatly influenced.

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