

Aggregation Behavior of 3,6-O-Carboxymethylated Chitin in Aqueous Solutions

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ABSTRACT: The aggregation behavior of 3,6-O-carboxymethylated chitin (3,6-O-CM-chitin) in aqueous solutions was investigated by viscometry, gel permeation chromatography (GPC), and GPC combined with laser light scattering (GPC-LLS) techniques. 3,6-O-CM-chitin has a strong tendency to form aggregates in NaCl aqueous solutions with the apparent aggregation number (N_{ap}) of about 27. There were three kinds of aggregates corresponding to different cohesive energies, the aggregates with low cohesive energy were first dissociated at 60°C, the aggregates with middle cohesive energy were then dissociated at 80 to 90°C, and the aggregates with high cohesive energy were difficult to be disrupted by heating. Decreasing polysaccha-

ride concentration (c_p) or increasing NaCl concentration (c_s) reduced the content of the aggregates. At the critical c_p of 2.5×10^{-5} g/mL, the aggregates were dissociated into single chains completely. The change of aggregation and disaggregation of 3,6-O-CM-chitin in water-cadoxen mixtures occurred from 0.1 to 0.4 of v_{cad} , and were irreversible. Intermolecular hydrogen bonding can be ascribed as main driving force for aggregation. © 2002 Wiley Periodicals, Inc. *J Appl Polym Sci* 86: 1838–1843, 2002

Key words: polysaccharides; gel permeation chromatography (GPC); viscosity; light scattering

INTRODUCTION

Polymer aggregations have been an extensive research attracting a great deal of attention in recent years because they determine the apparent molecular weight and polymer morphology in the aqueous solutions, which are closely related to the polymer properties and applications.^{1–6} Aggregations of amphiphilic nonionic polymers, block copolymers^{2,4,7,8} and hydrophobically modified polyelectrolytes including block polyelectrolytes in organic solvents or aqueous solutions^{1,3,9} have been reported over the past 2 decades. Because aggregation is mainly attributed to intermolecular hydrogen bonding⁴ and entanglement,¹⁰ control of aggregation behavior can be achieved by changing of such interchain interactions with methods of varying the solution temperature or modifying the aqueous solution quality with, for example, the addition of salts etc.^{1,5}

Carboxymethylated chitin (CM-chitin), a negatively charged ether derivative of chitin, has already been extensively used in a variety of applications such as bacteriostatic agent, wound dressings, artificial bone and skin, blood anticoagulants, and elements in drug delivery system due to its unique chemical, physical, and biological properties, especially its biocompatibility

and its ability to form film, fiber, and gel.^{11–16} It has been found that functions of CM-chitin such as moisture-retention ability and antimicrobial capacity depend on its chain conformation in solution and its molecular weight in our previous work;^{17,18} thus, GPC and viscometry methods were used to investigate these molecular properties. Aggregates were found to persist in solution, giving rise to “pseudo” high molecular weight fractions in GPC chromatograms. Because the driving force for aggregation, which may be attributed to interaction of active amino, hydroxyl, and carboxyl groups in CM-chitin changes with the surrounding environment, which leads to multifarious and complex apparent molecular weights, a full understanding of aggregation behavior of CM-chitin under different conditions is very important for the applications. However, published studies on aggregation of CM-chitin are scarce.

It is our objective in this study to investigate the effects of temperature, polysaccharide concentration, salt concentration, and cadoxen on the aggregation behavior of CM-chitin. The aggregating interaction was discussed.

EXPERIMENTAL

Preparation and analysis of sample

Chitin was supplied by Yuhuan Ocean Biochemistry Co., Ltd., in the Zhejiang province in China. The deacetylation degree was 0.1, determined by elemental analysis.¹⁹ Carboxymethylated chitin was prepared according to the literature.¹⁸ The substituting degree

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obtained from potentiometric titration²⁰ was 0.8. The ¹³C-NMR spectrum was recorded on a Varian Mercury 300 spectrometer; chemical shifts were given in D₂O at 323 K. It showed chemical shifts at δ 69.3 and δ 70.9, corresponding to $-\text{O}-\text{CH}_2\text{COOH}$ substituted on C-3 and C-6, respectively,²¹ indicating that the product prepared was 3,6-O-CM-chitin.

Viscometry

The viscosity of the 3,6-O-CM-chitin in aqueous solutions was measured with an Ubbelohde capillary viscometer at 30°C. The intrinsic viscosity, $[\nu]$, of 3,6-O-CM-chitin in the NaCl aqueous solutions and in water-cadoxen mixtures was estimated with Huggins and Kraemer plots. The $[\eta]$ of the sample in pure water was measured by the Fuoss experiential equation, which was suited to the polyelectrolyte solution:

$$(\eta_{sp}/c)^{-1} = ([\eta])^{-1} + B([\eta])^{-1}c^{1/2} \quad (1)$$

where c is the polymer concentration (g/mL).

GPC measurements

A HPLC instrument (Waters Co.) equipped with a TSK-gel 5000 PWXL column (7.5 × 300 mm), p100 pump (Thermo Separation products), RI-150 differential refractometer, and Jiangshen software was used for GPC analysis of aggregation behavior of 3,6-O-CM-chitin in aqueous solutions. The eluent was aqueous solutions of desired NaCl concentration, and the flow rate was 1.0 mL/min.

GPC-LLS measurements

GPC-LLS measurements were performed with a DAWN-DSP multiangle laser photometer combined with a P100 pump (Thermo Separation products) equipped with a TSK-gel 6000PWXL column (7.5 × 300 mm), and a differential refractive index detector (RI-150) at 30°C to determine the weight-average molecular weight (M_w) of each fraction. The eluent was a 0.2 mol/L NaCl aqueous solution with a flow rate of 1.0 mL/min. Astra software was utilized for the data acquisition and analysis.

IR spectra

3,6-O-CM-Chitin was dissolved in 0.1 M NaCl aqueous solutions and in cadoxen, respectively, with a polysaccharide concentration of 1×10^3 g/mL. Then half of 3,6-O-CM-Chitin in 0.1 M NaCl aqueous solution was heated to 90°C for 1 h, and the other half remained as a control. Ethanol was used to insolubilize and wash the samples, obtained as white, free-flowing powders. The IR spectra of 3,6-O-CM-Chitin

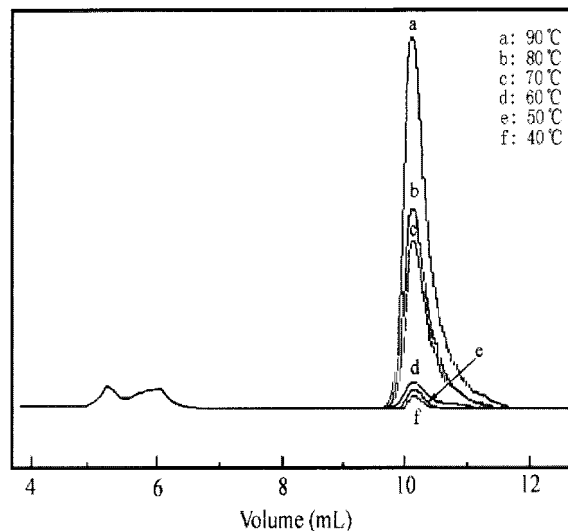


Figure 1 GPC chromatograms of 3,6-O-CM-chitin in a 0.1 M NaCl aqueous solution at different temperatures.

powders were recorded with a Nicolet 170 SX FTIR spectrometer.

RESULTS AND DISCUSSION

N_{ap} in 3,6-o-cm-Chitin

The GPC chromatograms of 3,6-O-CM-chitin in 0.1 M NaCl aqueous solutions contain two main peaks, indicating the occurrence of aggregation by the bimodal distribution. The peaks at the low elution volume correspond to the aggregates with high weight-average molecular weight ($M_{w,ag}$), and the others correspond to single chains with low weight-average molecular weight ($M_{w,s}$). However, the left side in the GPC patterns was almost a vertical line, because the exclusion limit of the column was lower than the highest apparent molecular weight of the sample used here. So the GPC-LLS technique equipped with a TSK-gel 6000 PWXL column was used to determine the weight-average molecular weight of each fraction. The $M_{w,ag}$ and $M_{w,s}$ were calculated to be 3.995×10^5 for the aggregate and 1.44×10^4 for the single chain, respectively. Thus, the apparent aggregation number (N_{ap}) was estimated to be about 27 by the formula $N_{ap} = M_{w,ag} / M_{w,s}$. But the peaks at the low elution volume were divided into two parts both in the GPC and GPC-LLS chromatograms, suggesting a broad distribution of aggregation number of 3,6-O-CM-chitin in NaCl aqueous solutions.

Dependence of disaggregation on temperature

Figure 1 shows the GPC chromatograms of 3,6-O-CM-chitin in 0.1 M NaCl aqueous solutions that were heated at different temperatures for 1 h but were

measured at 30°C. The polysaccharide concentration was 1.0×10^{-3} g/mL. As shown in Figure 1, aggregates were the main existing form at room temperature, and the content of aggregates was calculated to be about 75%. With increasing temperature, the area of the peak corresponding to the single chain increased, and that of the aggregates decreased. There were three regions and two transition points in the temperature range used. At temperature higher than 80°C, the trend of the decrease was the strongest, indicating there was a turning point near 80°C, which was called the transformation temperature. In explanation, the aggregates could be disrupted sharply when macromolecules obtained enough energy, namely, a cohesive energy of the aggregates in the solution. From 60 to 80°C, the trend of the decrease of the aggregates was also strong, suggesting another turning point at near 60°C. From 60 to 90°C, the content of the aggregates decreased about 80%. However, the area of the peak corresponding to the aggregates decreased just slightly in GPC chromatograms with further heating, and even after heating at 90°C for 5 h, the aggregates hardly disaggregated completely into single chains, suggesting that a small part of aggregates could not be dissociated by heating.

The viscometry data of 3,6-O-CM-chitin in 0.1 M NaCl aqueous solutions after heating to the desired temperature for 1 h were measured at 30°C. The inherent viscosity ($\ln \eta_r/c$) decreased with an increase in temperature. Two evident transformation temperatures were at 60 and 80°C, which can reflect the change of the transformation properties of the polysaccharide solution. The inherent viscosity of the 3,6-O-CM-chitin solution heated to 90°C, but measured at 30°C almost did not change with the storage time. This phenomenon indicated that once the aggregates of 3,6-O-CM-chitin decomposed at relatively higher temperatures, they hardly reaggregated at 30°C, suggesting that the aggregation was thermally irreversible.

It is likely that, to a large extent 3,6-O-CM-chitin exists in aqueous solution as aggregates and the increasing temperature leads to disruption of the aggregates by destruction of the aggregating interactions. The dissociation process of the aggregates can be ascribed as follows, taking reference to the literature.⁵ When the system obtained a certain energy above 60°C, the aggregates with relatively low cohesive energy were first dissociated, the aggregates with middle cohesive energy were then dissociated at 80 to 90°C, and a small part of aggregates with high cohesive energy were difficult to be dissociated by heating.

Effects of the concentration of polysaccharide and salt on aggregation

The GPC chromatograms of 3,6-O-CM-chitin in 0.1 M NaCl aqueous solutions with different polysaccharide

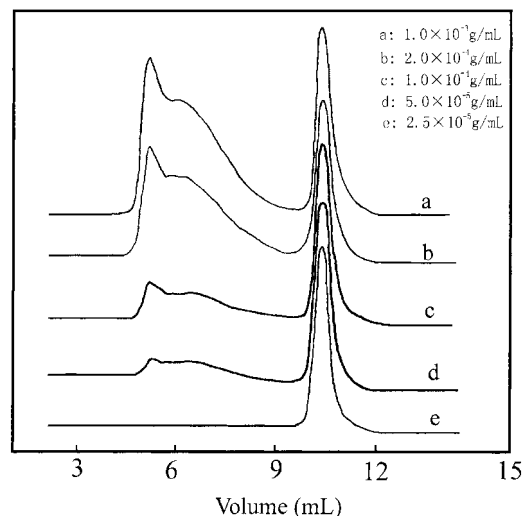


Figure 2 GPC chromatograms of 3,6-O-CM-chitin at different polysaccharide concentrations in a 0.1 M NaCl aqueous solution at 30°C.

concentrations (c_p) ranging from 2.5×10^{-5} to 1.0×10^{-3} g/mL at 30°C are shown in Figure 2. With c_p decreasing from 1.0×10^{-3} to 2.0×10^{-4} g/mL, the trend of the decrease of the aggregates was slight, but from 2.0×10^{-4} to 1.0×10^{-4} g/mL, content of the aggregates decreased sharply, and the peaks at the low elution volumes corresponding to the aggregates disappeared completely, showing a sharp peak of single-chain stranded, when c_p reached 2.5×10^{-5} g/mL. It can be interpreted that the lower polymer concentration in solution is more effective at interacting the solvent with the polymer. With increasing the polymer concentration, the solvent-polymer interaction will decrease, while the polymer-polymer interaction will increase, resulting in a great increase of aggregates.⁷ Respaud et al.²² reported that triblock copolymer in a selective solvent formed aggregates above the critical aggregation concentration of $(1.6 \pm 0.2) \times 10^{-3}$ g/mL. Apparently, 3,6-O-CM-chitin can form aggregates even in exceeding dilute solution, which has a critical aggregation concentration of 2.5×10^{-5} g/mL, showing a strong tendency of aggregation. This property is of interest in using 3,6-O-CM-chitin as viscosity increasing agent in cosmetics and in food.

Figure 3 shows salt concentration (c_s) dependence of the intrinsic viscosity $[\eta]$ for 3,6-O-CM-chitin in desired NaCl concentration aqueous solution. $[\eta]$ value increased significantly with decreasing c_s , exhibiting a strong polyelectrolyte character. When c_s was 0.05 M, the electrostatic repulsion effect was almost controlled. With c_s increasing from 0.05 to 0.5 M, $[\eta]$ value decreased gradually, suggesting that increasing NaCl concentration could disrupt the aggregates of 3,6-O-CM-chitin. Interestingly, there were two transformation NaCl concentrations near 0.1 and 0.3 M. With c_s increasing from 0.1 to 0.15 M, $[\eta]$ value decreased

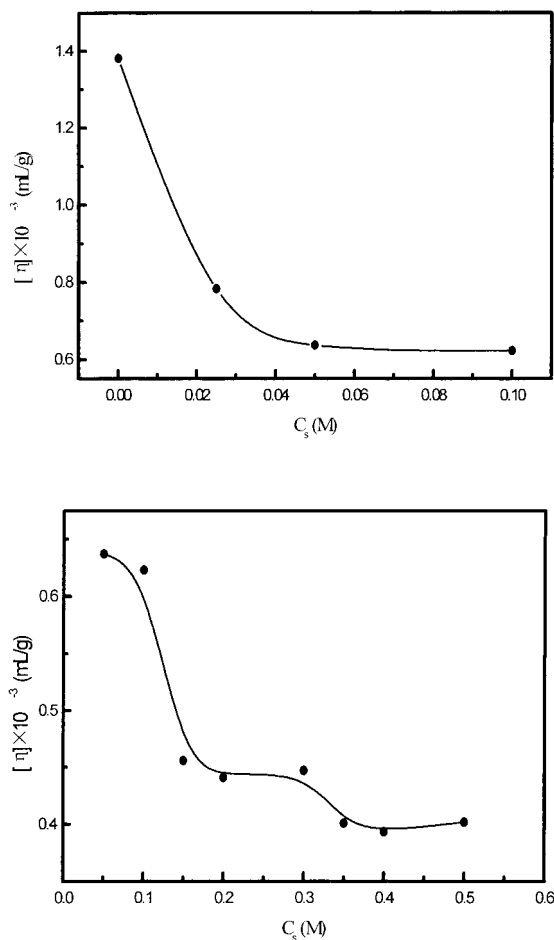


Figure 3 Dependence of the intrinsic viscosity $[\eta]$ of 3,6-O-CM-chitin on the NaCl concentration (c_s) at 30°C.

significantly, while when c_s increasing from 0.3 to 0.35 M, $[\eta]$ value decreased just slightly and leveled off when c_s was above 0.35 M. This result seems to be different from those obtained by Eisenberg and Zhang et al. Eisenberg et al.¹ reported two block polyelectrolyte samples as a function of NaCl concentration, and found that at low salt concentration ($c_s < 0.2$ M) aggregation numbers initially increased with c_s and then eventually leveled off. Zhang et al.⁵ reported that the aggregation behavior of Aeromonas gum was almost independent of the salt concentration, but reduced dimensions caused by decrease of electrostatic force was likely to have strong effect on $[\eta]$, thus intrinsic viscosity $[\eta]$ decreased with salt concentration. To verify the result obtained from viscometry method, 3,6-O-CM-chitin solutions of desired NaCl concentration were injected into the GPC column at 30°C. With c_s increasing, the area of the peak corresponding to the aggregates decreased, and that of the single chains increased, which are in accordance with the result got from viscometry method. This phenomenon can be explained that as a polyelectrolyte, 3,6-O-CM-chitin may change from a more expanded chain into a ran-

dom coil in high NaCl concentration solutions due to decrease of electrostatic repulsion effect. Therefore, the aggregating interaction will decrease because of a stronger steric hindrance among polymer chains.

Effect of cadoxen

Cadoxen has been used as a solvent, which breaks the strong intermolecular interaction of polysaccharides. Aeromonas Gum, xanthan, and β -D-glucan from *Poria cocos* dissolve in NaCl aqueous solution mainly as aggregates, which are sustained by intra- and intermolecular hydrogen bonds, but as single chains in cadoxen at room temperature.^{23–25} In this work water-cadoxen mixtures of different ratios of water to cadoxen were prepared to use as solvents for 3,6-O-CM-chitin.

Figure 4 shows the plot of $[\eta]$ for 3,6-O-CM-chitin in water-cadoxen mixtures against the volume fraction of cadoxen in water-cadoxen mixtures (v_{cad}). As v_{cad} increased from 0.1 to 0.3, the $[\eta]$ values decreased sharply, and then slowly decreased with continuous increase of cadoxen. But the $[\eta]$ value in pure cadoxen increased suddenly to above that of 3,6-O-CM-chitin in 30% cadoxen. It is suggested that the aggregates were destroyed into single chains mainly in a narrow range of solvent composition (v_{cad} between 0.1 to 0.3), which is in agreement with the results of Aeromonas gum²³ and that of β -D-glucan from *poria cocos*,²⁵ in which the change of aggregation and disaggregation occurred in the range of v_{cad} from 0 to 0.4, and from 0.2 to 0.3, respectively. Although the slight decrease of $[\eta]$ in the range of v_{cad} from 0.3 to 0.8 might be ascribed to be a decrease in chain stiffness resulted from increase of v_{cad} . Increasing of $[\eta]$ value in pure cadoxen can be explained that while the disruption of aggregates in cadoxen leads to an extensive decrease in $[\nu]$, the

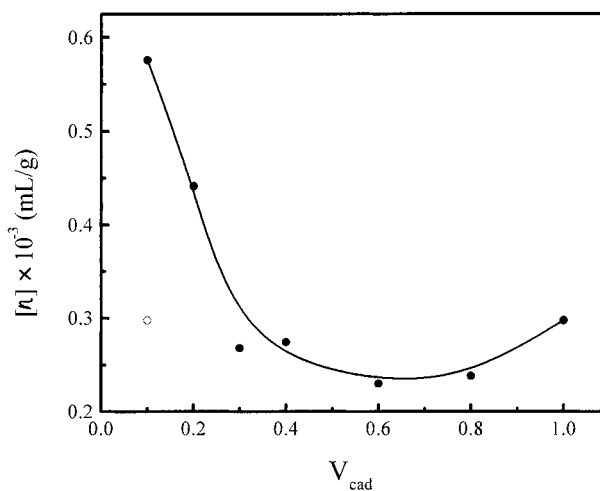


Figure 4 Plots of $[\eta]$ against v_{cad} for 3,6-O-CM-chitin in water-cadoxen mixtures at 30°C. See the text for open circle explanation.

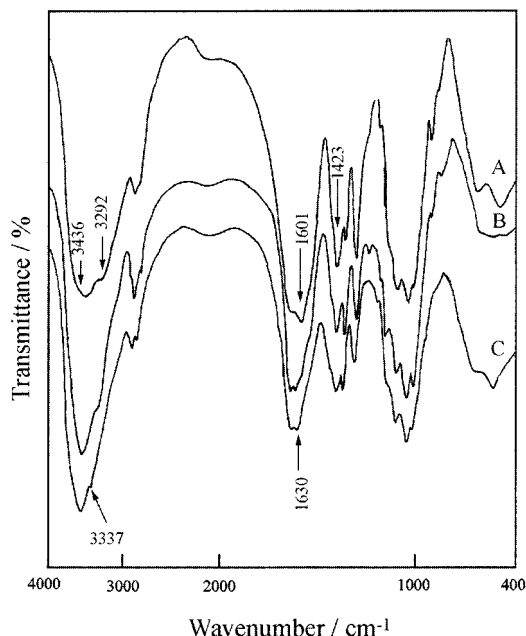


Figure 5 IR spectra of 3,6-O-CM-chitin. (A) Insolubilized from 0.1 M NaCl aqueous solutions. (B) Insolubilized from 0.1 M NaCl aqueous solutions after heating at 90°C for 1 h. (C) Insolubilized from cadoxen.

solvation in cadoxen causes the increase in chain dimension. The open circle in Figure 4 refers to the value of $[\eta]$ obtained for the 3,6-O-CM-chitin dissolved in pure cadoxen and then diluted to 10% cadoxen solution with water. This value was much lower than that of 3,6-O-CM-chitin, which was directly dissolved in 10% cadoxen solution, but a little higher than the $[\eta]$ values above $v_{\text{cad}} = 0.3$, indicating that the process of aggregation-disaggregation of 3,6-O-CM-chitin in water-cadoxen was irreversible, and the chain stiffness increased by diluting with water.

IR spectra

As demonstrated in Figure 5, the spectra of 3,6-O-CM-chitin show the characteristic absorptions at 1601 and 1423 cm^{-1} due to asymmetric and symmetric stretching vibration of COO^- , indicating a successful substitution of carboxymethyl groups.¹⁵ The strong, broad bands in spectrum A at 3436 and 3292 cm^{-1} are assigned to the hydrogen-bonded —OH and —NH bands.²⁶ It is important to note that when treated with heating and cadoxen the —OH stretching vibration bands were narrowed and shifted to a higher wave number, while the hydrogen-bonded —NH bands at 3292 cm^{-1} disappeared and was accompanied by the appearance of a weak N—H stretch at 3337 cm^{-1} . These strongly suggest that the hydrogen bonding in the polymer chains was disrupted by heating and cadoxen. Simultaneously, as we can see, the absorption of asymmetric stretching vibration of COO^-

groups at 1601 cm^{-1} decreased and also shifted to significantly higher wave number. It may be concluded that intermolecular hydrogen bonds ($\text{C=O} \cdots \text{HN}$, $\text{C=O} \cdots \text{HO}$) exist between —NH/—OH and COO^- groups in 3,6-O-CM-chitin in NaCl aqueous solutions, which can be ascribed as the main driving force for aggregation.

CONCLUSIONS

3,6-O-CM-chitin has a strong tendency to form aggregates in NaCl aqueous solution at room temperature with an N_{ap} of about 27. On the basis of the polysaccharide concentration, salt concentration, and temperature dependence of the aggregation, it is concluded that the driving force for the aggregation, which can be mainly attributed to intermolecular hydrogen bonding between polymer chains, increases with decreasing salt concentration, temperature, and increasing polysaccharide concentration. There were three kinds of aggregates corresponding to different cohesive energy: the aggregates with low cohesive energy were first dissociated at 60°C, the aggregates with middle cohesive energy were then dissociated at 80 to 90°C, and the aggregates with high cohesive energy were difficult to be disrupted by heating. At the critical polysaccharide concentration of 2.5×10^{-5} g/mL, the aggregates were broken into single chains completely. Cadoxen showed a fine capacity to disrupt the aggregating interaction. The change of aggregation and disaggregation of 3,6-O-CM-chitin in water-cadoxen mixtures occurred from 0.1 to 0.4 of v_{cad} and were irreversible.

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