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ORIGINAL PAPER

Effect of the ionic strength of the media on the aggregation behaviors of high molecule weight chitosan

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Abstract The objectives of this study are to explore the effect of the ionic strength of aqueous acidic media and the intrinsic characteristics of chitosan used on the formation of different types of aggregation. Two different degrees of acetylation (DA) of β-chitosan are prepared from β-chitin by stepwise alkali deacetylation. These two chitosans have high chain stiffness and typical good solvent behavior in a 0.20 M acetic acid/0.10 M sodium acetate buffer solution. However, a trace amount of aggregates forms in the higher DA chitosan dilute solution. At ionic strength (I) ≥ 0.20 M, the association of compact chitosan forms and/or the amount of the aggregates increases with increasing I of the media. At I=0.01 M, a considerable quantity of particle structure is observed in the low ionic strength solution and the electrodynamic coupling effect predominates on the mobile behavior of the extended and congested chitosan molecule in solution.

Keywords Chitosan · Degree of acetylation · Ionic strength · Aggregation

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Introduction

Chitin and chitosan are linear copolymer of β-1,4 linked 2-acetamido-2-deoxy-D- glucopyranose (GlcNAc) and 2-amino-2-deoxy-D-glucopyranose (GlcN). With the alkaline N-deacetylation of chitin, when the degree of deacetylation reaches about 50%, it becomes soluble in aqueous acidic media through the protonation of the -NH_2 group on the C-2 position of the D-glucosamine units and it is chitosan. In solution, the degree of protonation of chitosan depends on the pK and concentration of the acid used [1, 2].

Chitosan can be regarded as an amphilic macromolecule [3–6]. The proportion and distribution of acetylated and nonacetylated residues of chitosan govern the balance between hydrophilic and hydrophobic properties of it in solution. The amount and distribution of N-acetyl groups affect the formation of intra- and inter-molecular hydrogen bonds of chitosan and an increase of the inter-molecular hydrogen bond promotes the forming of aggregates in the chitosan solution [7]. The β-1,4 linked polysaccharides have high chain stiffness. In the aqueous acidic media, chitosan behaves as a semi-flexible polyelectrolyte. The model of worm-like chain had been used to calculate the persistence length of chitosan in an acidic aqueous solution [4, 8, 9]. Brugnerotto et al. [10] reported that the calculated

Lp increased with an increase in the percentage of N-acetylated residues, but was irrelevant to the backbone substitution pattern of random or block of N-acetyl groups. Lamarque et al. [9] reported that the conformation and persistence length of chitosan varied according to two distinct regions versus the degree of acetylation (DA) with a transition range in between (a triple behavior). However, Berth et al. [11] reported that the DA had no measurable effect on the conformation of chitosan.

Chitosans prepared from chitin by heterogeneous alkali deacetylation have a tendency to form aggregates in acidic solution [12, 13], and the aggregation phenomenon of a chitosan acidic solution may affect the preciseness of the molecular weight measurement [12, 14]. The aggregation phenomenon of chitosan in acidic aqueous media studied by light scattering has been attributed to the factors of residual N-acetyl groups [11–13]; dissociation (deprotonation) and DA of chitosan [4, 6], the effect of acetic acid concentration [15], and the heating effect of the solution [14]. Whether the DA affects the conformation, stiffness and aggregation of chitosan has been an ongoing topic of interest.

There are complex mutual interactions in low ionic strength polyelectrolyte solutions. Different types of aggregate polyelectrolyte, such as an anisotropic translational diffusion [16], the domain structure [17] and the particle structure [18], have been reported for low ionic strength polyelectrolyte solutions. Due to the existence of electrostatic interaction of polyelectrolyte in the aqueous solution, in studies on the hydrodynamic property of polyelectrolyte, salt has usually been added to the solution to screen the charges of ionic groups along the chains and to minimize the electrostatic interaction. Sodium chloride and Sodium acetate have been traditionally used when studying the physicochemical behavior of chitosans in aqueous solution, but they cannot prevent the formation of aggregation in the solutions [12, 19]; in addition, an increase in the ionic strength of chitosan solution favors the formation of a hydrophobic microdomain and favors the attractive van der Waals effect, and that induces the formation of inter-chain aggregation or association of chitosan molecules [8, 20–23]. Furthermore, an increase in the ionic strength of the media would promote the intra-chain compaction effect of chitosan [24, 25]. The aggregation of a group of molecules that held together in some way does not easily broken apart; moreover, the association colloid is a dispersion of colloidal-sized aggregates of small molecules and it is lyophilic [26].

This paper is focused on the effect of the ionic strength of the solution and the intrinsic characteristics of the chitosan on the hydrodynamic properties of chitosan, and examined the formation of aggregates in aqueous acidic media. The hydrodynamic properties and the formation of chitosan aggregates in aqueous acidic media were monitored mainly by dynamic light scattering. The intensity-mean, the distribution of relaxation times and the hydrodynamic radius of different relaxation modes that appeared in the chitosan solutions were used to depict the different types of aggregation that were effected by the above two factors.

Materials and methods

Preparation of chitosan

β-Chitin was extracted from squid pens (*Iuex argentinus)*. The squid pens were dried in an oven for 1 week at 40 °C and were then ground. To collect the ground squid pens powder resided between 40–80 mesh. The modified method of Kurita et al. [27] was used to obtain the initial β-chitin sample.

Heterogeneous deacetylation of β-chitin was prepared by alkali deacetylation with 50% NaOH for 100 min at 80 °C; the weight ratio of chitin to 50% NaOH was 1:10. The deacetylation processes (changing the processing time to 80 min) was repeated in the new alkali solutions to obtain the β_2 and β_4 chitosans; the Arabic numbers of subscript indicate the total number of deacetylation processes required. The samples were then washed to neutrality and freeze-dried.

Determination of DA

Infrared spectrometry was used to determine the degree of acetylation (DA) of the chitosans. Chitosan powder was mixed with KBr (1:100) and pressed into a pellet. The absorbances of amide 1 (1,655 cm^{-1}) and the hydroxyl band (3,450 cm^{-1}) were measured using a Bio-Rad FTS-155 infrared spectrophotometer (Hercules, CA). The band of the hydroxyl group at 3,450 cm^{-1} was used as an internal standard. The percentage of the DA was given by $A_{1655}/A_{3450}\times 115$. Herein, A_{1655} and A_{3450} were the absorbances at 1,650 cm^{-1} and 3,450 cm^{-1}, respectively [28].

Determination of Mw

Static light scattering was used to measure the weight-average molecular weight (Mw) of the chitosan. A concentration series (0.10–0.50 mg/ml) of chitosan in 0.20 M acetic acid/0.10 M sodium acetate buffer solution was prepared by diluting a stock solution (0.50 mg/ml) that had been filtrated with a 0.45 μm filter (Millipore, USA). The solvent was filtered through a 0.02 μm filter (Whatman, USA). Prior to the light scattering measuring, the test solutions were again filtrated with a 0.45 μm filter. The scattered light intensity of the solutions, between 30° and 120°, was measured by a Malvern light scattering photometer (Malvern 4700, UK) at 632.8 nm and 30±0.1 °C [29]. The Mw was calculated from the Zimm plot processed by Malvern software (version 1.61 for Windows).

Determination of intrinsic viscosity

A capillary viscometer (Cannon-Fenske, No 75, USA) was used to measure the passage time of solutions flowing through the capillary. The capillary viscometer was equilibrated in a water bath (Tamson TMV-40, Holland) at 30±0.1 °C. Different concentrations of chitosan were dissolved in two solvent systems. System 1 was 0.10 M acetic acid with various concentrations of NaCl (0.01–0.50 M) (The pH values of the acetic acid aqueous solutions slightly decreased from 2.97 to 2.63 with increasing the ionic strength of the media from 0.01 to 0.50 M); and system 2 was a buffer solution of 0.20 M acetic acid/0.10 M sodium acetate (pH=4.32). These solutions were filtered through a 0.45 μm filter (Millipore, USA) before measuring the passage time. Using the Huggins equation, the reduced viscosity was plotted against the concentration of chitosan and the intrinsic viscosity ([η]) was then determined by extrapolating the reduced viscosity to zero concentration [29].

Dynamic light scattering

The preparation process for the dynamic light scattering measuring of the chitosan solutions was the same as for the [η] measuring. The dynamic light scattering was probed for a scattering angle θ between 30–90°. The fluctuation of scattered light intensity of the chitosan solutions was measured by a Malvern light scattering photometer (Malvern 4700, UK) at 632.8 nm and 30±0.1 °C with a multi-tau sampling time correlator (7032) and exponential sampling software (PCS) (version 1.61 for Windows).

In the dynamic light scattering experiments, the normalized time correlation function (C(t)) of the scattered intensity can be expressed in terms of the field correlation function $g^{(1)}$(q, t) through

$$C(t) = A + B\left|g^{(1)}(q,t)\right|^2 \quad (1)$$

where A is the baseline, B the coherence factor and q the magnitude of the scattering vector, $q = \frac{4\pi n_0}{\lambda}\sin\theta/2$, n_0 is the refractive index of the scattering medium (solvent), λ is the wavelength of incident beam in vacuum. For the monodisperse system, the $g^{(1)}$(q, t) is quantified by the first-order auto correlation function (2), and for the polydisperse system, the $g^{(1)}$(t) of the polydisperse system can be written as an integral Eq. 3.

$$g^{(1)}(t) = \exp[-\Gamma t] \quad (2)$$

$$g^{(1)}(t) = \int_0^\infty G(\Gamma)e^{-\Gamma t}d\Gamma \quad (3)$$

In the above equation $\Gamma = Dq^2$, Γis the decay rate, the decay (relaxation) time $t = \frac{1}{\Gamma}$, D is the translational diffusion coefficient and $G(\Gamma)$ is the normalized distribution function of the decay rate. The hydrodynamic radius (R_h) of the particle can then be calculated from the translational diffusion coefficient by the Einstein-Stokes equation:

$$R_h = \frac{kT}{6\pi\eta_0 D} \quad (4)$$

where k is the Boltmann constant, T the absolute temperature and η_0 the viscosity of the solvent.

After the fitting process, the $g^{(1)}$(q, t) was resolved by two methods: the cumulant method and CONTIN [30]. In this experiment, the distributions of the relaxation times and the hydrodynamic radius of the different relaxation modes that appeared in the chitosan solutions were acquired by the resolving of CONTIN; in addition, the apparent diffusion coefficient (D_{app}) was resolved by the cumulant method.

Light microscopy

The light microscopy image was obtained with a microscope (Olympus BX61, Japan). The image was viewed with a CCD camera (Olympus DP71, Japan) and directly recorded by CCD image with no resolution enhancement [18]. Before the light microscopy measurement, different ionic strengths of chitosan solutions were filtrated with a 0.45 μm filter and then left standing for 30 min.

Results and discussion

Intrinsic characteristics of the chitosans used

The intrinsic characteristics of the chitosans used such as the DAs were 30.0% and 7.3% for β_2 and β_4, respectively, and the weight-average molecular weight (Mw), the radius of gyration (Rg) and the intrinsic viscosity [η] of the chitosans used in 0.20 M acetic acid/0.10 M sodium acetate buffer solution decreased from 1.75×10^6 to 1.00×10^6 dalton, from 164 nm to 110 nm and from 2.17×10^3 ml/g to 1.61×10^3 ml/g, respectively, when the steps of the deacetylation process were increased. The model of worm-like chain in the condition of Gaussian coil limit was used to calculate the total persistence length (L_{pT}) of these two high molecular weight chitosans.

$$3Rg^2 = L_{pT}L\alpha^2 \quad \text{for } L/L_{pT} >> 1 \quad (5)$$

where L is the contour length of the polymer, α the expansion factor and α is almost 1.2 for chitosan with ionic strength of 0.12 M [11].

The rough estimated values of the L_{pT} of β_2 and β_4 chitosan calculated from Eq. 5 were 110 and 80 Å in 0.20 M

acetic acid/0.10 M sodium acetate buffer solution. The monomeric unit length of chitosan is almost 5 Å, so it indicates that these two chitosans used in this experiment have high chain stiffness.

The results in Table 1 show that the second virial coefficient (A_2) of chitosan solutions increased from 3.01×10^{-3} to 3.91×0^{-3} cm^3 mol g^{-2} with decreasing DA of chitosans. A_2 reflects the solvent quality and the excluded volume effect of the polymer chains. A_2 of these two chitosans was positive and at the level of 10^{-3} cm^3 mol g^{-2} in 0.20 M acetic acid/0.10 M sodium acetate buffer solution, which is expected for polyelectrolyte in the thermodynamically good solvents [12]. Schatz et al. [3] and Lamarque et al. [9] reported that the decrease of A_2 (the loss of solubility) with an increase in DA was attributed to the fact that the increase of GlcNAc residues of chitosan enhanced the hydrophobic interactions and/or decreased the hydrophilic interactions (electrostatic repulsions) of the polymer chains.

Hydrodynamic radius distributions of chitosans used

The distribution of hydrodynamic radius resolved by the CONTIN program to indentify the effect of deacetylation process on the aggregation behavior of these two chitosans in 0.20 M acetic acid/0.10 M sodium acetate buffer solution. At the scattering angle $\theta=90^o$, the results in Fig. 1a show, the majority of the hydrodynamic radius distribution of the chitosans used were in the range of 10–400 nm and 10–200 nm, respectively for β_2 and β_4; the distribution range became narrower with an increase in the steps of alkali deacetylation process, and the peak value of the hydrodynamic radius distributions decreased from 90 nm to 60 nm with an increase in the number of deacetylation times. The results in Fig. 1a also show, in the β_2 diluted solution, the intensity fraction of the hydrodynamic radius within 200–400 nm was almost 5%. At the scattering angle $\theta=30^o$, as the results in Fig. 1b show, two modes of hydrodynamic radius distribution appeared in β_2 dilute solution; the relaxation time distributions of chitosan β_2 could be identified as two distinct relaxation modes and the intensity-mean hydrodynamic radius of the slow relaxation mode of β_2 was almost 1 μm, which was much larger than the radius of gyration of the chitosan β_2 (Rg= 164 nm). However, the hydrodynamic radius of β_4 was still in the range of 10–200 nm, much smaller than that of β_2. The results indicated that trace amounts of aggregates were formed in a higher DA chitosan solution.

From the results shown in Fig. 1, the amount of the aggregate structure (slow relaxation mode of β_2) in the aqueous acidic chitosan dilute solution should be only a trace quantity which could not be identified by number-weighted distribution of hydrodynamic radius, resolved by the CONTIN program, at the scattering angle $\theta=30^o$ (data not shown), and it could be decreased with increasing the steps of deacetylation process. Similar results have reported by Anthonsen et al. [12] and Yanagisawa et al. [13]. The aggregation or association of chitosan might be related to the consecutive unit of GlcNAc residual sequences (chitin-like structure) along the chains. Berth et al. [11] reported that a higher molar mass and higher acetylation fraction of chitosan formed a more compact species (aggregation) in solution, and that the source of the compact species was imperfectly deacetylated fractions in the heterogeneous population of chitosan. The higher DA chitosan (β_2) should have a small quantity of imperfectly deacetylated fragment with chitin-like structure and have a higher proportion of GlcNAc-GlcNAc diad in a molecular chain than the lower DA chitosan (β_4) [31]. Although the solvent (0.20 M acetic acid/0.10 M sodium acetate buffer solution) used in this experiment was a good solvent for these two chitosans (Table 1), it might be a poor solvent for those small quantity chitin-like structures with a higher DA backbone. The higher DA chitosan chains with higher number of NAc groups and consequently lower number of amino groups would have more hydrophobic force and lower electrostatic effect; moreover, increasing the amount of consecutive unit of GlcNAc not only favored the hydrophobic interaction of chitosan chains but also promoted the formation of inter-chain hydrogen bonding [3], thus causing the aggregation of those chitosan chains with higher proportion of consecutive unit of GlcNAc in the acidic solutions.

In the preparation process of chitosan solution, the inter-chain aggregate may have formed in the original solution; during the clarification processes, the shearing stress might disintegrate the inter-chain aggregate structure of the chitosan with chitin-like structure. This facilitated those chitosan passing through the filter (0.45 μm) and forming

Table 1 Physico-chemical characteristics of degree of acetylation (DA), weight-average molecular weight (Mw), radius of gyration (Rg), intrinsic viscosity ([η]), total persistence length (L_{pT}) and second virial coefficient (A_2) of chitosans used

Chitosan	DA (%)	Mw×10^{-6} (dalton)[a]	Rg (nm)	[η]×10^{-3} (ml/g)	L_{pT} (Å)	$A_2\times10^3$ ($cm^3\cdot mol\cdot g^{-2}$)
β_2	30.0	1.75	164.4	2.17	110	3.01
β_4	7.3	1.00	110.3	1.61	80	3.91

[a] The buffer solution used to obtain the Mw, Rg, [η] and A_2 was 0.20 M acetic acid/0.10 M sodium acetate, at 30 °C.

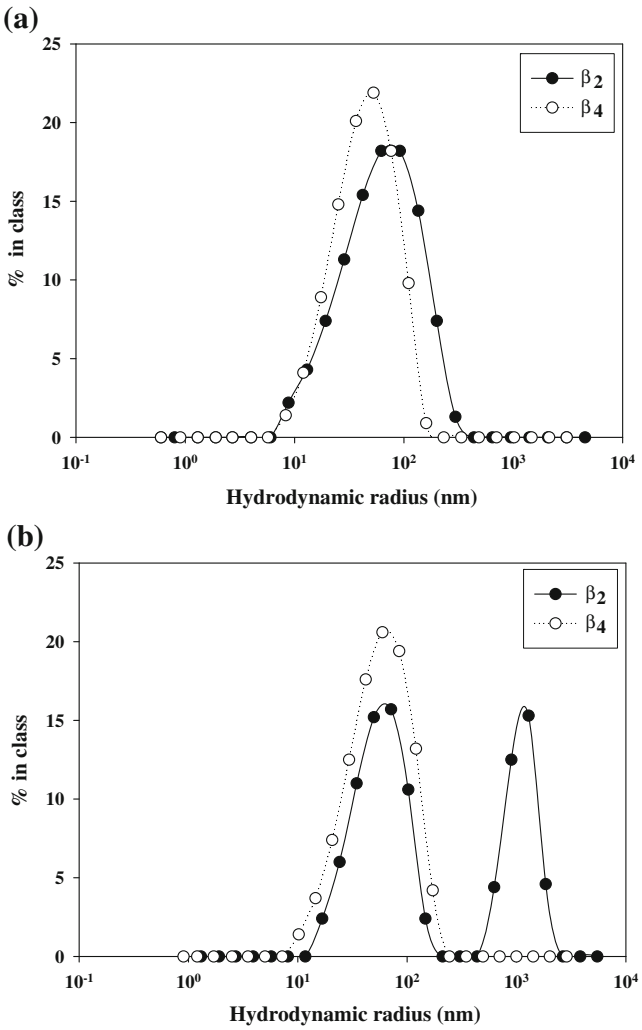

Fig. 1 Intensity fraction of hydrodynamic radius distributions at scattering angle θ=90° (**a**) and 30° (**b**) for two chitosans (β_2 and β_4) at 0.40 mg/ml in a 0.20 M acetic acid/0.10 M sodium acetate buffer solution

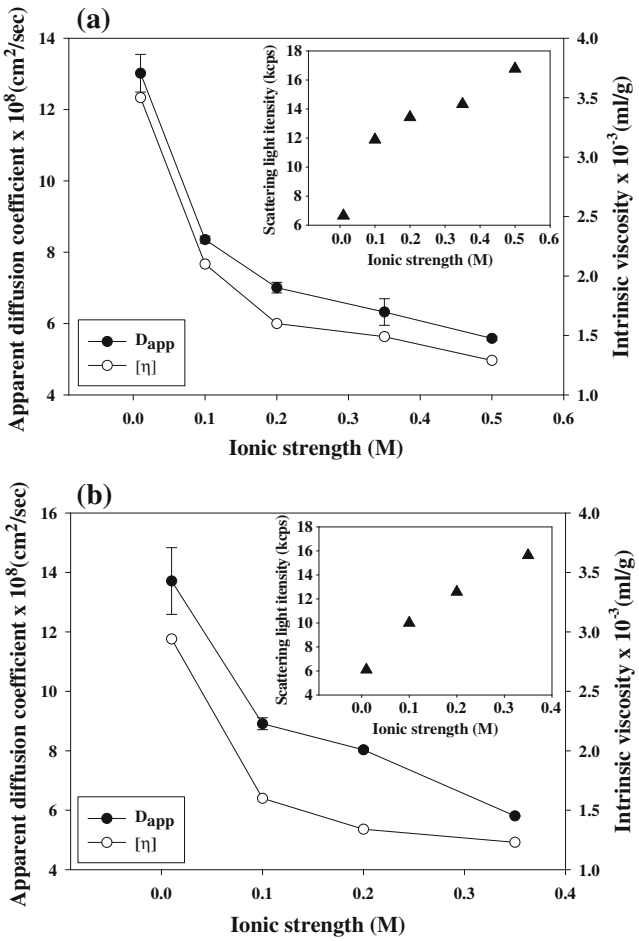

Fig. 2 Changes of the intrinsic viscosity ([η]), the apparent diffusion coefficient (D_{app}) and the scattering light intensity (insert figure) with an ionic strength between 0.01–0.50 M for chitosan β_2 (**a**) and between 0.01–0.35 M for chitosan β_4 (**b**) in 0.10 M acetic acid (the apparent diffusion coefficient and the scattering light intensity were measured at scattering angle θ=90° with concentration of chitosan=0.40 mg/ml)

aggregation or association later in the solution; however, the trace amount of the aggregates of chitosan solution (slow relaxation mode of β_2) were removed completely by using a 0.22 μm filter. The reasons for this maybe due to either the pore is too small for aggregate to pass through or the positive pressure (normal stress) may lead to the incomplete disintegration of aggregate chitin-like structure.

Effect of ionic strength on the hydrodynamic properties of chitosan

Figure 2a and b show that the D_{app} and [η] of chitosan β_2 decreased from 13.0×10^{-8} to 6.0×10^{-8} cm^2/s and from 3.50×10^3 to 1.29×10^3 ml/g, respectively, with increasing the ionic strength of the media from 0.01 to 0.50 M; and the D_{app} and [η] of chitosan β_4 decreased from 14.0×10^{-8} to 6.0×10^{-8} cm^2/s and from 2.94×10^3 to 1.23×10^3 ml/g, respectively, with increasing the ionic strength from 0.01 to 0.35 M. The reason for using lower I of 0.35 M for β_4 solution is that chitosan β_4 was not completely dissolved in an acidic aqueous media with ionic strength of 0.50 M. In addition, the results in the insert figure of Fig. 2 show the effect of ionic strength (I) on the scattering light intensity of β_2 and β_4 chitosan solutions. The results showed that the scattering light intensity of chitosan solution increased with increasing the ionic strength of the media at measuring angle θ=90°. The results of Figs. 2a and b, which show that the [η] of chitosan decreased with an increase in the I of the media, were in agreement with the results of Tsaih and Chen [25, 29]; however, that the diffusion coefficient decreased with ionic strength was contrary to the results of Errington et al.

[32] and Tsaih and Chen [33]. Their results showed that the diffusion coefficients of chitosan increased with increasing ionic strength of the solutions. The diffusion coefficient and the [η] are parameters of hydrodynamic properties of macromolecules in aqueous solutions. If the diffusion coefficient really reflected the motion of entire polyelectrolyte in dilute regime, the hydrodynamic volume of chitosan would increase with decreasing ionic strength (third electroviscous effect); in other words, the coil would expand with decreasing ionic strength and result in a decrease in its diffusion coefficient. However, the results of D_{app} in Fig. 2 were contrary to this line of thought and the reasons for this are discussed fully in the following section.

Small ion-macroion coupling effect

From the results shown in Fig. 2, the [η] of chitosan increased but the D_{app} of it also increased significantly with decreasing the ionic strength from 0.10 to 0.01 M. An increase of the D_{app} of chitosan in the low ionic strength (I=0.01 M) solution may be due to the small ion-macroion (electrodynamic) coupling effect overcoming the hydrocolloid extending effect [34]. The small ion-macroion coupling effect can either increase or decrease the D_{app} of polyion, which is depended on the predomination of Donnan equilibrium effect or electrolyte dissipation effect. With Donnan equilibrium effect, the decrease in the ionic strength leads to an increase in the diffusion coefficient; but with electrolyte dissipation effect, the retardation force reduces the mobility of the polyion with an asymmetric distribution of ionic groups. Except for the trace chitin-like component, the protonable -NH_2 group of the D-glucosamine units on the backbone of β-chitosan with DA ≤30% should distribute randomly [31]. In aqueous acidic media, the distribution of charges of these two chitosans could be seen as symmetric. It was reasonable to assume that the mobility of the two chitosan molecules (β_2 and β_4) followed the Donnan equilibrium effect in low ionic strength solution. Therefore, the extending of polyelectrolyte should be restricted at the reduced concentration of chitosans ≥1 (at a polymer concentration of 0.40 mg/ml with I=0.01 M, the reduced concentration of chitosan β_2 and β_4 was 1.4 and 1.2, respectively), and then the extending effect of polyelectrolyte was overwhelmed by the Donnan equilibrium effect which led to an increase of the D_{app} of chitosan in the low ionic strength (I=0.01 M) solution (Fig. 2).

Compaction effect and association behavior of chitosan

In the polyelectrolyte solution, the total persistence length (L_{pT}) that represents the chain rigidity of the polyelectrolyte can be summed by two contributions: $L_{pT} = L_{po} + L_{pe}$. L_{po} is the intrinsic persistence length and L_{pe} is the electrostatic persistence length. The electrostatic persistence length can be written as:

$$L_{pe} = \frac{1}{4}\frac{\lambda_D^2}{\lambda_B}, \text{ for } b \le \lambda_B \quad (6)$$

λ_D is the Debye-Hückel screening length, λ_B the Bjerrum length and b the mean distance between the two ionic groups of polyelectrolyte. In 0.10 M acetic acid, the pH values of these chitosan solutions used with an ionic strength between 0.01–0.50 M were below 3.4, the degrees of protonation of both chitosans should be 100% [1, 3], the ionic strength dependent electrostatic persistence lengths of chitosans with DA ≤ 30% ($b \le \lambda_B$) calculated from Eq. 6 were 33.7 Å at I=0.01 M, 3.4 Å at I=0.10 M and 0.7 Å at I=0.50 M. The rough values of persistence length of these two chitosans at I=0.10 M were 110 and 80 Å, respectively; so long as the ionic strength of the solution exceeded 0.1 M, the effect of electrostatic interaction on persistence length could be neglected [35]. Nevertheless, the [η] of chitosan β_2 at I=0.50 M ([η]=1.29×10^3 ml/g) and I=0.20 M ([η]=1.60×10^3 ml/g) were almost 0.6 times and 0.8 times of the value of it at I=0.10 M ([η]=2.17×10^3 ml/g), respectively (Fig. 2). The result indicated at I ≥ 0.20 M, the reducing of the hydrodynamic volume of chitosan was principally due to the compaction effect of chitosan [24, 25], and that the conformation of chitosan became more compact with increasing the ionic strength of solution. In other words, an excess of salt resulted in a decrease in the degree of hydration of chitosan in high ionic strength media.

Figure 3 shows the effect of ionic strength on the hydrodynamic radius distribution of chitosan β_2 and β_4. The results showed that the peak value and the range of hydrodynamic radius distribution of chitosan β_2 and β_4 increased with increasing the ionic strength of the media at measuring angle θ=90°. It implied that an increase in the ionic strength of media favored the formation of inter-chain aggregation or association of chitosan molecules. The Debye screening length of chitosan decreased with an increase in the ionic strength of media. In addition, the decrease in the electric double layer thickness of chitosan enhanced the attractive van der Waals effect which facilitated the interaction between polyelectrolytes [8]. The enhancement of the attractive van der Waals effect may favor the association of polyelectrolyte then led to the scattering light intensity as well as the hydrodynamic radius distribution of the associate chitosan being increased.

The results shown in Figs. 2 and 3 implied that the compaction effect of high molecule weight chitosan and the association of compact molecules occurred with the increase in the ionic strength of solution. Furthermore, the reason for a decreasing [η] with an increasing I, as shown

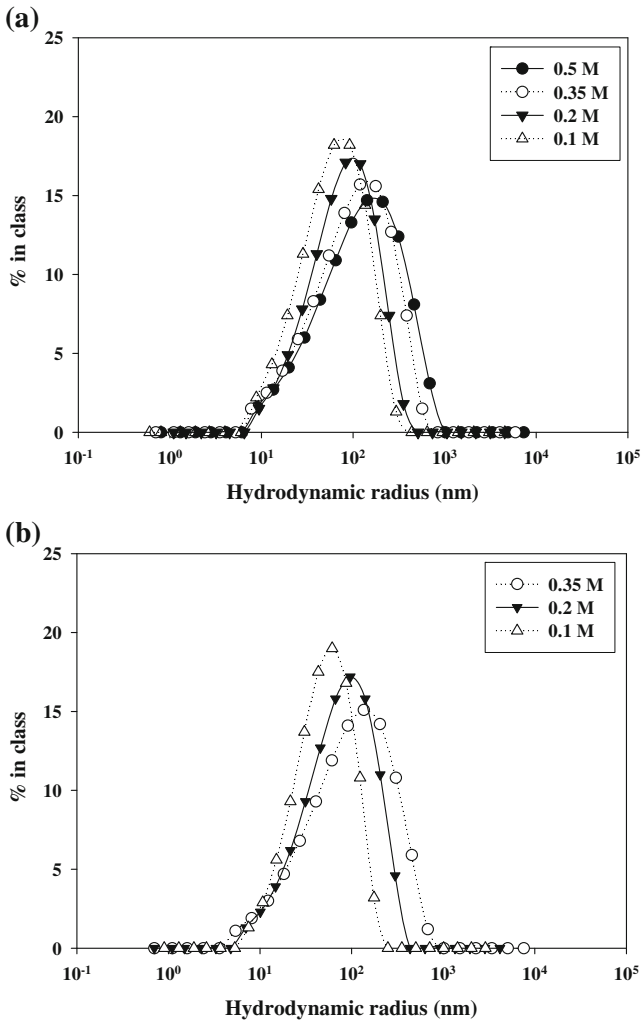

Fig. 3 Intensity fraction of hydrodynamic radius distributions at scattering angle θ=90° for chitosan β_2 (**a**) and chitosan β_4 (**b**) in 0.10 M acetic acid (0.40 mg/ml) at different NaCl concentrations

in Fig. 2, may have been due to the shear thinning effect. In the [η] measurement, when the sample solutions flowed through the capillary, the phenomenon of shear thinning may have occurred which disintegrated the weak interaction between the associate molecules. Besides, the concentration of the chitosan associates may be much lower than the concentration of isolated molecule of chitosan in the high ionic strength solution. Then the screening effect of counterions and/or the compaction effect of chitosan reflected on the variation of the [η] with an increase in the ionic strength.

The effect of deprotonation on the changes of physico-chemical characteristics of chitosan was reported by Sorlier et al. [5, 6], and their results showed that the D_{app} and [η] of chitosan decreased with an increase in the degree of deprotonation. Decreasing the proton density of chitosan and/or screening the electric charges of chitosan should decrease the effect of intra-chain and inter-chain electrostatic repulsion. On the other hand, increasing the amount of free -NH_2 groups of chitosan would favor the forming of inter- and intra-chain hydrogen bonding [5, 6]. Screening the electric charges of hydrocolloid might also promote the attractive van der Waals interactions; furthermore, increasing the ionic strength of chitosan solution would favor the formation of hydrophobic interactions [21, 22]. In any case, the outcome would be the compacting of intra-chain [24, 25, 31] and/or the associating of inter-chain [5, 6, 8, 23] in chitosan solution. The compacting of intra-chain would cause the decreasing of hydrodynamic volume of chitosan resulted in the decreasing of [η] and the association of inter-chain would cause the formation of multimer of chitosan resulted in the decreasing of D_{app}.

Aggregation behavior in high ionic strength chitosan solution

Figure 4 shows the effect of ionic strength on the intensity-mean hydrodynamic radius of fast relaxation mode and slow relaxation mode of chitosan β_2 and β_4 at measuring angle θ=30°. The results showed that the intensity-mean hydrodynamic radius of fast relaxation mode increased with increasing the ionic strength of the media. It indicated that the inter-chain associating of chitosan increased with increasing the ionic strength of the media. The results in Fig. 5 show that the ratios of the relative amplitude of slow relaxation mode to fast relaxation mode (A_{slow}/A_{fast}) of β_2 and β_4 increased from 0.25 to 1.10 and from 0 to 1.20 with increasing the ionic strength from 0.10 to 0.50 M and from 0.10 to 0.35 M for β_2 and β_4, respectively, at measuring angle θ=30°. The increase of A_{slow}/A_{fast} was used to indicate the increase in

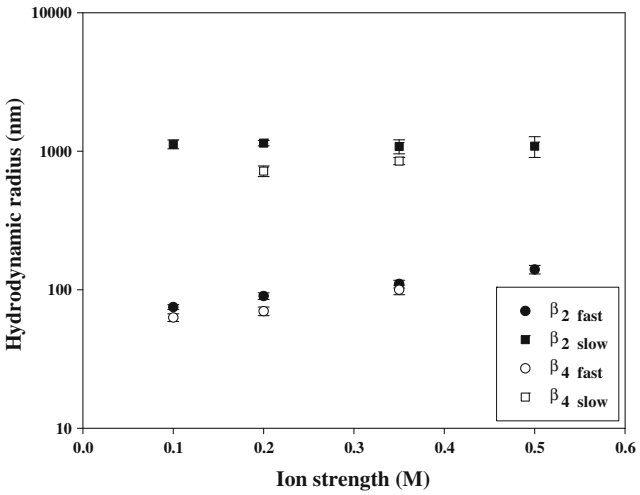

Fig. 4 Changes of the intensity-mean hydrodynamic radius of the fast relaxation mode and slow relaxation mode with ionic strength for 0.40 mg/ml chitosan β_2 and β_4 in 0.10 M acetic acid at scattering angle θ=30°

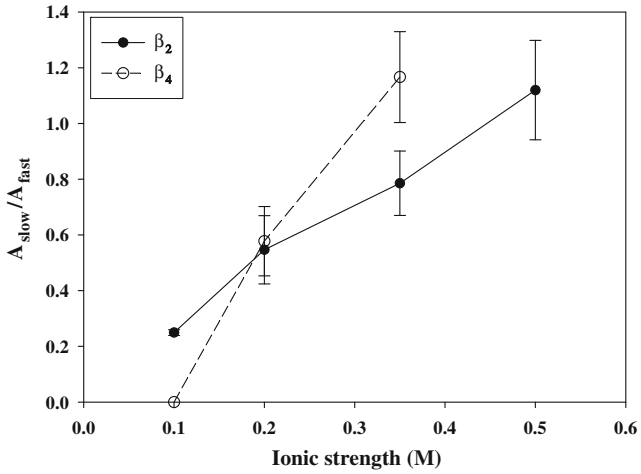

Fig. 5 Changes of the ratios of the relative amplitude of slow relaxation mode to fast relaxation mode (A_{slow}/A_{fast}) with ionic strength for 0.40 mg/ml chitosan β_2 and β_4 in 0.10 M acetic acid at scattering angle $\theta=30^o$

the concentration of or the hydrodynamic radius of the aggregates. In addition, the results in Fig. 4 show that the intensity-mean hydrodynamic radius of slow relaxation mode of β_2 was almost independent of I. The above results indicated that in the case of higher DA chitosan, an increase of I led to an increase in the concentration of aggregates with a constant intensity-mean hydrodynamic radius. At I=0.10 M, although a slow relaxation mode did not appear for chitosan β_4 with low angle measuring, it appeared at I≥0.20 M (Figs. 4 and 5). This indicated that an increase of I also facilitated the formation of aggregates of chitosan with a lower DA value.

The increases in the scattering light intensity and of the mean of hydrodynamic radius with an increase in the ionic strength of aqueous media may indicate the formation of association and/or aggregation of chitosan hydrocolloid (Figs. 2, 3 and 4). Furthermore, the A_{slow}/A_{fast} increased with an increase in ionic strength indicated that increasing the ionic strength of aqueous media promoted the formation of the aggregate structure (Fig. 5). As mentioned above, inhibiting electrostatic repulsion and enhancing the compaction effect of chitosan not only promoted the association of molecular colloid, but also facilitated the formation of a small amount of inter-chain aggregation of chitosan. Rinaudo et al. [19] indicated that the solvent of 0.10 M AcOH/0.20 M NaCl was a poor solvent for chitosan, which increased the Mw of chitosan and promoted the formation of aggregation. The result of Philippova et al. [21] also indicated that an increase in the ionic strength promoted the hydrophobic interaction of chitosan. Improving the inter-chain hydrophobic interaction may favor the inter-chain aggregating of chitosan molecules with block or random sequences of GlcNAc, and then the concentration of aggregate chitosan increased with an increase in the ionic strength of aqueous media.

Aggregation behavior in low ionic strength chitosan solution

The results in Fig. 6 show that at I=0.01 M, the relaxation time distributions of chitosan β_2 and β_4 could be identified as two distinct relaxation modes at the measuring angle $\theta = 90^o$; furthermore, the intensity-mean hydrodynamic radius of the slow relaxation mode of β_2 was also almost 1 μm (data not shown). In the case of chitosan β_2 and β_4, at a constant concentration (C_p=0.40 mg/ml), the mean relaxation times of the fast mode at I=0.01 M (β_2: τ_{fast}=200 μs, β_4: τ_{fast}=160 μs) were almost three times shorter than it at I=0.10 M (β_2: τ_{fast}=600 μs, β_4: τ_{fast}=450 μs) (data not shown). The fast mode displayed in the low ionic strength polyelectrolyte solution may not have reflected the diffusion motion of entire polyelectrolyte but resulted from the small ion-macroion coupling effect [17].

The slow mode displayed in the low ionic strength polyelectrolyte solution at the measuring angle $\theta = 90^o$ may indicate that the formation of the aggregate structures increased and/or the trace amount of the inherent aggregates appeared. The scattering intensity of chitosan solutions decreased significantly with the decrease in the ionic strength of media from 0.10 to 0.01 M (Fig. 2). As the ionic strength of media was low enough, the small amount of scattering signal from the trace amount of the inherent aggregates, which may have been hidden from the strong signal of the polyelectrolyte in a high ionic strength solution, was detected by decreasing the scattering signal of polyelectrolyte [36]. However, Pa and Yu [15] reported that the appearance of slow mode in

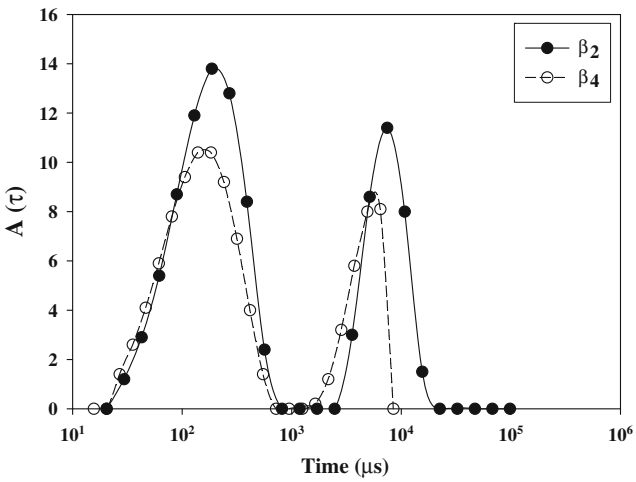

Fig. 6 Intensity fraction of relaxation time (τ) distributions at scattering angle $\theta=90^o$ for 0.40 mg/ml chitosan β_2 and β_4 in 0.10 M acetic acid/0.01 M NaCl

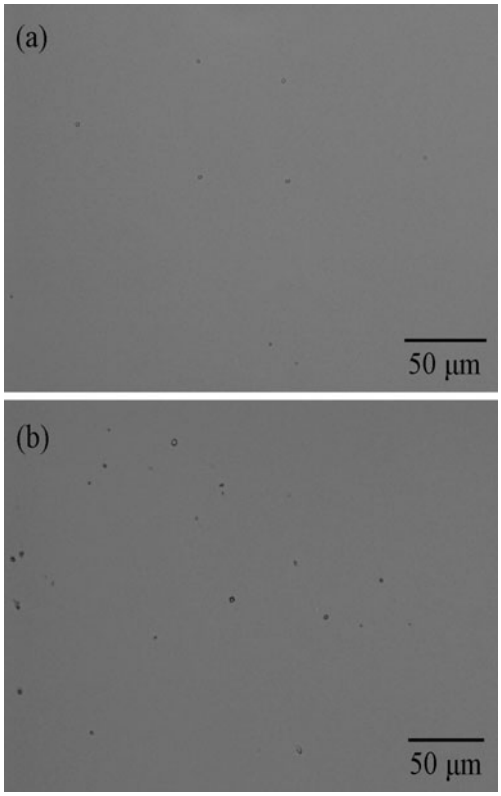

Fig. 7 Light microscope images of 0.40 mg/ml chitosan β_2 0.10 M acetic acid solution with an ionic strength of 0.10 M (a) and 0.01 M (b), after filtration with a 0.45 μm filter

chitosan salt-free solutions may be due to a cluster of several chitosan molecules and the hydrodynamic radius of the cluster increased with decreasing the pH value of solution.

The pictures from the light microscope (Fig. 7) showed considerable quantities of particle structure existing in the low ionic strength solution, but only a small amount of particle structure existing in the high ionic strength chitosan solution, despite both solutions having been filtered by 0.45 μm filters. From the results: (1) the scattering intensity of chitosan solutions decreased sharply from I=0.10 M to 0.01 M (Fig. 2); (2) the slow relaxation mode of chitosans with I=0.01 M appeared at a higher measured angle (Fig. 6) than those with I=0.10–0.50 M (Figs. 3 and 4); and (3) considerable quantities of particle structure existed in the chitosan solution with I=0.01 M (Fig. 7). The slow mode which appeared in the relaxation time distribution (scattering angle $\theta=90^{o}$) was assumed to be the result of not only the decreasing of scattering intensity but also the increasing of particle structure.

The formation of particle structure was observed in the low ionic strength (I=0.01 M) sodium poly-(styrene sulfonate) aqueous solutions with light scattering and light microscopy image [18]. Tanahatoe and Kuil [18] indicated that an attractive interaction of non-electrostatic origin may possibly play a role in the formation of aggregates of polyelectrolyte in the low ionic strength media (I=0.01 M). Furthermore, an attractive fluctuating dipolar interaction caused by asymmetric distribution of counterions was proposed by Schmitz [34]. In the clarification process, a small quantity of imperfectly deacetylated fragment of chitosan with chitin-like structure that suspended in the chitosan solution can pass through the filter with a pore size of 0.45 μm, and the protonable -NH_2 group of those chitosan with imperfectly deacetylated fragment may distribute asymmetrically. In low ionic strength media, although the electrostatic repulsion between intra-chain and inter-chain ionic group of polyelectrolyte are strong, we assume that the extending effect of chitosan molecule may favor the formation of inter-chain H-bondings of the chitin-like structure and the asymmetric distribution of protonable -NH_2 group of the imperfectly deacetylated chitosan also favored an attractive fluctuating dipolar interaction in this solution. The outcome would be the aggregating of chitosan

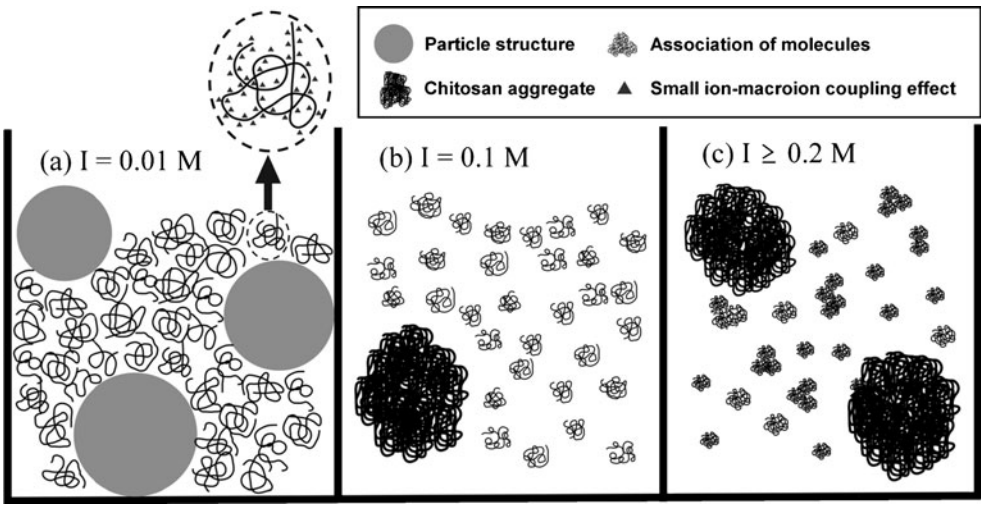

Fig. 8 Hypothesis model of different types of aggregate of 0.40 mg/ml higher DA chitosan (β_2) in 0.10 M acetic acid solution at different ionic strengths regions

molecules with chitin-like structure and the aggregating process was different from those found in high ionic strength solutions.

Hypothesis model of effect of ionic strength on different types of aggregate

From the results shown in Figs. 2, 3, 4, 5, 6 and 7, the effect of ionic strength on the hydrodynamic properties of higher DA chitoan (β_2) at a constant concentration of 0.40 mg/ml was schematically summarized, as shown in Fig. 8: (a) in low ionic strength media, the fast mode displayed in the chitosan solution may have resulted from the coupling of fast moving counterion and portion of chains; however, the slow relaxation mode was due to the formation of considerable quantities of particle structure; (b) in the middle ionic strength media, the hydrodynamic volumes of chitosan molecules were isolated, and a trace amount of aggregates were produced in the acidic dilute solution; and (c) in the high ionic strength chitosan solution, the association of compact macromolecules was due to the compaction effect and the screening effect. In addition, increasing the ionic strength of the chitosan solution also promoted the aggregates with a constant intensity-mean hydrodynamic radius produced in acidic dilute solution.

Conclusions

In the dilute region with I=0.1 M, at scattering angle $\theta = 30^{o}$, the higher DA chitosan (β_2) appeared two relaxation modes, but the lower DA chitosan (β_4) appeared one relaxation mode, indicating that higher DA chitosan prepared from chitin by heterogeneous alkali deacetylation had a tendency to form a small amount of aggregates in an acidic dilute solution. At $I \geq 0.2$ M, the screening effect of the counterions was overwhelmed by the compaction effect of the high molecule weight chitosan; moreover, the association of the compact chitosan hydrocolloid and the increase of aggregates occurred in the high ionic strength chitosan solution. At I=0.01 M, the scattering light intensity of chitosan solutions decreased significantly, the electrodynamic coupling effect predominated on the mobile behavior of the extended and congested chitosan molecule in a low ionic strength solution. Furthermore, a different origin of aggregate particle structure than those found in high ionic strength solutions was formed in the low ionic strength chitosan solution.

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References

1. Rinaudo M, Pavlov G, Desbrières J (1999) Polymer 40:7029–7032. doi:10.1016/S0032-3861(99)00056-7
2. Rinaudo M, Pavlov G, Desbrières J (1999) Int J Polym Anal Charact 5:267–276. doi:10.1080/10236669908009742
3. Schatz C, Viton C, Delair T, Pichot C, Domard A (2003) Biomacromolecules 4:641–648. doi:10.1021/bm025724c
4. Schatz C, Pichot C, Delair T, Viton C, Domard A (2003) Langmuir 19:9896–9903. doi:10.1021/la034410n
5. Sorlier P, Viton C, Domard A (2002) Biomacromolecules 3:1336–1342. doi:10.1021/bm0256146
6. Sorlier P, Rochas C, Morfin I, Viton C, Domard A (2003) Biomacromolecules 4:1034–1040. doi:10.1021/bm034054n
7. Rinaudo M (2006) Prog Polym Sci 31:603–632. doi:10.1016/j.progpolymsci.2006.06.001
8. Buhler E, Rinaudo M (2000) Macromolecules 33:2098–2106. doi:10.1021/ma991309+
9. Lamarque G, Lucas JM, Viton C, Domard A (2005) Biomacromolecules 6:131–142. doi:10.1021/bm0496357
10. Brugnerotto J, Desbrières J, Roberts G, Rinaudo M (2001) Polymer 42:9921–9927. doi:10.1016/S0032-3861(01)00557-2
11. Berth G, Dautzenberg H, Peter MG (1998) Carbohydr Polym 36:205–216. doi:10.1016/S0144-8617(98)00029-0
12. Anthonsen MW, Vårum KM, Hermansson AM, Smidsrød O, Brant DA (1994) Carbohydr Polym 25:13–23. doi:10.1016/0144-8617(94)90157-0
13. Yanagisawa M, Kato Y, Yoshida Y, Isogai A (2006) Carbohydr Polym 66:192–198. doi:10.1016/j.carbpol.2006.03.008
14. Domard A, Rinaudo M (1983) Int J Biol Macromol 5:49–52. doi:10.1016/0141-8130(83)90078-8
15. Pa JH, Yu TL (2001) Macromol Chem Phys 202:985–991. doi:10.1002/1521-3935(20010401)202:7<985::AID-MACP985>3.0.CO;2-2
16. Lee WI, Schmitz KS, Lin SC, Schurr JM (1977) Biopolymers 16:583–599. doi:10.1002/bip.1977.360160309
17. Ermi BD, Amis EJ (1998) Macromolecules 31:7378–7384. doi:10.1021/ma980579+
18. Tanahatoe JJ, Kuil ME (1997) J Phys Chem B 101:9233–9239. doi:10.1021/jp972227v
19. Rinaudo M, Milas M, Dung PL (1993) Int J Biol Macromol 15:281–285. doi:10.1016/0141-8130(93)90027-J
20. Amiji MM (1995) Carbohydr Polym 26:211–213. doi:10.1016/0144-8617(94)00095-B
21. Philippova OE, Volkov EV, Sitnikova NL, Khokhlov AR, Desbrières J, Rinaudo M (2001) Biomacromolecules 2:483–490. doi:10.1021/bm005649a
22. Wang PF, Wu SK (1998) Shi XY, Deng BM, Sun C. J Mater Sci 33:1753–1757. doi:10.1023/A:1004324531314
23. Cho J, Heuzey MC, Bégin A, Carreau PJ (2006) J Food Eng 74:500–515. doi:10.1016/j.jfoodeng.2005.01.047
24. Anthonsen MW, Vårum KM, Smidsrød O (1993) Carbohydr Polym 22:193–201. doi:10.1016/0144-8617(93)90140-Y
25. Tsaih ML, Chen RH (1999) J Appl Polym Sci 71:1905–1913
26. Laurier LS (2008) Dictionary of nanotechnology, colloid and interface science, 1st edn. Wiley-VCH, Weinheim
27. Kurita K, Tomita K, Tada T, Ishii S, Nishimura SI, Shimoda K (1993) J Polym Sci Part A Polym Chem 31:485–491. doi:10.1002/pola.1993.080310220
28. Baxter A, Dillon M, Taylor KDA, Roberts GAF (1992) Int J Biol Macromol 14:166–169. doi:10.1016/S0141-8130(05)80007-8
29. Tsaih ML, Chen RH (1997) Int J Biol Macromol 20:233–240. doi:10.1016/S0141-8130(97)01165-3
30. Štěpánek P (1993) In: Brown W (ed) Dynamic light scattering: the method and some applications. Oxford, Clarendon Press

31. Vårum KM, Anthonsen MW, Grasdalen H, Smidsrød O (1991) Carbohydr Res 217:19–27. doi:10.1016/0008-6215(91)84113-S
32. Errington N, Harding SE, Vårum KM, Illum L (1993) Int J Biol Macromol 15:113–117. doi:10.1016/0141-8130(93)90008-A
33. Tsaih ML, Chen RH (1999) J Appl Polym Sci 73:2041–2050
34. Schmitz KS, Lu M, Gauntt J (1983) J Chem Phys 78:5059–5066. doi:10.1063/1.445374
35. Dautzenberg H, Jaeger W, Kötz J, Philipp B, Seidel Ch, Stscherbina D (1994) Polyelectrolytes: Formation, characterization and application, 1st edn. Hanser, New York
36. Reed WF (1994) Macromolecules 27:873–874. doi:10.1021/ma00081a039