

Viscoelastic Sol–Gel State of the Chitosan and Alginate Solution Mixture

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ABSTRACT: The rheological behavior of chitosan/alginate solutions was investigated in relation to gelation and polyelectrolyte complex (PEC) formation. Before mixing, the chitosan and the alginate solutions were both homogeneous fluids. However, heterogeneity developed after mixing, accompanied by a serious increase of viscosity. To determine the sol–gel state of the solutions, the viscoelastic variables, such as the dynamic storage modulus (G') and loss modulus (G''), the loss tangent, and the viscoelastic exponents for G' and G'' , were obtained. Depending on the concentration, the chitosan/alginate solutions revealed unexpected rheological

behavior. At a polymer concentration of 1.0 wt %, the chitosan/alginate solution was in a viscoelastic gel state, whereas, at higher concentrations, viscoelastic sol properties were dominant. A viscoelastic gel state for the chitosan/alginate solution was induced based on the weak formation of fiber-shaped precipitates of a PEC at a low polymer concentration. © 2007 Wiley Periodicals, Inc. *J Appl Polym Sci* 104: 1408–1414, 2007

Key words: alginate; chitosan; gelation; polyelectrolyte; rheological behaviors

INTRODUCTION

A polyelectrolyte complex (PEC) is formed by the association of two or more polymers based on an electrostatic force. For example, a polycation interacts with a polyanion through a proton transfer, resulting in a PEC. The complex stability is dependent upon the charge density, solvent, ionic strength, pH, and temperature.^{1–7} Several kinds of polyelectrolytes with positive or negative charges exist among polysaccharides. Chitosan is a polysaccharide comprising the copolymer of glucosamine and *N*-acetylglucosamine, and it is soluble in an acidic solvent, being positively charged due to its number of amine groups. PECs have already been prepared using chitosan and a counter pair of polyanions, like an alginate composed of (1–4)-linked β -D-mannuronic acid and α -L-guluronic acid units. It is well-known that an insoluble layer is formed at the interface between the acidic chitosan and the aqueous sodium alginate solution. As such, PEC formation using chitosan and an alginate is an effective technique for preparing separating membranes⁸ and microcapsules^{9–12} as drug, cell, and protein carriers.

The phase transitions, including the sol–gel transition, order–disorder transition, and phase separation, have already been monitored through rheological experiments.^{13–18} In particular, the sol–gel transition for chemically or physically crosslinked systems can be described by the *gel equation* developed by Winter and Chambon as follows:¹³

$$\sigma(t) = S \int_{-\infty}^t (t - t')^{-n} \dot{\gamma}(t') dt' \quad (1)$$

where σ , $\dot{\gamma}$, S , and n are the shear stress, rate of deformation of the sample at the gel point, gel strength parameter, and viscoelastic relaxation exponent, respectively. S and n are the only material parameters characterizing linear viscoelastic properties. Oscillatory shear experiments provide the complex modulus; both the storage (G') and the loss (G'') are helpful in determining the frequency dependence of viscoelastic materials. The dynamic modulus is defined as the ratio of stress to strain for small cyclic deformations at a given frequency. The sol–gel transition is simply determined by the intersection of the G' and the G'' .^{13–16} A more general method for identifying the sol–gel transition is based on the fact that the loss tangent ($\tan \delta$) (G''/G') is constant, independent of the frequency.^{13–16}

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The viscoelastic behaviors of PEC solutions were studied by several research groups.^{19–22} Especially, Payet et al. investigated the temperature effect on the gelation kinetics of chitosan and alginate studied with rheology.²² In this study, we attempted to describe the concentration effects on the rheological behavior of a chitosan/alginate solution mixture in relation to gelation and PEC formation. As such, the viscoelastic properties of a mixed solution were investigated through a series of rheological experiments to determine the sol–gel state, where time and solution concentration were used as the variables governing the sol–gel state of the fluid. Plus, morphological examinations were conducted to explain the PEC formation in the chitosan/alginate solution.

EXPERIMENTAL

Materials and solution preparation

Chitosan was purchased from Aldrich and purified by the removal of all insoluble residues in a dilute acetic acid solution and the following precipitation in a sodium hydroxide solution. The viscosity-average molecular weight (M_v) of the purified chitosan was determined by solution viscometry (30°C, 0.2M CH₃COOH/0.1M CH₃COONa as solvent, $K = 6.6 \times 10^{-3}$, $a = 0.88$ as Mark–Houwink parameters).²³ The degree of deacetylation (DD) of the purified chitosan was determined by alkali-titration method.²⁴ Briefly, chitosan was dissolved in excess of acid and the solution was then titrated potentiometrically with NaOH. This gives a titration curve having two inflection points, the difference between the two along abscissa corresponding to the amount of acid required to protonate the amine groups. The M_v and the DD of the purified chitosan were 500,000 and 95%, respectively. Sodium alginate was purchased from Showa Chemicals (Tokyo, Japan) and used without further purification. The M_v of sodium alginate, determined by solution viscometry (25°C, 0.1M NaCl, $K = 6.9 \times 10^{-6}$, $a = 1.13$),²⁵ was 230,000. Chitosan and sodium alginate were dissolved in a 0.1M acetic acid aqueous solution and deionized water, respectively, then mixed together (10 mL, respectively) with stirring to prepare the chitosan/alginate solutions at each mixing time.

Rheological experiment

The dynamic viscoelastic variables of the chitosan/alginate solutions were measured using a Physica UDS 200 rheometer in a concentric cylinder at a maximum strain amplitude of 5%. The strain level was determined using a strain sweep test so that all the measurements could be carried out within a linear viscoelastic regime.

The G' and the G'' exhibit a power law frequency dependence at the sol–gel transition point (GP)

$$G'(\omega) \propto G''(\omega) \propto \omega^n \quad (2)$$

and can be described as

$$G' = G''/\tan \delta = S\omega^n \Gamma(1-n) \cos \delta \quad (3)$$

where ω and $\Gamma(1-n)$ are the frequency and Legendre gamma function, respectively. The phase angle (δ) between the stress and strain is independent of the frequency, yet proportional to n . Plus, $\tan \delta$ is independent of the frequency at the GP.

$$\delta = n\pi/2 \quad \text{or} \quad \tan \delta = G''/G' = \tan(n\pi/2) \quad (4)$$

Therefore, the GP can be determined by the dynamic viscoelasticity according to the following three criteria: (i) the intersection of the G' and the G'' ($G' > G''$ in a gel state), (ii) the constant value of $\tan \delta$, irrespective of the frequency, i.e., a frequency-independent $\tan \delta$ ($\tan \delta$ increases with the frequency in a gel state), and (iii) the intersection between the viscoelastic exponents for the $G'(n')$ and the $G''(n'')$ ($n' < n''$ in a gel state).

Morphological study

The precipitates that formed in the chitosan/alginate solutions were examined using an optical microscope. For the scanning electron microscope (SEM) observation, the precipitates were taken carefully from the mixed solution, washed with a dilute acetic acid solution followed by deionized water, and lyophilized for five days. The dried samples were then coated with gold and observed using a Hitachi S-4300 SEM.

RESULTS AND DISCUSSION

Generally, concentration and temperature dependences are considered in a phase study of a polymer solution. This study focused on the effect of the solution concentration on the rheological response. Before mixing, the chitosan and the alginate solutions were both homogeneous fluids. Yet, heterogeneity developed after mixing, accompanied with a serious increase of viscosity. To verify whether the complex solutions were a “sol” or “gel,” the G' and the G'' or the n' and the n'' were compared and the frequency dependence of $\tan \delta$ checked.

Figure 1 shows the dynamic moduli of the chitosan/alginate solutions at various mixing times. The 1.0 wt % solution was regarded as a gel irrespective of time, based on the fact that the G' was higher than the G'' [Fig. 1(a)]. However, the gelation of the chitosan/alginate solutions was found to slightly differ from that occurring by chemical crosslinking. A macroscopic

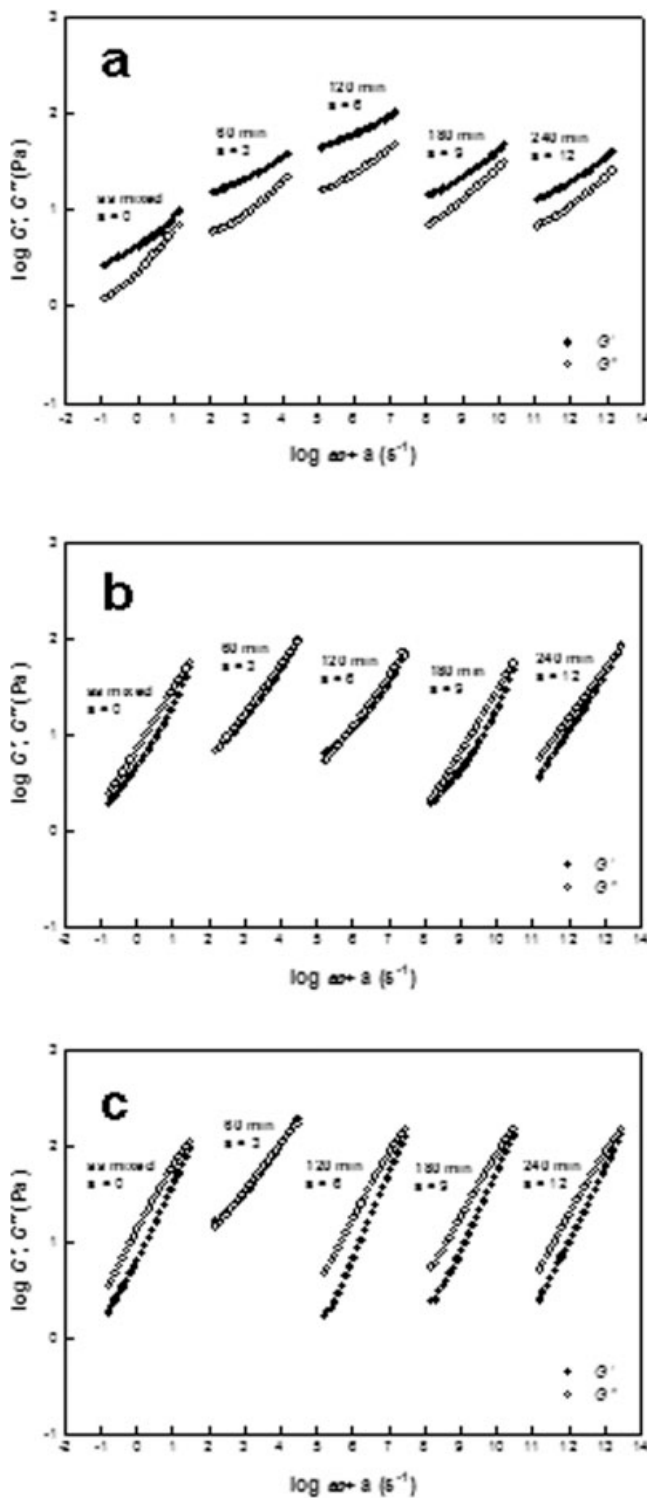


Figure 1 The dynamic moduli of the 1.0 wt % (a), the 2.0 wt % (b), and the 3.0 wt % (c) chitosan/alginate solutions as a function of frequency at various mixing times. (a (shift factor) = 0, 3, 6, 9, 12).

gel is conventionally defined as a state without any flow, yet this was not completely applicable to the 1.0 wt % solution, which did have a flow, even though it was rheologically defined as a gel. Similar examples have already been found in physical gela-

tion systems accompanying liquid–liquid phase separation or concentration fluctuation.⁹ The dynamic moduli of the 1.0 wt % solution increased up to 120 min, then fell off thereafter, implying a slight change in the phase behavior after gelation, which will be discussed later. Meanwhile, it was hard to say that the 2.0 wt % and the 3.0 wt % solutions were gels because the G' was not higher than the G'' , as shown in Figures 1(b) and 1(c).

The frequency dependence of the $\tan \delta$ or the comparison of the n' and the n'' provided more precise information on determining the sol–gel state of the polymer solutions. A homogeneous polymer solution typically has the characteristic of a viscoelastic liquid where the $\tan \delta$ decreases with the frequency. As such, a definite decrease of the $\tan \delta$ with the frequency denotes that the system is in a pregel regime, while a ready increase of the $\tan \delta$ as the frequency increases indicates a postgel regime and a viscoelastic solid property. A frequency-independent $\tan \delta$ is obtained at the GP. As shown in Figure 2(a), the $\tan \delta$ for the 1.0 wt % solution increased with the frequency, irrespective of time, indicating that the system was in a viscoelastic gel state. Conversely, the $\tan \delta$ for the 2.0 wt % solution revealed a time-dependent behavior, as shown in Figure 2(b). Up to 120 min, the 2.0 wt % solution was assumed to be a gel, owing to the overall increase of the $\tan \delta$ with the frequency, while a viscoelastic sol state was assumed at 180 and 240 min due to the reduction of the $\tan \delta$ with the frequency. Meanwhile, the 3.0 wt % solution was in a sol state at first, around the GP at 60 min, and then changed to a viscoelastic sol at 120 min [Fig. 2(c)].

The slopes of $\log G'$ and $\log G''$ against $\log \omega$ yield the n' , the viscoelastic exponent for the frequency dependence of the moduli. As a system goes through the gelation process, the n' falls off more seriously than the n'' , while G' increases above G'' . Figure 3 shows the n' and the n'' obtained at various mixing times. A comparison of the n' and the n'' supported the results obtained above that the concentration was a significant factor governing the sol–gel state of the chitosan/alginate solutions. The 1.0 wt % solution was regarded as a gel ($n'' > n'$) irrespective of the mixing time, while a viscoelastic liquid character ($n' > n''$) developed in the 2.0 and 3.0 wt % solutions at 180 and 120 min, respectively, implying that the system had changed to a viscoelastic sol phase. This coincided well with the results in Figures 1 and 2. A series of rheological experiments suggested that the 1.0 wt % solution was a viscoelastic gel, the 2.0 wt % solution was in between a sol and a gel, and the 3.0 wt % solution was much closer to a sol. Consequently, a viscoelastic gel phase was formed in the chitosan/alginate complex solution, yet at a high concentration a viscoelastic sol phase was prevalent, which was unexpected, as it is generally accepted that a higher concentration promotes gelation.

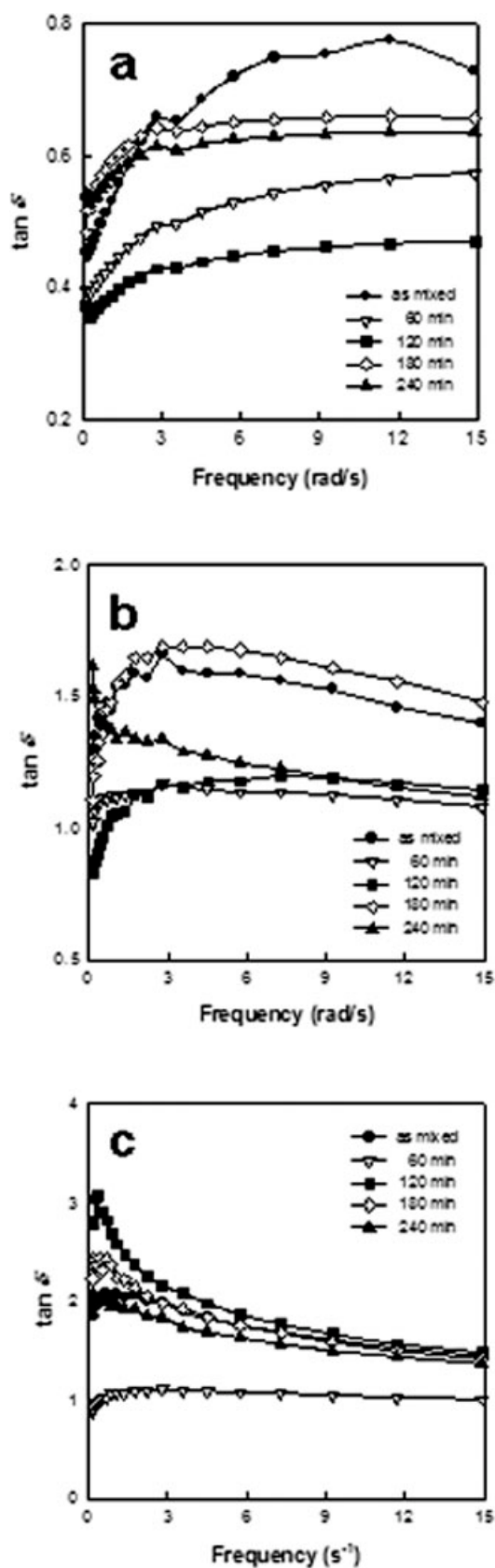


Figure 2 The loss tangents of the 1.0 wt % (a), the 2.0 wt % (b), and the 3.0 wt % (c) chitosan/alginate solutions as a function of frequency at various mixing times.

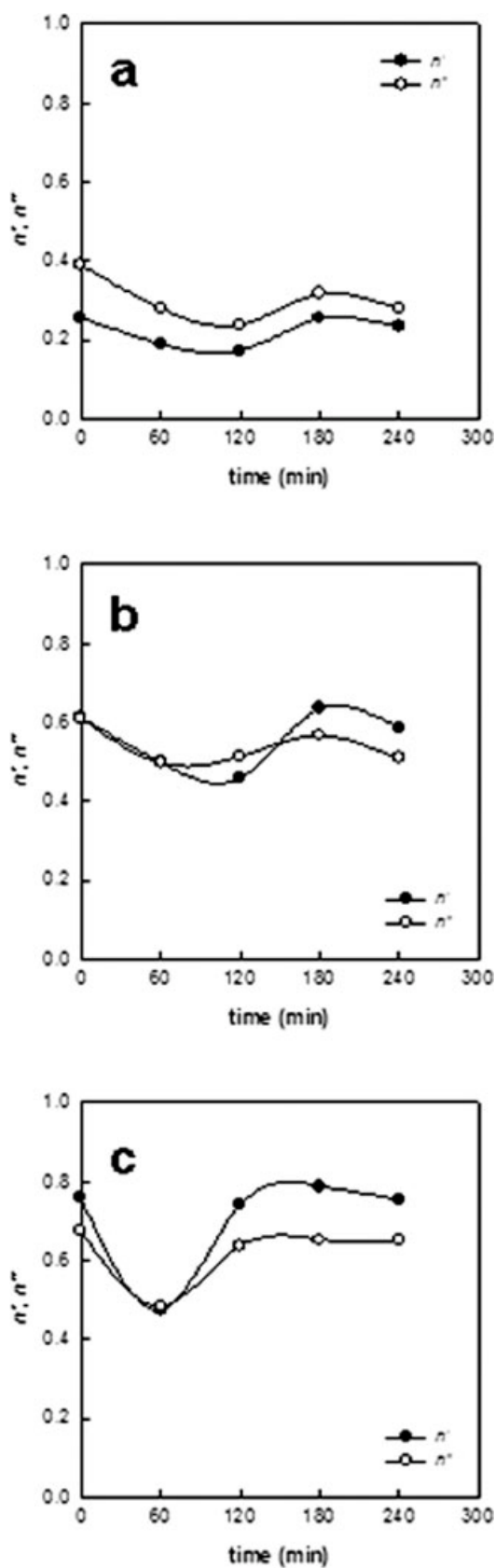


Figure 3 The viscoelastic exponents of the 1.0 wt % (a), the 2.0 wt % (b), and the 3.0 wt % (c) chitosan/alginate solutions as a function of mixing time.

The precursor solutions of chitosan and alginate revealed a typical behavior of "sol" irrespective of time, i.e., $G' < G''$, $\tan \delta$ decreases with frequency, and $n' > n''$. In addition, at the same concentration, the magnitudes of dynamic modulus and viscosity of the precursor solution (for example, in the case of chitosan, $G' = 5.07$ Pa, $G'' = 12.2$ Pa, $\eta^* = 1.43$ Pa s at 9.25 rad/s) were not very different from those of PEC solution at the initial stage of mixing. However, G' , G'' , and η^* of the PEC solution were higher than those of the precursor solution after mixing, implying that the viscoelastic sol properties declined and the gelation degree increased.

The reduction of the dynamic moduli after 120 min in Figure 1(a) indicates that the properties of the viscoelastic solid as a gel became weaker. This was verified from the increment of the $\tan \delta$ after 120 min in Figure 2(a). Hodgson and Amis explained the behavior of $\tan \delta$ in the course of gelation using the concept of clusters,²⁶ where a decrease of $\tan \delta$ occurs corresponding to the rapid growth of clusters. Thus, the decrease of the $\tan \delta$ in the 1.0 wt % solution up to 120 min was attributable to the growth of clusters in the postgel state, whereas the increase of the $\tan \delta$ after 120 min implies that the growth of clusters was surpassed and the viscoelastic gel properties declined. Figure 4(a) shows a plot of the complex viscosity (η^*) of the 1.0 wt % solution against the mixing time. The reduction of η^* after 120 min also indicates a decrease in the properties of the viscoelastic solid as a gel. A similar trend was found in the case of the 2.0 wt % solution, as shown in Figures 2(b) and 4(b). A slight decrease of the η^* [Fig. 4(c)] and increase of the $\tan \delta$ [Fig. 2(c)] after 60 min were also found in the 3.0 wt % solution, although the solution was mostly in a viscoelastic sol state.

The rheological behavior of the chitosan/alginate solutions was significantly related with the formation of a PEC. As shown in Figure 5, the white precipitates in the chitosan/alginate solutions indicated the formation of a PEC. The PEC formation in the chitosan/alginate solutions assumed different qualities according to the concentration. In the 1.0 wt % solution, the PEC formation was relatively slow and took place mostly at the interface of the two solutions [Fig. 5(a)]. Hence, during the initial stage of mixing, there were several domains of each solutions surrounded by the PEC formed at the interface. The domains entrapped by the PEC were highly elastic gel aggregates, leaving the solution in a viscoelastic gel state. Meanwhile, as shown in Figure 5(b), in the case of the 2.0 wt % solution, the higher concentration stimulated the PEC formation so that the PEC was formed inside the domains as well as at the interface. Therefore, the number and size of the gel domains were significantly reduced, thereby weakening the viscoelastic gel properties. In the 3.0 wt % solution, the PEC formation was

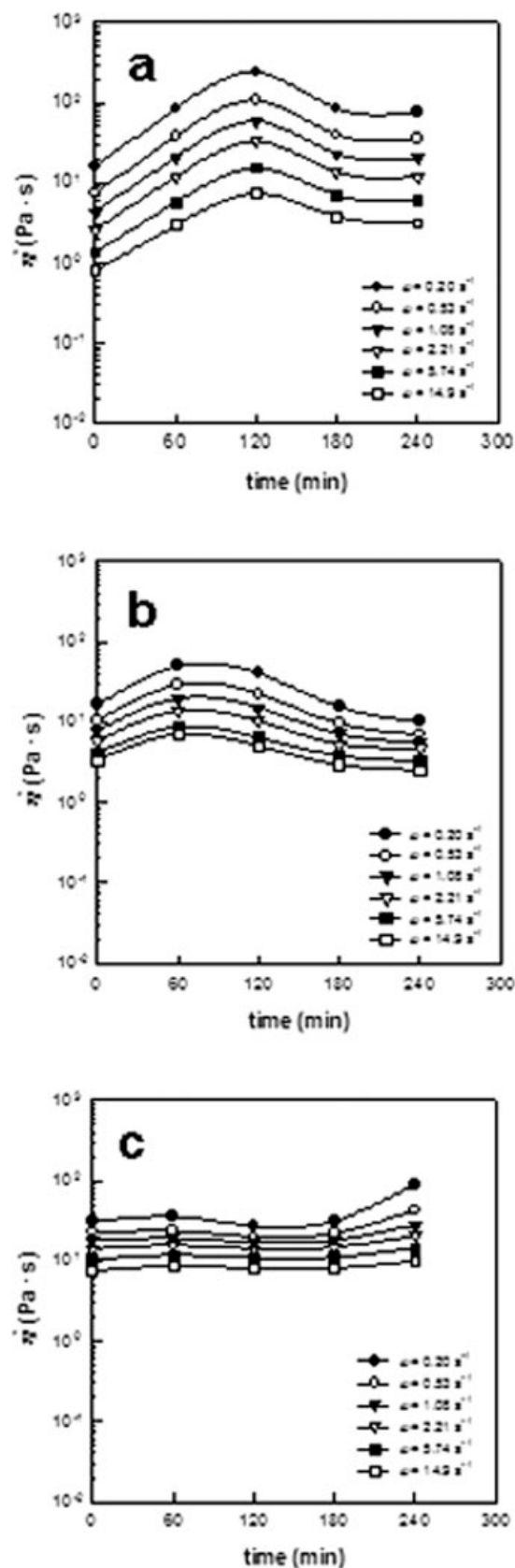


Figure 4 The complex viscosities of the 1.0 wt % (a), the 2.0 wt % (b), and the 3.0 wt % (c) chitosan/alginate solutions as a function of mixing time at various frequencies.

even faster so that the PEC was formed homogeneously without the existence of any gel domains [Fig. 5(c)]. As such, there were PEC precipitates and mixed solvents in the system and the solution was much closer to a viscoelastic sol. The conversion of chitosan/alginate into PEC precipitates became higher with the mixing time due to the reduced the number and size of the gel domains. For this reason, the viscoelastic gel properties decreased with time, along with a decrease of the η^* and the dynamic moduli, and an increase of the $\tan \delta$.

The PEC precipitates formed in the chitosan/alginate solutions had the shape of a fiber with a "stem and branch" structure, as shown in Figures 5(a) and 5(b). The stem-like fibers were the PEC formed at the interface of the chitosan and alginate solutions, while the branch-like fibers were the PEC formed inside the domains. The gel domains were actually maintained by the PEC in the initial stage, then gradually disappeared with the growth of the fibers. From the SEM photographs in Figure 6, it is clear that the chitosan/alginate PEC was composed of a number of micro-

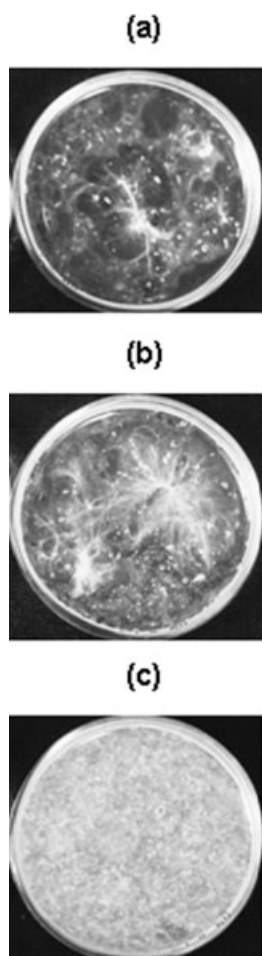


Figure 5 Photographs of the 1.0 wt % (a), the 2.0 wt % (b), and the 3.0 wt % (c) chitosan/alginate solutions after 30 min of mixing.

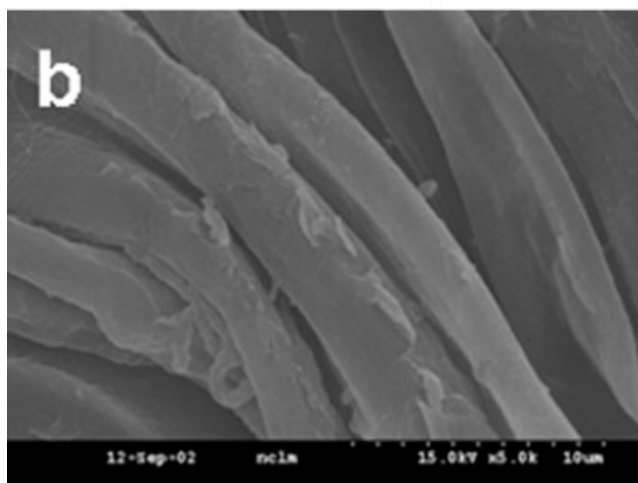
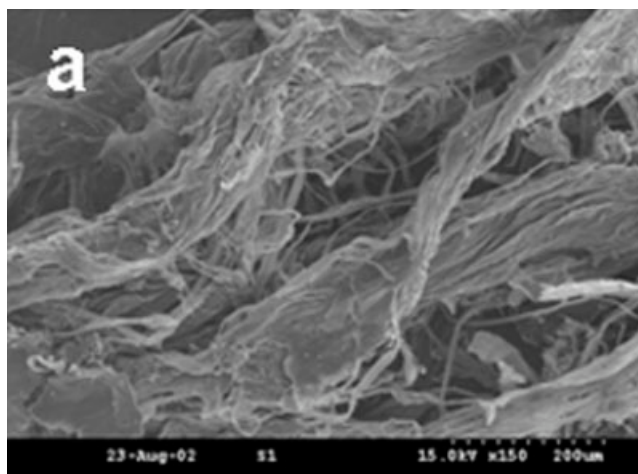


Figure 6 SEM microphotographs of PEC formed in chitosan/alginate solution: (a) $\times 150$; (b) $\times 5000$.

fibrils, suggesting that the chitosan and alginate molecules associated with each other based on an electrostatic interaction to form a PEC with a microfibrillar structure.

CONCLUSIONS

The viscoelastic gel state of chitosan/alginate complex solutions was successfully determined according to the following three criteria: (i) $G' > G''$, (ii) an increase of $\tan \delta$ with the frequency, and (iii) $n' < n''$. The sol-gel state was significantly affected by the solution concentration. At a lower concentration, the PEC formation was relatively weak and slow, taking place mostly at the interface of the two solutions. The domains entrapped by the PEC were found to be highly elastic gel-aggregates, leaving the solution in a viscoelastic gel state. Meanwhile, at a higher concentration, the conversion of chitosan/alginate into PEC precipitates was more active in reducing the number and size of the gel

domains, resulting in a viscoelastic sol state. The PEC precipitate formed in the chitosan/alginate solutions had the shape of a fiber with a microfibrillar structure.

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