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ORIGINAL CONTRIBUTION

Dynamic scaling for gelation of a thermosensitive chitosan-β-glycerophosphate hydrogel

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Abstract This paper investigates the thermal gelation of a chitosan-β-glycerophosphate system using rheometry. The gelation, performed for two polymer concentrations, was achieved in isothermal conditions to examine the effect of various temperatures (between 35 and 65 °C) on the sol-gel transition. The oscillatory shear data was used to verify the scaling behavior ($G' \sim G'' \sim \omega^n$) at the gel point, and the power-law index n was found to be dependent to some extent on chitosan concentration and temperature. The solutions zero shear viscosity (η_0) and the gels low frequency modulus (G_e) were analyzed in terms of the relative distance to gelation time (ε). The two properties showed power–law relationships with ε, i.e., $\eta_0 \sim \varepsilon^{-s}$ and $G_e \sim \varepsilon^z$, and the indexes slightly increased with temperature. The dynamic scaling theory predicts a relationship between the indexes, i.e., $n=z/(z+s)$, and this was in reasonably good agreement with the experimental findings.

Keywords Chitosan · Gelation · Rheometry · Dynamic scaling

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Introduction

For many years, hydrogels have gathered much attention due to their potential use in the preparation of drug delivery systems, foodstuffs, enzyme immobilization, wound dressings, and cosmetics. A good candidate for hydrogel formulation is chitosan, a non-toxic, biocompatible, and biodegradable material [1]. This biopolymer, generally derived from crustaceans, is soluble in acidic media below a pH of 6.5 due to the protonation of the free amine groups on the chitosan chains [2]. Aqueous chitosan solutions can be used to formulate homogenous thermosensitive gels by neutralizing the solution with a glycerophosphate (GP) salt and by heating above room temperature [3, 4]. According to Almdal et al. [5], a gel corresponds to the following definition:

1. It is a soft solid or solid-like material comprising two or more components, out of which one is a liquid (water in the case of hydrogels).
2. It is characterized by a storage modulus, $G'(\omega)$, which exhibits a pronounced plateau extending to times at least of the order of seconds, and by a loss modulus, $G''(\omega)$, which is considerably smaller than the storage modulus in the plateau region.

In opposition to chemical gels, physical gels occur through non-covalent inter- (and intra-) molecular bonding. The presence of various interactions such as ionic bridging, hydrogen bonding, and hydrophobic associations greatly complicates the physical description of their networks, as the number and positions of these bonds can fluctuate with time and temperature [6]. Physical gels can be triggered by pH and/or temperature variations and sometimes show reversibility [4, 7, 9], while chemical gels do not.

The general mechanisms of gelation of the chitosan-β-GP system have been investigated in heating–cooling cycles conditions in our previous work [4, 10, 11]. While gelation was promoted upon heating to 90 °C, reverse cooling to 5 °C caused partial thermoreversibility of this physical gel system due to the existence of remaining associations, confirmed by the spontaneous recovery of the gel after breakup at low temperature [4]. Increasing temperature up to 90 °C had no effect on the pH values that remained around 7, while conductivity and calculated ionic strength increased. Values from the pH measurements along with temperature dependence of the various acid dissociation constants, K_a's, were used to estimate the degree of protonation of each species as a function of temperature. The decreasing ratio of $-NH_3^+$ in chitosan and $-OPO(O^-)_2$ in β-GP suggested a reduced chitosan solubility along with a diminution of potential ionic interactions such as ionic bridging with increasing temperature. On the other hand, the increased ionic strength as a function of temperature in the presence of β-GP enhanced screening of electrostatic repulsion and increased hydrophobic effect, resulting in favorable conditions for gel formation. Hence, our previous studies suggested that hydrophobic interactions and reduced solubility were the main driving force for chitosan gelation above room temperature in the presence of β-GP [4]. Monitoring of the heat-induced gelation also showed that the data obtained at different β-GP concentrations could be superposed into a single master curve in the gel state, indicating that the gelation process and kinetics were identical regardless of the β-GP content, while measurements obtained at different chitosan concentrations could not be shifted onto a single curve [11].

Description of gels dynamic properties in the vicinity of the sol-gel transition can be quite difficult due to the complexity of the phenomenon; however, this task can be greatly simplified by using power laws derived from network theories [12]. Scaling of dynamic properties such as zero-shear viscosity η_0, dynamic moduli G' and G'', and equilibrium modulus G_e before, at, and beyond the gel point respectively, can be expressed as [12, 13]):

$$\eta_0 \sim \varepsilon^{-s} \text{ for } p < p_{\text{gel}} \tag{1a}$$

$$G'(\omega) \sim G''(\omega) \sim \omega^n \text{at } p_{\text{gel}} \tag{1b}$$

$$G_e \sim \varepsilon^z \quad \text{for } p > p_{\text{gel}} \tag{1c}$$

where ε $(=p/p_{\text{gel}}-1)$ is the relative distance from the gel point. The critical conversion p_{gel} occurs when there is a nonzero probability that a randomly chosen chain shows an infinite molar mass, while p represents the proportion of reacted groups [14]. Other parameters include ω the frequency, while s, n, and z are the positive indexes for the scaling laws. While these equations serve well the purpose of chemical gels, physical gels need to be treated more carefully. As the latter generally contain large amounts of solvent, it has been observed that, below a critical concentration, C_0, gelation can not occur even after long times. The apparent similarity with p_{gel} in step-growth systems suggests the following relationship [14]:

$$\frac{p}{p_{\text{gel}}} \approx \frac{C}{C_0} \approx \frac{t}{t_{\text{gel}}} \tag{2}$$

Close to the gel point, the power–law exponents calculated using time or concentration instead of the formal percolation expressions in terms of p and p_{gel} should be the same. Relaxation exponent n must be within 0 and 1 [15], while typical experimental values for s and z can be scattered, 0.75–1.5, and 1.9–3.5, respectively [13]. Theoretical values from the percolation theory in the Zimm–Rouse limits predicts n=2/3, s=0 to 4/3, and z=8/3 [13]. A scaling relationship has also been suggested, i.e. [12]:

$$n = \frac{z}{z+s} \tag{3}$$

Scaling laws have been applied to chemical gels and also with some success to physical gels, including systems, such as gelatin [16], methylcellulose [9, 17], and agarose [18].

In this work, we investigate the rheological properties of a chitosan-β-GP system in the vicinity of the gel point and beyond, considering the effect of temperature and polymer concentration. Dynamic scaling of this process has not so far been addressed; therefore, we test the validity of the scaling laws with respect to time as the gelation variable in isothermal conditions.

Materials and methods

Materials

The chitosan used in this study was purchased from Marinard Biotech (Rivière-aux-Renards, QC). Its degree of acetylation (DA) is 7% and weight-average molar mass ($\overline{M}_w$) 8.5×10^5 g/mol ($\overline{M}_w/\overline{M}_n = 2.8$).The degree of acetylation or fractional acetyl content was obtained from colloidal titration with polyvinyl sulfate potassium, initially standardized with cethylpyridinium chloride. For the determination of the molecular weight, size exclusion chromatography (SEC) measurements were conducted with an Ultrahydrogel™ 500 Column (Waters, Milford, MA) in 0.25 M acetic acid/0.25 M sodium acetate using dextran standards. The weight-average molecular weight obtained by SEC was in good agreement with the viscosity-average molecular weight previously determined, i.e., 1.1×10^6 g/mol ($[\eta]$=9.1 dl/g) [19]. Acetic acid (99.7%, Sigma-Aldrich Canada, Oakville, ON) was used to dissolve the chitosan, while disodium-β-GP (glycerol 2-phosphate disodium salt hydrate: $C_3H_7Na_2O_6P\ xH_2O$, formula weight=216.04 g/mol, Sigma-Aldrich Canada) was employed to adjust the pH of the chitosan solutions around neutrality.

Preparation of the chitosan solutions

Two chitosan solutions were prepared using 2 and 3 g of polymer in 98 and 97 ml, respectively, of a 1% v/v acetic acid aqueous solution using a laboratory magnetic stirrer (PC-420 Corning® Stirrer/Hot Plate, Corning, MA) at 50 RPM for 4 h at room temperature. At this point of the preparation, the pH of the solution was ≈4.5 (pH meter from Hana, Àvore-Vila de Conde, Portugal). For pH below its pK_a (pH<6.5) [2], chitosan is water-soluble and positively charged after the protonation of the free amine groups. Then, β-GP (16 g) was added very slowly to both solutions under rapid stirring to increase the pH around neutrality (i.e., pH≈7) and subsequently promote gelation. A mixing time of 0.5 h was used to homogeneously disperse the β-GP and to avoid forming local precipitates. During the stirring process, the containers were covered with aluminium foil to prevent evaporation. The prepared chitosan-β-GP solutions were left to rest 3 h to degas at room temperature. All the samples were stored at 5 °C and used within 1 week to avoid aging effects (it has been observed that the solutions viscosity increased with storage time, probably due to the formation of hydrogen bonds in this case of low protonation of chitosan at a pH≈7). The compositions of the two solutions are summarized in Table 1, along with their pH and the nomenclature adopted.

Rheological measurements

The rotational rheometer used in this study was a stress-controlled instrument (AR-2000, TA Instruments, New Castle, DE) with a Couette geometry (cup and bob diameters=30 and 28 mm, respectively). Mineral oil covered the surface of the chitosan solutions and gels to prevent evaporation during the tests. The effect of the mineral oil on the measurements was shown to be negligible. The rheological properties of the chitosan-β-GP system were characterized using small amplitude oscillatory shear. During the gelation process, the dynamic properties were monitored as functions of time in isothermal conditions at different temperatures (35, 45, 55, and 65 °C). The time sweep tests were performed over frequencies that ranged between 3.95 and 31.4 rad/s, probing several internal modes of the polymer to determine the relaxation exponent *n* (Eq. 1b). Each time, a fresh sample was used to avoid the effect of any previous thermo-mechanical history. During these tests, a small deformation (γ_0) of 0.01 was applied not to disturb the gel formation. Our previous work has shown that this applied deformation is well within the linear regime, both in the solution and gel states (Fig. 4 of reference [4]), in accordance with the importance of performing gelation tests in the viscoelastic linear regime recently discussed by Ross-Murphy [6]. At the lowest temperatures (35 and 45 °C), for which the slower kinetics allowed it, frequency sweeps were performed at regular intervals during the time sweeps to measure the dynamic properties on a reasonable scale of frequencies (0.1–19.95 rad/s) and determine the scaling indexes *s* and *z* (Eq. 1a and 1c, respectively). Tests at low frequency were avoided (i.e., <0.1 rad/s) to achieve dynamic measurements on a nearly constant gel structure.

Table 1 Nomenclature, solution compositions, and pH

Sample	Chitosan (g)	β-GP (g)	1% (*v*/*v*) AcOH (ml)	pH
2C16GP	2	16	98	6.9
3C16GP	3	16	97	7.0

Results and discussion

Determination of the gelation time and relaxation exponent "*n*"

Upon heating above room temperature, an initially clear chitosan-β-GP solution is transformed into a slightly opaque gel system, and the transition results in greatly enhanced mechanical properties, consequence of the three-dimensional network formation. Figure 1 shows the evolution at 45 °C of the storage (G') and loss (G'') moduli of sample 2C16GP, as functions of time for different oscillatory frequencies. It can be seen at early times that the storage modulus is lower than the loss modulus, a typical behavior of viscoelastic solutions. At a certain point, the two moduli crosses and G' becomes larger than G'', nearly one order of magnitude. Beyond this point, G' continues to slightly increase, consequence of cluster growth, and shows nearly no frequency dependence as opposed to the solution behavior. In the case of the loss modulus G'', a nearly asymptotic limit is seen, a frequently observed effect that is not easily explained by chain relaxation theories [6]. In early work, the moduli crossover has been extensively used to identify the gel point, but this approach results in a frequency-dependent value while this property should be unique. A more absolute criterion was proposed by Chambon and Winter [15], which defined the gelation point at that time when both G' and G'' show the same power–law exponents vs frequency, i.e.:

$$G' \sim G'' \sim \omega^n \tag{4}$$

In this work, gel times were determined at various temperatures (35, 45, 55, and 65 °C) for the two polymer concentrations using this procedure. Time sweeps were performed at different frequencies as shown in Fig. 1, with a reproducibility within 5% for the three lowest temperatures and within 15% at 65 °C in the early stage of gelation, and within 5% past the gelation point. The values of tanδ (=G''/G') are drawn in a log–log plot as functions of frequency for various experimental times (Fig. 2a), and a power–law function defined by Eq. 5 is fitted to the data [20]:

$$\tan(\delta) \sim \omega^q \tag{5}$$

Fig. 2b presents the dependency of parameter q on time, showing the gel point (q=0) at approximately 6.5 h for this particular case. The calculated gel times for all conditions are shown in Fig. 3a, and as expected, gel time decreased with polymer concentration, being 15 and 70% shorter at the highest concentration for 35 and 65 °C, respectively. Gel time also decreased with temperature, which is coherent with the behavior of a heat-induced system. The relaxation exponent n was calculated at the gelation point from the amplitude of tanδ:

$$\tan(\delta) = \tan(n\pi/2) \tag{6}$$

and these values are presented in Table 2 and in Fig. 3b, along with the value of 2/3 predicted by the percolation theory. Experimental data are shown to lay slightly above and below the percolation value. It has been hypothesized that experimental values of n in a range as large as 0.2 to 0.8 could be explained by a combination of percolation and partial or full extent of screening of excluded-volume effect, dependent on the nature of the specific system [21] or the omission of the effect of trapped entanglements on G' [6]. It has also been reported that the theoretical value of 2/3 could not to describe entirely the reality of polymer gelation [21].

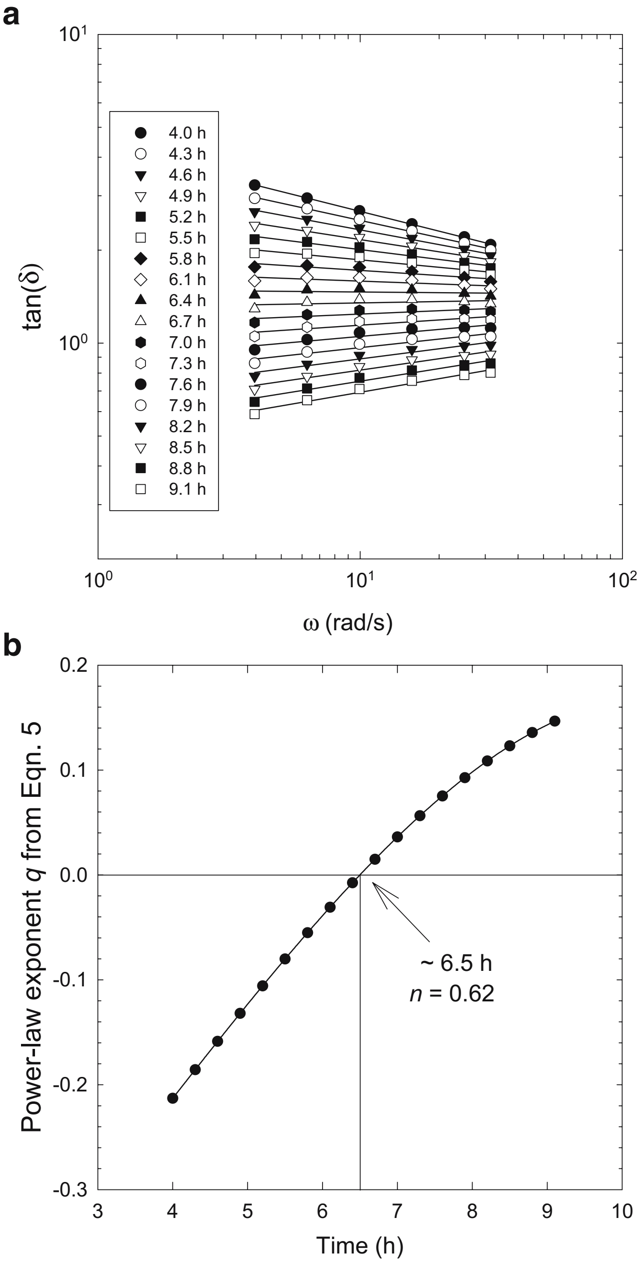


Fig. 2 **a** Log–log plot of tanδ–frequency curves at various times using data of Fig. 1, and **b** plot of power–law exponent q (Eq. 5) as a function of time. In **a**, *lines* are linear regressions

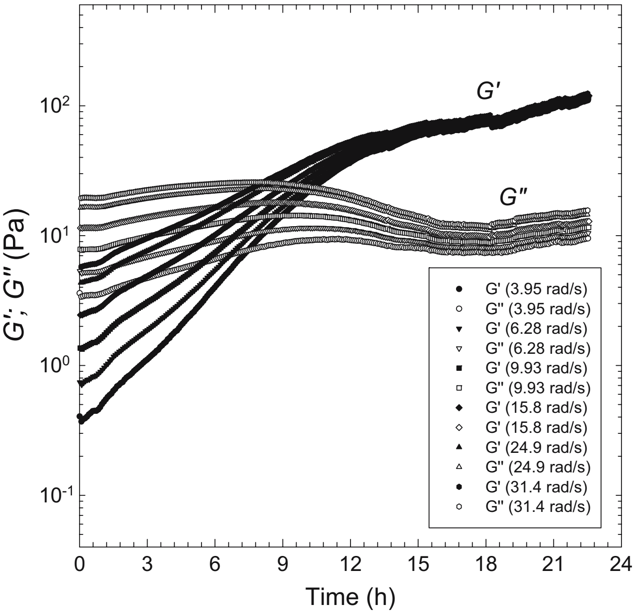


Fig. 1 Dynamic moduli as functions of time measured at different frequencies. Sample 2C16GP at 45 °C (γ_0=0.01)

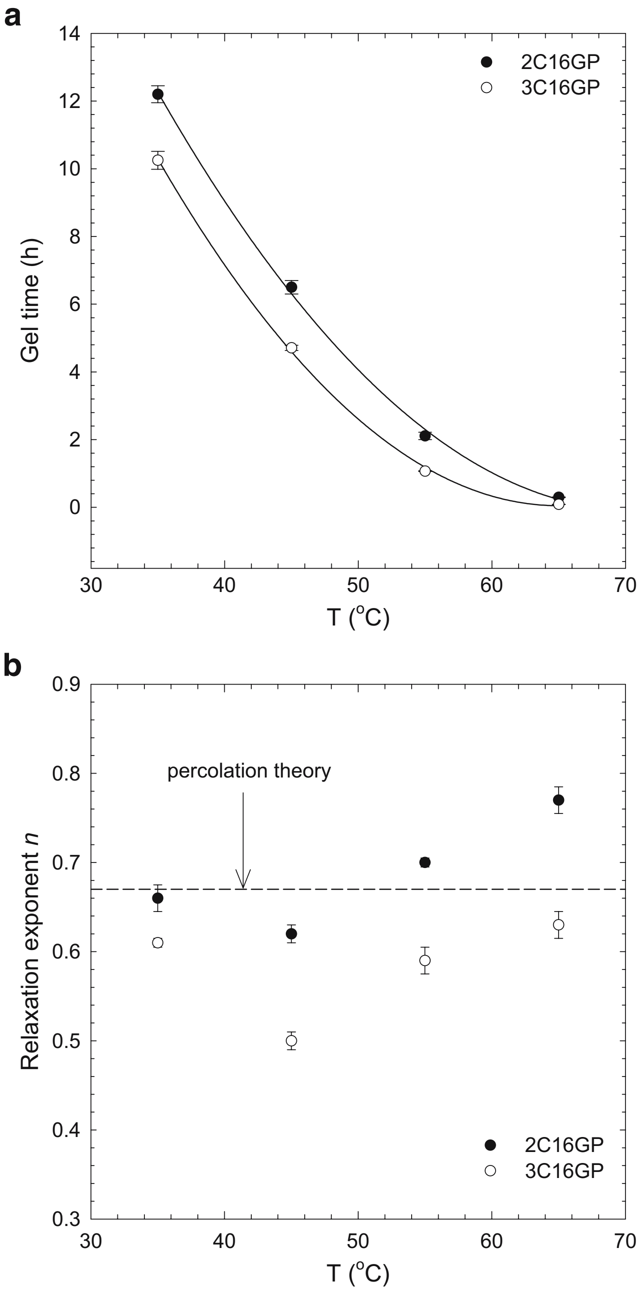


Fig. 3 **a** Determined gel times and **b** calculated relaxation exponent *n* for both polymer concentrations. *Lines* in **a** are to guide the eye

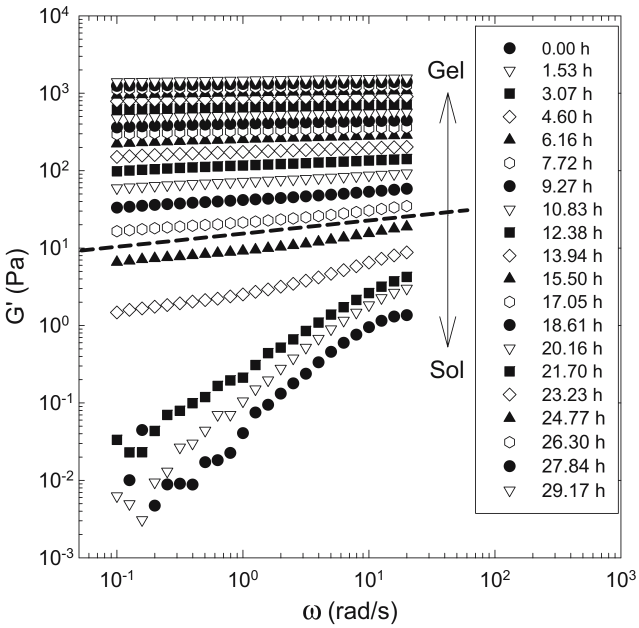


Fig. 4 Evolution of the storage modulus as a function of frequency and time (sample 2C16GP at 45 °C, γ_0=0.01)

Calculation of the scaling parameters before and beyond the gel point

Frequency sweeps were performed at regular intervals during the time sweeps to probe the dynamic properties on nearly three decades of frequencies (0.1–19.95 rad/s) and determine the scaling indexes *s* and *z*. As mentioned previously, these measurements were only performed at 35 and 45 °C, as the kinetics of gelation was too fast at 55 and 65 °C to allow the measurement time to be significantly shorter than the rate of change of the viscoelastic properties. The evolution of the storage modulus, as captured by the frequency sweeps during the gelation process, is shown in Fig. 4 for sample 2C16GP at 45 °C. The delimitation between the sol and the gel behaviors, shown by the dotted line, can be clearly observed as the dependence on frequency is gradually lost in the gel state. Figure 5 presents the complex viscosity as a function of frequency for different distances ε from the gel point (or different times), and a clear Newtonian plateau is observed in the range of frequencies investigated for ε>0.25. For $\varepsilon\leq$0.25, no plateau could be seen anymore, and the viscosity started

Table 2 Scaling exponents before (*s*), at (*n*), and beyond (*z*) the gel point at 35 and 45 °C and calculated values from the scaling relationship for *n*

Sample, temperature	*n*	*s*	*z*	*n*=*z*/(*z*+*s*) (variation with experimental value in %)
2C16GP, 35 °C	0.66±0.07	0.74±0.07	1.9±0.1	0.72 (+9%)
2C16GP, 45 °C	0.62±0.06	0.84±0.08	1.9±0.1	0.69 (+12%)
3C16GP, 35 °C	0.61±0.06	0.75±0.08	1.4±0.1	0.63 (+4%)
3C16GP, 45 °C	0.50±0.05	1.0±0.1	2.3±0.1	0.70 (+42%)

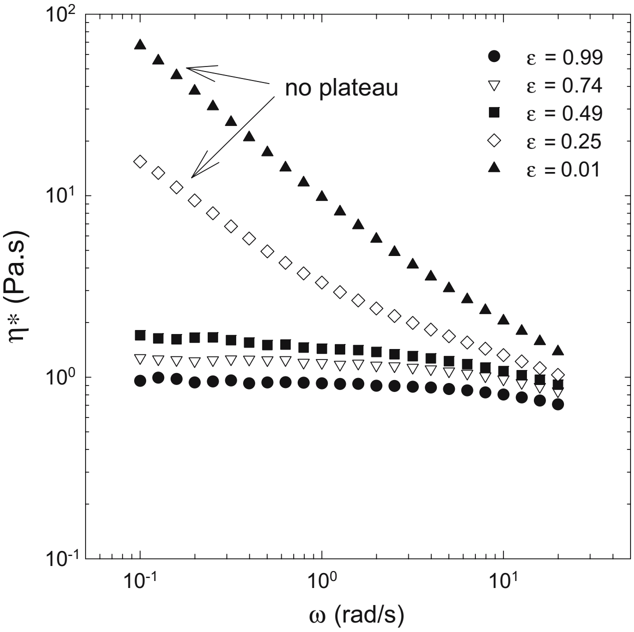
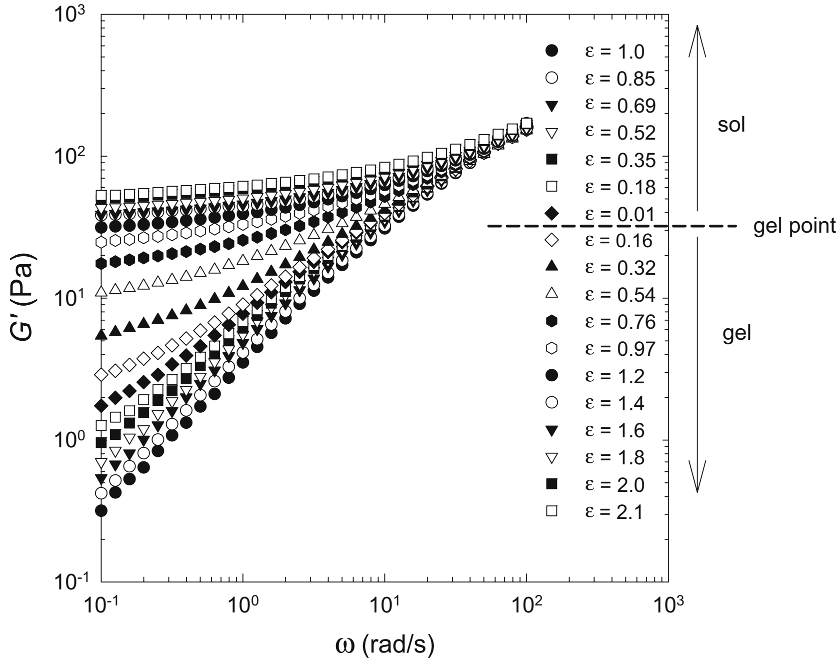


Fig. 5 Complex viscosity as a function of frequency for various distances ε before the gel point (γ_0=0.01)

to diverge as the system got closer to the gel point. Therefore, only the data for ε>0.25 was used for our analysis. This incorporates a complication in the determination of the scaling parameters, as recently reported by Ross-Murphy [14]. While the extent of the critical domain is still debated among theoreticians, a practical guide suggested by Stauffer et al. [22] restrains the critical regime to the region where $10^{-2} \leq p/p_{\text{gel}}-1 \leq 10^{-1}$. Ross-Murphy has shown that outside that range the assumption that $t/t_{\text{gel}}-1=p/p_{\text{gel}}-1$ does not hold, as the relationship between p and t is not linear [14]. As a result, exponents calculated outside the recommended critical regime will be lower. Obviously, it would be necessary to extend the frequency sweeps to lower frequencies (ω<0.1 rad/s) to investigate the scaling of the zero-shear viscosity at distances closer to the gel point, but these tests would be too long to guarantee that the systems would be probed on a constant gel structure. We need to keep this in mind when we compare the determined experimental parameters with the theoretical values. The zero-shear viscosity was evaluated from the oscillatory shear data at 0.1 rad/s and by fits from the Carreau and Cross–Williamson models [23], all in good agreement (results not shown). For all conditions investigated, the determined values of exponent s are presented in Table 2. These were shown to vary very slightly with temperature and concentration and to be overall in the experimental range reported in the literature (0.75–1.5) [13].

Past the gelation point, the appearance of an equilibrium modulus is indicative of the building 3D network. In this case, again, a minimum distance from the gel point (ε> 0.25) was used to get meaningful values, as the equilibrium modulus is zero at the sol-gel transition and remains below the detection limit at early times [12] or does not show clearly the non-terminal behavior, as seen in Fig. 6 for sample 3C16GP at 35 °C. Determined values of parameter z are presented in Table 2. All values lay within the low range of the reported experimented exponents (1.9–3.5)

Fig. 6 Storage modulus as a function of frequency for various distances ε before and beyond the gel point. Sample 3C16GP at 35 °C, (γ_0=0.01)



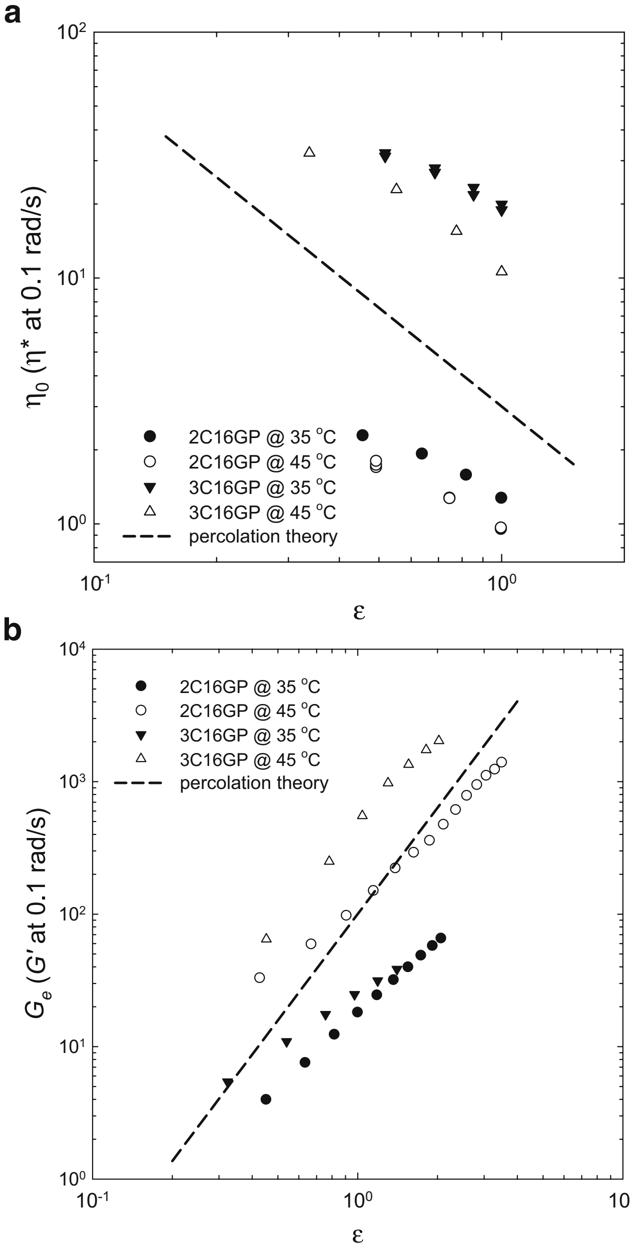


Fig. 7 **a** Zero-shear viscosity and **b** equilibrium modulus (properties recorded at 0.1 rad/s) as functions of distance from the gel point. Results from the percolation theory in the Rouse limit are shown as the *dotted lines* in **a** and **b**

[13], except for sample 3C16GP at 35 °C that showed a lower value (1.4) than the other conditions (~25% lower). This may be due to the fact that the parameters were calculated outside the recommended critical regime. Although there was a large increase of the index with temperature for sample 3C16GP, no effect of temperature could be detected for the lowest chitosan concentration. An increase with concentration was also observed at 45 °C. Figure 7a and b presents the overall data used to determine the scaling parameters *s* and *z* of Table 2, along with the predictions from the percolation theory in the Rouse limit (exponents=4/3 and 8/3, respectively for *s* and *z*). As for the relaxation exponent *n* determined earlier, the experimental data were in moderate agreement with the percolation theory. Finally, Table 2 shows calculated values of exponent *n* derived from the scaling relationship (Eq. 2). In all cases, the relationship overestimated the exponent, although three out of the four estimates were less than 12% away from the experimental value. Considering the overall experimental errors, Eq. 3 showed quite a good validity in the case of the chitosan-β-GP system.

Discussion

In isothermal conditions, gelation of this system may occur at room temperature but be quite long, considering the molecular interactions involved. Higher temperature, between 35 and 65 °C, can however promote quick heat-induced gel formation, in a time as short as 5 min at 65 °C. At the temperatures investigated here, the behavior towards and away from the gel point in terms of rheological properties follow scaling relationships. This behavior is typical of self-similar relaxation, i.e., of relaxation modes that are not independent of each other but coupled [12] and of self-similarity of the mass distribution of clusters. For the same system, Crompton et al. [24], using laser confocal microscopy (LSCM), have qualitatively interpreted a weakly segregated fractal structure from the slopes of the power spectra obtained from LSCM. However, as this gel has been observed to be slightly opaque, it cannot be self-similar over all relevant length scales. Quantitative information about the fractality of this system is therefore still needed. The low frequency behavior ($\eta_0 \sim \varepsilon^{-s}$ and $G_e \sim \varepsilon^{z}$) show slight concentration and temperature-dependency of the scaling of these gels, and hence the exponents not to be universal but to obey reasonably with the scaling relationship $n=z/(z+s)$. It confirms that the particular molecular architecture of every individual system plays an important role in affecting the scaling exponents [16]. Power–law exponents from the percolation theory in the Rouse limit, where no hydrodynamic interactions are assumed, are shown to be larger than the experimental data, but this is probably due to the fact that the calculated scaling parameters were determined outside the recommended critical region due to experimental difficulties as mentioned earlier. Nevertheless, it is relatively rare that all exponents are consistent with the percolation theory (we have encountered only one study until now [25]). The results presented in this paper may contribute in enlarging the potential applications of the chitosan-β-GP system in different industrial activities and controlling the gelation process.

Conclusions

In this work, dynamic scaling of a chitosan-β-GP system were investigated using small-amplitude oscillatory shear rheometry. Viscoelastic properties before, at, and beyond the gel point obeyed scaling relationships but presented lower values than the power–law exponents derived from the percolation theory, most probably because measurements were achieved slightly away from the gel point due to experimental limitations. Knowledge of the gelation evolution and gelling properties of this chitosan system may be helpful for control and industrial applicability of the material.

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