



Characterization of gelification of chitosan solutions by dynamic light scattering

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ABSTRACT

Gels consist of soft materials with vast use in several activities, such as pharmaceutical industry, food manufacturing, and coatings/textile applications. In order to obtain these materials, the process of gelification, which can be physical (based on physical interactions) and/or chemical (based on covalent crosslinking), has to be carried out. In this manuscript we used dynamic light scattering (DLS) and rheometry to monitor the covalent gelification of chitosan solutions by glutaraldehyde. Intensity correlation function (ICF) data was obtained from DLS and the exponential stretched Kohrausch–William–Watts (KWW) function was fitted to them. A correlation between the parameters of KWW equation and delay time was explained in terms of viscoelastic changes during the process of gelation, caused by, at a first stage, the increase of chitosan macromolecular dimensions and, at longer times, by the increase in chitosan crosslinking density and loss of ergodicity. The dependence between apparent viscosity on reaction time was used to support the discussion proposed in this work.

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1. Introduction

Slow dynamic effects in disordered, out-of-equilibrium systems consist in a stimulating subject that can be used to understand characteristic properties of systems with a broad range of composition, from polymer solutions to glasses, passing also by gels and foams (Cipelletti & Ramos, 2002). In the case of organic, polymeric gels, they can be a result of physical interactions (electrostatic forces or hydrogen bonding from polymer entanglements) as well as covalent crosslinking (Bordi, Paradossi, Rinaldi, & Ruzicka, 2002; Starodoubtsev, Kidoaki, & Yoshikawa, 2002). Physical gels are formed using processes such as gelification by a non-solvent and/or electrostatic virtual crosslinking (Carn, Djabourov, Coradin, Livage, & Steunou, 2008; Cheng, Yang, & Lin, 2011). Covalently crosslinked gels are obtained using chemical reactions involving specific chemical groups along the macromolecular chains and small molecules, the crosslinking agents (Testouri, Honorez, Barillec, Langevin, & Drenckhan, 2010).

The most basic form of characterizing the kinetics of gelification is by monitoring the variation of apparent viscosity as a function of time, as already pointed out in the 1960s (Davis & Tabor, 1963). Still rheologically speaking, the viscoelastic behavior during gelification has also been used to follow gelification thorough real and storage moduli-time monitoring (Johnson, Dunstan, & Franks, 2004). Dielectric properties, which also have a dependence on viscoelastic behavior, have been used via dielectric spectroscopy (Boucard,

Viton, & Domard, 2005; Hu, Fan, & Yue, 2001; Lefebvre et al., 2006; Mijovic, Han, Sun, & Pejanovic, 2003). Light scattering can also be used to follow gel crosslinking: Bulone et al. have used static light scattering to characterize different steps in agarose gelation (Bulone, Giacomazza, Martorana, Newman, & San Biagio, 2004) although Segre et al. have pointed out that, like the glass transition, static light scattering does not change during gelation, suggesting that a better monitoring of gelation kinetics can be achieved by using dynamic light scattering (Segre, Prasad, Schofield, & Weitz, 2001).

Dynamic light scattering (DLS) consists in a very effective tool in probing relaxation processes stimulated by random motion occurring in non-crystalline condensed matter (Degiorgio & Piazza, 1996). Shibayama has extensively used this technique with gels and given an excellent account about the theory involved in it (Shibayama, 1998, 2006), so that it will be briefly addressed here. DLS can be used to probe dynamic process occurring in dispersions using the intensity correlation function, $g^{(2)}(t_D)$:

$$g^{(2)}(t_D) \equiv g^{(2)}(t_D, q) = \frac{\langle I(0; q)I(t_D; q) \rangle_t}{\langle I(0; q) \rangle_t^2}, \quad (1)$$

where t_D is the delay time, $I(t_D; q)$ is the scattered intensity at time t_D , q is the scattering vector modulus, $\langle i \rangle_t$ denotes a time average of i , and $g^{(2)}(t_D)$ is the scattering field time-correlation function. The intensity correlation function (ICF) $g^{(2)}$ is related to $g^{(1)}$, the theoretical field autocorrelation function, according to

$$g^{(2)}(t_D) = 1 + \beta [g^{(1)}(t_D)]^2, \quad (2)$$

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where β is a constant that depends on the optical properties of the system. If there is a well defined, discrete, relaxation process, $g^{(1)}$ is defined as

$$g^{(1)}(t_D) = e^{-\Gamma t_D}, \quad (3)$$

where the relaxation rate, Γ is

$$\Gamma = q^2 D, \quad (4)$$

$$q = \frac{4\pi n_0}{\lambda} \sin\left(\frac{\theta}{2}\right), \quad (5)$$

D is the diffusion coefficient associated with this motion, n_0 is the refractive index of the sample, θ is the angle at which the detector is located to the sample cell, and λ is the radiation wavelength. If the relaxation process is better represented as a continuous relaxation rate distribution function, $G(\Gamma)$, Eq. (3) becomes

$$g^{(1)}(t_D) = \int_0^\infty G(\Gamma) e^{-\Gamma t_D} d\Gamma \quad (6)$$

The determination of $G(\Gamma)$, or parameters related to analytical functions related to $g^{(1)}(t_D)$ or $g^{(2)}(t_D)$ yields information on the dynamics of these systems.

DLS has been applied to follow the gelification of soft systems ranging from inorganic/hybrid gels to bio-synthesized materials (Kobayashi, Huang, & Lodge, 1999; Massey, Power, Manners, & Winnik, 1998). Among the macromolecules with biological origins, chitosan, an aminopolysaccharide present as a major compound in crustacean exoskeleton, is a very interesting subject for the application of this technique, since this macromolecule yields materials of wide application in biomedicine/pharmacy, due to its low toxicity and high biodegradability (de Vasconcelos, Bezerril, Dantas, Pereira, & Fonseca, 2007; Muzzarelli, 2009; Muzzarelli et al., 2007; Muzzarelli, Terbojevich, Muzzarelli, & Francescangeli, 2002; Neto et al., 2005).

When applied to chitosan samples, DLS has mainly been used to monitor the formation of (nano)microparticles for the controlled delivery of biologically active substances (Kim, Park, Nah, Choi, & Cho, 2004; Kim et al., 2001; Lee, Kwon, Kim, Jo, & Jeong, 1998; Yue et al., 2010; Zheng et al., 2011). To the best of our knowledge, DLS studies of chitosan solutions under covalent crosslinking have not been reported in the literature, although the crosslinking of chitosan has been used in numerous articles for the preparation of chitosan-based biomaterials (Crescenzi, Francescangeli, Taglienti, Capitani, & Mannina, 2003; Kildeeva, Perminov, Vladimirov, Novikov, & Mikhailov, 2009; Monteiro & Airoidi, 1999). The aim of the present work is to follow covalent crosslinking of solubilized chitosan using time-dependent DLS-related parameters and compare them with rheometry data.

2. Experimental

Chitosan used in this work was purchased from Polymar LTD (Brazil) and was purified as reported in the literature (Caroni, de Lima, Pereira, & Fonseca, 2009). It had a deacetylation degree of 88%, determined by CHN elemental analysis and conductometric titration as described elsewhere (dos Santos, Caroni, Pereira, da Silva, & Fonseca, 2009), and average viscometric molar mass, $\bar{M}_V = 1.6 \times 10^5 \text{ g mol}^{-1}$, was determined using Mark-Houwink-Sakurada equation (Bezerril, de Vasconcelos, Dantas, Pereira, & Fonseca, 2006; Rinaudo, Milas, & Ledung, 1993). Acetic acid (P.A., Cromato Produtos Químicos Ltd, Brazil), and glutaraldehyde (P.A., 25%, Cromoline Química Fina, Brazil Ltd, Brazil) were used as received. Bi-distilled water was used in all experiments.

A crosslinking reaction was carried out by mixing, at $t = 0$, 5.0 mL of a solution of chitosan [10 g L^{-1} in acetic acid 2% (v/v)] with

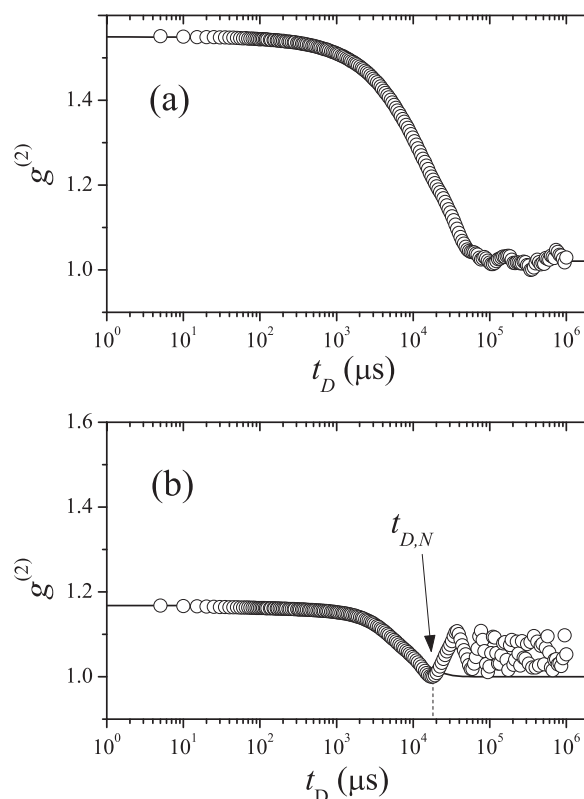


Fig. 1. Intensity function, $g^{(2)}$, as function of delay time, t_D , for (a) uncrosslinked chitosan solution and (b) solution of chitosan with glutaraldehyde after 28 min of reaction time. Open circles: experimental data. Continuous line: Eq. (7). The arrow indicates $t_{D,N}$ the time in which the ICF becomes noisy.

0.4 mL of a 25% aqueous solution of glutaraldehyde. After homogenization, the reactant mixture was submitted DLS or rheometry experiments. All solutions were filtered through $0.22 \mu\text{m}$ Durapore membrane filters.

Dynamic light scattering experiments were carried out using a 90 Plus Particle Analyzer (Brookhaven Instruments Corporation, USA). Data acquisition parameters were adjusted as: time of analysis, 3 min (average of 3 measurements of 1 min); wavelength, 659 nm; scattering angle, $\theta = 90^\circ$; temperature, $T = (25 \pm 1)^\circ\text{C}$. Rheometry was carried out using a Haake Mars rheometer (Germany, cup Z43 DIN 53018 and rotor Z41 DIN 53018). The temperature was kept at $(25.0 \pm 0.1)^\circ\text{C}$ using a thermostatic bath. In these experiments, apparent viscosity, η , was determined at a fixed shear rate of 25 s^{-1} . The volumes of chitosan and glutaraldehyde solutions were doubled for rheometry experiments.

3. Results and discussion

Firstly, we noticed the following occurrence in the ICF's of the chitosan solutions, as time went on:

- A rather noisy, oscillatory, random signal appeared for longer delay times, $t_{D,N}$.
- The initial intensity of the ICF decreased.

The occurrences (i) and (ii) can be visualized in Fig. 1, which shows the ICF's for a chitosan solution at two reaction times: $t = 0$ (just before crosslinking agent was added), and $t = 28 \text{ min}$. The appearance of strong fluctuations in the ICF has been assigned to the onset time of gelation (Takata, Norisuye, Tanaka, & Shibayama, 2000; Yoshida, Yamaguchi, Osaka, Endo, & Shibayama, 2010) and the decrease in ICF initial intensity, β , has been associated to

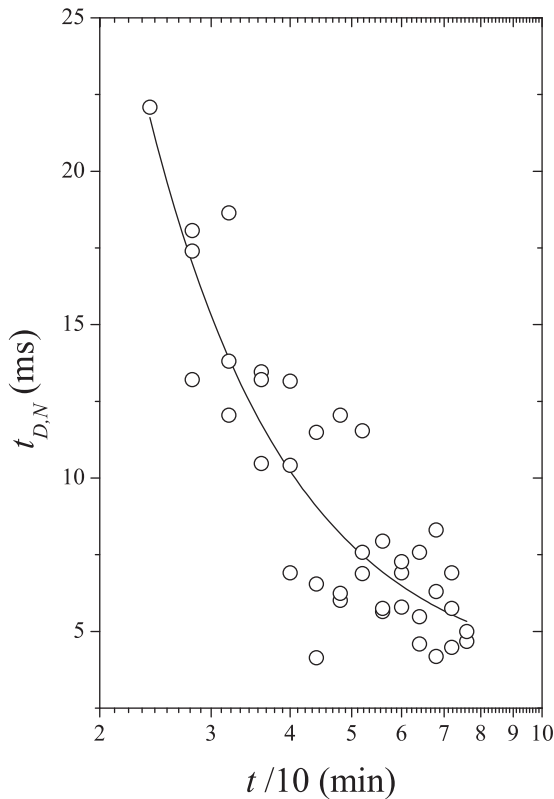


Fig. 2. Noise-associated delay time, $t_{D,N}$, as a function of reaction time, t , for solutions of chitosan with glutaraldehyde.

the occurrence of crosslinking (Joosten, Geladé, & Pusey, 1990). In the case of the chitosan gel studied in this work, however, it was difficult to determinate a well defined onset time of gelation only by considering $t_{D,N}$, since it shifted to longer and longer delay times, as reaction time tended to zero (i.e., it asymptotically tended to infinite as crosslinking degree tended to zero). This behavior can be straightforwardly perceived by a rapid analysis of Fig. 2, which shows the dependence of $t_{D,N}$ on reaction time.

A much more promising investigation, though, can be made if we use β as well as parameters obtained when using a KWW-based stretched exponential equation:

$$g^{(2)}(t_D) = 1 - \beta e^{-(2\Gamma_C t_D)^C}, \quad (7)$$

so that

$$g^{(1)}(t_D) = e^{-(\Gamma_C t_D)^C}. \quad (8)$$

Here, Γ_C is a characteristic relaxation rate and C is a dimensionless constant that has a value between 0 and 1. If we define a function $H(\Gamma) = \Gamma G(\Gamma)$, Eq. (6) can be rewritten as

$$g^{(1)}(t_D) = \int_0^\infty G(\Gamma) e^{-\Gamma t_D} \frac{d\Gamma}{\Gamma} = \int_{-\infty}^{+\infty} H(\Gamma) e^{-\Gamma t_D} d \ln \Gamma \quad (9)$$

Clearly, Eq. (9) can be treated in an analogous way as it has been done in stress relaxation experiments with the relaxation spectrum (Shaw & MacKnight, 2005):

$$E(t) = \int_{-\infty}^{+\infty} H(\tau) e^{-(t/\tau)} d \ln \tau, \quad (10)$$

where $E(t)$ is the relaxation modulus, τ is the relaxation time, and $H(\tau)$ is the relaxation spectrum. Indeed, a KWW equation,

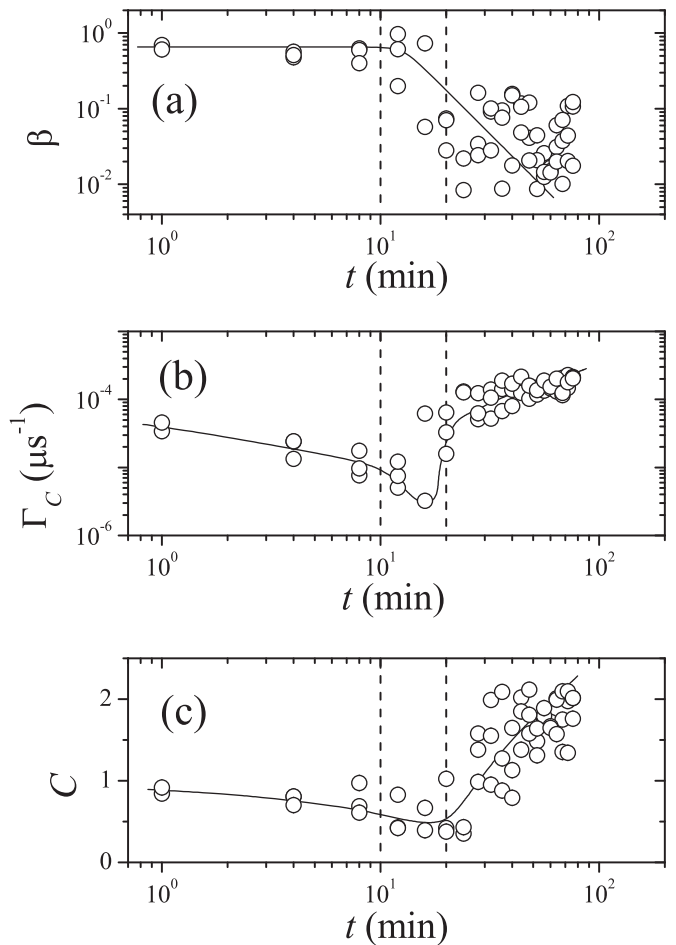


Fig. 3. Parameters related to Eq. (8) as a function of reaction time, t : (a) β , (b) Γ_C , and (c) C . The dotted lines delimit the time range in which gelification occurs.

analogous to Eq. (8), has been used to describe stress relaxation of poly(urethane-urea)s (Monteiro & Fonseca, 1997):

$$\frac{E(t)}{E_0} = e^{-(t/\tau_C)^C}, \quad (11)$$

where E_0 is the modulus at $t=0$, τ_C is a characteristic relaxation time, and C is a constant between 0 and 1. These authors found that, in terms of spectrum/distribution calculation, the most important parameters were:

- The parameter τ_C , which indicates the range in relaxation time in which most of the relaxation processes occur.
- The parameter C , which was limited to $0 < C < 1$. For $C=1$, one had a relaxation process with one defined relaxation time. As $C \rightarrow 0$, the relaxation spectrum is represented by a wider distribution of relaxation times. In other words, the parameter C can be viewed as a sort of “heterogeneity index” of the relaxation process: the closer to 1 it is, the more homogeneous the relaxation process is.

Hence Eq. (8) was fitted to ICF data and the parameters β , Γ_C , and C were determined via non-linear regression. Eq. (8) was applied only to the part of the data that was not scattered, in other words, for $0 < t_D < t_{D,N}$, as shown in Fig. 1. The resultant parameters are plotted in Fig. 3. The behavior of β , the initial amplitude of the ICF (shown in Fig. 3a), shows a clear dependence with the reaction time, t . As reaction time tends to zero, β tends to its maximum value. At a reaction time of ca. 10–20 min, the value of β starts to catastrophically decrease: after 50 min of reaction, the decrease in β is around

two orders of magnitude. Shibayama and Norisuye have reported that the suppression of the initial amplitude of the ICF characterize sol–gel transitions in soft matter (Shibayama, 1998; Shibayama & Norisuye, 2002), so that a strong decay in the initial amplitude of the ICF should be a good indication of threshold gelation time (Norisuye, Shibayama, Tamaki, & Chujo, 1999).

As Fig. 3b shows, the characteristic rate of relaxation, Γ_C , initially decreases as the crosslinking reaction follows. That could be due to predominant influence of intermolecular crosslinking, at early stages of the reaction (Torii, Ushiki, & Horie, 1993). Intermolecular crosslinking results in an increase in macromolecular dimensions, causing the increase in viscous, hydrodynamic interactions between the growing macromolecular coils and the solvent (de Vasconcelos, de Azevedo, Pereira, & Fonseca, 2000). If we think of the macromolecule as an isolated particle, a characteristic value of macromolecular radius, R_C , can be defined according to Stokes–Einstein relationship (Dorfschmid, Mullen, Zumbusch, & Woll, 2010):

$$R_C = \frac{kT}{6\pi\eta D_C}, \quad (12)$$

where k is the Boltzmann constant, T is the absolute temperature, D_C is a characteristic diffusion coefficient, and η is the viscosity of the continuous medium, so that substitution of (4) in (12) yields

$$\Gamma_C = \frac{kTq^2}{6\pi\eta R_C}. \quad (13)$$

Consequently, the increase in the dimensions of the macromolecular coils, due to intermolecular crosslinking, would lead, in the earlier stages of covalent crosslinking, to a decrease of Γ_C . As macromolecular dimensions become bigger, internal crosslinking becomes more important. The scattering centers are not the macromolecules anymore: they are regions within the macromolecule (that is becoming to be part of a gelified structure) with a characteristic dimension ξ_C (Shibayama, 2006). Substitution of R_C by ξ_C in (13), consequently, yields:

$$\Gamma_C = \frac{kTq^2}{6\pi\eta \xi_C}. \quad (14)$$

From this point, further crosslinking will result in a decrease in the dimensions ξ_C of the blobs formed by the macromolecular chains embodied by the crosslinks: therefore, Γ_C will increase. As one can see in Fig. 3b, the region in which it happens coincides with the region related to changes in β (10–20 min).

Finally, we analyze the parameter C . It can be seen that, as reaction time goes on, firstly C decreases: as previously pointed out in this paper, it means that the distribution of relaxation times (or rates) is becoming broader. It agrees with the formation of higher molecular weight coils (with broader molecular weight distributions), during the early stages of crosslinking. These coils with broader molecular weight distributions will be able to undertake more differentiated forms of entanglements, implying a broader relaxation rate distribution, as observed a long time ago for stress relaxation experiments carried out by the group of viscoelasticity pioneer A.V. Tobolski (Knoff, Hopkins, & Tobolsky, 1971). Sol–gel transition has also been detected as a peak broadening of the characteristic decay time distribution $G(\Gamma)$ in DLS experiments (Shibayama, 1998; Shibayama & Norisuye, 2002). Back to following Fig. 3b, a minimum is reached and then C starts to increase, indicating that the system becomes a gel and crosslinking homogenizes it: the distribution of the number of carbons between joint points in the crosslinked material becomes narrower and narrower, as crosslinking develops. According to this explanation, one would expect that, as $t \rightarrow \infty$, $C \rightarrow 1$; however, C continues to increase, reaching values as higher as 2. This sort of decay, in form of a “compressed” exponential ($C > 1$) has been interpreted

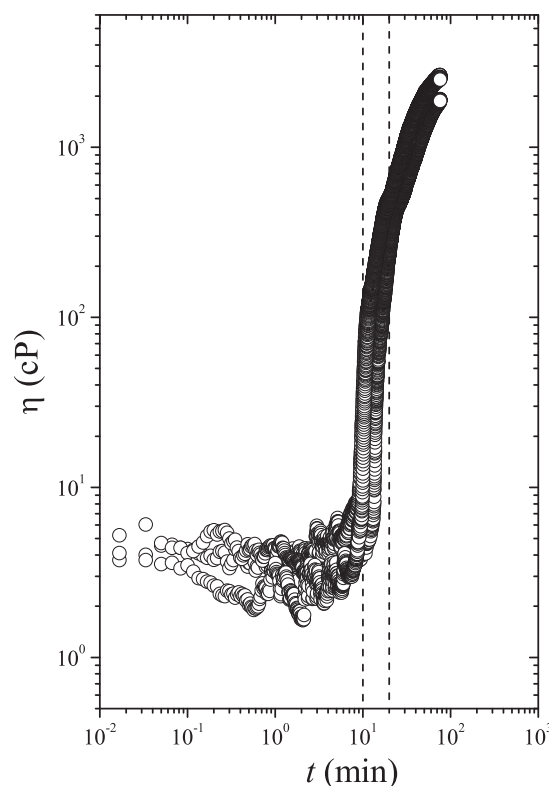


Fig. 4. Apparent viscosity, η , as a function of reaction time, t , during the reaction between chitosan and glutaraldehyde in aqueous solution. The dotted lines delimit the time range in which gelification occurs.

as extremely fast motions resulting from the relaxation of internal stresses, such as the ones resultant from syneresis (Bouchaud & Pitard, 2001; Cipelletti & Ramos, 2002, 2005). The occurrence of these compressed exponentials indicates, therefore, the occurrence of non-ergodicity (Bandyopadhyay, Liang, Harden, & Leheny, 2006). The minimum in C can indicate, consequently, the gelification of the system. As with the other parameters, it seems to be located at the range of 10–20 min.

Lastly, a comparison with a more conventional rheometric approach can strengthen our point. Fig. 4 shows the dependence of apparent viscosity, η_{app} , as a function of reaction time, t , during gelification of chitosan in solution by glutaraldehyde. The gelification time can be unequivocally characterized by the extreme growth in η_{app} that occurred at the same interval of time registered in DLS experiments (10–20 min). A second glance at Fig. 4 may emphasize the advantage of using DLS over rheometry for the determination of gelification time: as the solution becomes a gel, the very shear rate used to characterize gelification destroys the freshly obtained gel, as can be inferred by the decay in the rate of apparent viscosity increase for long reaction times. The non-destructive character of DLS becomes evident.

4. Conclusions

Dynamic light scattering can be used to monitor the covalent crosslinking of chitosan in solution by glutaraldehyde. The use of the parameters obtained from the fit of the stretched exponential decay KWW function to data from the ICF's allows one to determine gelification time according to the following parameters:

- The time at which the decrease in the amplitude of the ICF collapses.

- The time at which the relaxation rate distribution is centered at its minimum value.
- The time at which distribution of relaxation rates is the broadest. Values of the dimensionless parameter of the KWW equation above 1 also indicates the loss of ergodicity in the system.

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