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Effect of urea on solution behavior and heat-induced gelation of chitosan-β-glycerophosphate

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Abstract

In this study, we have investigated the effect of urea on the physicochemical (pH and conductivity) and rheological properties of the chitosan-β-GP system in order to assess the main polymer–polymer interactions at low and high temperature. The pH of the solutions was slightly increased due to the consumption of H^+ in solution through the hydrolysis of urea. Furthermore, the addition of urea considerably decreased the conductivity, and therefore the ionic strength of the solutions, and this effect was more important at high temperature. It indicated that urea strongly affects polymer–polymer interactions by weakening hydrogen bonds at low temperature, but in addition can hinder hydrophobic effect at high temperature since the reduction of ionic strength results in less screening of electrostatic repulsion between protonated glucosamine groups. At 15 °C, the addition of urea to chitosan-β-GP solutions decreased their elasticity, shortened relaxation times and simplified the relaxation process due to the disruption of hydrogen bonds. Heat-induced gelation of the chitosan system in the presence of urea showed higher gelation temperature (T_{gel}) in non-isothermal tests and longer gelation time (t_{gel}) in isothermal conditions. The activation energy for gelation also increased with increasing urea concentration. We concluded that the detrimental effect of urea on the gelation process was mainly related to a decrease in polymer-polymer hydrophobic effect, as shown by the decrease in conductivity.

Keywords: Chitosan; Sol–gel; Rheology

1. Introduction

Chitosan is a biopolymer derived through a series of chemical treatments from the chitin components of the shells of crustaceans. It is a copolymer of 2-amino-2-deoxy-D-glucose (glucosamine) and 2-acetamido-2-deoxy-D-glucopyranose (acetylglucosamine) (Roberts, 1992). An important chemical characteristic of the molecule is its degree of deacetylation (DDA), or the fraction of glucosamine units in the chemical structure. The copolymer is generally considered as 'chitosan' when the DDA is greater than 50% (Brugnerotto, Desbrières, Heux, Mazeau, & Rinaudo, 2001). Chitosan is a non-toxic, biocompatible and biodegradable material and has been used in many industries such as nutraceutic, medical, cosmetic and pharmaceutical (Kassai, 1999; Singla & Chawla, 2001; Kumar, 2000; Jackson, 1987; Chenite, Chaput, Wang, & Slemani, 2001; Chaput & Chenite, 2001).

Chitosan contains hydrophobic ($-CH_3$) and hydrogen bonding favoring groups (–OH, $-NH_2$ and –C=O) (Roberts, 1992). However, since the copolymer becomes positively charged due to the protonation of the free amine groups below a pH of 6.2, corresponding to the pKa of chitosan at high protonation (Park, Choi, & Park, 1983; Vachoud, Zydowicz, & Domard, 1997), polymer–polymer interactions via hydrophobic effect and/or hydrogen bonding junctions can be hindered due to electrostatic repulsion. For example, it is known that phase separation is triggered by the use of high salt concentration (Cho, Heuzey, Bégin, & Carreau, in pressa). Likewise, gels can be formed by controlling the pH. Chenite et al. Chenite, Buschmann, Wang, Chaput, and Kandani (2001) obtained a homogenous gel system by neutralizing chitosan solutions using a weak base, β-glycerophosphate (β-GP: $pK_{a,2}=6.65$ at 25 °C (Alberty & Silbey, 1996; Golberg, Kishore, & Lennen, 2002)) and controlling temperature. The system remained in solution at physiological pH (=7) and room temperature and changed into a gel upon heating at physiological temperature (=37 °C).

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We have investigated rheological and physicochemical properties of the chitosan-β-GP system in terms of temperature in a previous publication (Cho, Heuzey, Begin, & Carreau, in pressb). According to the evolution of the rheological properties under non-isothermal conditions, three regions were defined: (1) a liquid-like behavior at low temperature, (2) a fast gelation process near the gel point, and (3) a slow gelation beyond. The gel structure formed at high temperature was only partially thermoreversible upon cooling to 5 °C. Increasing temperature had no effect on the pH value of the chitosan-β-GP system, while conductivity (and calculated ionic strength) increased. With values from the pH measurements, we have shown that the estimated protonation of chitosan decreased with increasing temperature, but that of β-GP increased. It indicated a reduction of the ratio of $-NH_3^+$ in chitosan and $-OPO(O^-)^2$ in β-GP at high temperature, resulting in a decrease of potential ionic interactions such as ionic bridging. On the other hand, increased ionic strength as a function of temperature involved enhanced screening of electrostatic repulsion forces and hence more polymer–polymer hydrophobic interactions, resulting in favorable conditions for gel formation. Since it is generally assumed that hydrogen bonding interactions are not predominant at high temperature, we suggested that hydrophobic effect was the main driving force for the chitosan-β-GP gelation at high temperature.

In this work we investigate physicochemical and rheological properties of the chitosan-β-GP system in the presence of urea in order to expand our understanding of the role of hydrogen bonding and hydrophobic effect. Urea is generally known as a hydrogen bonding disrupting agent (Hammes & Schimmel, 1967; Kjønisken, Hiorth, Roots, & Nystorm, 2003; Kokufuta et al., 1998; Kim, Sarathchandra, & Mainwaring, 1996; McGrane, Mainwaring Carnell, & Rix, 2004) but it can also affect hydrophobic interactions (Philippova et al., 2001). The presence of urea on chitosan-β-GP solutions when the temperature is increased can consequently reduce furthermore hydrogen bonds and decrease the hydrophobic effect. In this study, the variations of pH and conductivity of the polymer system are measured as functions of urea concentration and temperature, and the solution and gelation behaviors are characterized through rheological measurements.

2. Temperature and presence of urea effects on gelation of chitosan

From its hydrophobic and hydrogen bonding favoring parts, chitosan can self-associate in aqueous solutions (Roberts, 1992; Chenite et al., 2001; Philippova et al., 2001; Amiji, 1995; Nyström, Kjøniksen, & Iverson, 1999; Wang, 1999; Tsaih & Chen, 1997). These two types of junctions, along with ionic bridging in the presence of divalent species (Hamdine, Heuzey, & Bégin, in press), are critical interactions involved in the formation of physical gel structures in chitosan-based systems. The balance between these interactions is critical to the formation of three-dimensional networks and can be controlled by temperature variation and/or in the presence of urea (Hammes & Schimmel, 1967; Kjønisken et al., 2003; Kokufuta et al., 1998; Kim et al., 1996; McGrane et al., 2004; Philippova et al., 2001; Cheng, Prud'homme, Chick, & Rau, 2002; Tako & Hanashiro, 1997). Urea has been extensively used to control the intensity of hydrogen bonding interactions in biomedical systems (Hammes & Schimmel, 1967; Kjønisken et al., 2003; Kokufuta et al., 1998; Kim et al., 1996; McGrane et al., 2004; Tsaih & Chen, 1997) as well as to control hydrophobic effect (Philippova et al., 2001). We briefly discuss below the general influence of temperature and urea on hydrogen bonds and hydrophobic interactions in the light of previous research.

2.1. Temperature effect

Water molecules are presumed to form enclosed structures that surround the polymer chain at low temperature (Li et al., 2001). Increasing temperature enhances the vibrational and rotational energy of water molecules and inhibits weak hydrogen bonds to orient dipolar water molecules around the polymer chains, causing the decrease of hydrogen bonding interactions. With increasing temperature, the energized water molecules enclosing the polymer chain are removed and the dewatered hydrophobic polymer segments start associating with each others. Thus, increasing temperature increases the hydrophobic effect and results in physical networks formed by enhanced association (Desbrieres, Martinez, & Rinaudo, 1996).

2.2. Urea effect on hydrogen bonding interactions

Urea, known as a hydrogen breaking agent (Hammes & Schimmel, 1967; Kjønisken et al., 2003; Kokufuta et al., 1998; Kim et al., 1996; McGrane et al., 2004), can hinder the formation of macromolecular structures. However, there are two different views about the effect of urea on hydrogen bonding interactions. One is that urea is unable to disrupt intramolecular interactions (Kim et al., 1996; McGrane et al., 2004; Cheetham & Tao, 1997) and only have impact on the intermolecular ones, while the other suggests that adding urea breaks intramolecular hydrogen bonds and causes a change in the molecular conformation (Tsaih & Chen, 1997; Chen & Tsaih, 2000). Two mechanisms are proposed to explain the denaturing function of urea in aqueous media (Hammes & Swann, 1967). The first is the binding of urea to specific groups on the polymer chains, thereby weakening hydrogen bonding between polymer chains (intermolecular), while the other possible mechanism is the breakdown of the hydrogen-bonded structure of the solvent, or the 'structuring breaking effect'. According to the literature (Hammes & Swann, 1967), both mechanisms concurrently produce the denaturing process.

Kjønisken et al. Kjønisken et al. (2003) showed that the dynamic moduli (G' and G'') of pectin solutions exhibited weaker values in the presence of urea, and that gel formation at 25 °C was hindered. High urea concentrations also prevented a network microstructure forming for proanthocyanidin (PA) polymer solutions and yielded nearly Newtonian characteristics of independent particle motions (Kim et al., 1996), while

for amylase gels in a mixture of water and dimethyl sulfoxide (DMSO), the use of urea reduced the gel strength significantly (McGrane et al., 2004). The previous examples illustrate the effect of urea on intermolecular hydrogen bonds. On the other hand, Tsaih and Chen Tsaih and Chen (1997) reported that the addition of urea to chitosan solutions resulted in an increase of the intrinsic viscosity and persistence length, therefore in a less compact structure, due to the hindrance of intramolecular hydrogen bonds.

2.3. Urea effect on hydrophobic interactions

The influence of urea on hydrophobic interactions is controversial. Kokufuta et al. Kokufuta et al. (1998) proposed that these interactions, involved in the formation of gels of poly(ethyleneimine), were not affected by the addition of urea. Furthermore, recent neutron light scattering experiments showed that urea caused no apparent disruption of the water structure even at high concentration of urea (Finny & Soper, 1994), indicating no effect on hydrophobic interactions. However, Dubin and Stauss Dubin and Stauss (1973) reported that urea weakened hydrophobic interactions between solute molecules. Urea preferentially solvates hydrophobic residues by breaking the hydrogen-bonded structures of water (Roseman and Jenks, 1975; Alonso and Dill, 1991), and this leads to an effective reduction of hydrophobic interactions. Computer simulations based on energy minimization of clusters supported that urea directly participates in the solvation of hydrophobic solutes by replacing water molecules in the hydration shells of the solute (Christianziana, Lelj, Amodeo, Barone, & Barone, 1989). Wallqvist and Covell Wallqvist and Covell (1998) monitored the effect of urea on hydrophobic interactions by computing the potential of mean force in the ternary system methane–water–urea. Urea appeared to enhance hydrophobic interactions and to act as a renaturant for the uncharged methane. However, it acted as a denaturant in the ternary charged methane–water–urea system by destabilizing the hydrophobic bonds between the solutes. Finally, Philippova et al. Philippova et al. (2001) investigated the influence of urea on hydrophobic effect in the association of chitosan molecules using fluorescence measurements. The authors showed reduced formation of hydrophobic domains in chitosan solutions in the presence of urea at a concentration of 7 M.

3. Experimental

3.1. Materials

The chitosan used in this study was purchased from Marinard Biotech (Rivière-aux-Renards, QC). The chemical structure of chitosan is shown in Fig. 1(a). The degree of deacetylation (DDA), or fraction of glucosamine units, was obtained from colloidal titration with polyvinyl sulfate potassium, initially standardized with cethylpyridinium chloride. Gel permeation chromatography measurements were conducted in order to characterize the molecular weight

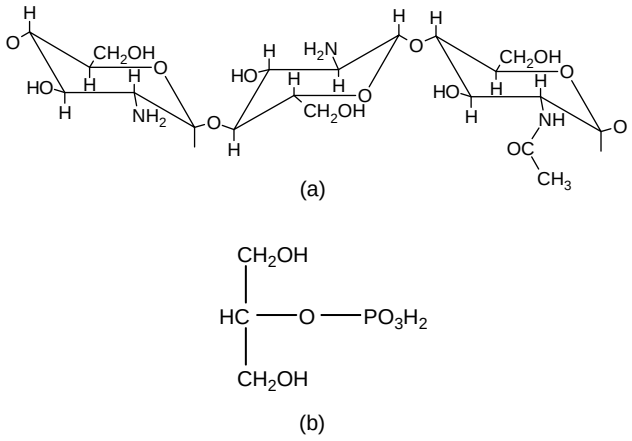

Fig. 1. Chemical structure of (a) chitosan and (b) β-glycerophosphate (β-GP).

using an Ultrahydrogel™ 500 Column (Waters Co., Milford, MA) and dextran standards. The determined weight-average molecular weight ($\bar{M}_w$) was 8.5×10^5 g/mol ($\bar{M}_w/\bar{M}_n = 2.76$) in a solvent of 0.25 M acetic acid/0.25 M sodium acetate. For the solutions preparation, acetic acid (AcOH) (99.7%, Sigma-Aldrich Canada Ltd, Oakville, ON) was used to dissolve the chitosan. Also disodium-β-GP (glycerol-2-phosphate disodium salt hydrate: $C_3H_7Na_2O_6P\cdot xH_2O$, FW 216.04, Sigma-Aldrich Canada Ltd, Oakville, ON) (chemical structure shown in Fig. 1(b)) was used to adjust the pH of the solutions. Lastly, urea (99.5%, Sigma-Aldrich Canada Ltd, Oakville, ON) was added in order to control the various interactions at low and high temperature.

3.2. Preparation of chitosan solutions

Two different chitosan quantities were added to 1 w/v% acetic acid aqueous solution. The solutions were mixed for 4 h in order to achieve complete solubilization of chitosan. The mixing was performed at room temperature at a rate of 50 rpm using a laboratory magnetic stirrer (PC-420 Corning® Stirrer/Hot Plate, Corning Inc., MA, USA). The β-GP was slowly added to the chitosan solutions in order to increase the pH around 7. A mixing time of 0.5 h was used to homogeneously disperse the β-GP and to avoid forming local precipitates. Finally, various amounts of urea (0–5 M) were added to the chitosan-β-GP solutions and mixed for 1 h. The volume difference caused by the addition of β-GP and urea was compensated in other solutions by adding deionized water. The final total volume of each solution was 60 mL. The composition in molarity and the nomenclature of the chitosan solutions are presented in Table 1. During the stirring process, the containers were covered with aluminium foil to prevent evaporation. The prepared chitosan-β-GP-urea solutions were left to rest 3 h for degassing at room temperature and kept in a refrigerator overnight at 5 °C. It was found that this overnight storage was essential to obtain reproducible results, possibly because of the presence of small remaining bubbles. All the samples were stored at 5 °C and used within one week in order to avoid aging effect due to polymer degradation.

Table 1
Nomenclature and solution compositions expressed in molarity

Sample	AcOH	Chitosan[a]	β-GP	Urea
U0	0.12	0.08	0.53	0
U1	0.12	0.08	0.53	1
U3	0.12	0.08	0.53	3
U5	0.12	0.08	0.53	5
14CU0	0.12	0.14	0.53	0
14CU3	0.12	0.14	0.53	3

[a] Concentration of glucosamine units or monomol/L.

ductivity measurements

All solutions were characterized in terms of pH and conductivity as a function of temperature. A water bath (model BT-15, Cole-Parmer, IL, USA) was used to control temperature at a constant heating rate (0.8 °C/min) and all measurements were recorded in a continuous manner in order to simulate the heat-induced gelation process. The temperature range used was 0–55 °C for pH measurements (pH-meter, Hanna Ltd, Àrvore-Vila do Conde, Portugal) and 0–75 °C for conductivity (conductance meter, model 35, YSI Inc., Yellow Springs, OH). Since the conductance meter used was linearly temperature compensated (referenced to 25 °C), we applied the following equation to recover the conductivity at a given temperature:

$$\kappa_T = \kappa_{25}[1 + \alpha(T - 25)] \quad (1)$$

with κ_{25} the conductivity referenced to 25 °C, κ_T the conductivity measured at a given temperature T, and α the temperature coefficient of variation (HonerKamp & Weese, 1993). The choice of different temperature ranges was a consequence of the different limitations of the respective electrodes.

3.4. Rheological measurements

The rotational rheometer used in this study was a stress-controlled device (AR-2000, TA Instruments, New Castle, DE) with a Couette geometry. Mineral oil covered the surface of the chitosan solutions to prevent evaporation during the tests. The effect of the mineral oil on the measurements was shown to be negligible. The chitosan concentration of 0.08 M was used to study the solution behavior and the gelation under non-isothermal conditions. The dynamic rheological properties of chitosan-β-GP solutions were characterized in terms of urea concentration at 15 °C. The oscillatory shear measurements were performed in the linear viscoelastic regime. The stress relaxation spectra were calculated from the obtained G' and G'' data, using a commercial software (NLREG®) (HonerKamp & Weese, 1993). The chitosan solutions zero shear viscosity (η_0) was determined from small amplitude oscillatory data using the Carreau-Yasuda model (Carreau, De Kee, & Chabra, 1997), assuming validity of the Cox-Merz rule (Cho et al., in pressa), and from the time-weighted stress relaxation spectra area.

During the gelation process in non-isothermal conditions, the evolution of rheological properties was monitored between 15 and 90 °C using a constant heating rate (1 °C/min). Small amplitude deformation γ_0 (0.01) and relatively low frequency ω (6.28 rad/s) were applied in order not to disturb the gel formation. The gelation temperature (T_{gel}) was determined as the crossover point of the storage (G') and loss (G'') moduli ($\tan\delta = 1$), despite of the slight dependency on frequency. The thermoreversibility of the gels was investigated by decreasing the temperature from 90 to 15 °C at the same rate (1 °C/min). After the cooling cycle, the resulting system was characterized by a frequency sweep at 15 °C.

For the isothermal gelation tests, the rheological properties of the chitosan-β-GP system were also investigated in the absence and presence of urea (3 M). A chitosan concentration of 0.14 M was used since the lower one (0.08 M) resulted in too large inertial effects. The moduli G' and G'' were reported as functions of time under various temperatures (40–60 °C). As for non-isothermal tests, a small deformation ($\gamma_0 = 0.01$) and frequency ($\omega = 6.28$ rad/s) were applied. The gelation time (t_{gel}) was also determined as the crossover of G' and G'', and the gelation activation energy was calculated from these specific times at various temperatures using an Arrhenius type relationship.

4. Results

4.1. Physiochemical measurements

The pH of various solutions was measured as a function of temperature and urea concentration (Fig. 2). The pH of a 0.15 M acetic acid aqueous solution was very sensitive to temperature due to the increased ionization of AcOH. Adding the polymer to the acetic acid solution (0.10 M chitosan-0.15 M AcOH) resulted in a pH increase from 3.2 to 4.2 at 25 °C, due to the protonation of the free amine groups in the

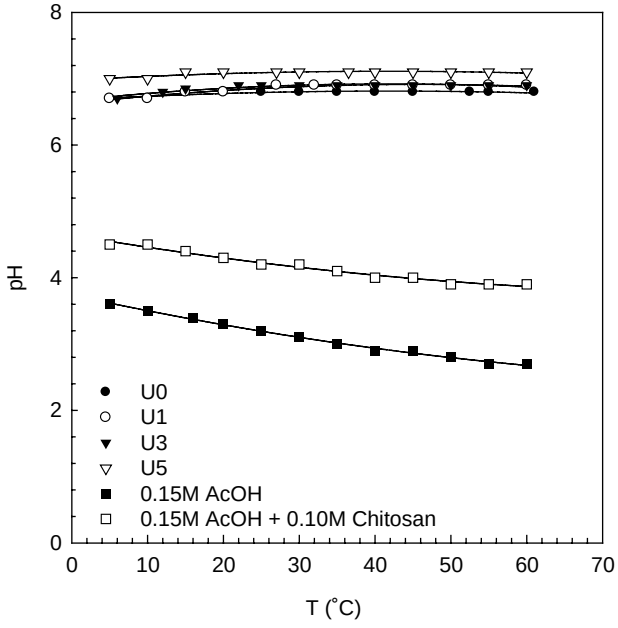

Fig. 2. Effect of urea content and temperature on the pH of various solutions.

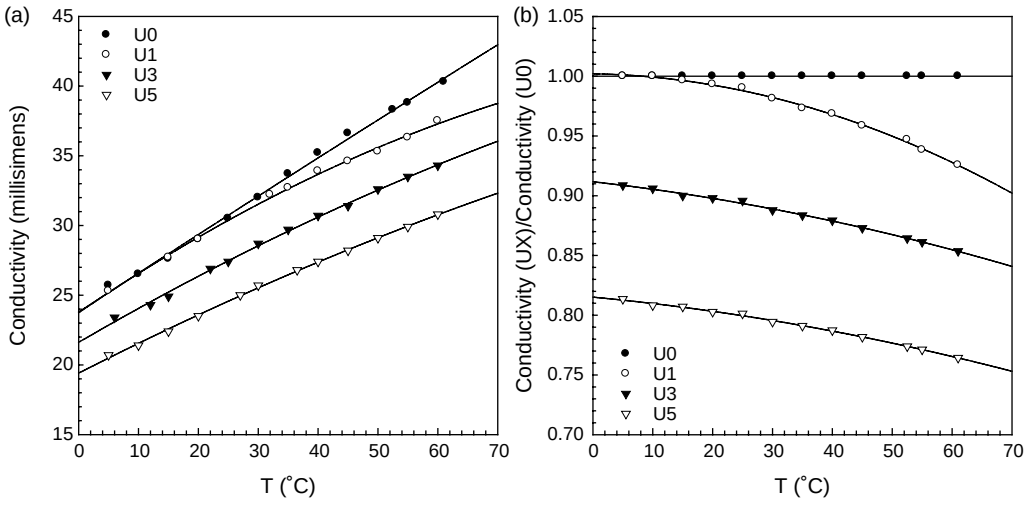

Fig. 3. Urea effect on (a) conductivity and (b) relative conductivity as functions of temperature.

chitosan molecules. For this chitosan-AcOH solution, the decrease in pH at high temperature resulted from the deprotonation of chitosan and the increased ionization of AcOH (Cho et al., in pressb). Upon the addition of β-GP (solution U0), the pH was increased around neutrality. Wang Wang (1999) also reported that the addition of β-GP to chitosan solution increased the pH value to a physiologically acceptable level, from 6.8 to 7.2, due to the high buffering capacity of β-GP in this range of pH. In the presence of β-GP, the pH was nearly independent of temperature, most probably because of its buffering capacity. It can also be observed in Fig. 2 that the pH slightly increased in terms of urea concentration (solutions U1, U3 and U5). The hydrolysis of urea causes the consumption of H^+ ions in solution (Shaw & Bordeaux, 1995), resulting in increasing pH value.

The conductivity of the chitosan-β-GP solutions was measured as a function of temperature in the presence of urea. Fig. 3(a) presents the evolution of the conductivity of each solution. We may interpret the effect of urea on the conductivity of solutions U1, U3 and U5 from the interaction of urea with the carboxylic groups (–COOH) of acetic acid by hydrogen bonding and/or possibly by the formation of ureanium ions (Jana & Moulik, 1993). It is known that higher ionic strength decreases electrostatic repulsion between chitosan protonated amines, therefore enhancing the hydrophobic effect (Desbrieres et al., 1996). Consequently, the observed decrease of the conductivity (or ionic strength) was directly correlated to lower polymer–polymer interactions, and this result agreed with that of Philippova et al. Philippova et al. (2001). Fig. 3(b) shows the relative conductivity ratio (i.e. solutions conductivity normalized by that of solution U0) as a function of temperature. Adding urea decreased the relative ratio, and this effect was more pronounced at high temperature. Thus the use of urea in the chitosan-β-GP system can affect polymer–polymer interactions by mainly weakening hydrogen bonds at low temperature (solution behavior), and predominantly hydrophobic interactions at high temperature (Desbrieres et al., 1996) (gel behavior). In the next sections, we look more closely at these two different regimes.

4.2. Urea effect on solution behavior

The effect of urea on the rheological properties of chitosan-β-GP solutions at 15 °C is shown in Fig. 4 in terms of the dynamic moduli (G' and G'') and tan δ (insert of Fig. 4) as functions of frequency (ω). The behavior is typical of a concentrated entangled polymer solution. The entanglement concentration C_e of this chitosan sample in acetic acid has been determined previously to be approximately 7 g/L (Cho et al., in pressa). Therefore, the concentration used in this work (0.08 M, corresponding to 14 g/L) is way into the entangled regime. The storage modulus (G') and loss modulus (G'') showed different responses to the addition of urea in solution.

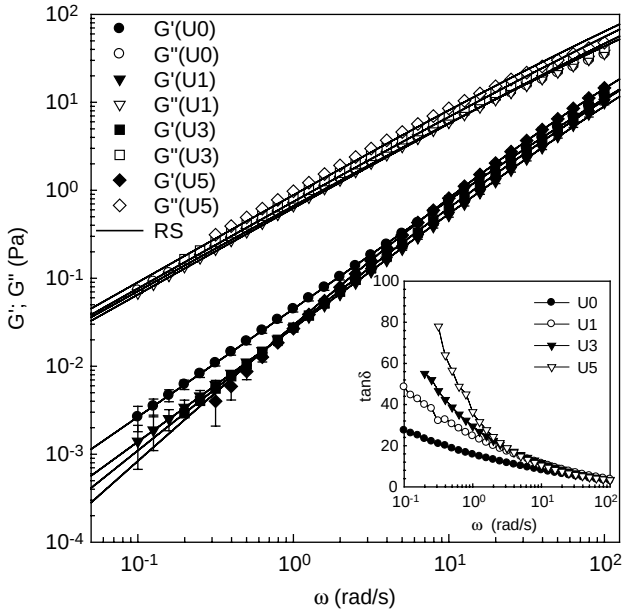

Fig. 4. Dynamic moduli G' and G'' of chitosan-β-GP solutions in the presence of urea at 15 °C. Insert: tan δ (γ_0 = 0.1).

G' decreased with increasing urea concentration while G'' slightly increased. This dissimilar behavior caused a decrease of the elasticity of the polymeric solutions, as shown by tan δ in the insert of Fig. 4. This decline has been related to the urea breaking intra-and/or intermolecular hydrogen bonding interactions (Tsaih & Chen, 1997; Chen & Tsaih, 2000; Hammes & Swann, 1967).

The complex moduli G^*, defined as $G^*(\omega) = \sqrt{G'(\omega)^2 + G''(\omega)^2}$, was used to calculate the stress relaxation spectrum ($H(\tau)$) according to (Roth, Maier, Friedrich, Marth, & Honerkamp, 2000):

$$G^*(\omega) = \int_{-\infty}^{\infty} (\omega\tau + i)H(\tau)\frac{\omega\tau}{1+(\omega\tau)^2}\mathrm{d}(\ln\tau) \quad (2)$$

with τ the relaxation time. The various spectra were normalized using the zero shear viscosity (η_0), corresponding to the area under the spectra curves:

$$\eta_0 = \int_{-\infty}^{\infty} \tau H(\tau)\mathrm{d}(\ln\tau) \quad (3)$$

in order to compare them on similar scales. Fig. 5 shows the normalized time-weighted stress relaxation spectra for various urea contents. All the solutions spectra are monomodal, and most of the relaxation process of the urea-containing solutions was completed within 10^2–10^3 s, while that of U0 was much slower, as expected for a more elastic system. In Fig. 4, we report the calculated values of the dynamic moduli using the relaxation spectra (solid lines). The agreement is excellent.

The dynamic moduli G' and G'' measured in the linear viscoelastic regime can be presented in a cole–cole plot, and most polymer systems will show a power-law relationship ($G' \sim G''^P$) (Lauten & Nystrom, 1999; Thuresson, Lindman, & Nyström, 1997). The exponent P is equal to 1/2 for a one-mode Maxwell fluid:

$$G^2 = GI(G_e - GI) \quad (4)$$

or

$$\log(G'') = \frac{1}{2}\log(GI) + \frac{1}{2}\log(G_e - GI),$$

where G_e represents the plateau value of G' at high frequencies. Therefore, cole–cole plots can be used to characterize deviations from the one-mode Maxwell model (Lauten & Nystrom, 1999; Thuresson et al., 1997), using the generalized equation:

$$\log(G'') = P\log(GI) + (1-P)\log(G_e - GI) \quad (5)$$

in analogy with Eq. (4), provided that $G^{2P} \ll G_e^{2P}$. Fig. 6 shows the cole–cole plots for solutions with various urea contents. For all solutions, the exponent P was larger than 1/2 (between 0.63 and 0.75) (insert of Fig. 6), indicating that the solutions deviated from the single mode Maxwell element simple relaxation, as observed from the dynamic data of Fig. 4. This deviation is often observed for cross-linked and entangled polymers that exhibit a spectrum of relaxation times. The exponent P decreases with increasing urea concentration, denoting that the stresses relaxed faster due to the reduction of molecular interactions. The plateau modulus G_e was also estimated from the cole–cole plots; it decreased from 2060 to 400 Pa when urea was added to the chitosan-β-GP solutions (solutions U0 and U5, respectively).

4.3. Urea concentration effect on gelation

The gelation behavior under non-isothermal conditions was investigated in the presence of urea. As mentioned before, the gelation of chitosan-β-GP solutions proceeded in three stages,

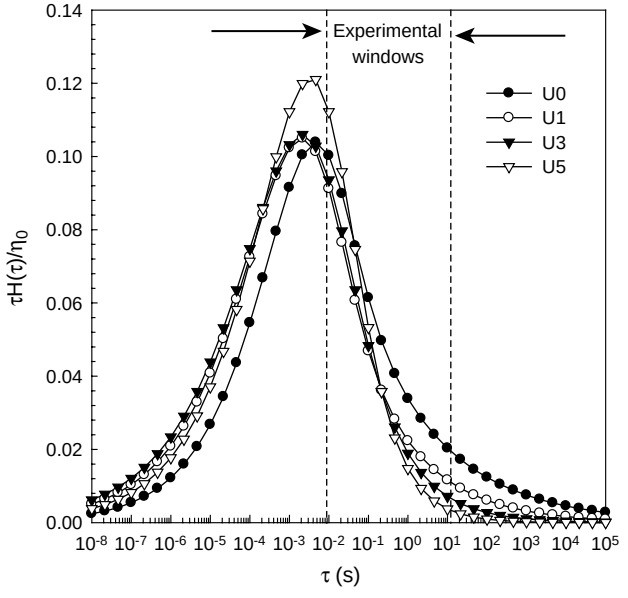

Fig. 5. Urea effect on the normalized time-weighted stress relaxation spectra.

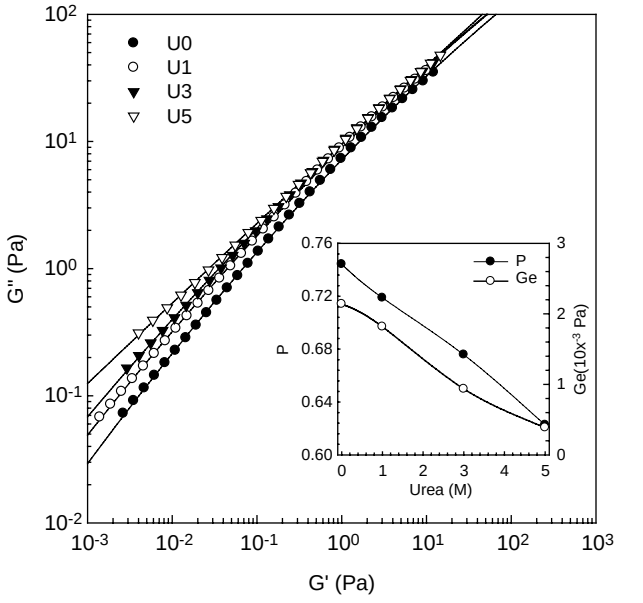

Fig. 6. Urea effect on the cole–cole plot. Insert: effect of urea on exponent P and plateau modulus G_e (Eq. (5)).

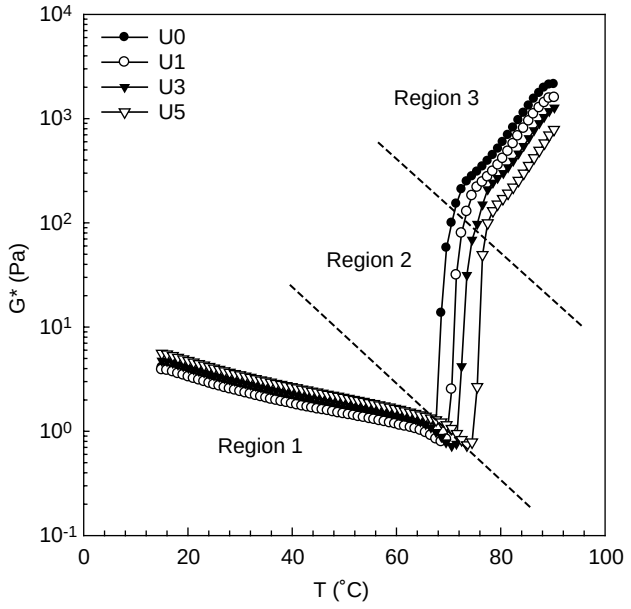

Fig. 7. Urea effect on heat-induced gelation. Complex modulus, G^*, reported as a function of temperature ($\gamma_0 = 0.01$, $\omega = 6.28$ rad/s, heating rate = 1 °C/min.).

and this behavior was unchanged by the presence of urea (Fig. 7). In region 1, the behavior was liquid-like. We are showing the complex modulus G^* since the storage modulus was extremely small below the gel point and interpreted as negative values by the rheometer software. G^* showed a very sharp decrease near region 2 for all samples, and this phenomenon has been attributed to some polymer precipitation (Sarkar, 1995). In region 2, the complex modulus rapidly increased with increasing temperature due to the formation of a three-dimensional network. In the last zone (region 3), the much slower gelation was caused by the lower diffusivity that resulted from the viscosity increase after gelation. The gelation temperature (T_{gel}) and the gel strength (G^* at 90 °C) were examined in terms of urea concentration (Fig. 8). While T_{gel} increased, denoting that urea retarded gelation, the gel strength decreased. As shown from our conductivity measurements, urea was detrimental to hydrophobic interactions and resulted in weaker gels.

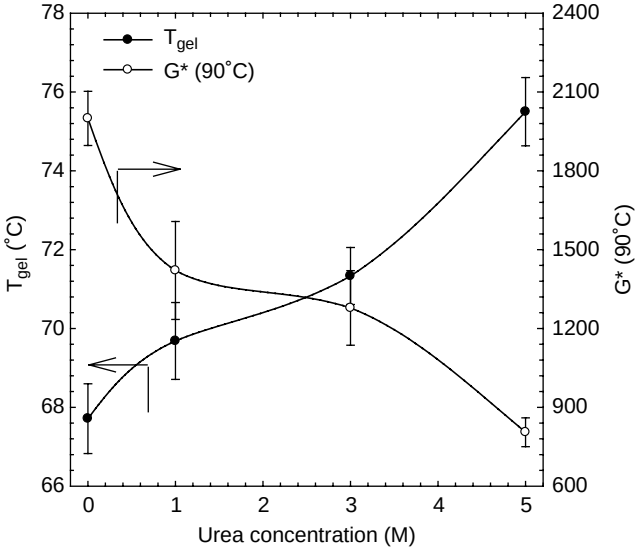

Fig. 8. Effect of urea on gelation temperature (T_{gel}) and gel strength (G^* at 90 °C).

4.4. Non-isothermal gelation kinetics

We investigated the non-isothermal gelation kinetics of the heat-induced gelation in order to quantify the negative effect of urea. We used a kinetic model that is a combination of Arrhenius and time-temperature relationships (Lopes da Silva, Gonçalves, & Rao, 1995; Fu & Rao, 2001; Ross-Murphy, 1991), yielding:

$$\ln\left(\frac{1}{G^n}\frac{dG'}{dt}\right) = \ln k_0 - \left(\frac{E_{ag}}{RT}\right) \quad (6)$$

For heat-induced gelation, the equation is rewritten as:

$$\ln\left(\frac{1}{G'^n}\frac{dG'}{dt}\right) = \ln k_0 + \left(\frac{E_{ag}}{RT}\right) \quad (7)$$

in order to account for a positive activation energy. In this equation n is the reaction rate, t the time, k_0 the Arrhenius frequency factor, E_{ag} the activation energy for gelation, and RT have their usual significance. The exponent n represents both the dimension r of the growing crystallites and the type of nucleation s ($n = r + s$) (Böhm & Kulicke, 1999). Parameter r is 1 for rods, 2 for discs and 3 for spheres, while s is either 0 for predetermined nucleation (nuclei already present) or 1 for sporadic nucleation (nuclei arise and their number increases linearly with time) (Böhm & Kulicke, 1999; McIver, Axford, Colwell, & Elton, 1968). We assumed that the reaction rate n was 2, as proposed by Lopes da Silva et al. Lopes da Silva et al. (1995), Fu and Rao (2001), Ross-Murphy (1991) for gelation.

The derivative term in Eq. (7) was obtained through the differentiation of a polynomial regression fitted to the kinetics data of Fig. 7. Fig. 9(a) shows the semi-log plot of $1/G'^2 dG'/dt$ vs. $1/T$. Gelation regions 2 and 3 were again clearly delimited. The activation energy for gelation determined from the slope of each zone is shown in Fig. 9(b) as a function of urea concentration. The energy increased from 2800 to 5000 kJ/mol in the fast gelation region in the presence of urea, indicating that the development of intermolecular interactions was not favored energetically. In region 3, E_{ag} was much lower (about 170 kJ/mol), and consequently the evolution of the physical network was energetically easier, however hindered because of the large viscosity increase. The activation energy for gelation was also nearly constant regardless of urea concentration in this last region, most probably because gelation was diffusion controlled.

4.5. Partial thermoreversibility of the gels

Physical networks are generally thermoreversible; therefore, the resulting gels were cooled down in order to evaluate their reversibility by examining the variations of the dynamic moduli with temperature. As shown in Fig. 10(a), G' gradually

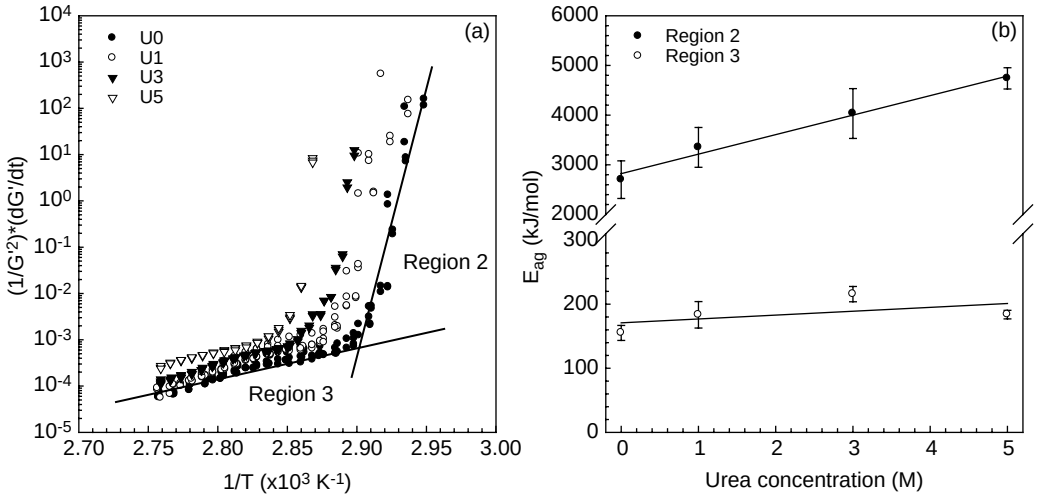

Fig. 9. Nonisothermal gelation kinetics under various urea concentrations. (a) Plot of $1/G'^2 \times dG'/dt$ vs. $1/T$ and (b) gelation activation energy as a function of urea content.

decreased with decreasing temperature while G'' showed a minimum and then increased during the cooling process. The temperature at the minimum was higher for larger urea concentration, i.e. from 24 °C for U0 to 37 °C for U5. As shown in Fig. 10(b), tan δ rapidly increased below that critical temperature. Two competing effects resulted in the minimum observed for G'': the destruction of the network junctions as the temperature was lowered, and the usual rheological properties increase with decreasing temperature due to reduced molecular mobility. This interpretation is in agreement with the variation of the critical temperature with urea concentration, as the second mechanism takes over earlier since there are fewer junctions to be destroyed. A minimum was not observed for G', probably because the continuous reduction of junctions overcame the impact of the decreased chain mobility at lower temperature. The gels were only partially thermoreversible since G' remained larger than G'', and this partial thermoreversibility was most probably due to the existence of remaining weak associations (Li et al., 2001).

The systems, cooled down at 15 °C, were characterized using small amplitude oscillatory shear (Fig. 11) in order to compare them with the initial solution state (Fig. 4). This time, all the systems showed a typical solid-like behavior ($G' > G''$) with a very slight frequency dependency ($G' \sim \omega^{0.03-0.15}$ and $G'' \sim \omega^{0.07-0.33}$), illustrating their non complete thermoreversibility, and showing a striking contrast with the data obtained before the heating–cooling cycle (Fig. 4). As expected, the gel strength (G') and the elasticity (tan δ, insert of Fig. 11) of the cooled gels were decreased in the presence of urea.

4.6. Isothermal gelation

We also investigated isothermal gelation in order to compare the activation energy with that obtained under non-

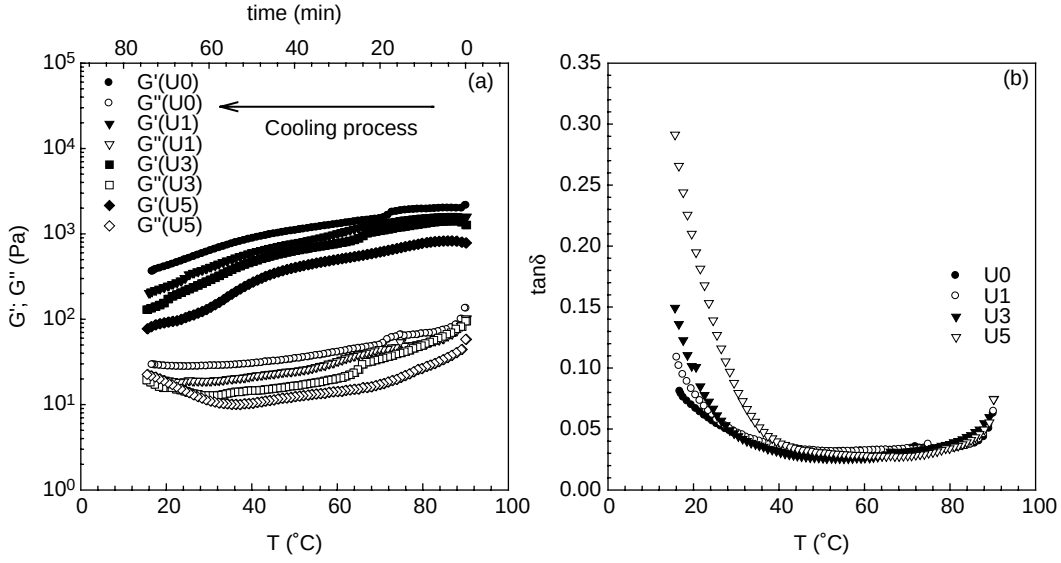

Fig. 10. Urea effect on the thermoreversibility of the gels. (a) Dynamic moduli G' and G'' and (b) tan δ during the cooling process ($\gamma_0 = 0.01$, $\omega = 6.28$ rad/s, cooling rate = 1 °C/min.).

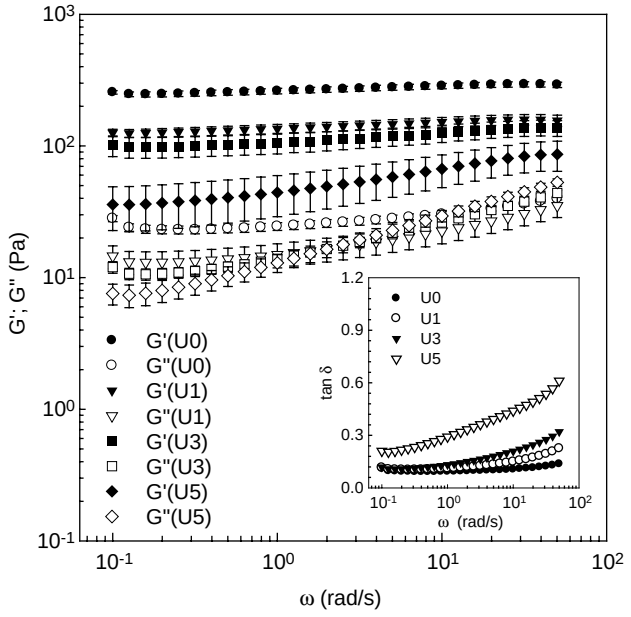

Fig. 11. Dynamic moduli G' and G'' for gels cooled at 15 °C, as functions of frequency. Insert: tan δ ($\gamma_0 = 0.1$).

isothermal conditions. For instances, it is often reported that there is large difference between the crystallization activation energies obtained in isothermal and non-isothermal conditions. For the crystallization of poly(3-hydroxybutyrate) (PHB), the reported activation energy was 170–220 kJ/mol from a non-isothermal kinetics study, while it was much lower, 9.8–10.8 kJ/mol for isothermal conditions (Ahmed, 2000). Li et al. Li, Zhang, Zhu, and Yan (2003) reported that the activation energy for crystallization was 2000 and 240 kJ/mol for Nylon 1012, a newly industrialized engineering plastic, in non-isothermal and isothermal conditions respectively. Therefore, the crystallization activation energy evaluated in non-isothermal conditions can be one order of magnitude higher that that obtained under isothermal studies. Since we were using gelation kinetics models derived from those used in crystallization, we expected to observe a similar difference.

As mentioned in the methodology part, solutions with larger chitosan concentrations (solutions 14CU0 and 14CU3) were used for these isothermal tests, and the evolution of G' and G'' was recorded as a function of time at various temperatures (Fig. 12(a)). The gelation time (t_{gel}) was longer in the presence of urea, illustrating again the retarding effect of the substance, and this time was also longer at higher temperature. The gelation time was used to estimate the activation energy for gelation in isothermal conditions, by plotting it as a function of $1/T$ (Fig. 12(b)). As observed for the non-isothermal data, urea had again a detrimental effect on gelation, requiring higher energy to proceed (170 and 200 kJ/mol for solutions 14CU0 and 14CU3, respectively) and resulting in lower modulus values. And as expected, the activation energy determined from isothermal kinetics was fairly small compared to that obtained in region 2 of the non-isothermal kinetics (one order of magnitude smaller). It was, however, very similar to that obtained in region 3, but this may be coincidental. Obviously, the energy required to achieve network formation in non-isothermal conditions is much larger since the sample is never at thermal equilibrium.

5. Discussion

The effect of urea on the solution and gel states of the chitosan-β-GP system has been widely illustrated by the previous data. Urea had a strong impact on the solution behavior, making the stresses relax considerably faster and the viscoelastic properties lower. Leibler et al. Leibler, Rubinstein, and Colby (1991) presented a model for the dynamics of entangled polymer networks made up of linear chains and temporary physical cross-links. Using a simplified assumption, i.e. that entanglement and physical cross-link contributions are additive and do not show any synergetic effects; they proposed the following equation for the plateau modulus:

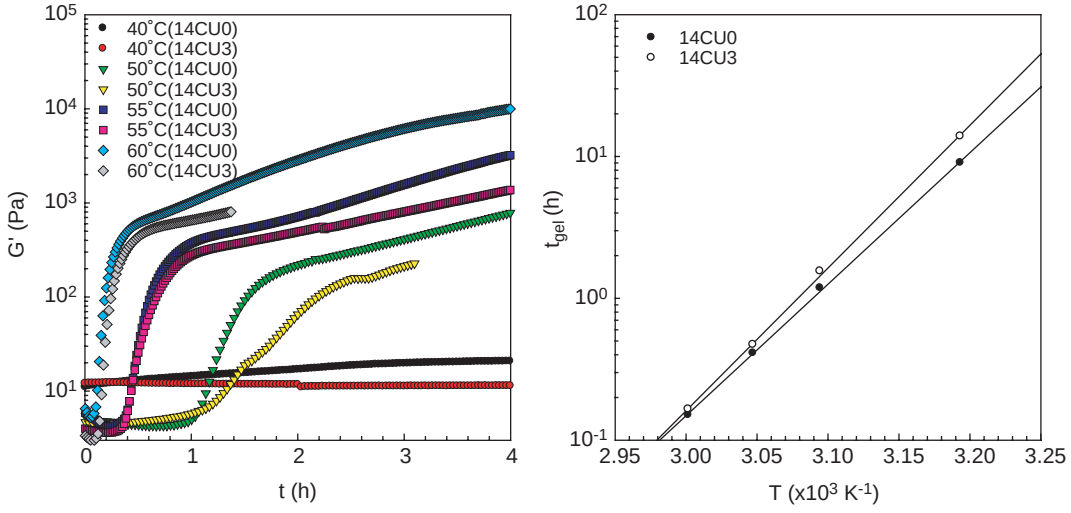

Fig. 12. (a) Storage modulus G' as functions of time under various temperatures and urea concentrations. (b) Effect of urea and temperature on gelation time (t_{gel}). Concentration of chitosan = 0.14 M, pH at 25 °C = 7 ($\gamma_0 = 0.01$, $\omega = 6.28$ rad/s).

$$G_e \cong cRT\left[\frac{p}{N_s}+\frac{1}{N_e}\right] \quad (8)$$

where c is the number concentration of monomers, p the fraction of physical cross-links (or 'stickers'), N_s the average number of monomers along the chains between stickers, and N_e the number of monomers in an entanglement. If we reasonably assume that N_s and N_e are unchanged by urea concentration, the decrease in the plateau modulus reported in the insert of Fig. 6 is directly related to a decrease of temporary junctions in the presence of urea.

The model proposed by Leibler et al. is a modified reptation model that considers a slower diffusion in the presence of reversible cross-links. This hindered reptation results in a delayed relaxation; however, the terminal relaxation of the modulus remained that of a reptation model as shown schematically in Ref. Leibler et al. (1991). Since Leibler et al. did not propose a predictive equation for $G(t)$, we have used the Doi-Edwards model Doi and Edwards (1978):

$$G(t) = \frac{8}{\pi^2}G_e\sum_{\text{odd}k}\frac{1}{k^2}\exp\left(-\frac{k^2 t}{\tau_{rep}}\right) \quad (9)$$

in order to compare the relaxation moduli calculated from the stress relaxation spectra of our various solutions (Fig. 5). The plateau modulus G_e used in this equation was that estimated from the cole–cole plots (insert in Fig. 6), while the reptation time τ_{rep} was taken as the mean relaxation time τ_m of Fig. 5 (or time at the maximum). It can be observed in Fig. 13 that even though urea had a 'simplifying' effect on the solutions relaxation by disrupting hydrogen bonds and hydrophobic interactions, as confirmed by the decreased exponent P of the cole–cole plot (insert in Fig. 6), the spectrum of relaxation times remained much broader than that predicted by reptation. 'sticky' or hindered reptation would only delay the occurrence of the terminal relaxation, but still not result in an appropriate representation of the modulus behavior. Obviously, additional relaxation mechanisms such as fluctuations in tube length and constraint release, known to be important for ordinary linear polymers, may also have to be accounted for. Likhtman and McLeish Likhtman and McLeish (2002) presented a model that combines self-consistently contour length fluctuations, constraint release and reptation for monodisperse linear polymers. Therefore, a combination of these mechanisms along with hindered reptation may improve the prediction of reversible networks relaxation such as that observed in Fig. 13. However, this is beyond the scope of the present work.

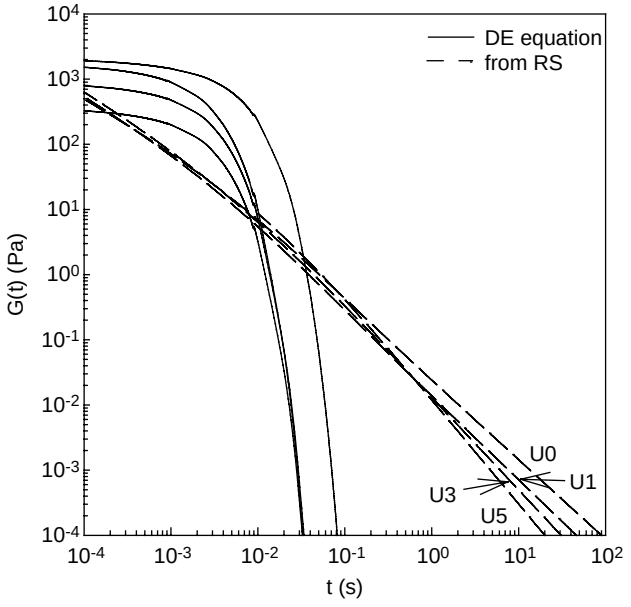

Fig. 13. Calculated relaxation moduli using Doi–Edwards equation (DE) and the relaxation spectra of Fig. 4 (RS).

The large influence of urea on the gelation process was demonstrated according to different characteristics: (1) higher gelation temperature in non-isothermal conditions, (2) longer gelation time in isothermal ones, and in both cases (3) larger gelation activation energies and (4) lower mechanical properties. The heat-induced gelation of the chitosan-β-GP system can be the result of hydrogen bonding, hydrophobic and/or ionic interactions. Our previous work (Cho et al., in pressb) showed that among the three types of junctions, hydrophobic interactions were dominant since hydrogen bonds are known to weaken rapidly with temperature, and our ionization calculations showed that ionic interactions also decreased with increasing temperature (Cho et al. in pressb). Therefore, the effect of urea in the heat-induced gelation process was mostly related to a decrease of polymer–polymer hydrophobic interactions, as shown by the decreased conductivity as a function of temperature (Fig. 3). Lower ionic strength involves less screening of electrostatic repulsion between protonated chitosan glucosamine groups, and hence reduced possible hydrophobic effect, resulting in detrimental conditions for gel formation. Therefore, the present results strengthen our suggestion that hydrophobic interactions are the main driving force for chitosan-β-GP gelation at high temperature (Cho et al., in pressb).

6. Conclusions

In this study, physicochemical and rheological properties of chitosan-β-GP solutions and gels were investigated in terms of urea concentration and temperature. Though the presence of urea is not mandatory for the chitosan-β-GP-acetic acid gel system, these properties provided information related to the function of hydrophobic and hydrogen bonding interactions.

In terms of physicochemical properties, the pH values of the chitosan-β-GP solutions slightly increased in the presence of urea due to the consumption of H^+ ions in solution through urea hydrolysis process, but they were constant regardless of temperature increase due to the buffering capacity of β-GP. The measured conductivity decreased with increasing urea concentration and temperature, most probably resulting from ionic interactions between ammonium ions and monovalent and divalent β-GP anions. Thus, the use of urea in the chitosan-β-GP system mainly affected polymer–polymer interactions by weakening hydrogen bonds at low temperature (solution

behavior), and predominantly decreasing hydrophobic effect at high temperature (gel behavior).

In the solution state, urea resulted in lower elastic properties and shorter relaxation times. The relaxation process was also simplified (narrower spectrum of relaxation times) due to the disruption of hydrogen bonds and hydrophobic interactions.

For the heat-induced gelation, urea increased the gelation temperature in non-isothermal tests and the gelation time in isothermal conditions. From the non-isothermal and isothermal gelation kinetics, the activation energy to form the gel structure increased in the presence of urea, indicating a detrimental effect. Upon cooling all gel systems presented partial thermoreversibility, most probably due to the existence of remaining weak associations.

Our previous results on the gelation of the chitosan-β-GP system illustrated that hydrophobic interactions were dominant since hydrogen bonds are known to weaken rapidly with temperature and ionization calculations showed that ionic interactions also decreased with increasing temperature (Cho et al., in pressb). Therefore, the detrimental effect of urea in the heat-induced gelation process studied in this work was mostly related to a decrease of hydrophobic effect, as confirmed by the decrease in the measured conductivity (or ionic strength) in the presence of urea and at high temperature. Therefore, the present results confirm that hydrophobic effect is the main driving force for the chitosan-β-GP heat-induced gelation.

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