Rheological study of the chitosan/glutaraldehyde chemical gel system

W. Argüelles-Monal[a,*], F.M. Goycoolea[b], C. Peniche[c], I. Higuera-Ciapara[b]

[a]*IMRE, Universidad de La Habana, La Habana 10400, Cuba*
[b]*Centro de Investigación en Alimentación y Desarrollo, A.C. Apdo. Postal 1735, Hermosillo, Sonora 83000, Mexico*
[c]*Centro de Biomateriales, Universidad de La Habana, La Habana 10400, Cuba*

Abstract

Chitosan dissolved in 0.1 moll^{-1} acetic acid shows an apparent yield stress at very low frequencies, probably due to a structuring process yielding gel-like response. It reflects complex relaxation mechanisms once chains disentangle and relax, presumably due to incipient hydrophobic contacts reinforced by the relative stiffness of the chitosan chains, which tend to slow down reptation. When chemical cross-linkages are introduced, the weak self-associated network of chitosan is gradually replaced by a permanent covalent network as the molar ratio of aldehyde/amine groups, R, is increased. At $R = 0.4$ a glass-to-rubber-type transition is observed, while at $R = 0.5$ the form of the mechanical spectrum suggests the co-existence of a chemically cross-linked gel 'dissolved' in a second entangled network formed by chitosan chains of restricted mobility. At higher cross-linking levels ($R > 1$) a strong permanent gel is formed. The observed frequency dependence near the rheological gel point suggests several modes of relaxation processes.

*Correspondence address: Centro de Investigación en Alimentación y Desarrollo, A.C. Apdo. Postal 1735, Hermosillo, Sonora 83000, Mexico. E-mail: waldo@cascabel.ciad.mx

1. Introduction

Chitosan is a highly specialised biopolymer established as the main industrial derivative of chitin, a polysaccharide of widespread abundance in the world. Chitosan is a linear cationic polysaccharide, which at low pH can be dissolved behaving as a stiff worm-like chain. Chemically, chitosan is made out of two kinds of $\beta(1 \rightarrow 4)$-linked monosaccharide residues, namely N-acetyl-D-glucosamine (**A**) and D-glucosamine (**D**). Whether these exist in blockwise or random structure, has been subject to controversy [1, 2]. Nevertheless, regardless of the microstructure, it is firmly established that **D**-residues at acidic pH provide protonated $-NH_3$ groups which render chitosan soluble, whereas **A**-residues have an important role on the stiffness of these chains, as well as in propitiating hydrophobic associations in aqueous solution [3].

In turn, $-NH_2$ functional groups provide enormous possibilities for chemical modifications. Among others, the reaction between chitosan amino group and aldehydes has been described. This reaction involves the formation of a Schiff base and it is accompanied by colour formation, which can be readily monitored by increase in measured absorbance in the 400–700 nm region. The increase in rate of colour formation is accompanied by an onset of gel formation [4]. The proposed explanation for this increase in rate of colour formation is that a three-dimensional network is established by each polymer chain forming one or more cross-links. The rate of gelation of this system is affected by temperature, acetic acid concentration, added electrolyte, and by the overall concentration of amine and aldehyde functional groups [4]. In general, kinetic studies of chemical gelation are complicated due to the convolution of time and frequency, as chemical cross-linking is still proceeding during the time required for the frequency sweep [5]. To circumvent this problem, in the study of the chemical gelation of polygalacturonate with 1,6-dibromohexane, Matricardi et al. [5] carried out time-independent dynamic rheological determinations. This was achieved by 'freezing' the gelation process by carefully controlling the amount of cross-linking agent added, and then frequency sweeps were recorded in the vicinity of the rheological gel point. These authors have emphasised the advantages of using a 'lean' cross-linking agent in facilitating the study of chemical polysaccharide gels, namely their strain independence. We have adopted a similar approach in this investigation as well. The emphasis of our study has been on the effect of the intrinsic rigidity and possible self-association behaviour of chitosan chains on the viscoelastic properties of gels formed, particularly at degrees of cross-linking in the vicinity of the critical gelation condition.

Chemically cross-linked gels formed via covalent association of chitosan with glutaraldehyde have received considerable attention over the past decades, with many published reports regarding their uses now available. Nevertheless, the nature of the chemical bonding between chitosan amine groups and glutaraldehyde has been subject of controversy, and very little work has been carried out on the viscoelastic behaviour of this system. Moreover, it has recently been indicated that the role of **A**-groups in chitosan self-association behaviour during chemical cross-linking with glutaraldehyde is more important than originally suspected [6]. So the aim of this paper was to revisit the chitosan/glutaraldehyde gelling system, using small deformation (low-amplitude) oscillation-sensitive rheology.

2. Experimental

Chitosan from lobster cephalotorax *Panulirus argus* was prepared industrially in a Cuban enterprise, with an N-acetyl content (expressed as the molar fraction of N-acetyl groups, F_A) of 0.201 as determined by ^{1}H-NMR. Viscosity-average molecular weight was 2.3×10^5, estimated from the value of intrinsic viscosity at 25°C in 0.3 mol l^{-1} acetic acid/0.2 mol l^{-1} sodium acetate [7].

Glutaraldehyde 50% Grade I solution was purchased from Sigma Chemicals Inc. and was kept below 0°C and checked to be free of α/β-unsaturations by UV-spectroscopy prior to use.

The rheological properties of chitosan solutions at varying concentration in 0.1 mol l^{-1} acetic acid, were investigated using a strain-controlled rheometer (Rheometrics Mod. RFSII Fluids Spectrometer, Piscattaway, NJ, USA), fitted with a cone-plate tool (cone angle : 0.0397 rad, diameter : 50 mm, gap : 53 μm) and a circulating environmental system for temperature control. In order to prevent drying of the samples during experiments with long duration times, a plastic ring of diameter $\sim$ 60 mm was fitted around the measuring geometry, and the annulus was filled with silicone oil of low viscosity.

Large-deformation steady-shear rheological measurements were performed in the shear-rate ($\dot{\gamma}$) mode of the motor from 0.1 to 100 s^{-1}. Low-amplitude oscillatory measurements were made within the linear viscoelastic strain (γ) region, as verified by strain sweeps on all solutions and gels tested.

Chemically cross-linked chitosan gels for rheological measurements were prepared by mixing accurately measured aliquots of glutaraldehyde and chitosan solutions 1% (w/w) (0.0224 mol l^{-1}) in 0.1 mol l^{-1} acetic acid. The molar ratio of aldehyde/amine groups, defined as the R value ($R = [-CH{=}O]/[-NH_2]$), varied in the range between 5 and 0. Once glutaraldehyde was rapidly mixed in the chitosan solution, it was loaded on to the plate of the rheometer previously set at 35°C. The evolution of the gelation process was monitored by measurements of storage, $G'(t)$ and loss, $G''(t)$, moduli ($\omega = 1$ rad s^{-1}), recorded at strain values varying in the range $\gamma = 0.15$–0.7 over a period of 260 min. Afterwards, frequency sweeps in the range between 0.06 and 100 rad s^{-1} were recorded at the same strain as for the time sweeps.

Capillary viscometric measurements were performed with Ubbelohde capillary viscometer immersed in a thermostated bath at 25 ± 0.01°C. Prior to measurements, both the solvent and solutions were filtered through 0.45 μm Sartorius membranes.

3. Results and discussion

3.1. Chitosan solution in 0.1 mol l^{-1} acetic acid

The characteristics of the networks yielded by the covalent cross-linking of polymer chains are bound to be highly affected by the rheological behaviour of the starting polymer solution, particularly at low degrees of cross-linking. For this reason, we

began this study exploring the viscoelastic properties of the chitosan solution. Obviously, a deep investigation on this subject requires understanding how the type of polymeric salt in solution, ionic strength, pH, acetylation degree of the polysaccharide and other factors affect the viscoelastic characteristics of chitosan. However, in this paper we shall limit our efforts to study the rheological behaviour of chitosan solution in 0.1 $\mathrm{mol\,l^{-1}}$ acetic acid, without addition of any low molecular weight electrolyte, because it is under these conditions that chemical gelation will be studied.

The intrinsic viscosity of chitosan dilute solution in 0.1 $\mathrm{mol\,l^{-1}}$ acetic acid was first determined from capillary viscometric measurements. A value of $[\eta] = 4715\ \mathrm{ml\,g^{-1}}$ was calculated by averaging the extrapolated values obtained by the well-known equations of Huggins, Kraemer, and 'single-point' procedure. Such a high value of intrinsic viscosity corresponding to a big hydrodynamic volume, could be due to intramolecular electrostatic repulsions allowing polymer chains to adopt very expanded coil conformations. Moreover, this unusually high value of intrinsic viscosity agrees well with data collected from measurements made in our laboratories during many years with different chitosan samples in various solvent systems [7–9] at 25°C as shown in Fig. 1. At low degrees of space occupancy, a dependence of $\eta_{sp} \sim C[\eta]^{1.16}$ is observed, which is slightly lower than the common slope of ~ 1.4 observed for most linear polysaccharides in the disordered state [10]. Moreover, departures from the linear dependence at a value of the coil overlap parameter very near to unity ($C[\eta] \approx 1.45$), are clearly observed, supporting the idea that some type of interaction arises between macromolecular domains at the onset of incipient contact and interpenetration. This also indicates very low degree of contraction of the chains before the onset of entanglement.

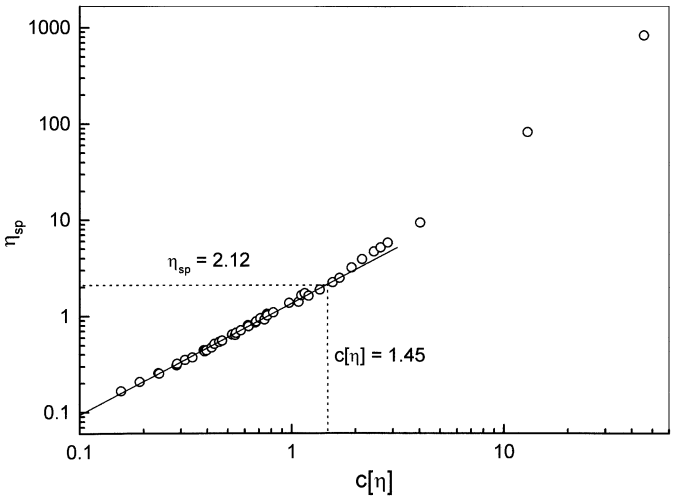

Fig. 1. Variation of specific viscosity (η_{sp}) with space occupancy ($C[\eta]$) for chitosan samples in different solvents at 25°C. See the text for details about the solvents.

In Fig. 2 is shown the mechanical spectrum of a 1% (w/w) chitosan solution in 0.1 mol l^{-1} acetic acid. It can readily be noticed that the solution does not exhibit the characteristic terminal flow region at low frequencies. Instead, the storage and loss moduli manifest a tendency to constancy and the dynamic viscosity displays an apparent yield stress. This plastic-type behaviour could be attributed to a structuring process in chitosan solution at very low shear frequencies probably due to superslow relaxation processes with varying weight in the relaxation spectrum, favoured by chain stiffness and intermacromolecular interactions at high degree of overlapping between macromolecular domains ($C[\eta] \approx 47$) exhibited by this solution [11].

In order to confirm the interpretation offered here to the rheological behaviour of chitosan in acetic acid, mechanical spectra of a chitosan solution (2% w/w, $C[\eta] \approx 92$) were recorded at various temperatures between 9 and 45°C. Time–temperature superposition of the spectra is shown in the master curve at 35°C (Fig. 3). Inspection of the frequency dependence of the superposed values of G', G'' and η^*, shows that the solid-like character of this solution at frequencies lower than 1 rad s^{-1} is reinforced at this concentration, showing weak gel behaviour as should be expected. Similar plastic-type flow behaviour has been reported for N-(carboxymethyl) chitosan at concentrations higher than 1%, which has been interpreted in terms of association of ordered chains to develop a cohesive network [12].

An additional confirmation about the gel-like response of chitosan solution has been obtained from the Cole–Cole plot (Fig. 4). This type of graphic representation of $\eta'(\omega)$ and $\eta''(\omega)$ in the complex plane has been suggested to be a useful tool for discrimination among gel and sol states in rigid polymer chains [13]. In Fig. 4, is

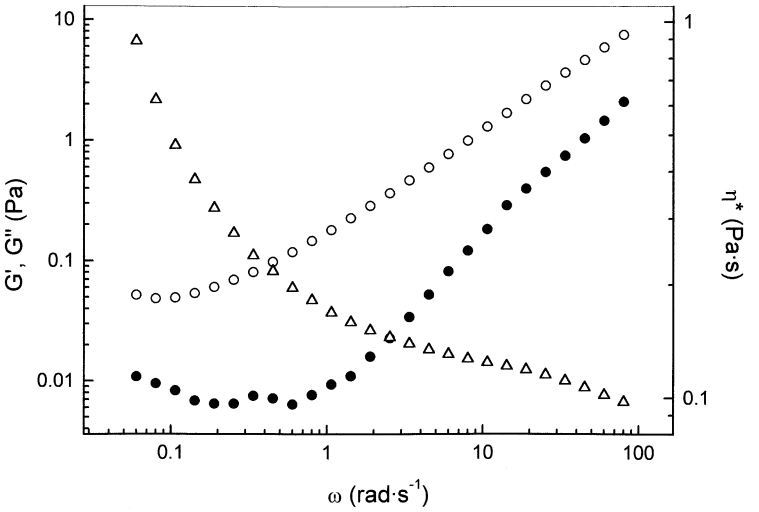

Fig. 2. Mechanical spectrum of 1% (w/v) chitosan solution in 0.1 mol l^{-1} acetic acid at 35°C ($\gamma = 50\%$). G', filled circles; G'', open circles; η^*, triangles.

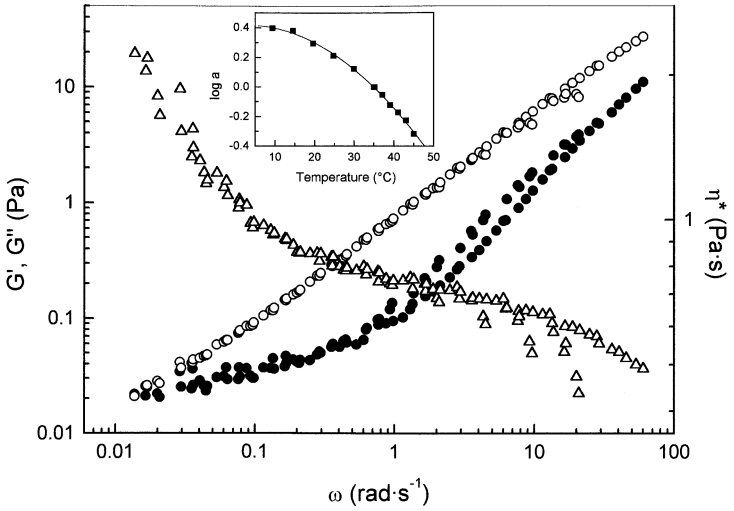

Fig. 3. Mechanical spectrum obtained from time–temperature superposition of 2% (w/v) chitosan solution in 0.1 moll^{-1} acetic acid; reference temperature: 35°C. Measurements were recorded by combined temperature-frequency sweeps from 9 to 45°C at intervals of 2°C ($\omega = 0.06$ to 60 rad s^{-1}; $\gamma = 15\%$). Inside the figure is shown the temperature dependence of shift factor. Symbols as in Fig. 2.

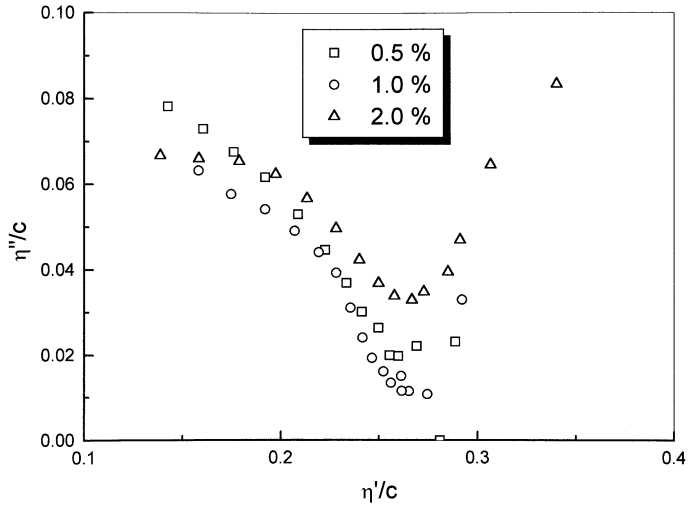

Fig. 4. Reduced Cole–Cole plot for chitosan solutions in 0.1 moll^{-1} acetic acid at 35°C. The polymer concentrations are indicated in the graph.

displayed a reduced form of Cole–Cole plots for chitosan solutions at three different concentrations. These have been scaled by their corresponding concentration values. It is readily noticeable that in no case, the data describe the classical half circular arc, characteristic of a polymer sol. By contrast, the three chitosan solutions describe

saddle-shaped curves which find a minimum in η''/C values at equivalent (reduced) loss viscosities. Overall these are markedly different from those characteristic of a weakly structured worm-like chain such as xanthan [13].

As already stated, chitosan repeating units are linked by $\beta(1 \rightarrow 4)$ glycosidic bonds. This fact already determines certain degree of chain stiffness characterised by a persistence length (L_p) value of 50 Å, as determined in non-aggregating conditions [7]. Nevertheless, regardless of the nature of these bonds it has been established that the rigidity of chitosan chain is decidedly influenced by the presence of **A**-residues. This is presumably determined by hydrogen bonding between two adjacent hexopyranose rings along the chain hindering the free rotation around glycosidic bonds and reinforced by an increment on the hydrophobic macromolecular interactions with the degree of N-acetylation. Then, these gel-like properties may be explained in terms of the worm-like character of chitosan chains in solution favouring macromolecular ordering and self-association probably through hydrophobic interactions due to the presence of N-acetyl units rendering a weak network. It has recently been demonstrated [3] that hydrophobic association phenomena occur in chitosan with high N-acetyl substitution. The role of hydrophobic association phenomena in the viscoelastic behaviour of aqueous solutions of model polymers has been the subject of recent studies [14–17]. At low frequencies, we suggest the existence of a network of temporarily associating hydrophobic junctions, which dissociate and reassociate dynamically, which underlies to a great extent the viscoelastic behaviour observed in the terminal region of the mechanical spectra. This may also imply at least two different relaxation processes, which would account for the results obtained from the Cole–Cole analysis.

The extent of associative network formation, depends on both the junction density and the strength of hydrophobic associations. Furthermore, the role of associating phenomena due to hydrophobic interactions in highly acetylated chitosan/glutaraldehyde chemical gels has also been noticed [6].

The absence of a terminal flow region in the low-frequency range suggests the existence of a very long relaxation process (10–100 s), possibly associated with the reptation-type motion of chitosan chains. In an entangled system of this type, the polymer chain can be visualised as being constrained within an imaginary tube formed by the surrounding molecules at entangled points leading to a slow diffusion of polymer chains by worm-like motion through this tube. When a deformation is imposed, the stress is dissipated through the reptation of the chains in the confining tube. The relaxation time of polymers by the reptation process is expected to be several orders of magnitude larger than the segmental relaxation time ($t \sim <r^2>^4$) and would dominate the terminal behaviour [18] as is the case in our system.

The existence of a network due to 'hyperentanglements' of sequences with a high N-acetyl content (i.e. associating to give chitin-like junctions), could not be ruled out either. In other linear polysaccharides sharing $\beta(1 \rightarrow 4)$ backbone geometry (e.g. galactomannans) hyperentanglement formation has been rationalised in terms of intermolecular association in addition to normal topological entanglement [10].

3.2. Chitosan/glutaraldehyde chemical gel

The rheological investigation of the chemically cross-linked chitosan network, focused the evolution of the viscoelastic characteristics of chitosan/glutaraldehyde of varying degree of cross-linking as well as on the mechanical properties of the formed gels, both under sensitive dynamic oscillatory testing. The degree of chitosan network cross-linking, was controlled by varying the stoichiometry of functional aldehyde groups per mole of chitosan (R value), in the range between $R = 5$ and 0. A linear viscoelastic response ($\gamma = 0.15$–0.7) in all gels persisted to low R values ($R = 0.32$), near to the rheological gel point.

Fig. 5 shows gelation curves of chitosan gels of varying R at 35°C, as recorded at a frequency window of $\omega = 1\ \mathrm{rad\,s^{-1}}$. It is established that the rate of chemical gelation of chitosan chains with glutaraldehyde, depends on various parameters, namely pH, ionic strength, temperature, chitosan concentration and degree of cross-linking [4]. In our investigation, all variables but R, were maintained constant. Typical gelation curves for gels of varying R, are included in Fig. 5, showing that gels were fully cured before recording frequency sweeps. While cross-linking density depends directly on R, the cross-linking rate can be regulated by changing the ratio between charged and uncharged reactive primary amino groups of the glucosamine units (**D**-residues). It has been suggested that the overall degree of N-acetyl substitution also determines the concentration of available uncharged, reactive amino groups [6]. Thus effect the of pH and amount of glutaraldehyde, on the rate and density of cross-linking depends on F_A as well [6]. In this report, chitosan gels of high degree of acetylation covalently cross-linked with glutaraldehyde, seem to continue curing for longer time than do those made of fully deacetylated chitosan [6]. Based on this, the

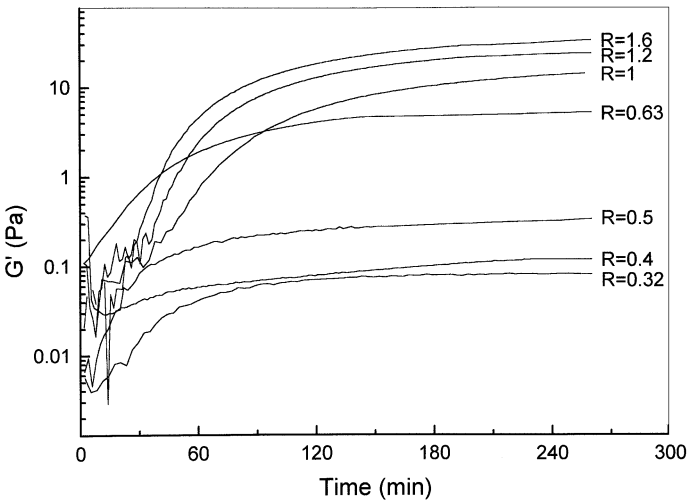

Fig. 5. Variation of storage modulus, G', with time for chitosan/glutaraldehyde chemical gels with stoichiometric ratios between 0.32 and 1.6. Measurements were made at $\omega = 1\ \mathrm{rad\,s^{-1}}$ at 35°C.

author argues in favour of additional chain association phenomena besides chemical cross-linking involving hydrophobic interactions between acetyl groups. No mention is provided in this study on the frequency dependence of the structures formed. As we show below, there are major changes in frequency dependence close to the rheological gel point, which lead to pose the question whether what is 'seen' in the long time-domain holds true at shorter time scales (i.e. at high frequency)? Central to our interpretation of rheological data of small deformation tests are the changes in frequency dependence of the networks formed observed as a function of R.

It is worth pointing out, that there is a step in G' moduli values to which the gels tend to, occurring within a very narrow range of R or cross-linking density (i.e. between $R = 0.5$ and 0.63). This again confirms that changes in frequency dependence of the formed gels are convoluted with the evolution of gelling process. Moreover, the setting curves in Fig. 5 seem to fall into two 'families' with a qualitative change in the rate of increase in G' at $\omega = 1\ \mathrm{rad\,s^{-1}}$ during the first 30–40 min, which could be interpreted as a consequence of complex network contributions to the storage modulus of physical and chemical type progressively changing in time.

In Fig. 6, are shown mechanical spectra of chitosan gels cross-linked with glutaraldehyde at different R, varying in the range $R = 5$ and 0. For clarity, the spectra are shown in two different scales on the y-axis. Fig. 6a, includes four spectra of chitosan gels of progressively lower degree of cross-linking ($R = 5$ and 0.5). At high degrees of cross-linking ($R = 5$), it is clear that $G'(\omega)$ shows almost no dependence with frequency (i.e. $G'(\omega) \sim \omega^1$) characteristic of a permanent gel network. As the gel network density decreases down to $R = 1.0$, G' values decrease by about 3 orders of magnitude, but show hardly any change in frequency dependence with respect to the strong gel. The overall network structure weakening in the spanned region, is accompanied by a gradual change in G'' frequency dependence. Notice that at $R = 1$, the mechanical spectra corresponds to a 'lossy', yet permanent gel network. As R decreases to $R = 0.5$, notice that the form of the mechanical spectrum merges the viscoelastic behaviour of a weak gel in the frequency range 0.06–6 $\mathrm{rad\,s^{-1}}$ (i.e. $G' > G''$ and both moduli increase with frequency) with that of a dissolved polymer dominated by entanglements of greater lifetime than 6 $\mathrm{rad\,s^{-1}}$. Presumably at this point the gel network density is just below its percolation point. Hence, this spectrum suggests the existence of a chemically cross-linked gel network 'dissolved' in a second entangled network formed by chitosan chains of restricted mobility. At $R = 0.4$ (Fig. 6b), the overall shape of the mechanical spectrum is similar to that of an amorphous vitrified system. This may be consistent with the suggestion that at low degrees of cross-linking, a slight increment in the restriction of chitosan chains mobility (by chemical cross-linking), increases the relaxation time drastically, so segmental motions predominate over long-range ones [19]. As the network density decreases further, namely at $R = 0.32$, although the overall mechanical strength of the system weakens, there is a sort of glass-to-rubber transition type of process, indicated by the frequency dependence of G' and G'' with a cross over at ca. 1 $\mathrm{rad\,s^{-1}}$. Although this behaviour is unusual for biopolymer gelling system in the presence of solvent, recent experimental evidence shows that rubber-to-glass transition can be induced in polysaccharide networks at high degrees of space occupancy [20, 21]. This again, may confirm the

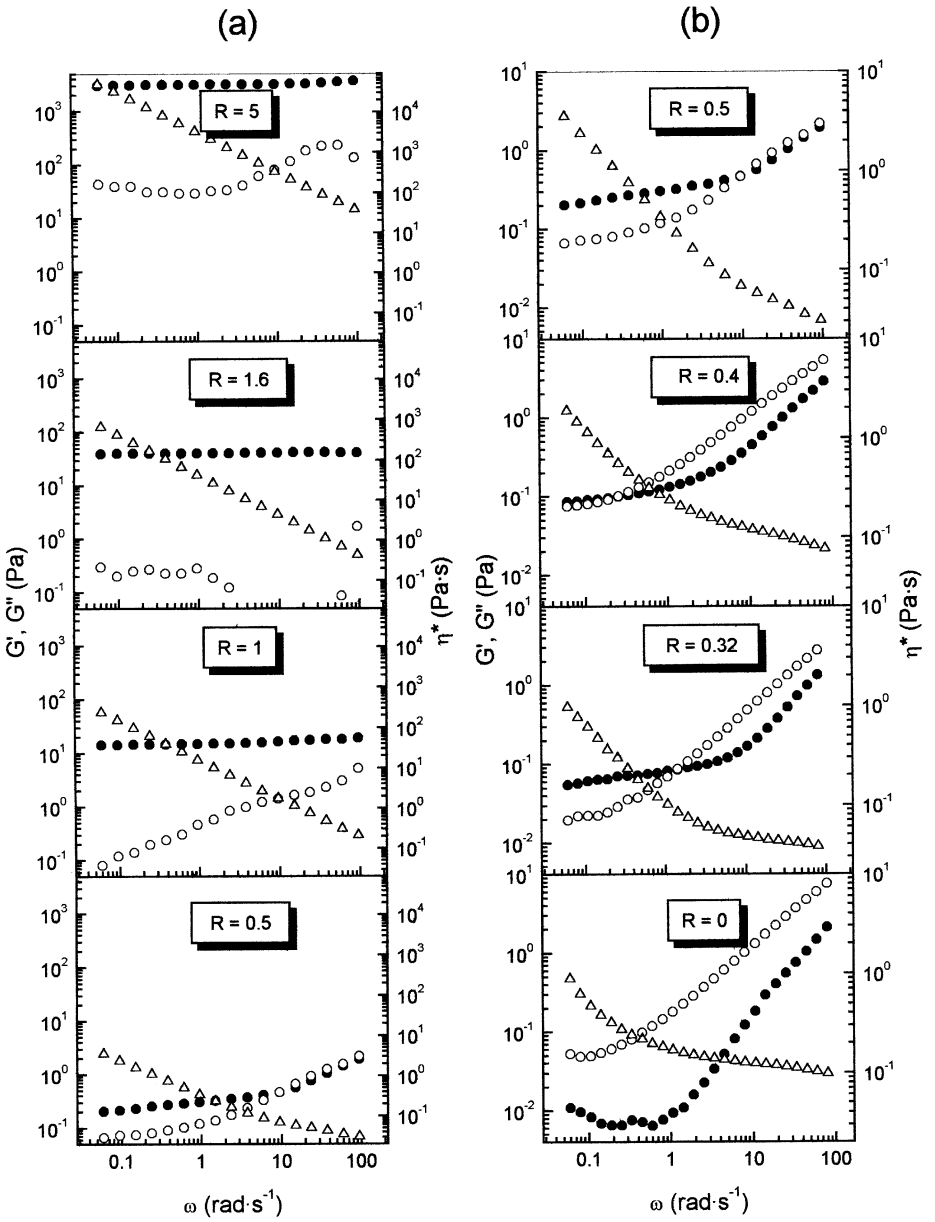

Fig. 6. Mechanical spectra of chitosan (1% (w/v) solution in 0.1 mol l^{-1} acetic acid) chemically cross-linked with glutaraldehyde (50% solution) with stoichiometric ratios, *R*, over the range 0.32–5 at 35°C. Symbols as in Fig. 2.

presence of a weakly structured network of self-associated chitosan (stabilised by hydrophobic associations of N-acetyl groups), of further restricted mobility by few covalent cross-linking points, thus effectively behaving as an amorphous elastomer.

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References

[1] Aiba S-I. Studies on chitosan : 3. Evidence for the presence of random and block copolymer structures in partially N-acetylated chitosans. Int J Biol Macromol 1991; 13 : 40–4.
[2] Vårum K, Anthonsen MW, Grasdalen H, Smisdrød O. Carbohydr Res 1991; 211 : 17–23.
[3] Amiji MM. Pyrene fluorescence study of chitosan self-association in aqueous solution. Carbohydr Polym 1995; 26 : 211–3.
[4] Roberts GAF, Taylor KE. Chitosan gels, 3. The formation of gels by reaction of chitosan with glutaraldehyde. Makromol Chem 1989; 190 : 951–60.
[5] Matricardi P, Dentini M, Crescenzi V, Ross-Murphy SB. Gelation of chemically cross-linked polygalacturonic acid derivatives. Carbohydr Polym 1995; 27 : 215–20.
[6] Draget KI. Associating phenomena in highly acetylated chitosan gels. Polym Gels and Networks 1996; 4 : 143–51.
[7] Rinaudo M, Milas M, Le Dung P. Characterization of chitosan. Influence of ionic strength and degree of acetylation on chain expansion. Int J Biol Macromol 1993; 15 : 281–5.
[8] Roberts GAF, Domszy JG. Determination of the viscometric constants for chitosan. Int J Biol Macromol 1982; 4 : 374–7.
[9] Lee VF. University Microfilms, Ann Arbor, 74/29446, 1974.
[10] Morris ER, Cutler AN, Ross-Murphy SB, Rees DA, Price J. Concentration and shear rate dependence of viscosity in random coil polysaccharide solutions. Carbohydr Polym 1981; 1 : 5–21.
[11] Yanovsky YG. Polymer rheology – Theory and Practice. Chapman & Hall: London, 1993.
[12] Delben F, Lapasin R, Pricl S. Flow properties of N-(carboxymethyl) chitosan aqueous systems in the sol and gel domains. Int J Biol Macromol 1990; 12 : 9–13.
[13] Nakamura K. Interactions between solvent molecules and networks in biogels – viscoelasticity, strength. In: De Rossi D, Kajiwara K, Osada Y, Yamauchi A, editors. Polymer gels. New York: Plenum Press, 1991. p. 57–75.
[14] Tirtaatmadja V, Tam KC, Jenkins RD. Rheological properties of model alkali-soluble associative (HASE) polymers: effect of varying hydrophobe chain length. Macromolecules 1997; 30 : 3271–82.
[15] Lee KY, Jo WH, Kwon IC, Kim Y, Jeong SY. Structural determination and interior polarity of self-aggregates prepared from deoxycholic acid-modified chitosan in water. Macromolecules 1998; 31 : 378–83.
[16] Tanaka F. Polymer–surfactant interaction in thermoreversible gels. Macromolecules 1998; 31 : 384–93.
[17] Klucker R, Schosseler F. Stress relaxation and reconstruction of the associating network in semidilute aqueous solutions of hydrophobically modified random block copolymers. Macromolecules 1997; 30 : 4927–33.
[18] De Gennes PG. Reptation of a polymer chain in the presence of fixed obstacles. J Chem Phys 1971; 55 : 572–9.

[19] Ferry JD. Viscoelastic properties of polymers. 3rd Ed. New York: Wiley, 1980.
[20] Kasapis S. Review: phase separated, glassy and rubbery states of gellan gum in mixtures with food biopolymers and co-solutes. Int J Food Sci Technol 1995; 30:693–710.
[21] Ross-Murphy SB. Rheological methods. In: Ross-Murphy, SB, editor. Physical techniques for the study of food biopolymers. Chapman & Hall: London, 1994. p. 344–92.