

Guar Gum and Scleroglucan Interactions with Borax: Experimental and Theoretical Studies of an Unexpected Similarity

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Guar gum is a galactomannan that assumes a very flexible conformation in solution, while Scleroglucan is a very rigid polysaccharide that dissolves in water as triple helices. Both polymers can form gels in the presence of borax. Despite their structural differences, the freeze-dried gel systems of both polymers, when compressed to form tablets, show a peculiar anisotropic swelling in water that reflects an amazing similarity in terms of their molecular properties. In this paper the behavior of the Guar/borax gel is compared with that of Scleroglucan/borax. The macroscopic properties of the two systems were characterized in terms of rheological measurements. Atomic force microscopy images and molecular dynamics simulation allowed to evaluate, at molecular level, the effect of borax addition to the Guar polymer. Both experiments show that an increasing of the polymer rigidity is produced by borax. The role played by galactose in the side chain was also discussed.

Introduction

Guar gum (GG) is a neutral, water-soluble galactomannan, extracted from the seeds of *Cyamopsis tetragonoloba*.¹ It consists of a linear backbone of $\beta(1\rightarrow 4)$ -linked D-mannopyranose units (Man) and with the presence of randomly attached $\alpha(1\rightarrow 6)$ -linked galactopyranose units (Gal) as side chains^{2,3} (Figure 1). The ratio Man/Gal ranges from 1.5:1 to 1.8:1 due to climate variations.

Scleroglucan (ScLg), a polysaccharide secreted by fungi of the genus *Sclerotium*, has a backbone built up by $(1\rightarrow 3)$ -linked β -D-Glucopyranose (Glc) units with a single $\beta(1\rightarrow 6)$ -D-Glc side chain linked to every third residue of the main chain (Figure 1). ScLg exhibits a triple helix conformation (triplex) both in aqueous solution and in the solid state.^{4,5}

The different structure of the two polymers leads to a very different behavior in aqueous solution: ScLg, with a persistence length of about 200 nm, is considered as one of the most rigid polysaccharides, while it is known that GG, with a persistence length ranging from 3 to 8 nm, assumes a flexible coil conformation.^{6–12}

Both polysaccharides have been widely investigated and proposed for a variety of industrial applications in different fields, such as in food,¹³ in oil recovery,^{14–16} in personal care industries,¹⁷ as well as in pharmaceuticals.^{11,18}

It is well known that borax is an efficient cross linker for polymers bearing hydroxyl groups and its presence can induce the formation of gel, but the type of linkage is still debated, and up to now, at least two models have been proposed. According to the first one, the borax ions hold together the polymeric chains by means of mixed physical/chemical linkages (Figure 2A). This model, proposed by Shibayama for poly vinyl-alcohol,¹⁹ has been recently suggested also for ScLg.^{20–23}

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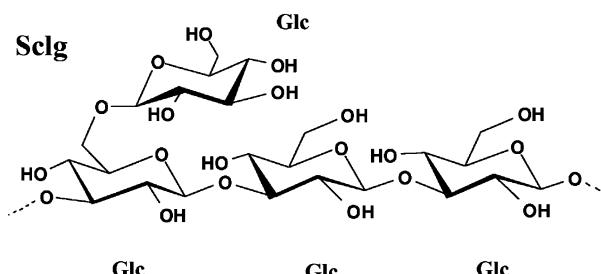
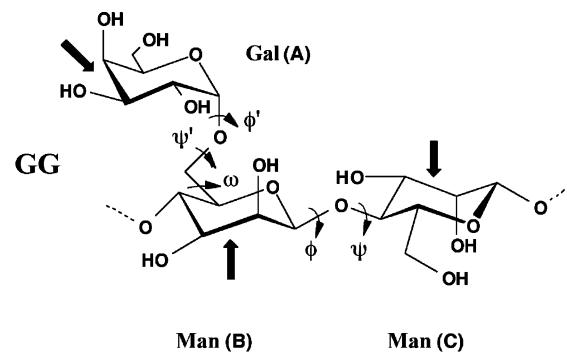


Figure 1. Repeating unit of GG (top) and Scleroglucan (bottom), in this case the angles $[\phi, \psi]$ (Man(B) \rightarrow Man(C)) and $[\phi^I, \psi^I, \omega]$ (Gal(A) \rightarrow Man(B)) are seen; the arrows indicate the alternative sites involved in the O2–O3 (Man) or O3–O4 (Gal) linkages with borax.

The second and more popular model implies only chemical cross-links between the polymeric chains and borax^{24–26} (Figure 2B), and it was proposed for the GG–borax interactions. Despite the large amount of data acquired in four decades, other relevant aspects are still under investigation, such as the formation of five- or six-membered rings in the presence of borax.^{27–31}

As far as the different behavior of GG and ScLg is concerned, it is interesting to point out that borax quickly promotes the

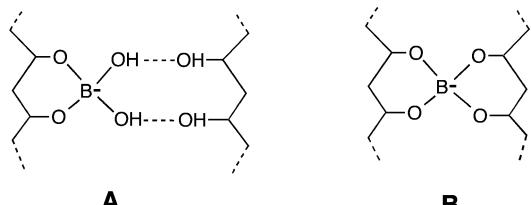


Figure 2. Schematic representation of the chemical/physical (A) and chemical/chemical (B) linkages.

GG gelation by means of cross-links characterized by a lifetime of the order of seconds,³² leading to self-healing properties of the network (i.e., the gel when pulled apart it will reassemble upon manipulation). On the other side, Sclg requires several hours for gelation in the presence of borax and no self-healing occurs.

Among the various techniques reported in the literature, the rheological approach appeared to be particularly useful for the characterization of these systems.^{32–34}

On the other hand, the atomistic details of polysaccharides are difficult to be obtained by means of X-ray and NMR because of their flexibility and poor tendency to crystallize. For this reason, the coupling of experimental techniques (such as atomic force microscopy) and computational analysis represents a useful alternative to more traditional approaches.²³

In particular, the atomic force microscopy (AFM) can provide structural information on polysaccharides, such as the persistence length in diluted solutions and the aggregation degree near the gel point.^{12,23,35–39} On the other hand, the atomic resolution, that cannot be obtained using this technique, can be acquired by means of computational analysis, as molecular mechanics (MM) and molecular dynamics (MD). New force field parameters for polysaccharides have been developed in recent years, leading to the possibility to apply MD techniques not only to proteins and nucleic acids as in previous times (see reference⁴⁰ for an exhaustive review).

As far as GG is concerned, adiabatic maps of the mannose and epimelibiose disaccharides (representing the GG repeating unit) were obtained by means of MM calculations,^{3,41–43} semiempirical techniques,⁴⁴ and also using short MD trajectories.⁴⁵ Furthermore, QM methods were applied for the study of mannose analogues, where the hydroxyl groups were replaced by H atoms.⁴⁶ Sometimes these data were used to extrapolate, via Monte Carlo approach, the general features of the polymer.^{3,45,47} Also the guar/borax (GG/borax) system was investigated by means of MM and density functional theory (DFT) calculations²⁹ and the theoretical results, compared with NMR data, allowed to conclude that borax coordinates the guar molecules via the 3,4-cis-diols of the side-chain galactose residue and via the 2,3-cis-diols of the mannose on the backbone.

The disaccharides gentiobiose and laminarabiose, which represent the repeating unit of the Sclg, were studied by means of computational techniques to predict the conformational features of the whole polymer.^{48–51}

Furthermore, MD simulations carried out on Sclg-triplex tracts^{51,52} showed that a network of interstrands H bonds stabilizes the Sclg triplexes in water and the borax^{22,23} is able to stabilize the interactions between different triplexes, favoring a parallel alignment also in the absence of chemical–chemical intertriplex cross links.

In spite of the above-reported differences between Sclg and GG, they show some similar and peculiar features when they interact with borax. In particular, both systems exhibit an anisotropic swelling when compressed in the form of

tablets.^{18,20–23,53} In this paper we propose a molecular explanation of this unexpected similarity. A comparison with dextran, a flexible polymer with a backbone of D-glucose linked predominantly $\alpha(1 \rightarrow 6)$, is also reported.

Experimental Section

Materials. Scleroglucan (Degussa, Germany; $M_w = 1.1 \times 10^6$, from viscometric measurements) and GG (CarboMer, USA; $M_w = 1.2 \times 10^6$, from viscometric experiments) were used after dialysis and freeze-drying. The ratio Man/Gal in GG was estimated to be about 1.5 according to ¹H NMR data.

Dextran (average molecular weight 4×10^4) was provided by Fluka (Italy). Borax was a Carlo Erba (Italy) product. Distilled water was always used.

Hydrogel and Tablet Preparation. For the preparation of the hydrogels, an appropriate amount of polymer (about 200 mg) was magnetically stirred in water for 24 h. Then, the calculated amount (i.e., moles of borax = moles of repeating units of polymer, $r = 1$) of 0.1 M borax solution was added, and the system was left under magnetic stirring for 5 min. In the case of dextran, the same amount of borax used for Sclg was added. It must be pointed out that the pH value remained constant at 9.0 during salt addition because of the self-buffering effect of borax. The obtained sample ($cp = 0.7\%$ (w/v)) was kept two days at 7 °C for gel setting and was then freeze-dried.²¹

Tablets were then prepared from the freeze-dried sample with an IR die (Perkin-Elmer hydraulic press) using a force of 5.0 kN for 30 s. The weight of the tablets was 230 ± 10 mg; the diameter was 13.0 ± 0.1 mm, and the thickness was 1.4 ± 0.2 mm.

Some Sclg/borax and GG/borax tablets, once prepared, were pressed again (using always a force of 5.0 kN for 30 s) along a direction perpendicular to the previously applied pressure direction; hereafter, such tablets will be called Sclg or GG double compressed (Sclg-dc and GG-dc).

Hydrogel Preparation for the Rheological Measurements. The hydrogels were prepared according to the following procedure: 35 mg of each polymer were dissolved, under magnetic stirring, in distilled water for at least 24 h. An appropriate amount of borax 0.1 M solution was then added and the solutions were stirred for 5 min. The samples, prepared with a $cp = 0.7\%$ into beakers with a diameter larger than the plate–plate geometry of the rheometer (4.5 cm), were kept at 7 °C for 48 additional hours, for the gel settling. To prepare a sample easy to manipulate and no so thick to invalidate the rheological measurements, the volume of the polymer solution was appropriately chosen, so that a hydrogel thickness in the range of 1.0–3.0 mm could be obtained.

Methods

Water Uptake and Dimensional Increase Studies. The swelling of tablets prepared with all systems (Sclg/borax, GG/borax, and dextran/borax) was carried out by soaking the tablets in distilled water at 37 °C. At fixed time intervals, the tablets were withdrawn, the excess of water removed with soft filter paper for 5 s, and then the corresponding dimensional variations determined by means of a screw gauge with an accuracy of ± 0.1 mm.

All analyses were performed on three replicate samples. Mean values are reported in Table 1, and in each case, all experimental values lay below 10% of variation of the mean.

Rheological Measurements. The rheological characterization of the samples was performed by means of a controlled stress rheometer (Haake Rheo-Stress RS300; Thermo Haake DC50

TABLE 1: Relative Increase of Height for Tablets of GG/Borax and Sclg/Borax, and for Tablets of GG/Borax-dc and Sclg/Borax-dc

sample	$(h - h_0)/h_0$				
	2 h	4 h	6 h	8 h	24 h
Sclg/borax	7.31	11.02	12.55	14.39	21.77
Sclg/borax-dc	3.76	5.19	6.14	7.10	9.95
GG/borax	5.48	9.58	11.27	13.11	20.69
GG/borax-dc	8.29	11.62	13.29	14.71	19.48

water bath). Two geometries were used: a cone–plate (Haake C60/1 Ti, cone diameter = 60 mm and cone = 1°; plate MP60 steel 8/8'', diameter = 60 mm) for the Sclg and GG samples and a grained plate–plate device (Haake PP35/S; diameter = 35 mm) for the hydrogel samples, to reduce the extent of the wall slippage phenomena.⁵⁴ To perform the measurements, the hydrogel, with a thickness of 1.0–3.0 mm, was removed with the aid of a small spatula from the beaker where it was settled and laid with care on the lower plate of the rheometer. The upper plate was then lowered until it reached the hydrogel surface. Gap setting optimizations have been undertaken according to the procedure described elsewhere.⁵⁵ Samples were loaded at a fixed temperature, 25 °C, and coated around their periphery with light silicone oil to minimize loss of water. When only Sclg or GG was tested, an appropriate amount of the polymer solution was spread out on the plate geometry to obtain a sample of the appropriate height. Rheological properties were studied in oscillatory experiments; mechanical spectra were recorded in the frequency range 0.001–10 Hz.

The linear viscoelastic region was assessed at 1 Hz by stress sweep experiments, and in the subsequent measurements, a constant deformation of $\gamma = 0.01$ was used.

Scanning Electron Microscopy (SEM). The morphology, surface appearance, and inner structure of the samples were examined by SEM analysis. Prior to examination, the samples were fixed on a stainless steel stub using a double-sided tape. The SEM images were obtained with a FEI Quanta 400 FEG apparatus. The polymers were examined under vacuum (50 Pa), with no need of the gold-coating technique, at an accelerating voltage of 15 kV. All images were acquired digitally using xTmicroscope Control software. The measurements were carried out on the freeze-dried polymer solutions and on the freeze-dried polymer–borax systems.

AFM Measurements. A small volume of GG or GG/borax solutions ($c_p = 0.07\%$, $r = 1$) was dropped onto a freshly prepared mica surface, allowed to dry overnight, and then analyzed in air environment at room temperature, by using AFM. AFM measurements were performed with Veeco Multimode microscopy (Veeco Instruments, Inc. Edina MN, USA) in tapping-mode configuration with nonconductive Si tips. The horizontal resolution was about 1 nm, while the vertical one was 0.01 nm. Both height and phase signals were recorded. AFM images were elaborated by using the WSxM software.⁵⁶ Persistence lengths were estimated using the “2D single molecule” software.⁵⁷

MD Simulation. The parameters used to describe our system were obtained starting from the 45A4 parameter set;⁵⁸ this set was recently recognized as one of the best performing in the saccharide simulations, by comparison with crystallographic and ab initio data.⁴⁰ For the borax moiety, the previously proposed parameters were used.²² The MD simulations were performed using GROMACS software package.⁵⁹ Two simulations were performed, for each GG and GG/borax system, starting from elongated and random coil conformations. In all cases, 10

repeating units of GG (corresponding to 30 pyranose rings) in explicit water solution (SPC model)⁶⁰ were considered. Two methyl protecting groups were added to the first O4 and last O1 atoms of the backbone.

The starting conformations of the elongated structure were obtained by setting, for all the Man–Man nearest neighbor residues, torsional angles (ϕ and ψ , see Figure 1) values close to those measured in the mannan fibers by electron,⁶¹ and X-ray⁶² diffraction. Such values are similar to those reported for the mannobiose⁶³ and mannotriose⁶⁴ crystal structures and also to the absolute minimum obtained for mannobiose, by using the MM3 force field.^{3,41} Three dihedral angles define the orientation of the galactopyranose residue in the side chain (ϕ^I , ψ^I , and ω , see Figure 1). The ϕ^I and ψ^I values of the starting structure were set in the absolute minimum found by Petkowicz and co-worker;³ the ω angles were initially set to 60°.

The trajectory of the elongated conformation, containing roughly 45,000 water molecules, lasted 40 ns. During these simulations, three further relative minima in the ϕ – ψ map were populated (see Discussion). Such minima, already predicted for mannobiose,^{3,41} were used to obtain the starting coiled structure for GG and GG/borax simulations. The same conformation was used to simulate both systems; about 18,500 water molecules were considered, and the trajectories lasted 10 ns.

The borax groups were randomly linked on the backbone or on the side-chain of each repeating unit because the experimental stability of the more stable borax adducts, (O2–O3 for mannopyranose and O3–O4 for galactopyranose), is roughly the same.^{29,30}

The relative rigidity of GG/borax with respect to GG has been estimated in terms of the ratio between the persistence length (P) of the two systems, that can be calculated according to the equation

$$\frac{P_{\text{GG/borax}}}{P_{\text{GG}}} = \frac{\ln(\langle \cos \vartheta \rangle_{\text{GG/borax}})}{\ln(\langle \cos \vartheta \rangle_{\text{GG}})} \quad (1)$$

where θ is the angle between the vectors connecting equivalent C1 atoms of two consecutive units of the backbone, considering that the distance between two consecutive C1 is the same for GG and GG/borax.

The root mean square deviation (rmsd) was calculated, according to usual equation, using the tool included in the GROMACS distribution.

According to the IUPAC-IUB,⁶⁵ the ϕ and ψ angles were defined starting from the atoms O5-C1-O4-C4 and C1-O4-C4-C5 in the backbone, respectively.

Analogously, the ϕ^I , ψ^I and ω angles were defined from the atoms O5(A)-C1(A)-O6(B)-C6(B), C1(A)-O6(B)-C6(B)-C5(B), and O6(B)-C6(B)-C5(B)-O5(B), respectively.

Results

Water Uptake and Dimensional Increase Studies of Tablets. It is well known that usually the gel systems swell isotropically in all directions,⁶⁶ but as already reported,²² when the gel of Sclg/borax is freeze-dried and compressed to prepare tablets, an anomalous anisotropic swelling is observed. When the tablets are soaked in distilled water at 37 °C, the compressed matrix elongates essentially along its axial direction (the direction of compression), while radial swelling is almost negligible. It must be pointed out that no anomalous swelling is detected in the case of tablets prepared with only Sclg.²⁰

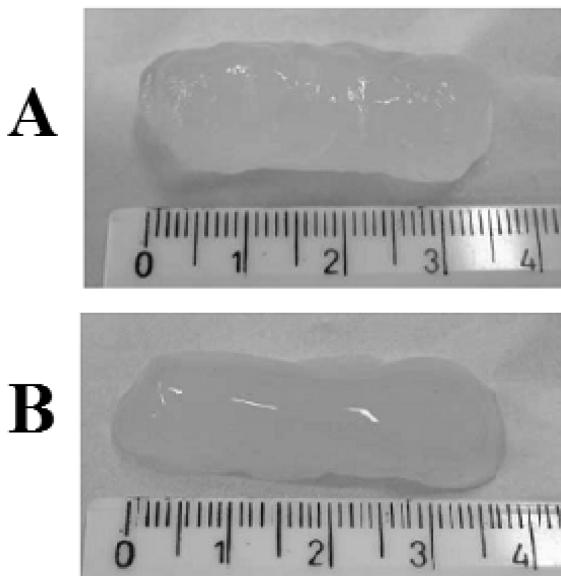


Figure 3. Tablets of Sclg/borax (A) and GG/borax (B) after swelling for 24 h in distilled water at 37 °C.

Surprisingly, despite the very different structures (see Figure 1) and conformational features of GG and Sclg, a similar swelling behavior is observed for the two polymers in the presence of borax (see Figure 3).

The relative height increases of tablets of Sclg/borax and GG/borax are reported in Table 1. For an appropriate comparison the experiment was carried out also with tablets prepared with the dextran/borax freeze-dried hydrogel. In this case, the tablets dissolved within 5–10 min, and therefore the obtained data are not reported. Dextran was chosen because in aqueous media its chains show high flexibility, due to the presence of β -(1→6) linkage among the sugar units, that leads to a viscometric behavior similar to that of GG.

As it results from Table 1, no significant differences between GG/borax and Sclg/borax were found when the anisotropic elongation is considered. The data relative to GG/borax-dc and Sclg/borax-dc show an intriguing and peculiar characteristic of the two systems. Actually, while for GG/borax the double compression did not affect significantly the elongation of the tablets, in the case of Sclg/borax system an elongation reduction of about 50% was monitored.

Rheological Analysis of the Hydrogels. The rheological characterization of the investigated systems is shown in Figure 4.

The mechanical spectra are completely different in the two cases. The Sclg sample shows the typical behavior of a weak gel, and the addition of borax does not change significantly the trend of the two moduli that increase only slightly. The formation of a macroscopic self-sustaining network is not accompanied by a significant storage modulus variation. This means that the number of the elastically active chains that contribute to the dynamic modulus is rather low, and the mixed physical and chemical nature of the linkage between borate ions and hydroxyl groups of the polymer²³ can explain and support this experimental evidence.

A different situation is observed for GG. The polymer sample has very low values for the two moduli with G'' always higher than G' and both show a strong dependence on the applied frequency. The spectra of the GG solution do not show the characteristics of the steady flow behavior due to the presence of inter- and intramolecular hydrogen bonds that allows to describe the polymer in terms of a hyperentangled system.^{10,67}

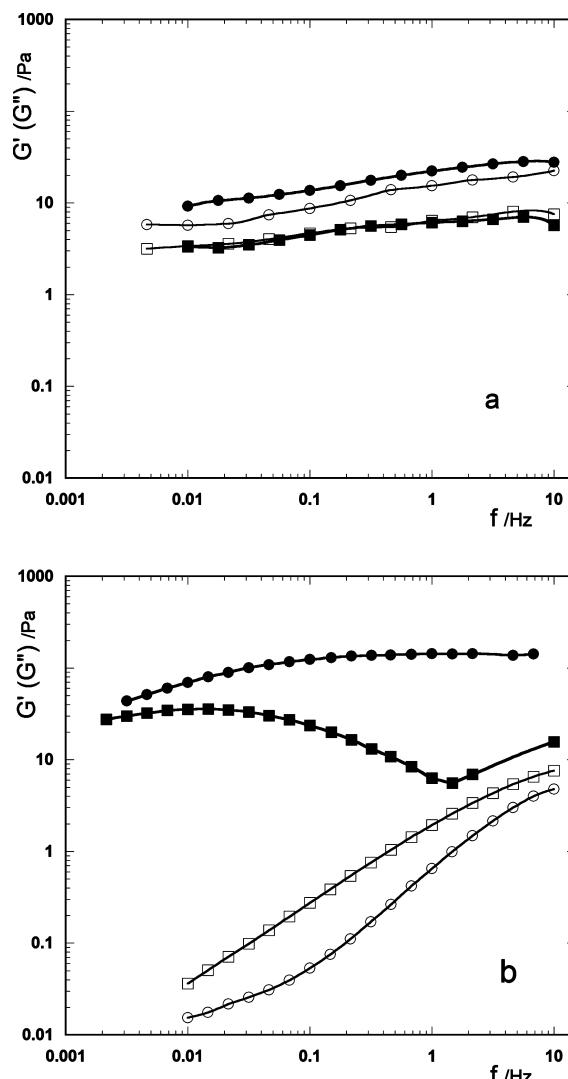


Figure 4. (a) Frequency sweep for Sclg/Borax (G' (●) and G'' (■), and Sclg (○) and G'' (□)); b) Frequency sweep for GG/Borax (G' (●) and G'' (■), and GG (○) and G'' (□)); $\gamma = 0.01\%$; cp = 0.7% (w/v). The lines are only a guide to the eyes.

As expected, GG cross-linking (by borate ion complexation) leads to an increase in both moduli. For the GG/borax gel, the typical features of a plateau characterized by a weak frequency dependence of $G'(\omega)$ and an increase of the storage modulus at least of a factor two, can be observed. The G'' spectrum is peculiar with the occurrence of a maximum and a minimum. These features of GG/borax complex gel are very close to those previously reported for concentrated polystyrene solutions^{68–70} where the rubbery zone is affected by the entanglement couplings between molecular chains.^{68,69} Thus, according to the modified Rouse⁷¹ and the Chomppff–Duiser theories,^{72,73} the relaxation spectrum is predicted to show a maximum characteristic of the entanglement slipping mechanism.

As a result, the curve of G'' vs ω should have a maximum, and that of G' versus ω should show a very flat plateau.

Actually, the same relaxation behavior was already observed for PVA/borate,^{74–76} konjac/borax,⁷⁷ and GG/borax (in the presence of salts)^{32,33,78} complex gels. In these systems, the shape of the G'' modulus cannot be simply ascribed to a disentanglement process but should be related to the labile nature of the chemical cross-links between the borax molecules and the polymeric chains.

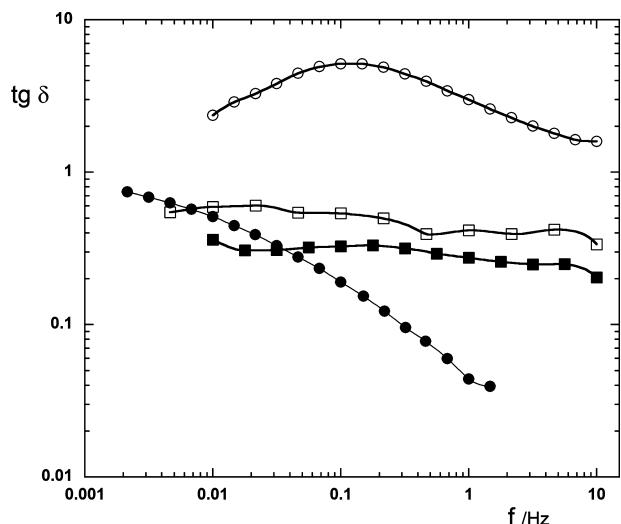


Figure 5. Viscoelastic loss tangent as a function of frequency for the GG/borax (●), GG (○), Sclg/borax (■), Sclg (□).

Furthermore, from Figure 4, it can be inferred that an intersection point of G' and G'' will occur at ca. $f \approx 0.001$. This crossing does not correspond to the maximum of G'' (G''_{\max}), suggesting that the viscoelastic behavior cannot be described by only one relaxation time and therefore by one Maxwell element.⁷⁹ According to the reptation model^{80,81} the characteristic frequency corresponding to G''_{\max} gives a good indication of the longest relaxation time $\tau_r (1/\omega_{\max})$, i.e., the time for a given macromolecule to disengage by a snakelike motion from a tube made up of neighboring chains. Actually, the motion of the whole chain is slowed down due to entanglement coupling. The longest relaxation time has often been considered equal to the reptation time; however, in the GG/borax gel the reptation of a polymer chain should be hampered by complexation since any monomer may be reversibly linked to a neighboring chain by borate ion. The system forms a loose reversible gel with temporarily cross-linked chains. Thus, the longest relaxation time represents the reciprocal of the exchange rate for the formation of intermolecular cross-links. Actually, from our experimental data it is not correct to assign a single longest relaxation time for the gel; still the frequency ω_{\max} corresponding to the maximum of the loss modulus yields a good idea of the characteristic time of the chief relaxation mechanism.

For our sample $\tau_r \approx 11$ s, which is the same value already found for GG/borax system studied at similar polymer concentration in 1 M NaCl.³² Furthermore, this value is of the same order of magnitude of that of the konjac/borax system ($\tau_r \approx 20$ s)⁷⁷ and higher than that of PVA/borax ($\tau_r \approx 0.24$ s).⁷⁹

The fact that the values of these mentioned relaxation times are on the order of seconds or less, whereas in other gels induced by ion complexation (PVA-congo red,⁸² PVA-pyroantimoniate,³² Cr³⁺-polyacrilamide⁸³) they may be counted in hours, comes from the particular lability of diol-borate cross links. Related to this aspect is the intriguing peculiarity of the self-healing and flowing nature of GG/borax gel that was not observed in the case of Sclg/borax system.

Also the behavior of the $\tan \delta$, reported for the two systems in Figure 5, appears to be interesting. In the case of Sclg, the addition of borax induces a slight decrease of $\tan \delta$ value that remains almost constant with the frequency. $\tan \delta$ values for Sclg and Sclg/borax are smaller than 1, thus indicating a gel behavior for both systems.

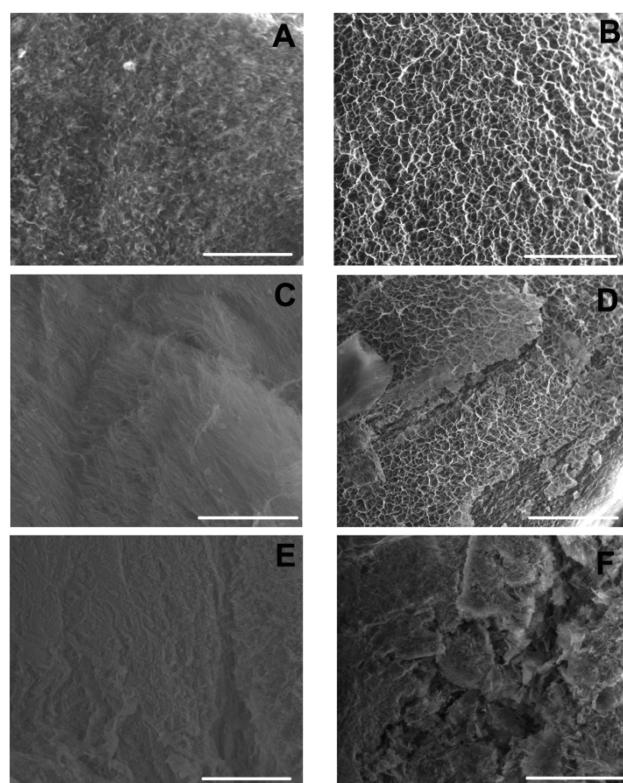


Figure 6. SEM images with 1 mm scale bar of Sclg without (A) and with borax (B), GG without (C) and with borax (D), and dextran without (E) and with borax (F).

On the other hand, while $\tan \delta$ values of the GG sample are rather high, thus indicating a solution behavior, in the case of the GG/borax system, $\tan \delta$ values are appreciably reduced, and they show a marked dependence on the applied frequency. This result supports the reversible nature of the GG and borax linkages. From Figure 5 it is also possible to observe that at low frequencies similar values for Sclg/borax and GG/borax gels are obtained, while increasing the frequency the more elastic nature of the GG/borax network is evidenced.

SEM Characterization. Figure 6 shows SEM micrographs of the freeze-dried polymer solutions and the freeze-dried hydrogels. While in the case of dextran the presence of borax does not affect appreciably the morphology of the sample, for both Sclg and GG systems significant texture variations can be detected with the appearance of “porelike” structures, indicating the presence of many entanglements among the chains. It is worth noting that in the case of GG without borax these structures are totally absent; on the other hand the Sclg shows similar structure, in a minor extent respect to Sclg/borax, reflecting an intrinsic ability to promote supramolecular organization.

AFM Measurements. As already reported,²³ the AFM images of Sclg samples show that the presence of borax tends to favor the triplexes aggregation. To investigate the influence of borax on GG samples, AFM images on GG and GG/borax were acquired (Figure 7). According to previously reported data,^{34,84} in our conditions (polymer concentration low enough to impair possible interactions between different GG chains, absence of strain) GG clearly shows a random coil organization, as evidenced by the roughly circular spots present in the Figure 7.

The presence of borax induces the transition toward elongated chains, as those evidenced in Figure 7. In our conditions, these structures, in which the polymer is complexed with borax,

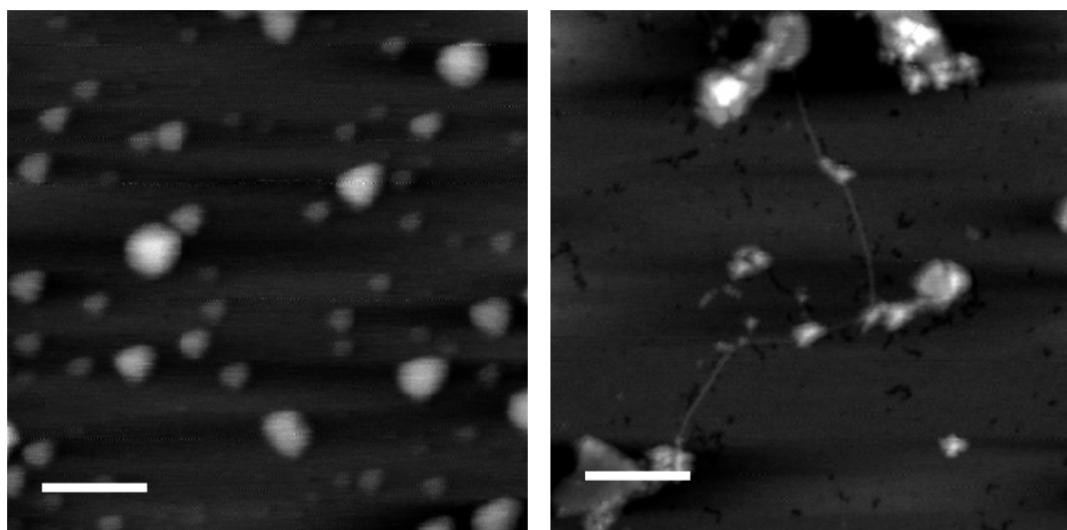


Figure 7. AFM images with 200 nm scale bar of GG in the absence (left) and in the presence (right) of borax.

coexist with the circular spots produced by chains poorly bound to borax. AFM images were also acquired with dextran, but in this case no significant changes were observed (data not shown).

The persistence length of the elongated structure detected in the GG/borax sample was estimated, and the obtained value (157 ± 31 nm) is quite similar to that obtained from diluted scleroglucan solution (ranging from 150 to 200 nm).^{12,38,85}

MD Simulation. To evaluate the influence of borax on the local conformation, oligomers of GG and GG/borax starting from elongated and coiled conformations were simulated.

In all simulations significant effects of borax on the orientation of the galactose rings in the side chains, in terms of the three dihedral angles ϕ^l , ψ^l and ω , are not observed. As a general rule, the results are similar to those obtained for the epimelibiose except for one of the two predicted relative minima.^{3,44} In particular, the $[\phi, \psi]$ minimum at about [90, 270] is absent. However, an inspection of the GG structure shows that such minimum, allowed in the dimer, in the case of the polymer is impaired by a strong steric hindrance between galactose and the subsequent mannose ring in the backbone. The ω angles populate, in all cases, two minima, centered at 60° and -60° , and numerous transitions are observed.

Backbone Conformation (Starting from Extended Conformation). In our simulations of GG and GG/borax starting from the elongated structures, the ϕ and ψ angles of nearest neighbor mannopyranoses, defining the backbone local conformation, essentially maintain their starting values corresponding to the absolute minimum [280, 225]. As a consequence, there are no global parameters (i.e., end-to-end distance) that show significant difference between simulations with and without borax. However, localized distortions are detected, and other three minima can be evidenced. These minima are centered at [197, 213], [14, 230], and [287, 85], respectively. The only significant difference between GG and GG/borax simulations is the population of the minimum centered at [287, 85]. In the case of GG the 3.5% of the analyzed conformations fall into this minimum, while in the case of GG/borax such percentage is reduced to only 0.5%; this difference is further emphasized in the simulation starting from coiled structures, as better discussed below.

The minima above-reported roughly correspond to those identified in the adiabatic maps of mannobiase.^{3,41} Other authors proposed a different set of minima, validating their results by comparison with NMR data.⁴⁴ However, they did not identify

the global minimum detected by X-ray diffraction of mannobiase⁶³ and mannotriose⁶⁴ and in the fibers of mannan by electron⁶¹ and X-ray⁶² diffraction. For this reason we consider reliable our data, and we used these local conformations to randomly generate a coiled conformation for the GG and GG/borax oligomers.

Backbone Conformation (Starting from Coiled Conformation). The rmsd values between couples of structures sampled each 10 ps during the simulations were calculated and used to obtain the pairwise rmsd matrices (not shown).

From these data, it can be estimated that GG/borax reaches an equilibrium after about 8 ns, and in the last 2 ns conformational changes cannot be appreciated. Furthermore, the rmsd value respect to the starting structures (coiled) in the last 2 ns is higher than 2.5 nm, indicating that the sampled conformation is very different with respect to the starting one (coiled).

On the contrary, in the case of GG system not clear transitions are detected, and the polysaccharide samples different conformations. Furthermore, for GG, the rmsd values from the starting conformation (coiled) are systematically lower than those of GG/borax.

To detect collective conformational changes the end-to-end distances have been estimated for GG and GG/borax (Figure 8). In the case of the GG/borax, the value, initially of 1.7 nm, increases until about 9 nm (close to the maximum allowed elongation), and it is then retained until to the end of the simulation. This value is comparable to those registered in the case of simulations performed starting from the extended polymers. The end-to-end distance behavior nicely correlated with the rmsd matrix analysis. According to the rmsd analysis, the end-to-end value of GG fluctuates spanning between about 1 and 4 nm during simulation suggesting that the GG does not retain any sampled conformation, as predicted for random coil polymers.

To investigate the local conformations two parameters were analyzed: (i) the complementary angles of those formed by three consecutive C1 atoms, related to the persistence length; (ii) the $\phi-\psi$ occurrence maps that can be compared with the same data of smaller molecules (e.g., mannobiase).

The former analysis showed that in the first nanoseconds of simulation the average angle values was $(33 \pm 13)^\circ$ for both GG and GG/borax. In the last nanoseconds the average value becomes $(19 \pm 8)^\circ$ for GG/borax, but it remains close to the starting value, in the case of GG simulation $(31 \pm 13)^\circ$. The

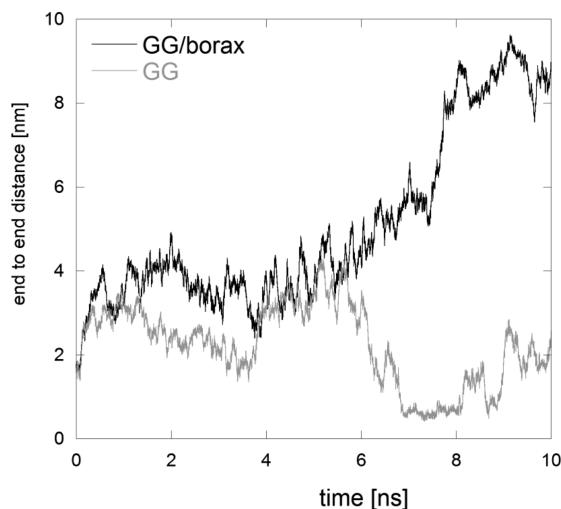


Figure 8. End-to-end distances evaluated for GG and GG/borax simulations. The values are obtained considering the distance between the two protective methyl groups added to the first O4 atom of the first residue and the O1 atom of the 10th residue.

average values of these angles, measured in the last nanoseconds of simulation, lead to a ratio between the persistent lengths, calculated according to the eq 1, of the two systems of about 2.8. This value cannot be compared with the corresponding experimental data (the simulated chain is definitely shorter than the real polymer), nevertheless it clearly reflects an increasing of rigidity induced by borax.

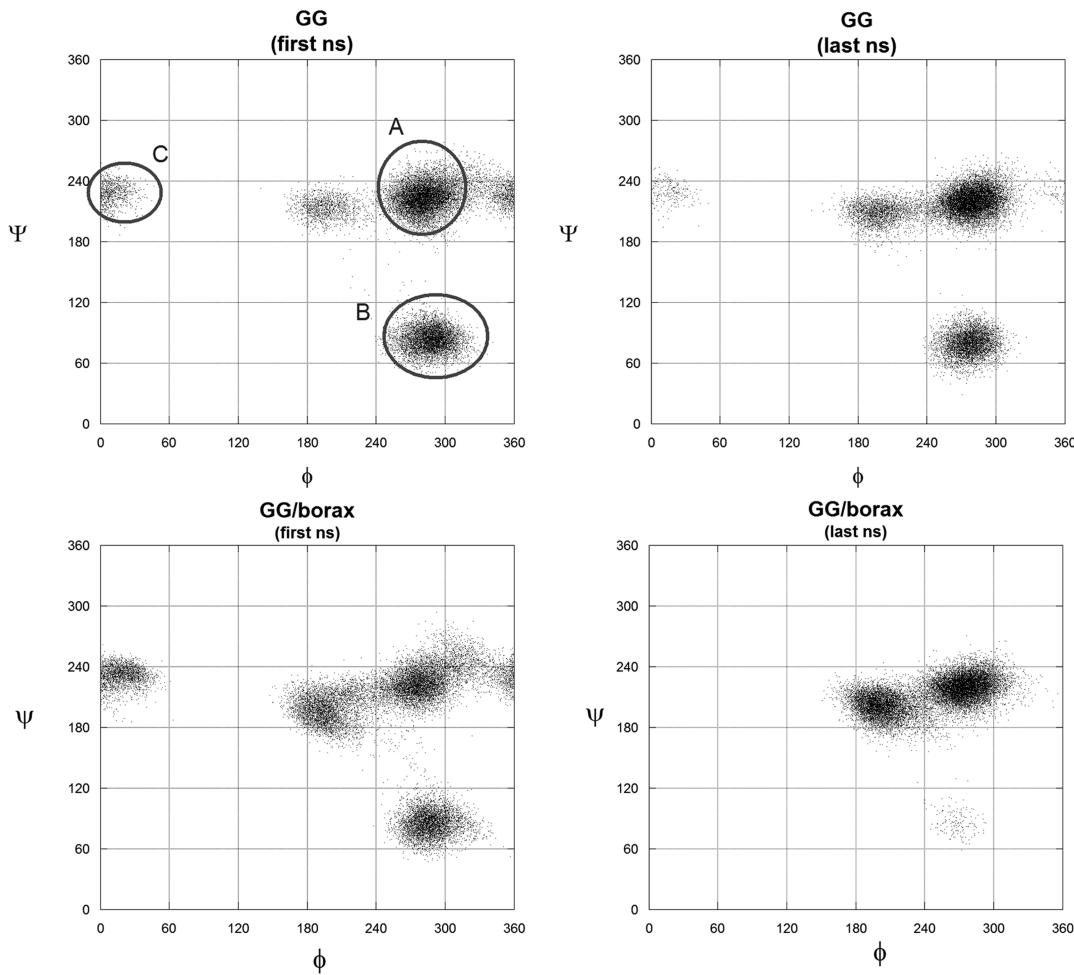


Figure 9. $\phi-\psi$ occurrence maps for the GG (above) and GG/borax (below) simulations starting from coiled structures.

In Figure 9 the $\phi-\psi$ maps of GG and GG/borax, acquired in the first and in the last ns of the simulations are reported. In the case of GG no significant differences are detected. On the other side, in the case of GG/borax simulation an evident variation in the local conformation is promoted by borax, leading to the almost complete loss of the minima roughly centered at [287, 85] (region B) and [14, 230] (region C).

Region A contains the experimental $[\phi-\psi]$ values obtained by electron ([279, 199])⁶¹ and X-ray diffraction ([270, 211])⁶² for the mannan chains. Furthermore, the angles obtained from crystallographic experiments for mannobiose ([266, 212])⁶³ and mannotriose ([266, 214] and [288, 225])⁶⁴ fall in the same region. The evidence that the populated minima during the simulation are practically coincident with those predicted for mannose, suggests that the presence of galactose side-chain plays a second order effect on the backbone conformation of the guar.

Such a finding is in good agreement with the results obtained by Petkowicz et al.³

Discussion

In this paper we studied the peculiar and similar behavior of Sclg and GG in terms of anisotropic swelling of tablets, in the presence of borax (Figure 2 and Table 1). Swelling experiments, carried out in our laboratory with other polymers, such as dextran, PVA, starch, pullulan,⁵³ alginate xanthan, and gellan (data not published), indicated that borax was never able to promote this anomalous swelling. Thus, it is evident that the anomalous swelling is promoted by borax only in the case of

some polymers, specifically some polysaccharides bearing side chains. Furthermore, the number of the side chains along the backbone appears to be crucial: if the branching degree is too low, the relative increase of height is drastically reduced, as evidenced by the different behavior of Locust Bean Gum (Man/Gal about 3.5) in comparison to GG (Man/Gal about 1.5).⁵³

Also the stiffness of the polymer plays an important role in the process leading to the anomalous swelling. As previously reported,^{22,23} the ability of Sclg triplexes to organize themselves in bundles is enhanced by borax. During the compression, these domains, with parallel triplexes, increase, both in number and dimension, and they produce the observed anomalous swelling.

A similar mechanism is very unlikely for a flexible polymer as GG. Nevertheless, some evidences showing that GG can assume an extended conformation were already reported in the literature.^{34,86} Also our AFM experiments showed how the presence of borax promotes the transition of some coiled chains toward an extended conformation (Figure 7). The same results were also confirmed by MD simulations, where an increase of rigidity is clearly observed for GG/borax (Figure 8). The observed rapid increase of the end-to-end distances (almost 500%) is particularly significant, especially considering that the chosen alternating distribution of the Gal units along the backbone leads to the most flexible polymer conformation with respect to moreover possible block and random sequences.^{3,47}

The stiffness induced by borax could be related to an electrostatic repulsion between the negatively charged groups introduced in the polymer chain, but it could be also a consequence of variations in the local conformations induced by borax. In the former case, stiffening should be observed whenever a linkage between borax and a polysaccharide takes place; in the other case, the observed stiffness should be peculiar of only few polymers.

The analysis of the electrostatic contribution to the global energy during the GG/borax simulation (not shown) does not show any relaxation correlated with the increase of the end-to-end distance. Thus, the relaxation of the electrostatic strain cannot be related to the stiffening of the GG/borax system.

On the other hand, the $\phi-\psi$ maps (Figure 9) show that the accessible conformational space is remarkably reduced in the presence of borax. Only two minima are populated in the last ns of the GG/borax simulation (very close to each other), and the more populated, centered at about [280, 225], corresponds to an elongated conformation. The minima B and C in Figure 9, which in the GG produce a bending of the polymer, are not populated.

Looking at the conformation of pyranose linked by borax, it is evident that the presence of the fused five member ring (involving the borax) induces some distortions on the canonical 4C_1 chair (boat or twisted conformations) mostly during the transitions from relative toward global minimum.

Overall, we can conclude that the addition of borax to GG increases the polymer rigidity, making quite similar Sclg/borax and GG/borax. This is also confirmed by the SEM micrographs reported in Figure 6, where the promotion of ordered micro-domain, as a consequence of borax addition, is evidenced.

The role of borax in the interchain interactions and, as a consequence, in the supramolecular organization was also investigated by means of rheological experiments. It is worth noting that other cross-linkers, promoting more stable linkages between chains, do not promote the anomalous swelling here reported (i.e., the glutaraldehyde with GG and Sclg⁸⁷).

The mechanical spectra (Figure 4) show a remarkable difference between the two polysaccharides and also for the

different kind of polymer cross-links that take place in the two systems (labile chemical/chemical for GG and chemical/physical for Sclg). In addition, the swelling behavior observed when the tablets are double compressed (GG/borax-dc and Sclg/borax-dc) is notably different. The relative increase of height for GG/borax-dc is similar to that of GG/borax, while in the case of Sclg/borax-dc tablets, it results reduced to one-half. Such difference can be explained in terms of transient linkages as reported below.

From our data, the estimated lifetime for cross-links in GG/borax is definitively lower (about 11 s) with respect to the swelling process (hours), as a consequence, such cross-links can be considered *dynamic* in the swelling time-scale. For Sclg, the dynamic character derives from the chemical–physical linkage that prevents the formation of permanent and stable bridges among the polysaccharidic chains.

Actually, the transient nature of the GG/borax linkages allows a microscopic reorganization of the chains nullifying the disturbing effect due to the second compression. On the other side, the local rearrangement of the bundles, for Sclg/borax-dc tablets, is clearly hampered when the anisotropic force is applied perpendicularly to the first one, probably due to the intrinsic stiffness of the Sclg triplexes.

Furthermore, the increasing of the GG rigidity observed in the MD simulations was obtained considering a short tract of GG with a high concentration of linked borax. This could not be the general case, as a nonhomogeneous distribution of borax (i.e., tracts with more or less borax) should better represent the system. This situation is also supported by the AFM images (Figure 7) that show extended and coiled chains.

This ambivalent and peculiar feature of GG chains (coiled and stretched) can explain both the similarity and the differences with respect to Sclg.

Nevertheless some aspects still remain to be clarified, such as the role played by the side-chains in the anomalous swelling. Two possible explanations can be proposed for the peculiar GG behavior. The first one takes into consideration, that, in the GG, the linkage of borax, occurs on the side chain pyranose; in this case it is difficult to imagine that the borax could induce a stiffness of GG/borax, determinant in promoting the formation of ordered domains in the sample. The other hypothesis, in our opinion more probable, is that the borax links everywhere along the GG chain, but, mainly the borax linked on the side-chain is involved in the formation of the interchain cross-links.

Conclusions

Both GG and Sclg are capable of forming gels in the presence of borax, and the tablets obtained by compression of the freeze-dried hydrogels show a similar anisotropic swelling in water, although GG assumes a flexible random coil conformation in water and Sclg is one of the more rigid polymer in nature. Also the interaction with borax takes place in a different way. In the case of Sclg the borax promotes mixed (chemical and physical) interactions between triplexes; on the contrary, in the case of GG the borax forms chemical bridges between chains by means of reversible linkages.

In spite of these differences, both systems are characterized by the presence of a high number of single pyranose side-chains. Furthermore, MD data and AFM images indicate that a high concentration of borax groups increases the stiffness of GG making such system more similar to Sclg-borax. During the swelling the labile nature of the borax cross-links in the GG makes the interchain interactions able to undergo the needed rearrangement, similar to Sclg where full chemical bridges are

not present. These similarities explain the strange parallel in the swelling between GG and Sclg, in the presence of borax.

On the other hand, the diversities between the two systems are reflected in the detected differences.

For example, when the tablets undergo a second compression in a direction perpendicular to the first one, the Sclg/borax system shows a remarkable reduction in the anisotropic swelling; for the GG/borax system swelling followed almost the same trend.

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