ELSEVIER

Carbohydrate Polymers 45 (2001) 363–372

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

Melting behaviour of schizophyllan extracellular polysaccharide gels in the temperature range between 5 and 20°C

A. Bot[a,*], H.E. Smorenburg[a], R. Vreeker[a], M. Pâques[a], A.H. Clark[b]

[a]*Unilever Research Vlaardingen, Olivier van Noortlaan 120, NL-3133 AT Vlaardingen, The Netherlands*
[b]*Unilever Research Colworth, Colworth House, Sharnbrook, Bedford MK44 1LQ, UK*

Received 30 November 1999; revised 8 May 2000; accepted 19 June 2000

Abstract

Gels of the glucan schizophyllan, consisting of a 1,3-β-D-linked backbone of glucose residues with 1,6-β-D-glucosyl side groups, were found to show melting behaviour in the temperature range between 5 and 20°C, depending on the glucose concentration in the solvent (0–50 wt% glucose). While the qualitative features of the modulus-versus-concentration and modulus-versus-temperature rheological data for the gels can be modelled using modified cascade theory (which implicitly assumes that no sub-level of organisation exists in the gel structure), a consistent quantitative fit cannot be achieved. The inconsistencies found are consistent with the idea that the gel is composed of bundles (consisting of many triple helices of schizophyllan) with strong intra-bundle attraction and weak inter-bundle forces. Transmission Electron Microscopy (TEM) micrographs of diluted samples indicate that schizophyllan polymers engage in lateral aggregation of triple helical strands at temperatures below the melting temperature, suggesting that indeed bundles of polymers will be present in the gel state.

Keywords: Fungus polysaccharide; Schizophyllan; Scleroglucan; Curdlan; Rheology; DSC; RI-TRALLS; TEM; Cascade theory

1. Introduction

Schizophyllan is a neutral extracellular polysaccharide produced by the fungus *Schizophyllan commune*. It consists of a 1,3-β-D-linked backbone of glucose residues, to which 1,6-β-D-glucosyl residues are attached. The backbone is identical to that of the polysaccharide curdlan, which lacks the glucosyl side chains, however (Sutherland, 1990). The molecular weight of native schizophyllan is typically of the order 10^6 Da (Norisuye, Yanaki & Fujita, 1980; Grisel & Muller, 1996; Tako, 1996).

Native schizophyllan occurs as triple helices in water (Norisuye et al., 1980) that are stable up to approximately 145°C. The material dissociates into single helices upon heating above this temperature, and the triple helices reform only partially upon subsequent cooling (Yanaki, Tabata & Kojima, 1985). The temperature of the transition from triple to single helices is reduced in solutions containing DMSO or concentrated NaOH (Tako, 1996; Brigand, 1993). Native schizophyllan is often used as a model polymer to study the viscosity of neutral linear rods (see e.g. Enomoto, Einaga & Teramoto, 1985; Chun & Park, 1994). Partially dissociated schizophyllan, however, forms a gel at room temperature at concentrations of typically 1 wt%.

The arrangement of schizophyllan chains in the triple helical structure is such that the glucosyl sidechains are on the outside of the helix. These sidechains prevent the formation of large insoluble aggregates of triple helices through hydrogen bonding. As a result, schizophyllan can be dissolved in water, in contrast to curdlan. Only at temperatures below 5°C, do associations between the triple helices dominate the structure. This results in the formation of a weak gel under these conditions. Many other polysaccharide gels show melting behaviour (e.g. agarose, κ- and ι-carrageenan, gellan), but usually at much higher temperatures.

Because of these peculiar melting properties, a rheological characterisation of schizophyllan gels was performed. As part of this, the possibility of shifting the transition around 5°C was investigated by changing the solvent quality through addition of various low molecular weight compounds: salts, glucose, HCl or NaOH. An attempt was also made to model the variation of the modulus with concentration and temperature using modified cascade theory. The results of this suggest that schizophyllan gels display two levels of organisation at low temperatures: schizophyllan triple helices, and aggregates of these in bundles, which in turn can form a physical network. This

* Corresponding author.
E-mail address: arjen.bot@unilever.com (A. Bot).

0144-8617/01/$ - see front matter
PII: S0144-8617(00)00270-8

interpretation is supported by a qualitative microstructural characterization of gels below and above the melting temperature.

2. Materials and methods

Schizophyllan (not purified, but without mycelia) was kindly supplied by Taito Co. Ltd.

The refractive index n at 20°C was found to vary as $n = 1.3328 + 0.1525w$, where w is the weight fraction of schizophyllan in the solution. From the density of a 1% solution, 1.00128 g/ml, we deduce that $dn/dc = 0.119$ ml/g. From refractive index/tri-angle laser light scattering experiments (RI-TRALLS) a molecular weight M_w of 2.0×10^6 Da ($M_n = 1.9 \times 10^6$ Da) was obtained. This value is in agreement with the data from the literature (Grisel & Muller, 1996; Tako, 1996), but lower values as a result of degradation during purification can also be found (Tako, 1996; Müller, Pretus, McNamee, Jones, Browder & Williams, 1995).

Differential Scanning Calorimetric (DSC) experiments were performed using a Perkin–Elmer 1020 Series DSC-7 Thermal Analysis System. Aluminum sample pans were filled with 10–20 mg schizophyllan solution, and sealed and loaded in the DSC-7 machine. DSC traces were obtained using a scanning rate of 2.5°C/min. Heat capacities in this study are expressed per gram of schizophyllan. A smooth background correction was applied to the data in order to obtain a constant base line.

Small deformation experiments were performed using a Carrimed CSL^2-50 stress-controlled rheometer, equipped with a cone and plate geometry (cone: diameter 4 cm, angle 2°). The maximum strain during oscillation was set to 0.01. The temperature was controlled within 0.1°C by a Peltier element in the plate. Evaporation of the sample was prevented using a water-lock. Temperature sweeps (1–80°C) were performed in typically 15 min frequency sweeps (2×10^{-2}–10^2 rad/s) and shear rate sweeps (3×10^{-3}–10^3 l/s) for the Cox–Merz test in typically 30 min.

The microstructural characteristics involved in the formation of the biopolymer network were studied on 1 and 10 wt% schizophyllan solutions. Structural characteristics of isolated triple helices were studied in highly diluted molecular solutions (0.0001, 0.0005, 0.001 wt%). The original biopolymer structure was replicated by application of a heavy metal coating using a shadowing procedure (Stokke & Elgsaeter, 1994). The heavy metal coat was stabilized with a carbon layer. The replicated structure of the biopolymers was transferred to a support grid and observed in the Philips CM12 Transmission Electron Microscope (TEM) at 120 kV. Digital images were taken by a Gatan 694 CCD type camera.

Highly diluted schizophyllan solutions. For characterization of the polymer structure above the melting temperature of the gel, 50 ml aqueous solutions of 0.0001, 0.0005, and 0.001 wt% schizophyllan at neutral pH were sprayed (35 cm spray distance) onto freshly cleaved mica (Tyler & Branton, 1980). Solutions of schizophyllan in pure water were investigated, as well as in 0.1 mol/l NH_4OH solutions and/or 50% glycerol solutions (Gekko, Mugishima & Koga, 1987). The schizophyllan strands showed preferred orientations on mica when dissolved in pure water or in solutions containing 50% glycerol, reflecting the crystalline symmetry of the mica. Such interactions between the biopolymers and the surface were not observed in solutions containing NH_4OH, and a homogeneous distribution of the orientation of the schizophyllan strands results. Mica pieces containing the sprayed solutions were placed on a specimen holder and transferred into the Cressington CFE50 freeze fracture and shadowing instrument. Vacuum drying was performed at 10^{-4} Pa and room temperature. Next, the specimen stage was cooled down to −180°C and the anti-contaminator to liquid nitrogen temperature. The specimens were rotated during shadowing with Pt/C (1.5 nm layer thickness) at an oblique angle (6°) relative to the specimen surface. Carbon (90°) backing (7 nm layer thickness) was used to stabilize the replica during transfer.

The specimens were transferred from the CFE50 to ambient conditions. By moving the specimen through an air/water interface the replica is separated from the mica. The floating replica was picked up with a 700 mesh Cu support grid, and transferred to the TEM.

For structure characterization below the gel melting temperature, the preparation steps up to vacuum drying were performed in a conditioned room at 2°C. All instruments, solutions, and gases, were equilibrated at this temperature before use. Transfer into the CFE50 was done on ice, and in a gaseous nitrogen atmosphere. The rest of the procedure followed was identical to the procedure at room temperature.

Concentrated schizophyllan solutions. The concentrated solutions were physically fixed using High Pressure Freezing (HPF) as a fixation technique (Studer, Michel, Wohlwend, Hunziker & Buschmann, 1995). Sample preparation involves the preparation of a sandwich consisting of a schizophyllan solution between two 100 μm specimen discs. The sandwich was left to rest in a humid chamber to prevent air-drying. This was done either at room temperature or by flotation on melting ice (0°C) to study the resulting network at those temperatures. During resting, the specimen was left unsheared for 1 h. Subsequently, the sandwich was covered with 1-hexadecene to exclude air bubbles, inserted in the HPF specimen holder, and frozen immediately at approximately 0.21 GPa. The frozen specimens were stored under liquid nitrogen.

To allow freeze fracturing and shadowing, the HPF sandwich was separated. This created a fracture surface through the frozen sample. The sample discs were transferred into the Cressington CFE50 freeze fracture and shadowing instrument.

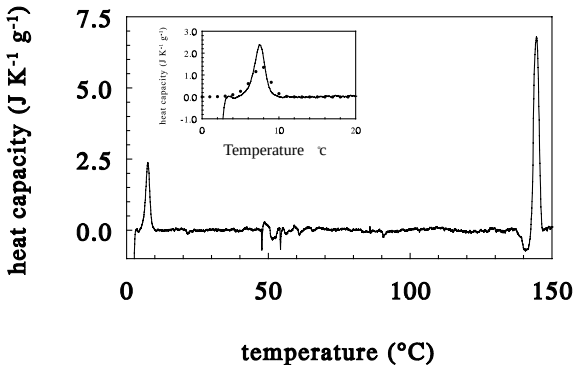

Fig. 1. DSC trace for a 10 wt% schizophyllan solution at a scanning rate of 2.5°C/min. The weight in the unit for the heat capacity refers to the weight of the polymer. The inset shows the DSC curve (solid line) and the prediction from the modified cascade model using the fitting parameters obtained from the rheological study (filled circles).

The frozen water was freeze-etched from the samples in the vacuum system of the CFE50. The 1 wt% gel was etched for 30 min, whereas the 10% gel was etched for 60 min. The resulting open network was rotary shadowed using 1.5 nm Pt\C, and in stationary mode using 8 nm carbon as a backing for the replica. The replica was floated and cleaned on distilled water and examined in the TEM.

3. Results and discussion

3.1. DSC

First, a qualitative DSC study was performed to confirm the existence of two transitions in the schizophyllan gels. Indeed, two enthalpic transitions were found from DSC on 10 wt% solutions: the first at 5°C has $\Delta H \approx 4$ J/g schizophyllan, the second at 145°C has $\Delta H \approx 13$ J/g schizophyllan (see Fig. 1). The first transition is associated with the dissociation of aggregates of triple helices (Tako, 1996) and is investigated in detail in the present study. The second transition should be interpreted in terms of melting of the triple helices themselves (Norisuye et al., 1980). The fitted line (dots) in the inset of Fig. 1 will be discussed in Section 3.3.

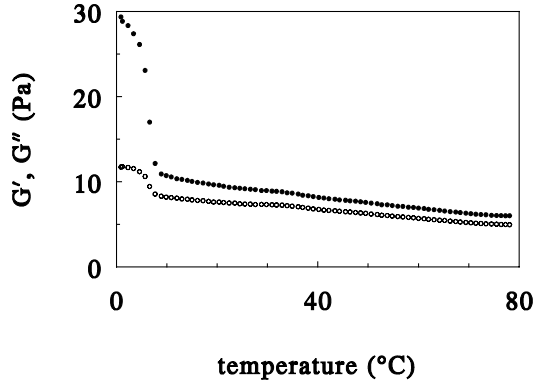

Fig. 2. Dynamic moduli as a function of temperature for a 1 wt% schizophyllan gel: (●) storage modulus G' (○) loss modulus G''. Temperature scanning rate 5°C/min.

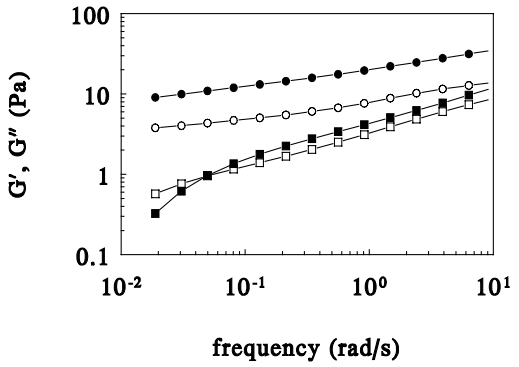

Fig. 3. Dynamic moduli as a function of frequency for a 1 wt% schizophyllan gel below and above the melting temperature: (●,○) 2°C; (■,□) 20°C. Filled symbols G', open symbols G''.

3.2. Rheology

Fig. 2 shows the storage modulus G' and the loss modulus G'' over a temperature range from 1 to 80°C. A melting transition is observed around 5°C, originating from associations between the triple helices in the solution (Tako, 1996). The transition temperature does not show any hysteresis, and occurs at the same temperature during heating and cooling.

Fig. 3 shows G' and G'' as a function of frequency ω. The data for the sample at 20°C show a cross-over at small frequencies and a pronounced frequency dependence, which is characteristic of a viscoelastic solution. The data for the sample at 2°C show a somewhat weaker frequency dependence, and $G' > G''$ over the full frequency range, as is characteristic of a weak gel.

Fig. 4 shows a Cox–Merz plot for schizophyllan. It can be seen that the $\eta(\mathrm{d}\gamma/\mathrm{d}t)$ and $\eta^*(\omega)$ curves obtained at 20°C superimpose, confirming that schizophyllan behaves as a viscous solution and does not form a (weak) gel at this

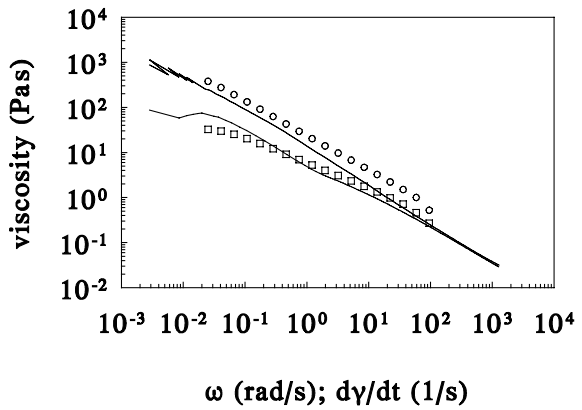

Fig. 4. Cox–Merz plot for a 1 wt% schizophyllan gel: (○) $\eta^*(\omega)$ at 2°C; (…) $\eta(\mathrm{d}\gamma/\mathrm{d}t)$ at 2°C; (□) $\eta^*(\omega)$ at 20°C; (—) $\eta(\mathrm{d}\gamma/\mathrm{d}t)$ at 20°C.

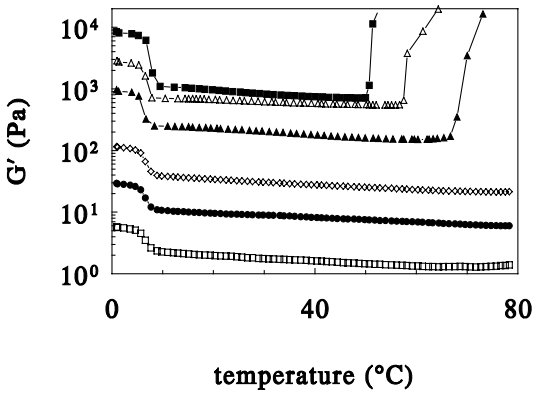

Fig. 5. Effect of schizophyllan concentration on the melting behaviour of schizophyllan gels. From bottom to top: 0.5, 1, 2, 5, 10 and 20 wt%. Temperature scanning rate 5°C/min.

temperature. Curves obtained at 2°C do not superimpose, especially at higher shear rates and frequencies. The differences are relatively small but reproduce in different experiments. The failure of superposition indicates the presence of a weak gel structure at this temperature (Clark & Ross-Murphy, 1987).

Fig. 5 demonstrates that the melting transition at 5°C is independent of schizophyllan concentration. Below the melting temperature, G' increases roughly with the square of the schizophyllan concentration. At higher concentration a new transition can be observed at high temperatures. This transition is probably related to an isotropic-liquid crystalline transition as described by Van, Norisuye and Teramoto (1981). The modulus shows clear hysteresis during a temperature cycle through this transition, in contrast to the nearly negligible hysteresis observed for the transition at 5°C. (The behaviour of G' as a function of schizophyllan concentration above the melting temperature is at the threshold of the reproducibility of our measurements, and we did not investigate this in any detail.)

In Fig. 6 the effect of glucose on the melting transition is shown. It can be seen that an increase in glucose concentration from 0 to 50 wt% shifts the transition from 5 to approximately 20°C. This effect should be attributed to a reduction in solvent quality; apparently, the solubility of schizophyllan decreases with increasing glucose concentration. The magnitude of the effect of glucose on the melting temperature is comparable to that of the addition of D-sorbitol to schizophyllan solutions (Fuchs, Richtering & Burchard, 1995). (Similar effects on scleroglucan gels were also observed, but not studied further in any detail.)

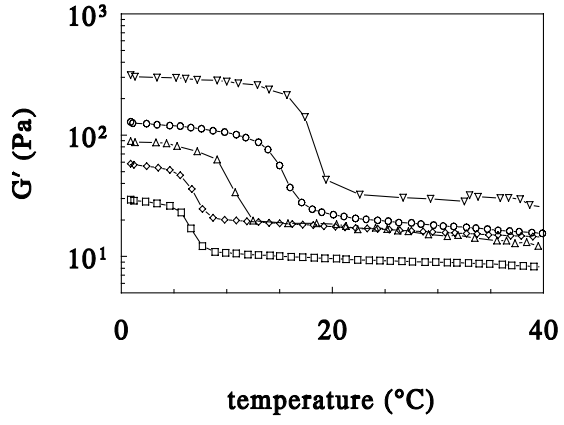

Fig. 6. Effect of glucose concentration (in wt%) on the melting behaviour of 1 wt% schizophyllan gels. Glucose concentration on total weight: (□) 0%, (◇) 1%, (△) 30%, (○) 40%, (▽) 50%. Temperature scanning rate 5°C/min.

Finally, this rheological section is concluded with some qualitative results on the presence of ions. The presence of certain salts is known to have a dramatic effect on the behaviour of schizophyllan (e.g. borate ions (Grisel & Muller, 1996), chromium salts (Brigand, 1993)). We investigated the effect of other additives to this transition also: KCl (0–3 mol/l) and pH (1.6–11.4). At extreme salt concentration associations between the triple helices are prevented. The transition at 5°C disappears at a KCl concentration of 3 mol/l, but at lower concentrations (0–1 mol/l) the transition remains basically unaffected. Associations between the triple helices are also prevented at very low or very high pH (1.6 or 11.4), but the transition remains essentially unaffected at intermediate values of the pH (2.8,4.5,7.5). We did not test whether the changes at very low or high pH were reversible upon re-neutralisation of the samples. However, schizophyllan is expected to be relatively resistant to hydrolysis because of the resistance shown by the chemically identical polysaccharide scleroglucan, produced by the fungus *Sclerotium rolfsii* (Brigand, 1993). Prolonged exposure to a strongly acidic environment will cause removal of a part of the glycosyl sidechains, however, resulting in a gel having curdlan-like properties.

3.3. Modelling

Cascade theory as modified by Clark (1993) and Clark, Evans and Farrer (1994) was used to model the melting profiles of schizophyllan gels for different concentrations. As already shown by Clark et al., the melting profile of a gel is characterised by a cross-link melting enthalpy ΔH_0 and entropy ΔS_0 (Clark et al., 1994). To first order, the steepness of the profile is determined by ΔH_0, whereas the melting temperature is determined by the balance between ΔS_0 and ΔH_0. In this approach the frequency dependence of the dynamic modulus is ignored, which is deemed justified in view of the steep melting profile of the gel (see Figs. 2 and 5) and the weak frequency dependence of the modulus (see Fig. 3).

To fit the present experimental data, a baseline subtraction had to be performed to correct for the residual modulus at temperatures above the melting point. This modulus is assumed to originate from entanglements in the polymer solution, which are not accounted for in the cascade theory (i.e. the modulus is assumed to be zero above the melting point).

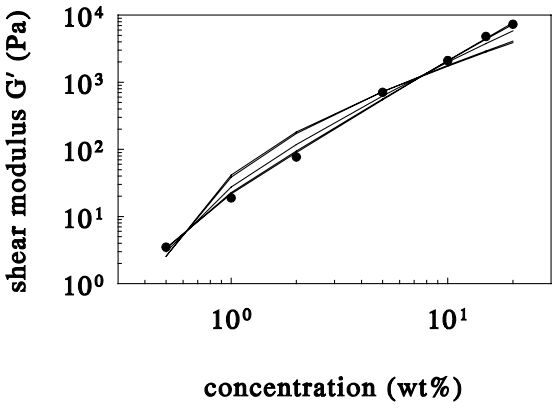

Fig. 7. Modulus versus schizophyllan concentration fits using the modified cascade model for gels at 1°C. Solid lines are fits for $f = 3$, 7, 100, 500 and 1000. The curves in this log–log plot become straighter as the functionality f of the model increases. Note that the residual modulus at temperatures above the melting point has been subtracted from the experimental data.

The data were fitted in two ways: modulus versus concentration and modulus versus temperature. Both approaches should result in similar gelling parameters. First we turn our attention to the modulus versus concentration fits. In this analysis, the concentration dependence of the modulus was investigated at temperatures below the melting point of the gel.

One of the input parameters for the cascade model is the 'functionality' f of the polymers composing the gel network (i.e. the number of potential sites along each polymer available for cross-linking). The qualities of fits obtained using low values for f were much lower than for fits using very high f values (see Fig. 7). Unfortunately, the nature of the cross-links in schizophyllan gels is not known, and therefore it is not possible to determine whether values for f up to 1000 are realistic. Results for gels at 1°C are shown in Table 1 and Fig. 7. The fitting results were not strongly temperature dependent in the range 0–5°C.

For the highest functionalities, the minimal concentration c_0 required to form a gel (i.e. the critical concentration) is found to be around 0.2 wt%. This is not inconsistent with experimental results, although experiments below 0.5 wt% were not performed. However, the front factor a representing the stiffness of the network strands is more problematic being very small for high f. The values obtained are in fact very near to the typical rubber elasticity value of unity, which seems improbable for a network based on stiffened ordered polysaccharides.

It is possible to surmount this difficulty by assuming that the basic network units are pre-formed thick bundles of strands (e.g. formed by 'crystallisation' during phase separation of the schizophyllan polymers) which randomly percolate to form a gel. In this case, the 'molecular' weight of the bundles should be used in the fits rather than the molecular weight of individual polymers. In terms of the cascade approach, this means that if the value of the 'molecular' weight is increased by, say, a factor 100, a increases in proportion without a change in fit quality or value of the critical concentration. The formation of strands is a cooperative process, and happens in a preliminary step. Subsequent formation of a random network from these building blocks then requires high values for the functionality, and for the molecular weight in a consistent and physically meaningful way.

Next we turn our attention to the modulus versus temperature analyses. From the discussion below it will emerge that it is not really possible to get results completely consistent with the modulus-versus-concentration fits. This is directly evident from the fact that only fits to the melt rheology using functionality $f = 7$ and melting enthalpy $\Delta H_0 = -1.2$ MJ/mol cross-links also reproduce the experimentally determined melting heat of approximately 4 J/g schizophyllan. The melting enthalpy per cross-link is fixed by the steepness of the melting profile observed in the rheological melt-down curve, and the total melting enthalpy of the transition by the value obtained from DSC measurements. The fitting results are listed in Table 2 and plotted in Fig. 8.

The fact that only $f = 7$ models realistically describe melting is inconsistent with the results obtained from the modulus versus concentration fits in Table 1. In these last fits, it was found that the $f = 7$ models do not fit particularly well. This inconsistency will be discussed at the end of the present section. First the conclusions from the fits in Table 2 are summarised:

- The enthalpy per cross-link ΔH_0 of -1.2 MJ/mol cross-links is very large, but consistent with the steep melting profile. In this sense the systems behave somewhat like gelatin (Clark et al., 1994).

Table 1
Fitting results at 1°C for the modulus G' as a function of concentration in the range 0.5–20 wt%. Σ is the sum of squared deviations based on $\log(G')$ values

	Temperature (°C)				
	1	1	1	1	1
Assumed M_w (g/mol)	2×10^6	2×10^6	2×10^6	2×10^6	2×10^6
Functionality f	3	7	100	500	1000
c_0 (wt%)	0.3109	0.3180	0.2507	0.2082	0.1970
K (l/mol)	4.289×10^5	2.156×10^4	82.25	3.866	1.018
Front factor a	16.03	6.329	1.467	0.8947	0.7925
Quality of fit Σ	0.3498	0.4110	0.08952	0.02669	0.02204

Table 2
Fitting results for the modulus G' as a function of temperature in the range 0–100°C. Σ is the sum of squared deviations based on $\log(G')$ values

	Polymer concentration (wt%)						
	0.5	1	2	5	10	15	20
Assumed M_w (g/mol)	2×10^6	2×10^6	2×10^6	2×10^6	2×10^6	2×10^6	2×10^6
Functionality f	7	7	7	7	7	7	7
Front factor a	0.168 ± 0.002	0.461 ± 0.003	0.9 ± 0.2	3.5 ± 0.1	4.7 ± 0.2	8.2 ± 0.2	8.9 ± 0.2
ΔH_0 (kJ/mol)	−1254 ± 42	−1254	−1254	−1254	−1254 ± 42	−1254 ± 42	−1254 ± 42
ΔS_0 (kJ/mol K)	−4.397	−4.397	−4.397	−4.414	−4.410	−4.410	−4.414 ± 0.265
Melting temperature T_m (°C)	7.36	7.67	8.04	7.46	8.11	8.33	8.30
Quality of fit Σ	0.018673	0.057669	0.036157	0.059097	0.15731	0.081986	0.026097

- The entropy per cross-link ΔS_0 of −4 kJ/mol cross-links is also large, but consistent with the very low melting temperature of the system. Again, this behaviour is similar to that of gelatin, but even more extreme. This large value for ΔS_0 is somewhat surprising, as the original triple helices were assumed to be quite rigid (Enomoto et al., 1985; Chun & Park, 1994) and little conformational change is expected to occur on formation of the bundles.
- The values of ΔH_0 and ΔS_0 are nearly concentration independent, in line with expectation.
- The melting temperature increases only slightly with concentration, which is consistent with steep co-operative melting. Again, similar behaviour is observed for gelatin.
- The parameter a increases markedly with concentration, which can only be explained in the simplest cascade approach in terms of a marked thickening of the network strands with concentration. This is not impossible, though it is not the most likely explanation for the present system (see below). In addition, a is much smaller than the corresponding result from the modulus versus concentration fits (for same functionality and molecular weight), except at the highest concentrations.
- The equilibrium constant K estimated from $\exp((\Delta S_0 - \Delta H_0/T)/R)$ (see Clark et al., 1994) is *much* greater than the corresponding value for $f = 7$ (using the same molecular weight of 2×10^6) shown in Table 1 for the modulus versus concentration fits; typically $K \approx 2\times10^8$ l/mol.

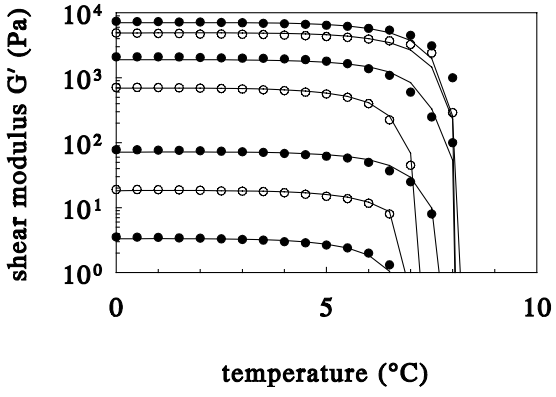

Fig. 8. Modulus versus temperature fits using the modified cascade model. Note that the residual modulus at temperatures above the melting point have been subtracted from the experimental data. From top to bottom: 20, 15, 10, 5, 2, 1, 0.5 wt% schizophyllan.

These last two points obviously cause some concern with regard to the use of the cascade model to interpret the present data. A possible explanation could be however that the parameters derived from the melt-down curves primarily reflect the melting of bundles of schizophyllan strands, whereas the concentration dependence reflects the concentration dependence of the number of associations between such bundles.

The fact that the melt-down parameters describe the melting behaviour of the bundles is illustrated in Fig. 1, which shows the DSC curve for a 10 wt% schizophyllan gel. The inset in Fig. 1 shows the low-temperature melting DSC peak, and a theoretical profile obtained from modified cascade theory, using input parameters derived from the rheological melt-down data. Agreement is fair: the experimental peak seems to be slightly sharper than the calculated peak. A small shift of 2.6°C in the temperature axis for the experimental data was applied to allow for inaccuracies in the calibration of the DSC machine. (The high-temperature peak at 145°C is caused by the melting of the triple helices, and appears to have both an exothermic and endothermic component. The wiggles around 50°C might be related to the sudden increase in modulus near that temperature as observed in Fig. 5. The extremely sharp endothermic spikes in that region are, however, electronic artifacts.)

Clearly, the cascade model in its current simple form is unable to provide a self-consistent fit to the concentration dependence of the melt-down rheology of schizophyllan gels. This leads to the situation where the enthalpy and entropy derived from the melt-down data imply very strong network cross-links, and a very low critical concentration, if taken literally. The parameter a is then set anomalously low to compensate. The fact that the cascade theory can describe the modulus–concentration relationship shows that inter-bundle cross-linking does change roughly quadratically with concentration and happens at random. The melting seems to take place sufficiently cooperatively that the inter-bundle cross-links vanish at the same time as the bundles melt; in this view, the high enthalpy of melting of

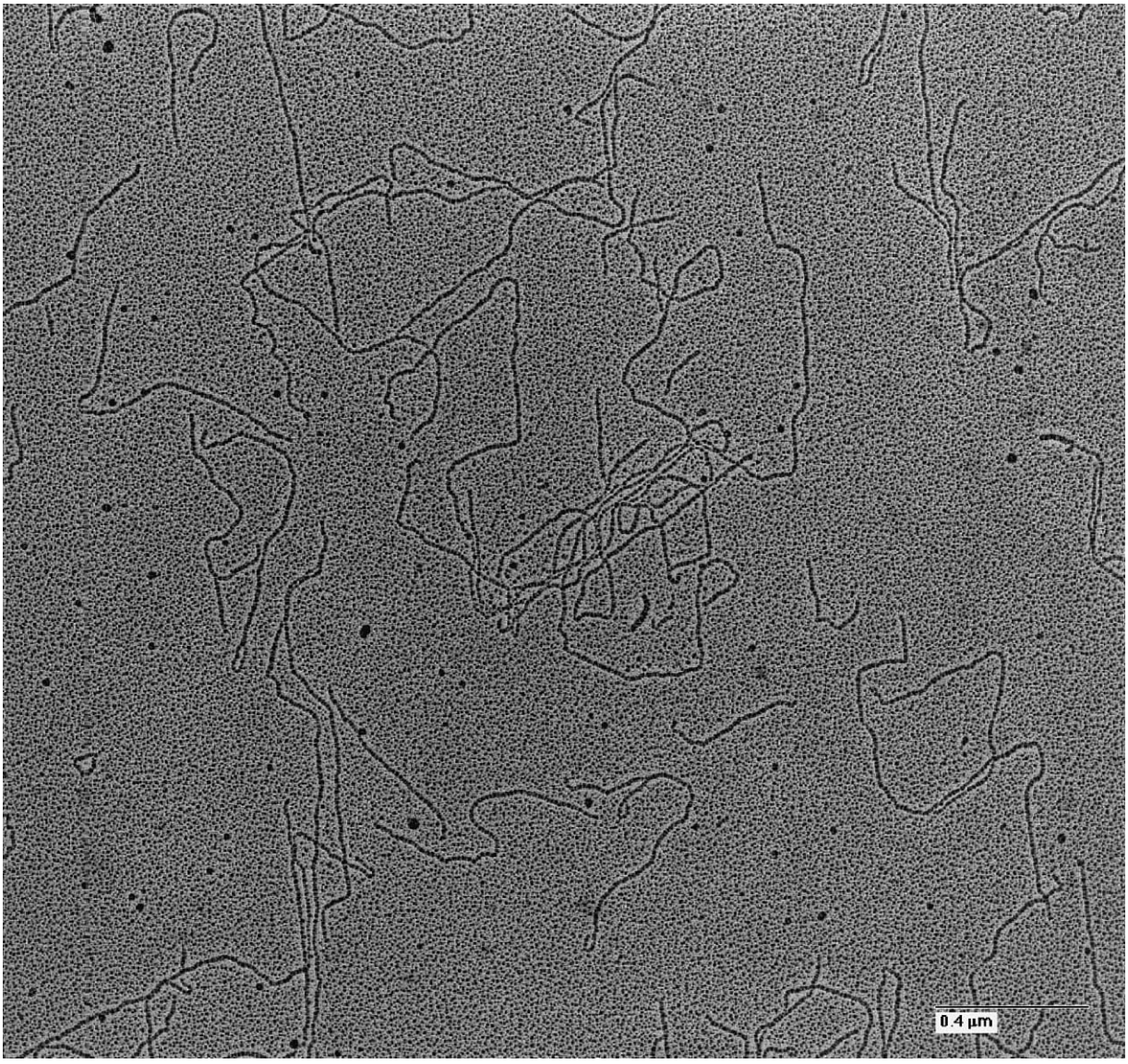

Fig. 9. TEM picture of dilute schizophyllan solution above the melting temperature.

the gels demonstrates the large number of intra-bundle associations involved in melting of the bundles. The inconsistency in the parameters f and a derived from both types of fits, shows that the model is not sophisticated enough to deal with this kind of melting in a satisfactory way. The analysis does, however, provide insight into the nature of schizophyllan gelation and indicates the direction in which improvement of the model has to proceed.

3.4. Microscopy

A qualitative microscopy study on dilute spray-dried schizophyllan solutions was performed to confirm the existence of interactions between the polysaccharide strands at low temperatures. The vacuum drying procedure that is applied to the samples requires that individual polysaccharides show some interaction with the substrate, in order to prevent the creation of artifacts by the moving water front during evaporation of the water. Extreme inhomogeneities may indicate that the interaction of the polymers with the substrate is insufficient. Thus, the observation of inhomogeneities in the polysaccharide distribution on micrographs of spray-dried schizophyllan solutions does not necessarily imply interactions between the polymers. However, any difference between micrographs obtained for samples spray-dried above and below 5°C should serve as a very strong indication of the existence of interactions between the strands.

Fig. 9 shows a homogeneous distribution for schizophyllan strands obtained from solutions spray-dried at room temperature. In contrast, micrographs from samples spray-dried at 2°C show a clearly inhomogeneous distribution of strands over the mica surface (Fig. 10). Parallel alignment between composite strands is observed frequently in this micrograph, suggesting that lateral aggregation of

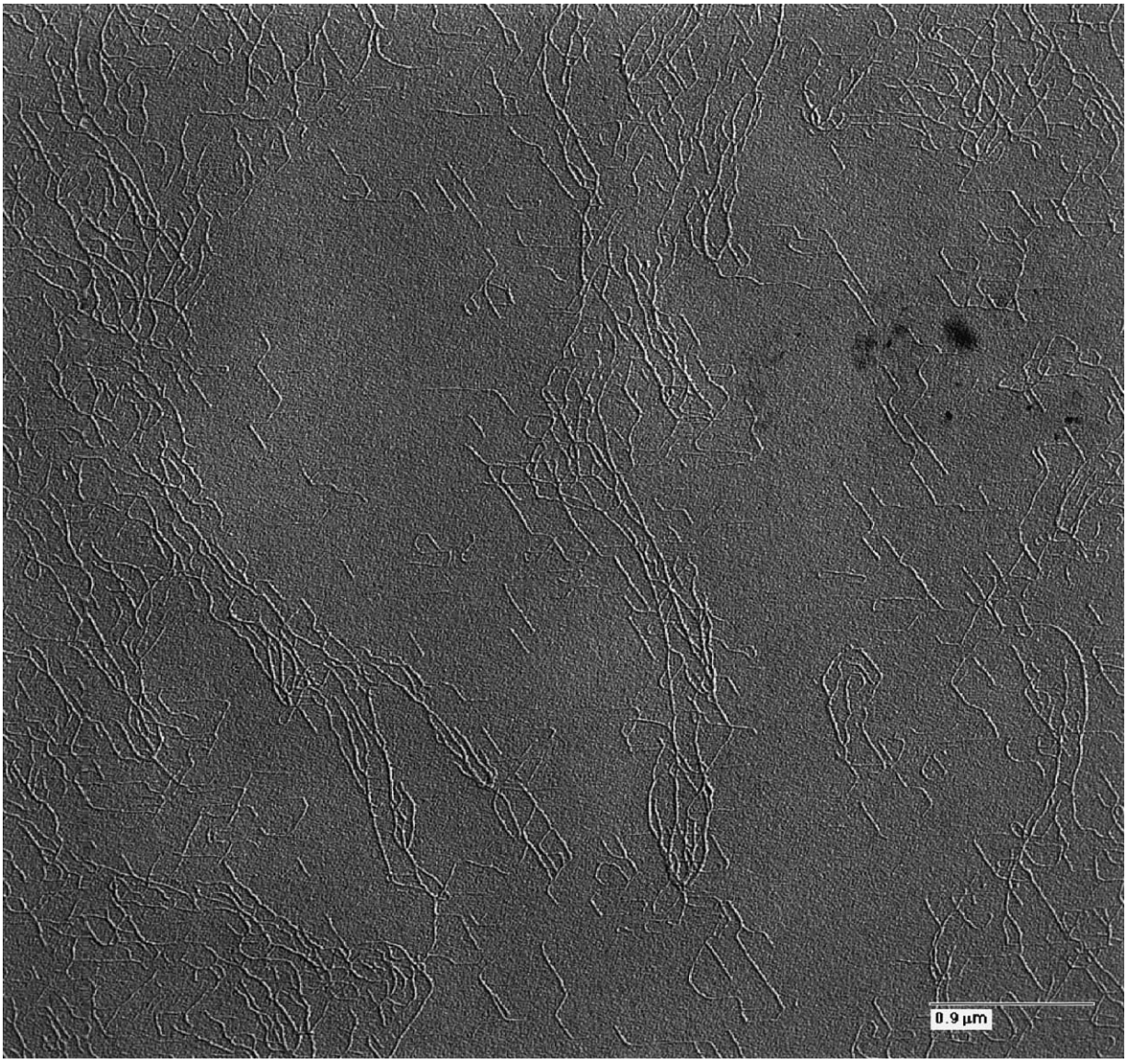

Fig. 10. TEM picture of dilute schizophyllan solution below the melting temperature: inhomogeneous distribution in sprayed sample.

schizophyllan strands is the basis for gelling. Such interactions are reminiscent of the schematic representation of schizophyllan interactions proposed by Fuchs, Richtering, Burchard, Kajiwara and Kitamura (1997), and would be consistent with the concept of bundles of polymers as developed in Section 3.3.

It should be emphasized that the micrographs obtained for dilute solutions do not contain information regarding the gel structure above the critical concentration, which is approximately 0.2 wt%. Those micrographs were primarily intended to derive information on the interactions between the schizophyllan strands. TEM micrographs of concentrated schizophyllan solutions at room temperature show a disordered highly entangled solution of loosely packed strands (Fig. 11) with relatively little orientational order on short length scales. Locally, structures occur consisting of several strands instead of single strands. These micrographs are quite similar to those of other polysaccharide systems (see e.g. Brigham, Gidley, Hoffmann & Smith, 1994).

4. Conclusions

Gels from schizophyllan were found to show melting behaviour in the temperature range between 5 and 20°C, depending on the glucose concentration in the solvent (0–50 wt% glucose). Melting behaviour has been reported for many other gelling polysaccharides, but usually in a completely different temperature range. Transmission Electron Microscopy micrographs of diluted samples suggest that schizophyllan polymers engage in lateral aggregation of strands at temperatures below the melting temperature,

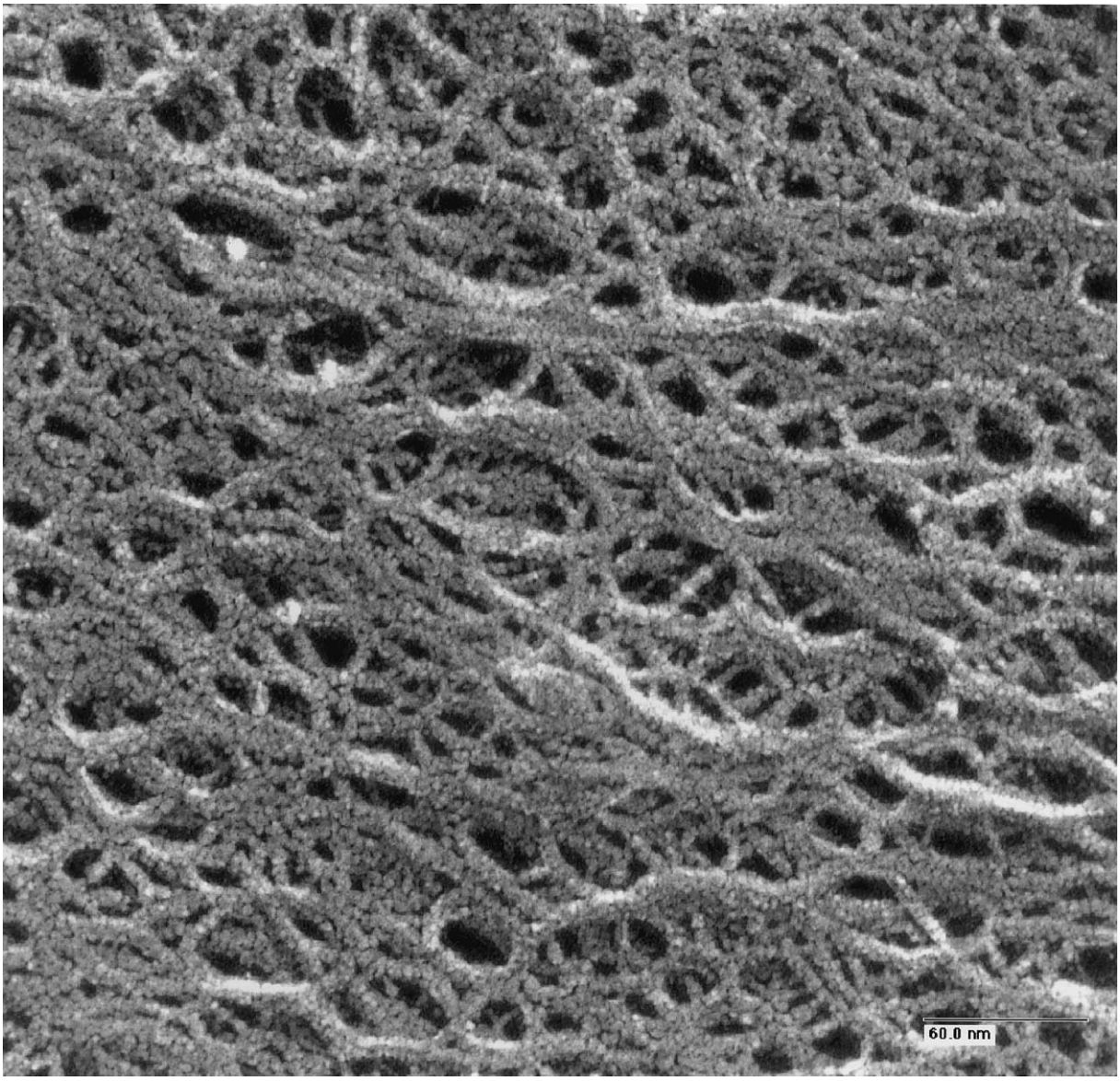

Fig. 11. TEM picture of a concentrated 10 wt% schizophyllan solution at room temperature.

suggesting that bundles of polymers will be present in the gel state. Indeed, the qualitative features of the modulus-versus-concentration and -temperature data for the gels can be modelled using modified cascade theory (which implicitly assumes that no sub-level of organisation exists in the gel structure), but a consistent overall quantitative fit cannot be made. The inconsistencies in modelling suggest that the gel is composed of bundles (consisting of many individual polymers) with strong intra-bundle attractions and weak inter-bundle forces. Such a description of schizophyllan (and most likely scleroglucan) gels appears to be consistent with their microscopic images.

Acknowledgements

Schizophyllan was kindly supplied by Taito Co. Ltd. (Kobe, Japan). The authors would like to thank R. den Adel, W.M. du Chatinier, J. Hazekamp and H. Meder for experimental support. W.G.M. Agterof is thanked for fruitful discussions.

References

Brigand, G. (1993). Scleroglucan. In R. L. Whistler & J. N. BeMiller, *Industrial gums* (3rd ed.). (pp. 461–474). London: Academic Press (chap. 17).

Brigham, J. E., Gidley, M. J., Hoffmann, R. A., & Smith, G. C. (1994). Microscopic imaging of network strands in agar, carrageenan, locust bean gum and kappa-carrageenan locust bean gum gels. *Food Hydrocolloids*, *8*, 331–344.

Chun, M. S., & Park, O. O. (1994). On the intrinsic viscosity of anionic and nonionic rodlike polysaccharide solutions. *Macromolecular Chemistry and Physics*, *195*, 701–711.

Clark, A. H. (1993). Biopolymer gelation: comparison of reversible and irreversible cross-link descriptions. *Polymer Gels and Networks*, *1*, 139–158.

Clark, A. H., & Ross-Murphy, S. B. (1987). Structural and mechanical properties of biopolymer gels. *Advances in Polymer Science*, *83*, 57–192.

Clark, A. H., Evans, K. T., & Farrer, D. B. (1994). Shear modulus-temperature meltdown profiles of gelatin and pectin gels. A cascade theory description. *International Journal of Biological Macromolecules*, *16*, 125–130.

Enomoto, H., Einaga, Y., & Teramoto, A. (1985). Viscosity of concentrated solutions of rodlike polymers. *Macromolecules*, *18*, 2695–2702.

Fuchs, T., Richtering, W., & Burchard, W. (1995). Thermoreversible gelation of a polysaccharide with immunological activity: rheology and dynamic light scattering. *Macromolecular Symposia*, *99*, 227–238.

Fuchs, T., Richtering, W., Burchard, W., Kajiwara, K., & Kitamura, S. (1997). Gel point in physical gels: rheology and light scattering from thermoreversibly gelling schizophyllan. *Polymer Gels and Networks*, *5*, 541–559.

Gekko, K., Mugishima, H., & Koga, S. (1987). Effects of sugars and polyols on the sol–gel transition of kappa-carrageenan: calorimetric study. *International Journal of Biological Macromolecules*, *9*, 146–152.

Grisel, M., & Muller, G. (1996). Rheological properties of schizophyllan in presence of borate ions. *Progress in Colloid and Polymer Science*, *102*, 32–37.

Müller, A., Pretus, H. A., McNamee, R. B., Jones, E. L., Browder, I. W., & Williams, D. L. (1995). Comparison of the carbohydrate biological response modifiers krestin, schizophyllan and glucan phosphate by aqueous size exclusion chromatography with in-line argon-ion multi-angle laser light scattering photometry and differential viscometry detectors. *Journal of Chromatography B*, *666*, 283–290.

Norisuye, T., Yanaki, T., & Fujita, H. (1980). Triple helix of a *Schizophyllum commune* polysaccharide in aqueous solution. *Journal of Polymer Science: Polymer Physics Edition*, *18*, 547–558.

Stokke, B. T., & Elgsaeter, J. A. (1994). Conformation, order–disorder conformational transitions and gelation of non-crystalline polysaccharides studied using electron microscopy. *Micron*, *25*, 469–491.

Studer, D., Michel, M., Wohlwend, M., Hunziker, E. B., & Buschmann, M. D. (1995). Vitrification of articular-cartilage by high-pressure freezing. *Journal of Microscopy (Oxford)*, *179*, 321–332.

Sutherland, I. W. (1990). *Biotechnology of microbial exopolysaccharides*, 3. Cambridge: Cambridge University Press (chap. 3, pp. 21–22).

Tako, M. (1996). Molecular origin of the thermal stability of schizophyllan. *Polymer Gels and Networks*, *4*, 303–313.

Tyler, J. M., & Branton, D. (1980). Rotary shadowing of extended molecules dried from glycerol. *Journal of Ultrastructure Research*, *71*, 95–102.

Van, K., Norisuye, T., & Teramoto, A. (1981). Liquid crystal formation in aqueous solutions of a polysaccharide schizophyllan. *Molecular Crystals and Liquid Crystals*, *78*, 123–134.

Yanaki, T., Tabata, K., & Kojima, T. (1985). Melting behaviour of a triple helical polysaccharide schizophyllan in aqueous solution. *Carbohydrate Polymers*, *5*, 275–283.