ELSEVIER

Carbohydrate Polymers 46 (2001) 241–248

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

Gelation of periodate oxidised scleroglucan (scleraldehyde)

B.E. Christensen[a,*], E. Aasprong[a], B.T. Stokke[b]

[a]NOBIPOL, Department of Biotechnology, Norwegian University of Science and Technology, N-7491 Trondheim, Norway
[b]Department of Physics, Norwegian University of Science and Technology, N-7491 Trondheim, Norway

Received 10 May 2000; revised 29 August 2000; accepted 27 September 2000

Abstract

Polysaccharides such as scleroglucan and schizophyllan contain periodate-resistant β-1,3-linked D-glucan backbones with regularly distributed side chains consisting primarily of single β-1,6-linked D-glucose residues that are susceptible to periodate oxidation. Side chain oxidation results in the formation of dialdehyde groups and the production of formic acid. During the initial periodate oxidation an increase in the storage modulus (G') associated with the formation of gels, was observed when the reduced concentration, $C_p[\eta]$ was above 3. These gels appeared to be physically stable, whereas aldehyde reduction (e.g. with $NaBH_4$) or oxidation to carboxyl (e.g. with $NaClO_2$ in acetic acid) dissolved the gels. The mechanism of cross-linking can be explained by the formation of *inter*molecular hemiacetal linkages. The gels were further characterised rheologically in terms of the initial increase in the storage modulus and the stability when stored in seawater at 55 and 90°C, respectively.

Keywords: Scleroglucan; Periodate oxidation; Scleraldehyde

1. Introduction

Scleroglucan belongs to a family of high molecular weight, neutral, water-soluble fungal β-1,3-D-glucans containing short branches, in this case a single β-1,6-linked D-glucose residue linked to every third residue of the glucan backbone (Johnson, Kirkwood, Misaki, Nelson, Scaletti & Smith, 1963) (Fig. 1). Such glucans adopt a higher order structure in aqueous solutions consisting of a triple-stranded structure where the side chains are exposed towards the exterior (Bluhm, Deslandes, Marchessault, Perez & Rinaudo, 1982; Yanaki, Tabata & Kojima, 1985). The resulting structure becomes very stiff, with a persistence length in the range of 150 nm (Kashiwagi, Norisuye & Fujita, 1981). Combined with a weight average molecular weight (M_w) well above 10^6 g/mol, scleroglucan becomes a powerful viscosifier. Scleroglucan exhibits pronounced shear thinning, and the viscosity is largely insensitive towards variations in pH and ionic strength (Stokke, Elgsaeter, Bjornestad & Lund, 1992). Grassi and co-workers reported changes in rheological behaviour that were interpreted in terms of a concentration dependent transition from sol to weak gel (Grassi, Lapasin & Pricl, 1996). Moreover, a transition towards more solid-like behaviour occurs in aqueous scleroglucan solutions upon reducing the temperature towards 0°C (Bluhm et al., 1982). Branched β-1,3-glucans of the scleroglucan type also exhibit interesting immunostimulating properties (Bohn & BeMiller, 1995). The triple-stranded structure also leads to enhanced stability towards depolymerisation (Hjerde, Stokke, Smidsrød & Christensen, 1998).

The 1,6-linked glucose side chains are susceptible to periodate oxidation, a simple and well-understood reaction which is normally used as an analytical tool in structural elucidation of complex carbohydrates (Perlin, 1970). Periodate oxidised starch (starch dialdehyde) is one of the very few examples where periodate oxidation is used to obtain new properties in industrial polysaccharides (Veelaert, deWit, Gotlieb & Verhe, 1997a,b). Periodate oxidation results in the formation of a reactive dialdehyde between vicinal hydroxyl groups. In scleroglucan the kinetics and stoichiometry of this reaction are expected to be analogous to those of simple glycosides such as methyl β-D-glucopyranoside (Aalmo & Painter, 1981). Fig. 1 shows a general scheme for periodate oxidation of the side chains in scleroglucan. Two different dialdehydes (S and S′) may be formed after a single attack of periodate, whereas a second attack on either of these yields another dialdehyde (D) with a concomitant release of formic acid. The reaction is a competitive, consecutive, second-order reaction. However, the intermediates S and S′ may further exist in unreactive, cyclic, hemiacetal forms (Aalmo & Painter, 1981 and references herein). By analysing the molar proportions of periodate consumed (P_t) and formic acid released

* Corresponding author.

0144-8617/01/$ - see front matter
PII: S0144-8617(00)00327-1

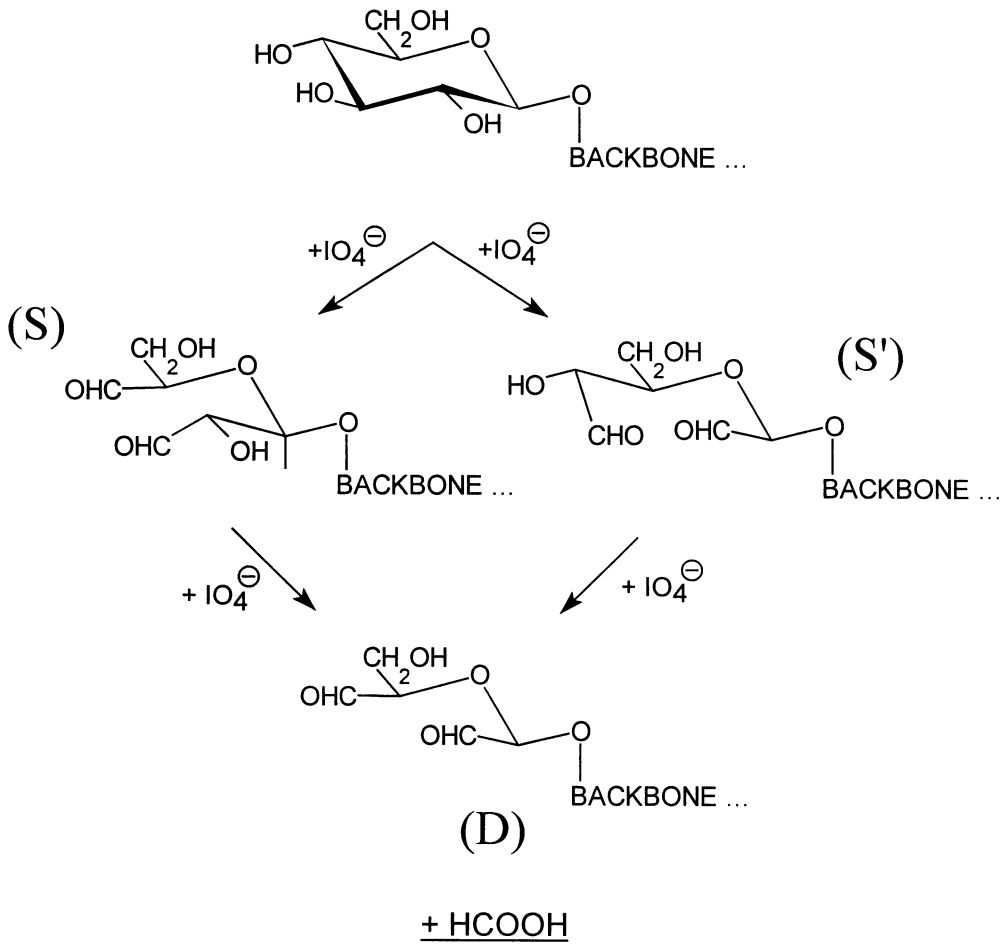

Fig. 1. Selective periodate oxidation of the side chain (a single D-glucose residue) in scleroglucan. The two singly oxidised intermediates (S and S′) may be further oxidised to form a doubly oxidised residue (D′).

(F_t), the proportions of singly ($S + S'$) and doubly (D) oxidised residues may be calculated (Aalmo & Painter, 1981). Partially oxidised scleroglucan with a predetermined degree of oxidation may be produced by controlling the stoichiometry between the periodate and the scleroglucan side chains.

The reactive aldehyde groups of partially or fully oxidised scleroglucan may react further, for instance by reduction with $NaBH_4$ to form the corresponding 'polyalcohol' (Schulz & Rapp, 1991) or oxidation with $NaClO_2$ (in acetic acid) to form 'sclerox', the corresponding polycarboxylate (Crescenzi, Gamini & Paradossi, 1983). The latter has been studied in considerable detail by Crescenzi and co-workers (Bosco, Sussich, Gamini, Reisenhofer, Adami & Rizzo, 1995; Coviello, Dentini & Crescenzi, 1995a; Coviello, Dentini, Crescenzi & Vincenti, 1995b; Crescenzi, Imbriaco, Velàsquez, Dentini & Ciferri, 1995; Gamini, Crescenzi & Abruzzese, 1984). Reaction of scleraldehyde with polyamines such as chitosans leads to gelation (Crescenzi et al., 1995; Guo, Elgsaeter & Stokke, 1998). Scleroglucan or scleraldehyde based gels and networks are of particular interest in the biomedical or pharmaceutical area because they may combine the immunostimulating properties of scleroglucan with the gel state. Such gels may also be useful in various oil field processes.

Crescenzi et al. (1983) also noted that soft gels were formed upon treatment of scleroglucan with $NaIO_4$, although the gels dissolved upon subsequent oxidation with $NaClO_2$. The gelation mechanism was not investigated, and in the present paper we reinvestigate the gelation, which occurs during periodate oxidation. It is argued that gelation occurs by the formation of intermolecular hemiacetals. Data on the gelation kinetics and stability of the gels are presented.

2. Experimental

2.1. Samples

Scleroglucan was obtained as a concentrated fermentation broth from Norferm ASA (Stavanger, Norway). The broth was purified as described earlier (Hjerde, Kristiansen,

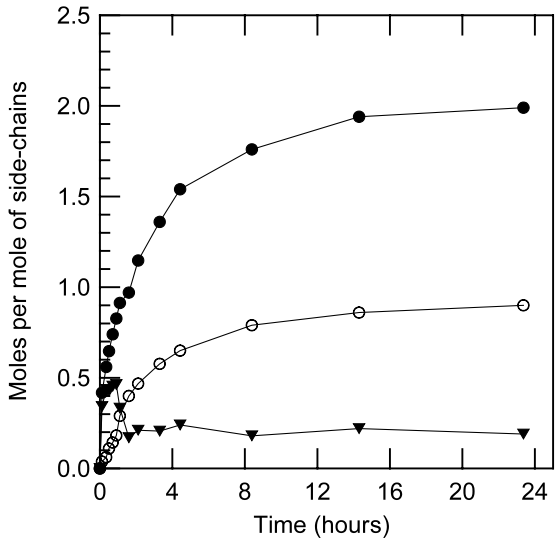

Fig. 2. Consumption of periodate (●), formation of formic acid (○), and the calculated proportion of singly (▼) oxidised intermediates formed upon periodate oxidation of scleroglucan at 20°C. All values are expressed as moles per mole of glucose side chains.

Stokke, Smidsrød & Christensen, 1994). Alternatively, powdered scleroglucan (Actigum CS11, Sanofi), $[\eta] = 3300$ ml/g (Guo et al., 1998) was used without further purification.

2.2. Chemicals

All reagents except urea were p.a. grade

2.3. Analytical periodate oxidation

Analysis of the amount of periodate consumed and formic acid released was carried out as described by Aalmo and Painter (1981).

2.4. Preparation of gels

Scleroglucan CS 11 stock solutions were prepared by dispersing the powder in water to a final concentration of 4–20 mg/ml and stirring for 24 h at 37°C or 48 h at room temperature. An aqueous solution of 0.3 M $NaIO_4$ was added to obtain a final concentration of 1 mol of $NaIO_4$ per mole of repeating units (648 g) of scleroglucan. The viscous solution was poured into a cylindrical tube ($d = 10$ mm, $l = 300$ mm), sealed and left overnight at room temperature. The resulting gel was removed from the tube and cut into pieces of 20 mm length. Gels were also made in the wells of Costar tissue culture plates ($d = 16$ mm, $l = 18$ mm), or loaded onto the sample stage of the rheometer to determine the storage and loss moduli at a selected frequency as function of time. Some of the gels were prepared at final salt concentrations corresponding to seawater by adding five times concentrated synthetic seawater (27.8 g/l NaCl, 9.54 g/l $MgCl_2{\cdot}6H_2O$, 1.62 g/l $CaCl_2{\cdot}2H_2O$, 0.027 g/l $SrCl_2$ and 7.73 g/l $Na_2SO_4{\cdot}10H_2O$) with a volume of 1/5 of the final volume.

2.5. Stability/dissolution tests

Cylindrical gels ($d = 10$ mm, $l = 20$ mm) were immersed in 28 ml of each of the following reagents: pure water, 1 M $NaBH_4$, 0.1 M HCl, 1 M CH_3COOH, 0.01 and 0.1 M NaOH, 6 M urea, 1 M LiI, 4 M KSCN, pure DMSO. Samples were prepared in duplicate and stored at room temperature and 80°C (except for $NaBH_4$), respectively. Changes in the structure and integrity of the gels were observed visually for a period of up to seven months. Semi-quantitative determination of changes in elasticity of gels (cured for 48 h) stored in synthetic seawater were carried out by determination of the storage modulus of the gels firmly fixed to the PP15 geometry of a Bohlin VOR rheometer using acrylate based glue (Moe, Draget, Skjåk-Bræk & Smidsrød, 1992). The average storage modulus, $\langle G' \rangle_{\omega,n}$ represents the mean values of the practically frequency-independent G' for $\omega \in (0.0628\text{–}6.28)\ s^{-1}$ for $n = 3\text{–}5$ independent gels. Various extents of swelling were corrected for as described by Moe et al. (1992).

2.6. Rheological investigations

Moduli of rigidity (E) of the cylindrical gels described above were measured using a Stevens texture analyser. The time dependence of the storage and loss moduli of the developing network structure was determined using the following procedure. Two millilitres of the scleroglucan stock solution was mixed with freshly dissolved, 0.3 M $NaIO_4$ solution. An aliquot of 1 ml of this mixture was rapidly transferred to the 30 mm diameter serrated parallel plate geometry (SP30) of a Bohlin VOR rheometer. A gap distance of 0.9–0.96 mm, and a 4 g cm torsion bar were employed for determination of the rheological properties at a fixed frequency and temperature at intervals of 2–5 min. The maximum strain was kept well below 0.01 in all rheological determinations. Possible artefacts associated with evaporation of the solvent during the gelation experiments were minimised using a low density, low viscosity silicon oil to seal the sample.

3. Results

3.1. Kinetics of periodate oxidation

Fig. 2 shows results obtained from the oxidation of scleroglucan (2 mg/ml) by an excess of $NaIO_4$ (10.5 mM) at 20°C. As expected from the structure, scleroglucan consumes up to 2 mol of periodate per mole of repeating unit, with a concomitant release of up to 1 mol of formic acid. The kinetics and stoichiometry of the reactions are basically similar to those of e.g. methyl-β-D-glucopyranoside (data not shown).

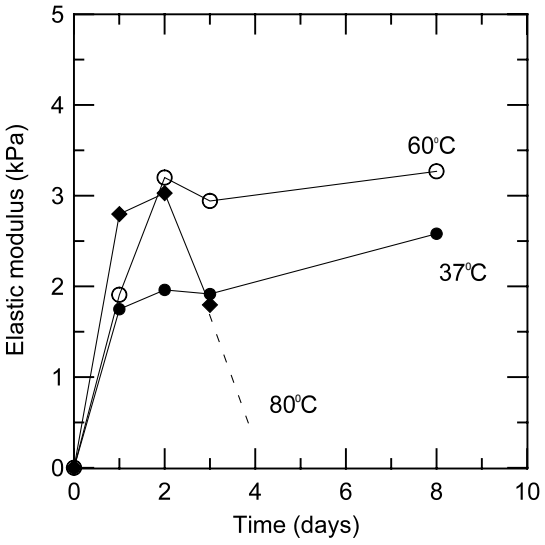

Fig. 3. Dependence of the gelation time and gelation temperature on the elastic modulus of scleroglucan gels (20 mg/ml) formed upon oxidation with periodate (4.9 mM).

3.2. Gel formation — initial observations

The addition of $NaIO_4$ to aqueous solutions of scleroglucan at relatively high concentrations (4–20 mg/ml) led to the formation of soft gels within a few hours at room temperature. The gels were transparent with a tendency to syneresis. The elastic modulus (measured at room temperature) for 20 mg/ml scleroglucan gelled in the presence of $NaIO_4$ (4.9 mM) was in the range of 2–3.5 kPa (Fig. 3). The modulus showed only a marginal increase after one day of reaction, and depended little on the temperature of gelation up to 60°C. At 80°C gelation initially followed the same increase in elastic modulus up to two days. For longer times the modulus decreased and the gels shrunk and started to disintegrate.

Gelation at room temperature appeared to be independent of pH in the range 1–12, whereas above pH 12 no gelation occurred in the presence of $NaIO_4$. Addition of lead tetraacetate, an agent that also cleaves vicinal diols, to solutions of scleroglucan in DMSO, also yielded a gel-like precipitate. The reaction appeared to be too rapid to obtain homogeneous gels.

3.3. Rheological investigations

Fig. 4 shows the storage modulus, G' ($\omega = 0.628\ s^{-1}$) at 30°C versus time after addition of $NaIO_4$ to the scleroglucan solutions for final polymer concentrations (C_p) from 1 to 12.5 mg/ml. The stoichiometric ratio between scleroglucan and periodate was constant in this particular series of experiments. The dependency of C_p on the gelation process is somewhat complex. Initially, G' increases monotonously

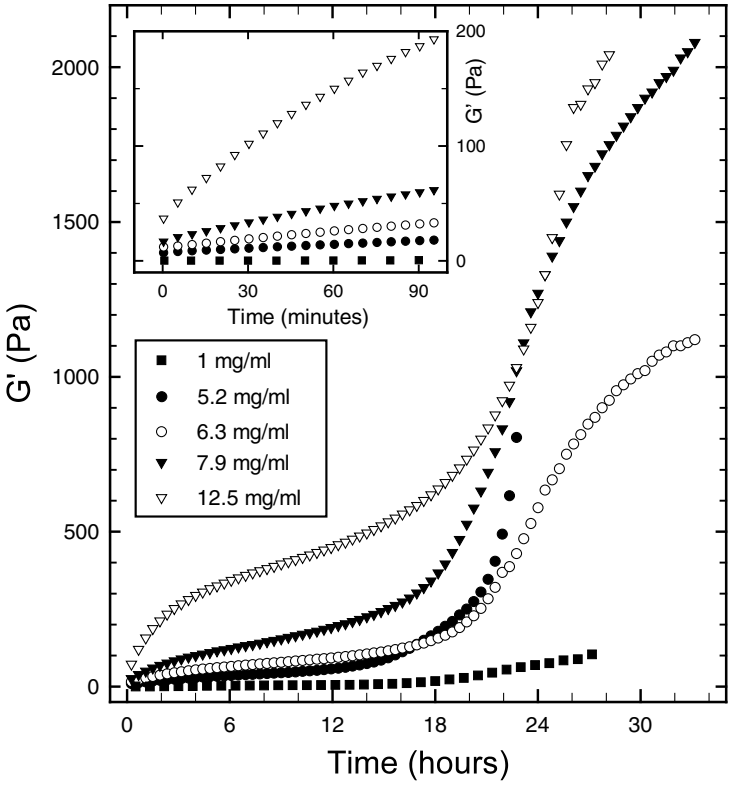

Fig. 4. Storage modulus G' ($\omega = 0.628\ s^{-1}$) versus incubation time at $T = 30$°C for $NaIO_4$ induced gelation of scleroglucan ($[\eta] = 3300$ ml/g) at polymer concentration $C_p = 1.0$ mg/ml (■), 5.2 mg/ml (●), 6.3 mg/ml (○), 7.9 mg/ml (▼) and 12.5 mg/ml (▽) in synthetic seawater. The stoichiometric molar ratio of $NaIO_4$ to scleroglucan side chains [$NaIO_4$]/[Scl] was 2.5.

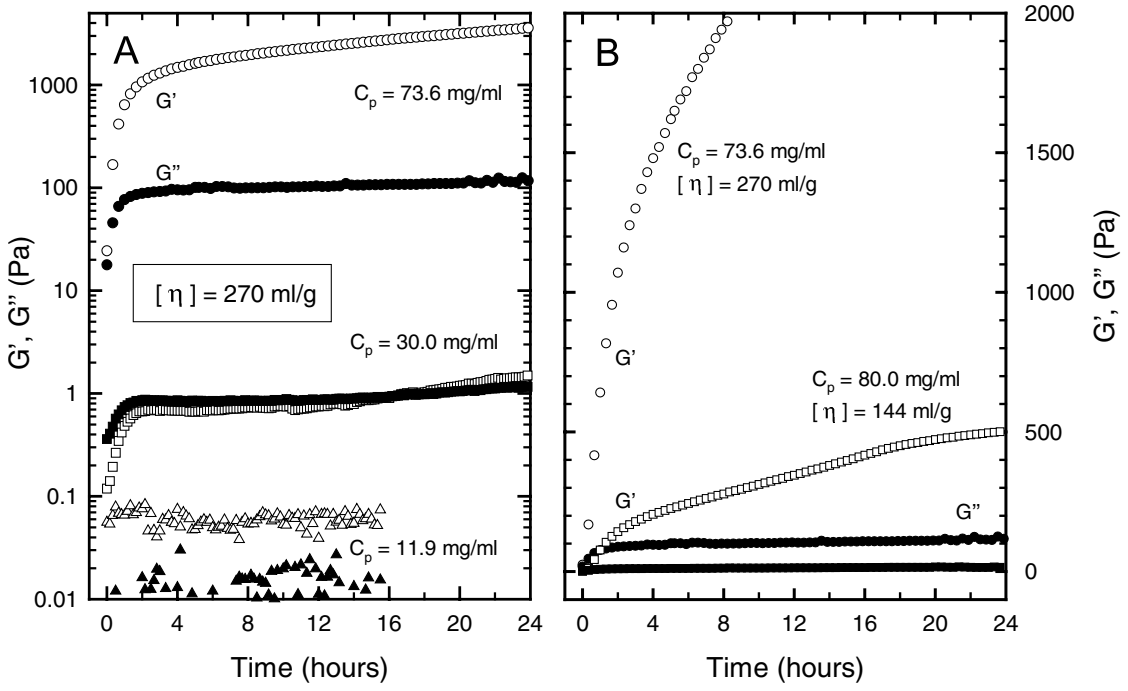

Fig. 5. Storage G' ($\omega = 0.628\ s^{-1}$) (open symbols) and loss moduli G'' ($\omega = 0.628\ s^{-1}$) (filled symbols) versus incubation time at $T = 30°C$ for $NaIO_4$ induced gelation of (A) scleroglucan with intrinsic viscosity $[\eta] = 270$ ml/g at polymer concentration $C_p = 11.9$ mg/ml (△, ▲), 30.0 mg/ml (■, □), and 73.6 mg/ml (●, ○), and (B) scleroglucan, $[\eta] = 270$ ml/g at 73.6 mg/ml (●, ○) and scleroglucan, $[\eta] = 144$ ml/g at 80.0 mg/ml (■, □). Synthetic seawater and $[NaIO_4]/[Scl] = 1$ was employed.

with time, and the rate of gelation clearly increases with increasing C_p (Fig. 4, insert). Thereafter, the gelation rate levels off until about 15–20 h, where it again appears to increase. However, the influence of C_p on the rate of gelation is less systematic in this region except for the lowest C_p, which only show a marginal increase in the gelation rate. The data presented in Fig. 4 was obtained using synthetic seawater as solvent. Similar results were obtained using distilled water as the solvent, thus suggesting that the ion concentrations or type of ions does not have a strong influence on the gelation kinetics.

Fig. 5 shows results obtained for scleroglucan with lower molecular weights ($[\eta] = 270$ and 144 ml/g) and polymer concentrations in the range of 11.9–80 mg/ml. Comparison

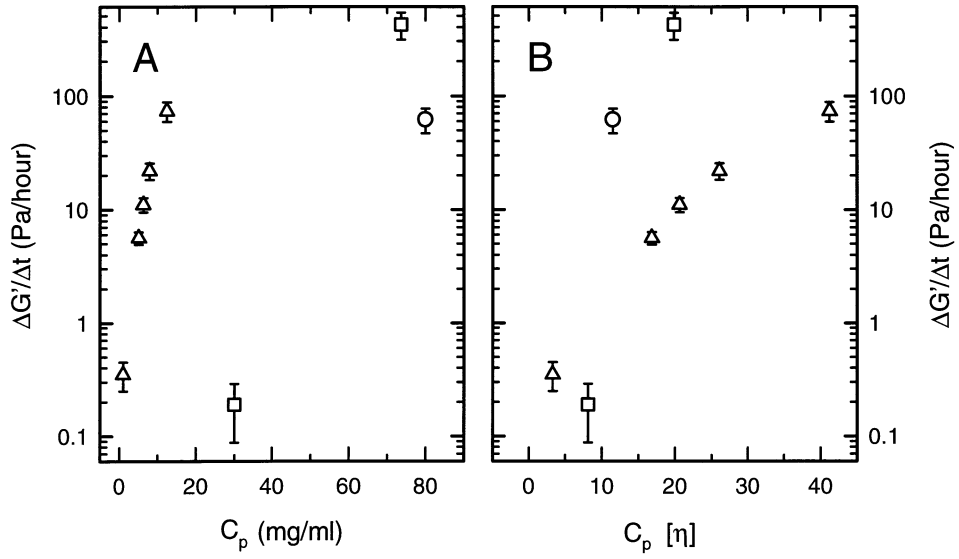

Fig. 6. Initial increase in the storage modulus per unit time, $\Delta G'/\Delta t$ at $T = 30°C$ versus (A) scleroglucan and (B) reduced scleroglucan concentration $C_p[\eta]$ at $T = 30°C$. The initial slopes of G' versus t were estimated for a duration of 2–5 h, and the mean and SD estimated for the scleroglucans with $[\eta] = 3300$ ml/g (△), $[\eta] = 270$ ml/g (□), and $[\eta] = 144$ ml/g (○).

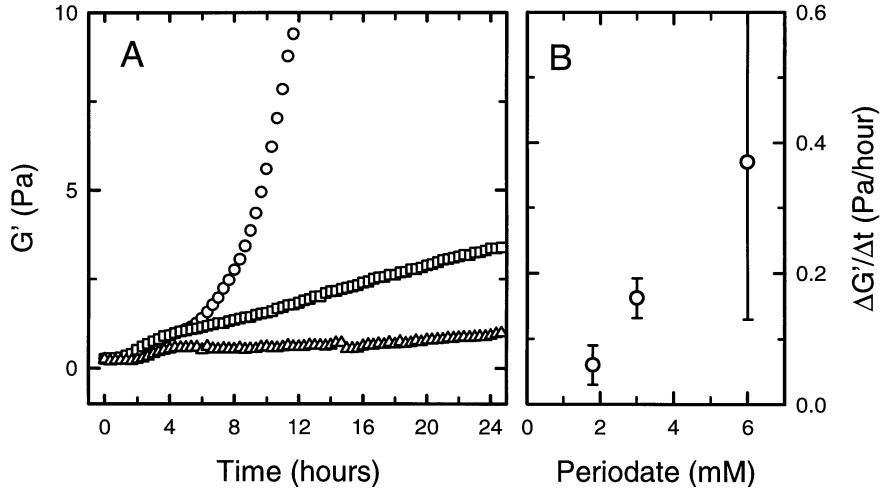

Fig. 7. (A) Storage modulus G' ($\omega = 0.628\ s^{-1}$) versus incubation time at $T = 30°C$ for $NaIO_4$ induced gelation of 1.0 mg/ml scleroglucan with intrinsic viscosity $[\eta] = 3300$ ml/g at $NaIO_4$ concentration 1.8 mM (△), 3 mM (□) and 6 mM (○). (B) Initial increase in the storage modulus per unit time, $\Delta G'/\Delta t$ at $T = 30°C$ versus $[NaIO_4]$. The mean and SD of the initial slopes of G' versus t was obtained as described in Fig. 6.

of the development of G' for the $[\eta] = 3300$ ml/g sample at $C_p = 12.5$ mg/ml (Fig. 4) with the data for the $[\eta] = 270$ ml/g sample at $C_p = 11.9$ mg/ml (Fig. 5A) demonstrates a strong influence of the chain length on the $NaIO_4$ induced gelation of scleroglucan. For the highest molecular weight sample, the data reveal an increase in excess of 200 Pa the first two hours, and a storage modulus in excess of 2 kPa is observed within one day. This contrasts the low and constant value of the storage modulus (G' ($\omega = 0.628\ s^{-1}$) ~0.05–0.08 Pa) observed for the $[\eta] = 270$ ml/g sample at $C_p = 11.9$ mg/ml (Fig. 5). Increasing the polymer concentration of the $[\eta] = 270$ ml/g sample, does however, yield conditions that allow periodate induced gelation (Fig. 5A). An additional sample with even lower molar mass ($[\eta] = 144$ ml/g) further demonstrates the importance of chain length in the gelation.

The initial rate of gelation ($\Delta G'/\Delta t$) depends on the polymer concentration as summarised in Fig. 6A. $\Delta G'/\Delta t$ is 0.3 Pa/h at $C_p = 1$ mg/ml, and increases strongly with C_p to a value of 76 Pa/h obtained at $C_p = 12.5$ mg/ml, all for the sample with the $[\eta] = 3300$ ml/g. Reduction of chain length displaces the $\Delta G'/\Delta t$ (C_p) relation towards higher C_p (Fig. 6A). Plotted as a function of the reduced concentration, $C_p[\eta]$, the rate of gelation did not collapse onto one master curve (Fig. 6B). The reason for this is a possible lack of correspondence between the critical overlap concentration,

Table 1
Stability of scleraldehydes (gels were prepared from 15 mg/ml scleroglucan with 1 mol of $NaIO_4$ per repeating unit; the 80°C treatment lasted for 17 days, further storage took place at room temperature) upon storage in various solutions

Treatment		After 1 h	After three days	After seven months
Distilled water	Room temp.	Intact	Intact	Intact
	80°C	Intact	Intact	Intact
1 M $NaBH_4$	Room temp.	Dissolved	–	–
0.5 M $NaClO_2$ (in acetic acid)	Room temp.	Dissolved	–	–
0.1 M HCl	Room temp.	Intact	Intact, turbid	Intact, turbid
	80°C	Intact	Intact	Intact, slightly turbid
1 M acetic acid (CH_3COOH)	Room temp.	Intact	Intact, turbid	Intact, turbid
	80°C	Intact	Intact	Intact, slightly turbid
0.01 M NaOH	Room temp.	Intact	Intact, slightly swollen	Intact, slightly swollen
	80°C	Intact	Intact, slightly swollen	Intact, slightly swollen
0.1 M NaOH	Room temp.	Intact	Intact, slightly swollen	Intact, slightly swollen
	80°C	Intact	Intact, slightly swollen	Intact, slightly swollen
6 M urea	Room temp.	Intact	Intact	Disintegrated/dissolved
	80°C	Intact	Intact	Disintegrated/dissolved
1 M LiI	Room temp.	Intact	Intact	Intact
	80°C	Intact	Intact	Intact
4 M KSCN	Room temp.	Intact	Collapsed	–
	80°C	Intact	Disintegrated	–
DMSO	Room temp.	Intact	Intact, contracted	Intact, contracted
	80°C	Intact	Intact, contracted	Intact, contracted

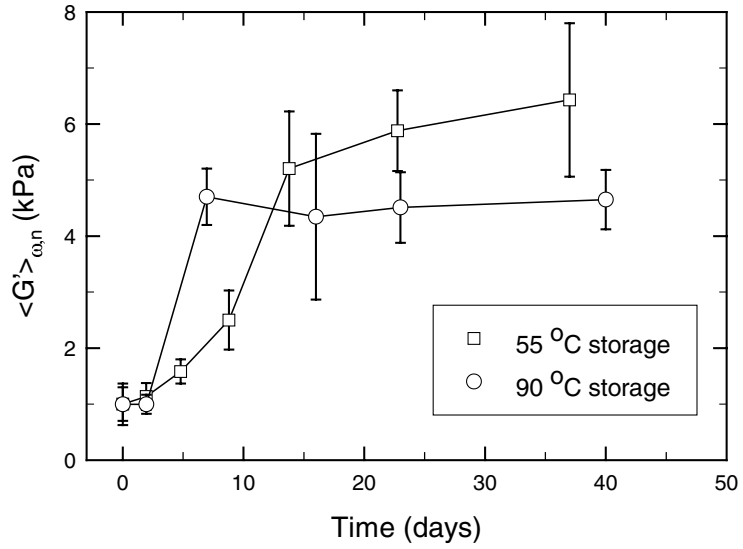

Fig. 8. Mean storage moduli $\langle G' \rangle_{\omega,n}$ versus incubation time of 10 mg/ml scleroglucan gels in synthetic seawater at 55 and 90°C, respectively.

and lower critical concentration needed for gelation. These data show that gelation readily occur when the reduced concentration is in the range 10 and above, whereas C_p $[\eta]$ below 3 does not appear to yield a macroscopic network.

Fig. 7 shows the effect of changing $NaIO_4$ concentration at a fixed polymer concentration $C_p = 1.0$ mg/ml. The data show that the storage modulus increases nearly linearly with time at $[NaIO_4] = 1.8$ and 3 mM. Increasing $[NaIO_4]$ to 6 mM yields an increase in G' similar to that observed for $[NaIO_4] = 3$ mM during the first 4 h, followed by an increasing rate of change compared to the 3 mM case. $\Delta G'/\Delta t$ is proportional to $[NaIO_4]$ within experimentally determined slopes. Extrapolation of $\Delta G'/\Delta t$ to zero does not yield a limiting periodate concentration different from zero within the uncertainty.

3.4. Chemical and physical stability of scleraldehyde gel

Table 1 summarises the behaviour of scleraldehyde gels in the presence of acids, bases, reducing and oxidising agents, as well as some salts and solvents which influence the solubility or solution properties of polymers. The gels are generally very stable and rapid dissolution was only obtained by $NaClO_2$ (in acetic acid) or by $NaBH_4$. Collapse or disintegration occurred in 6 M urea (after seven months) or 4 M KSCN. In DMSO, a solvent which denatures triple-stranded scleroglucan into single chains (disordered conformation), dissolution was not observed, but the gels shrunk considerably in this solvent.

Fig. 8 shows the average storage modulus versus time of 10 mg/ml scleroglucan gels stored in synthetic seawater, $T = 55$ and 90°C. The data show a transition of $\langle G' \rangle_{\omega,n}$ from the initial value of about 1–4.5 kPa (90°C stored samples) and about 6 kPa (55°C stored samples). This change occurs within a limited time interval of 3–6 days and 7–15 days for the 90 and 55°C stored samples, respectively. The main mechanism underlying the observed increased storage moduli is a decrease in the equilibrium gel volume in the excess solvent (syneresis).

4. Discussion

The finding that scleroglucan forms soft, elastic gels during periodate oxidation is in agreement with the observations reported by Crescenzi et al. (1983). The gels appear to be quite stable, and dissolve only by chemical reactions involving the aldehyde groups. Aldehyde reduction with $NaBH_4$, yielding the corresponding polyalcohol (Schulz & Rapp, 1991), rapidly dissolves the gel, as does oxidation with $NaClO_2$ to give the carboxylated derivative. These observations suggest that the aldehyde groups are directly involved in crosslinking, preferentially by the formation of intermolecular hemiacetals. Gelation also took place in DMSO lead tetraacetate. The latter oxidises the side chains into dialdehydes as for periodate (Perlin, 1970). In DMSO triple-stranded scleroglucan dissociates into individual strands. Nevertheless, gels are formed. Moreover, gels prepared in water did not dissolve when transferred to DMSO, although a substantial and reversible decrease in the gel volume has been observed (Guo et al., 1998). These observations lend support to the hypothesis regarding intermolecular hemiacetals, and the formation of cross-links apparently does not depend on the conformation of the scleroglucan. The gels dissolved or disintegrated following initial gelation at high temperature (80°C) when periodate or its reduction product (iodate) was present, whereas washed (periodate/iodate-free) gels appeared to be stable. We attribute this behaviour to depolymerisation of the scleroglucan, presumably by a free radical mechanism initiated by periodate/iodate in the presence of oxygen.

Such side reactions are normally suppressed by performing the periodate oxidation at low temperatures, in the absence of light, oxygen and heavy metal ions, and sometimes by adding a free radical scavenger such as *n*-propanol (Painter & Larsen, 1970).

Acknowledgements

Professor Terence J. Painter is thanked for valuable discussions. This work has been supported by Statoil (grant no. T182564) and VISTA (projects V6312 and V6314). Sanofi (France) is acknowledged for supplying samples of scleroglucan.

References

Aalmo, K. M., & Painter, T. J. (1981). Periodate oxidation of methyl glycopyranosides: rate coefficients and relative stabilities of intermediate hemiacetals. *Carbohydrate Research*, *89*, 73–82.

Bluhm, T. L., Deslandes, Y., Marchessault, R. M., Perez, S., & Rinaudo, M. (1982). Solid-state and solution conformation of scleroglucan. *Carbohydrate Research*, *100*, 117–130.

Bohn, J. A., & BeMiller, J. N. (1995). (1 → 3)-β-D-glucans as biological response modifiers: a review of structure–functional activity relationships. *Carbohydrate Polymers*, *28*, 3–14.

Bosco, M., Sussich, F., Gamini, A., Reisenhofer, E., Adami, G., & Rizzo, R. (1995). Divalent cation interactions with a carboxylated derivative of scleroglucan. *Macromolecular Chemistry and Physics*, *196*, 3979–3989.

Coviello, T., Dentini, M., & Crescenzi, V. (1995a). Conformation and thermal stability of oxidized scleroglucan chains in aqueous NaOH. *Polymer Bulletin*, *34*, 337–343.

Coviello, T., Dentini, M., Crescenzi, V., & Vincenti, A. (1995b). Ionic strength and temperature dependence of oxidized scleroglucan solution properties: optical activity and viscosity data. *Carbohydrate Polymers*, *26*, 5–10.

Crescenzi, V., Gamini, A., & Paradossi, G. (1983). Solution properties of a new polyelectrolyte derived from the polysaccharide scleroglucan. *Carbohydrate Polymers*, *3*, 273–286.

Crescenzi, V., Imbriaco, D., Velàsquez, C. L., Dentini, M., & Ciferri, A. (1995). Novel types of polysaccharidic assemblies. *Macromolecular Chemistry and Physics*, *196*, 2873–2880.

Gamini, A., Crescenzi, V., & Abruzzese, R. (1984). Influence of the charge density on the solution behaviour of polycarboxylates derived from the polysaccharide scleroglucan. *Carbohydrate Polymers*, *4*, 461–472.

Grassi, M., Lapasin, R., & Pricl, S. (1996). A study of the rheological behavior of scleroglucan weak gel systems. *Carbohydrate Polymers*, *29*, 169–181.

Guo, B., Elgsaeter, A., & Stokke, B. T. (1998). Gelation kinetics of scleraldehyde–chitosan co-gels. *Polymer Gels and Networks*, *6*, 113–135.

Hjerde, T., Kristiansen, T. S., Stokke, B. T., Smidsrød, O., & Christensen, B. E. (1994). Conformation dependent depolymerisation kinetics of polysaccharides studied by viscosity measurements. *Carbohydrate Polymers*, *24*, 265–275.

Hjerde, T., Stokke, B. T., Smidsrød, O., & Christensen, B. E. (1998). Free-radical degradation of triple-stranded scleroglucan by hydrogen peroxide and ferrous ions. *Carbohydrate Polymers*, *37*, 41–48.

Johnson Jr., J., Kirkwood, S., Misaki, A., Nelson, T. E., Scaletti, J. V., & Smith, F. (1963). Structure of a new glucan. *Chemical Industry (London)*, *1*, 820–822.

Kashiwagi, Y., Norisuye, T., & Fujita, H. (1981). Triple helix of *Schizophyllum commune* polysaccharide in dilute solution. 4. Light scattering and viscosity in dilute aqueous sodium hydroxide. *Macromolecules*, *14*, 1220–1225.

Moe, S. T., Draget, K. I., Skjåk-Bræk, G., & Smidsrød, O. (1992). Temperature dependence of the elastic modulus of alginate gels. *Carbohydrate Polymers*, *19*, 279–284.

Painter, T., & Larsen, B. (1970). Formation of hemiacetals between neighbouring hexuronic acid residues during the periodate oxidation of alginate. *Acta Chemica Scandinavica*, *24*, 813–833.

Perlin, A. S. (1970). Glycol-cleavage oxidation. In W. Pigman & D. Horton, *The carbohydrates. Chemistry and biochemistry* (pp. 1167–1215). , Vol. IIB. New York: Academic Press.

Schulz, D., & Rapp, P. (1991). Properties of the polyalcohol prepared from the β-D-glucan schizophyllan by periodate oxidation and borohydride reduction. *Carbohydrate Research*, *222*, 223–231.

Stokke, B. T., Elgsaeter, A., Bjornestad, E. O., & Lund, T. (1992). Rheology of xanthan and scleroglucan in synthetic seawater. *Carbohydrate Polymers*, *17*, 209–220.

Veelaert, S., deWit, D., Gotlieb, K. F., & Verhe, R. (1997a). Chemical and physical transitions of periodate oxidized potato starch in water. *Carbohydrate Polymers*, *33*, 153–162.

Veelaert, S., deWit, D., Gotlieb, K. F., & Verhe, R. (1997b). The gelation of dialdehyde starch. *Carbohydrate Polymers*, *32*, 131–139.

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