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Synthesis and preliminary characterisation of charged derivatives and hydrogels from scleroglucan

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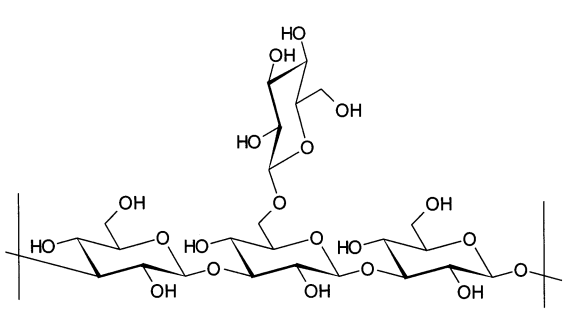
Abstract

The synthesis of negatively and positively charged polyelectrolytes from scleroglucan is described. Polycarboxylates were synthesised through nucleophilic substitution with chloroacetic acid or through a selective 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO)-mediated oxidation of the primary alcohol groups. Amine groups were introduced through nucleophilic substitution with 2-chloroethylamine or 3-chloropropylamine. Reaction conditions were varied to obtain insight into the influence of variables on the degree of substitution. The conformational behaviour of the obtained polyelectrolytes was studied as a function of pH, temperature and solvent. For the products with a low degree of modification, evidence of an ordered conformation was found, whereas the polymers with a higher degree of modification behaved as random coils in solution. The negatively charged polymers were reticulated using the Ugi four-component condensation, obtaining negatively charged hydrogels. The positively charged polymers were reticulated using diethyl squarate (3,4-diethoxy-3-cyclobutene-1,2-dion, DES) to obtain positively charged hydrogels.

Keywords: Polysaccharide modification; Carboxymethylation; TEMPO-mediated oxidation; Ugi four-component condensation; Conformation

1. Introduction

Scleroglucan is a neutral polysaccharide produced by micro-organisms, especially by fungi of the genus *Sclerotium* [1]. It consists of a main chain of (1→3)-linked β-D-glucopyranosyl units where every third unit bears a (1→6)-linked β-D-glucopyranosyl monomer [2] (Scheme 1). This structure is chemically identical to that of schizophyllan, a polysaccharide secreted by the basidiomycete *Schyzophyllum commune* [3]. The monomeric branches ensure that the polysaccharide is soluble [4] in solvents like water and dimethylsulfoxide since aggregation, as is observed in the case of the (1→3)-linked β-D-glucan curdlan [5], is unfavourable. In aqueous solution, the polysaccharide adopts a stable triple-stranded helical conformation held together by hydro-



Scheme 1.

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gen bonds [6]. This accounts for the unusual rigidity of this polysaccharide as quantified by its persistence length of about 200 nm [7]. The triple helix is destroyed at pH > 12 [8] or at a temperature exceeding 135 °C [9], yielding a random coil in solution. In dimethylsulfoxide solution, the polysaccharide adopts a random coil conformation [6]. Due to the rod-like character of native scleroglucan in aqueous solution and its resistance to hydrolysis, temperature and electrolytes, it has several commercial applications [4], the most important being in the field of enhanced oil recovery. Furthermore, (1→3)-β-D-glucans with (1→6) single unit branches in general enhance the immune system, which results in antitumour, antiviral and anticoagulatory activities [10].

An important line of research involving scleroglucan (and schizophyllan) is the study of its solution properties, since the polysaccharide can be used as a model polymer for a soluble rigid rod [11,12], which is relatively rare. Another line of research deals with the conditions influencing the conformation of scleroglucan in solution, that is, conditions of denaturation and renaturation of the triple helix [13]. For example, it is possible to denature and renature native scleroglucan in such a way that mainly macrocycles are obtained [14–16]. Chemical modification of scleroglucan, on the other hand, is still scarcely explored. To our knowledge, only a few derivatives of scleroglucan have been prepared. Polyelectrolytes have been obtained by periodate oxidation of the diol functionality in the β-D-glucopyranosyl branch and subsequent oxidation of the resulting aldehyde groups with sodium chlorite to obtain a polycarboxylate [17–19]. The conformational behaviour in solution of this modified polysaccharide has been studied extensively [17–22]. Another polycarboxylate has been synthesised through esterification with phthalic acid [23]. Acetylation of scleroglucan [24] was possible under strongly acidic conditions and the resulting product was only slightly soluble in dimethylsulfoxide and not at all in water, which prevented further studies. New derivatives may show interesting properties and may add to the understanding of the conformational behaviour of scleroglucan.

In this paper, we report on the synthesis of new ionic derivatives of scleroglucan. Negatively charged modified scleroglucans have been prepared by carboxymethylation with chloroacetic acid or by a selective 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO)-mediated hypohalite oxidation of the primary alcohol functions present in the polymer. Positively charged polyelectrolytes were prepared by substitution with aminoethyl chloride or aminopropyl chloride. Reaction conditions have been varied in order to obtain insight into the efficiency of the reactions. The solution properties of the obtained polyelectrolytes have been studied focusing on the presence of an ordered conformation in the modified polymers. By an ordered conformation, we mean any interaction leading to associations like double- or triple-stranded helixes, as can be measured, for instance, by an enhanced viscosity with respect to the non-ordered coil.

Finally, charged hydrogels, which find widespread use as absorbants, for example, have been prepared from the modified samples using two recently developed methods [25,26]. From the negatively charged polymers, hydrogels were prepared using the Ugi four-component condensation [25] and for the positively charged polymers, diethyl squarate (DES) was used as a coupling agent [26]. The influence of the cross-link density and pH on the swelling behaviour of the hydrogels is discussed.

2. Experimental

Materials.—Scleroglucan (Actigum CS 11) was obtained from CECA, Carentan, France. The value for the intrinsic viscosity of the polysaccharide used was 10.1 dL/g, which corresponds to a molecular weight of $\sim 8 \times 10^5$ g/mol, using literature values of the Mark–Houwink constants [7]. Pullulan was obtained from Hayashibara, Okayama, Japan. All other chemicals were commercially available products and were used without prior purification.

Carboxymethylation.—Scleroglucan (3.0 g) was dissolved in a solution of NaOH in water (see Table 1). After 30 min a certain amount

Table 1
Influence of the reaction conditions on the carboxymethylation

Entry	Polysaccharide	Concentration (w/V)	[CA]/[ps] [a]	[NaOH]/[ps] [b]	DS (%) [c]	[η] (dL/g) [d]
1	Scleroglucan	10	0.67	1.67	8	9.2
2	Scleroglucan	10	1	2.5	16	7.4
3	Scleroglucan	10	1.33	3.33	22	6.2
4	Scleroglucan	6.7	1.67	4	20	6.3
5	Scleroglucan	6.7	2	5	26	5.8
6	Scleroglucan	6.7	5	10	71	5.3
7	Pullulan	20	0.67	1.67	24	
8	Pullulan	20	2	5	56	

[a] Ratio of the initial concentration of chloroacetic acid to polysaccharide monomer units.
[b] Ratio of the initial concentration of NaOH to polysaccharide monomer units.
[c] Degree of substitution with respect to the monomer units of the polysaccharide.
[d] Intrinsic viscosity in 0.1 M NaCl.

of chloroacetic acid (Table 1) was added and the solution was stirred for 90 min at 80 °C. The solution was subsequently cooled to room temperature (rt), diluted and neutralised with glacial acetic acid. The product was isolated after extensive dialysis and lyophilization. The yield of product was always around 90% and the degree of substitution was determined by titration (see below).

TEMPO-mediated oxidation.—Scleroglucan (1.0 g) was dissolved in water (500 mL) and TEMPO (0.01 g) and NaBr (0.1 g) were added. This dilution was necessary to prevent gel formation before and during the oxidation. The solution was cooled in an ice-bath and sodium hypochlorite (15% solution) was slowly added. The pH was maintained at 9–9.5 during the oxidation by adding 0.5 M NaOH. This also allowed the determination of the degree of oxidation. The amount of sodium hypochlorite added depended on the desired degree of oxidation. After the reaction was complete sodium borohydride (0.2 g) was added to reduce carbonyl groups present. The reaction mixture was left overnight at rt and was neutralised with glacial acetic acid. The products were isolated after extensive dialysis and lyophilization. The yield of product was always around 90%.

Aminoethylation (aminopropylation).—Scleroglucan (3.0 g) was dissolved in a solution of NaOH in water (30 mL). The amount of NaOH, 2-chloroethylamine (or 3-chloropropylamine) and the reaction temperature and time were varied (see Table 3). After the reaction, the mixture was cooled, diluted, neutralized the product was isolated as described above. The yield appeared to depend strongly on the reaction conditions (see Table 3) and the degree of substitution was determined by elemental analysis.

Ugi four-component condensation.—Carboxymethyl scleroglucan was dissolved in water (4 mL) to obtain a viscous solution (conditions are given in Table 4, entries 1–9). Subsequently were added L-lysine methylester (the amount of this bifunctional reagent determining the theoretical cross-link density) and a small molar excess of formaldehyde. The pH of the solution was brought to 3.5–4 with diluted HCl and a small molar excess of cyclohexyl isocyanide was added. The solution was stirred at rt until it became too viscous to be stirred, which generally happened within 30 min. The gel was left overnight and subsequently dialysed for 48 h against 0.1 M Na_2CO_3 in order to hydrolyse the methylester. The transparent gel was then swollen against distilled water until constant weight.

Diethyl squarate (DES) network.—The amine-containing polysaccharide was dissolved in water (4 mL) to obtain a viscous solution (conditions are given in Table 4, entries 10–15). Dilute NaOH was added to the solution to obtain a pH of 9.5–10 and a known amount of DES was added (the amount of DES determining the theoretical

cross-link density). The solution was stirred at rt until it became too viscous to be stirred, which generally happened within several minutes. The gel was left overnight and was subsequently dialysed against distilled water until constant weight.

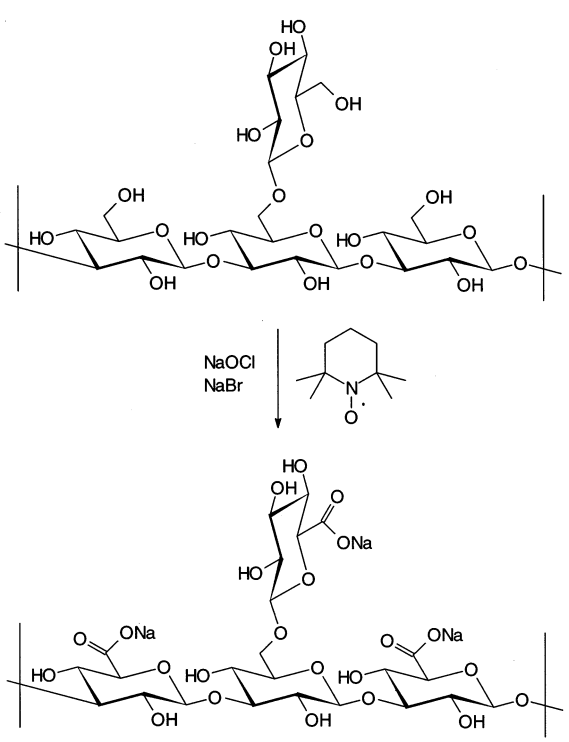
Titration of carboxymethyl scleroglucan.—A volume of dialysed carboxymethyl scleroglucan was directly brought on a Dowex 50×8 H^+ column to obtain the carboxylic acid. The polyacid was collected and freeze dried. A known amount was dissolved in water and titrated against an NaOH solution.

Viscosity.—The viscosity of the polymers was determined on a Schott–geräte apparatus with automatic dilution using an Ubbelohde capillary ($\varnothing = 0.53$ mm) at 25 °C.

Optical rotation.—The optical rotation at 302 nm as a function of temperature was measured on a Perkin–Elmer 241 polarimeter equipped with a thermostat.

3. Results and discussion

Synthesis of scleroglucan polyelectrolytes.—Carboxymethylation of polysaccharides, most notably cellulose, with chloroacetic acid is a well-documented reaction [27]. This nucleophilic substitution is generally performed in aqueous solution at such a pH that at least part of the alcohol functionalities of the polysaccharide are dissociated (i.e., pH > 13). However, the efficiency of the reaction strongly depends on the applied conditions, that is, the concentration of polysaccharide, heterogeneous versus homogeneous conditions etc. We performed several experiments in order to determine the influence of variables on the degree of substitution (Table 1). At least 2 equivalents of NaOH with respect to the molar amount of chloroacetic acid were necessary (one equivalent to neutralise chloroacetic acid and one equivalent consumed during the reaction) and we generally used a small excess. Note that with all experiments, the pH was high enough to denature the triple helix of scleroglucan. Increasing the ratio of chloroacetic acid–scleroglucan yielded products with a higher degree of substitution. The carboxymethylation was performed in a highly concentrated solution because it was found earlier [28] that the amount of water present had an important influence on the efficiency of the reaction. The less water present, the more efficient the reaction. This is probably due to side reactions such as hydrolysis of the etherifying agent. In order to get a clearer view of the influence of water, two experiments were carried out with pullulan as a substrate. This polysaccharide is more soluble than scleroglucan and consequently less water had to be used to obtain a clear viscous solution. Under the same conditions as used for scleroglucan, but at a pullulan concentration of 20%, the DS of pullulan was at least twice that found for scleroglucan, as shown in Table 1. Although the comparison of two different polysaccharides may be questionable, these results confirm earlier findings [28]. In Table 1, and in the following Tables, the intrinsic viscosity at an added salt concentration of 0.1 M (NaCl) is also given for several prepared polyelectrolytes. These results will be discussed below.



Scheme 2.

Using the TEMPO-mediated oxidation (Scheme 2), highly selective for primary alcohols, five polyelectrolytes were prepared with different degrees of oxidation (Table 2). The

Table 2
Characteristics of the oxidised scleroglucan derivatives

Entry	DO (%) [a]	[η] (dL/g) [b]
1	20	12.7
2	40	6.3
3	60	3.9
4	80	4.1
5	100	3.5

[a] Degree of oxidation with respect to the primary alcohol functions present.
[b] Intrinsic viscosity in 0.1 M NaCl.

degree of oxidation could be exactly monitored during the oxidation through the addition of sodium hydroxide [29]. A highly diluted solution was used because scleroglucan forms a physical gel in water at low temperature. Furthermore, at initial stages of the oxidation the viscosity of the solution increased appreciably, presumably through formation of intermolecular hemiacetals and acetals by reaction of the intermediate C-6-aldehyde formed during the oxidation [30] with hydroxyl functionality. The pH was kept at 9–9.5 since at higher pH degradation of the polysaccharide occurs due to β-elimination induced by the intermediate C-6-aldehyde [31]. When the oxidation was complete, a small amount of sodium borohydride was added to reduce the remaining C-6-aldehydes and ketones, which may possibly be present due to non-selective oxidation. It was shown, using starch as a substrate, that this reduction yielded products that were stable at 100 °C in water, whereas non-reduced products yielded slightly yellow solutions under these conditions.

Substitution reactions with amine-containing electrophiles are commercially applied on, for example, starch [32], however, the degree of substitution is always quite low (of the order of 5%). We tried to obtain materials with a higher degree of substitution with 2-chloroethylamine and 3-chloropropylamine as the etherifying agents (Table 3) using similar conditions, as previously described for the aminoethylation of dextran [33]. As in the case of the carboxymethylation, two equivalents of sodium hydroxide were necessary since we used the HCl salt of the amine. In general, a small excess of sodium hydroxide was used. We could not detect any difference in efficiency comparing 2-chloroethylamine and 3-chloropropylamine. From Table 3, it is clear that highly substituted products can be obtained using a molar excess of amine at relatively harsh conditions. However, these products were highly degraded as was indicated by viscosity measurements and the yields were very low. Clearly, under these

Table 3
Influence of the reaction conditions on the aminoalkylation of scleroglucan

Entry	Amine	[Amine]/[scle] [a]	[NaOH]/[scle] [b]	T (°C)	Reaction time (h)	Yield (%)	DS (%) [c]	[η] (dL/g) [d]
1	Aminoethyl	2	5	80	24	40	48	
2	Aminoethyl	3.2	6.5	80	24	35	60	
3	Aminoethyl	4	10	80	24	18	90	0.5
4	Aminoethyl	3	6	80	12	50	27	
5	Aminoethyl	3	6	80	6	61	10	
6	Aminopropyl	2	5	80	24	40	54	
7	Aminopropyl	3.2	6.5	80	24	33	61	
8	Aminopropyl	4	10	80	24	22	85	0.6
9	Aminopropyl	2	5	45	7	91	16	4.3
10	Aminopropyl	2	5	45	5	90	11	
11	Aminopropyl	2	5	45	2	97	<5	
12	Aminopropyl	2	5	80	5	65	25	3.1

[a] Ratio of the initial concentration of amine to scleroglucan monomer units.
[b] Ratio of the initial concentration of NaOH to scleroglucan monomer units.
[c] Degree of substitution with respect to the monomer units of scleroglucan.
[d] Intrinsic viscosity in 0.1 M NaCl.

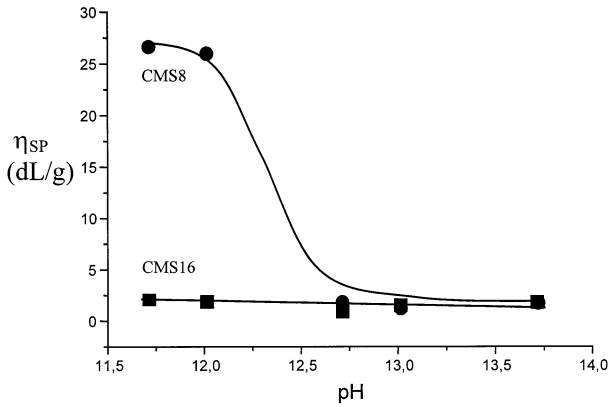


Fig. 1. The specific viscosity as a function of pH at 0.5 M added salt (NaCl) for two carboxymethylated scleroglucan derivatives (CMS8 (●) and CMS16 (■), lines are drawn to guide the eye).

conditions side reactions become important, which are not seen under similar conditions with chloroacetic acid as the etherifying agent. For example, in several cases a precipitate formed, which remained insoluble at low pH. Possibly, a polymerisation of the amine occured, which was not investigated further. To improve the yield and the molecular weight of the products, several experiments were carried out at lower temperatures or shorter reaction times with a lower amine–scleroglucan ratio. The obtained degree of substitution was lower under these conditions, but yields and molecular weights were much better. In short, using this one-step etherification, we were not able to obtain high molecular weight products with a degree of substitution exceeding ca. 25%.

Viscosity and conformational analysis of modified scleroglucan.—We focused on the samples obtained by carboxymethylation and oxidation, since using these reactions, series with higher degrees of substitution (or oxidation) could be prepared, which, in addition, were better controllable. Of these polyelectrolytes, we measured the intrinsic viscosities at an added salt strength of 0.1 M (NaCl), as shown in Tables 1 and 2. In all cases, except for the 20% oxidised sample, the intrinsic viscosity at this concentration of added salt is lower than for native scleroglucan (10.1 dL/g). Clearly, some degradation and/or loss of the triple helix occured during the reactions. Furthermore, for both modifications, a decrease in intrinsic viscosity at 0.1 M NaCl was observed when the degree of substitution (or oxidation) increased. If this decrease in intrinsic viscosity was only due to the loss of ordered conformation, this trend would be reversed since an increase in charge would yield polyelectrolytes with a higher hydrodynamic volume at 0.1 M NaCl. It may thus be concluded that both reactions are degradative towards the polymer chain. In the case of the carboxymethylation, degradation is more severe at higher degrees of substitution, probably due to the fact that a higher concentration of sodium hydroxide had to be used. The effect of the TEMPO-mediated oxidation on pullulan has been investigated before [31] and probably most degradation occurs due to β-elimination of the intermediate C-6-aldehyde, which becomes more important for higher degrees of oxidation since longer reaction times are necessary.

It is known that the transition from the ordered triple-helical conformation to a disordered chain in native scleroglucan can be determined using viscosity as a function of pH [8]. A sudden drop in the viscosity is seen at pH ~ 12.5, which is caused by the denaturation of the triple helix. We measured the viscosity of various carboxyl-containing samples as a function of pH at constant ionic strength (0.5 M NaOH + NaCl, depending on the desired pH). The results for the two lowest carboxymethyl scleroglucan samples are shown in Fig. 1. It seems that the sample with a DS of 8% (CMS8) is, at least partly, in an ordered conformation that is destroyed at pH ⩾ 12, whereas the pH has no influence on the viscosity of the other samples, of which only the sample with a DS of 16% (CMS16) is shown. In Fig. 2, a similar plot is shown for the three lowest oxidised samples. In the 20 (SOX20) and 40% (SOX40) samples a sudden drop in viscosity is also observed, whereas in the other samples, of which only the SOX60 is shown, the pH had no influence on the observed viscosity. Thus, in this case also, there seems to be some ordered conformation for the lower degrees of modification.

Another method used to detect a possible conformational transition was optical rotation as a function of temperature. As mentioned above, native scleroglucan shows a transition at a temperature exceeding 135 °C. For this

polysaccharide, the optical rotation at a wavelength of 302 nm shows a sharp change in going from the ordered triple-helical state to a disordered coil state. We measured $[\alpha]_{302}$ in the range 20–90 °C. A non-linear change was only found for the CMS8 and SOX40 in this range; all the other samples showed a slight linear increase on increasing the temperature. In Figs. 3 and 4, the results are shown for CMS8 without and with added salt (0.01 M NaCl), respectively. Every point pertains to a measurement carried out 10 min after the temperature became stable. The fact that, in this temperature range, a conformational transition occurs indicates that any ordered conformation is less stable than in the case of native scleroglucan. Furthermore, the ordered conformation of SOX20, a sample of which showed a linear behaviour in this temperature range (not shown), is more stable than that of SOX40. In these Figures also, the behaviour is shown going from a high temperature, that is, a disordered state, to room temperature. Clearly, an ordered state reforms, which seems, judging from the $[\alpha]_{302}$ value, less ordered than the initial state. After 48 h, the value of $[\alpha]_{302}$ returned to its value before heating, indicating that the ordering of these modified polysaccharides (CMS8 and SOX40) in aqueous solution is dominated by thermodynamic rather than kinetic principles. As seen in Figs. 3 and 4, CMS8 in water showed a transition at about 40 °C, whereas in 0.01 M NaCl this transition occurred at about 65 °C. This is in agreement with the expected behaviour, since added salt partly screens the charges diminishing their repulsion, thus, stabilising interstrand interactions.

Finally, it should also be possible to observe a transition from an ordered to a disordered conformation using solutions with varying ratios of water–dimethyl sulfoxide, since in dimethyl sulfoxide scleroglucan is present in its disordered form. This is shown in Fig. 5 for native scleroglucan and the three samples that showed an ordered conformation in water as indicated by the measurements described above. The transition was followed again using optical rotation. As expected, all samples showed a sigmoidal curve. The transition of native scleroglucan takes place at a percentage of dimethyl sulfoxide of about 70%, whereas the modified samples showed a transition at a

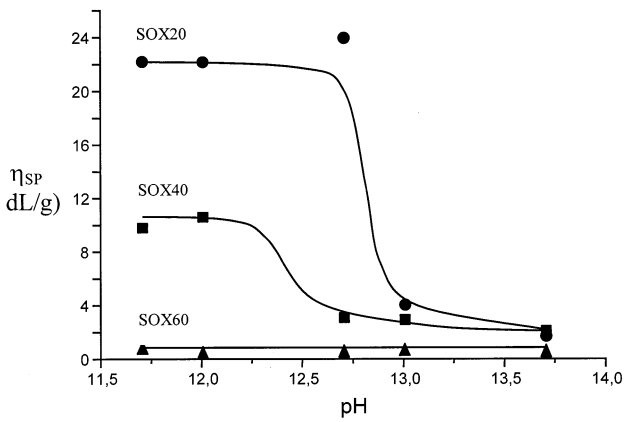


Fig. 2. The specific viscosity as a function of pH at 0.5 M added salt (NaCl) for three oxidised scleroglucan derivatives (SOX20 (●), SOX40 (■) and SOX60 (▲), lines are drawn to guide the eye).

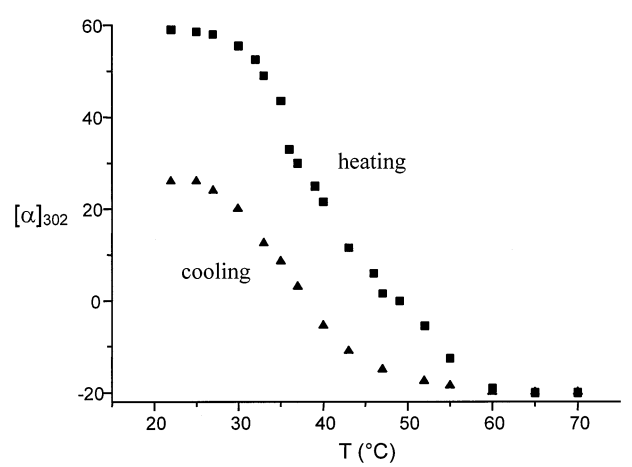


Fig. 3. The optical rotation (in deg/dm dmol) as a function of the temperature in water for CMS8.

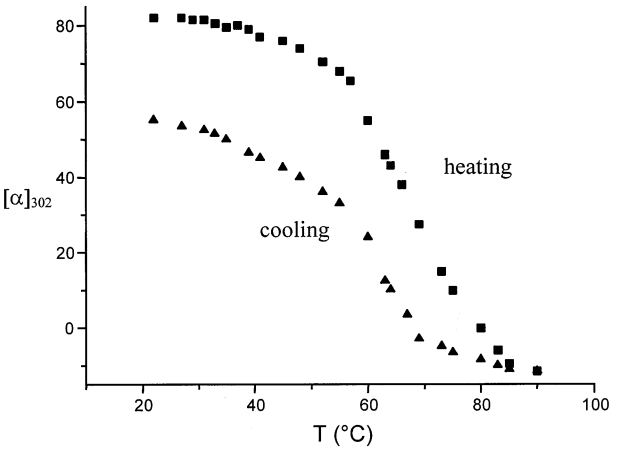


Fig. 4. The optical rotation (in deg/dm dmol) as a function of the temperature in 0.01 M NaCl for CMS8.

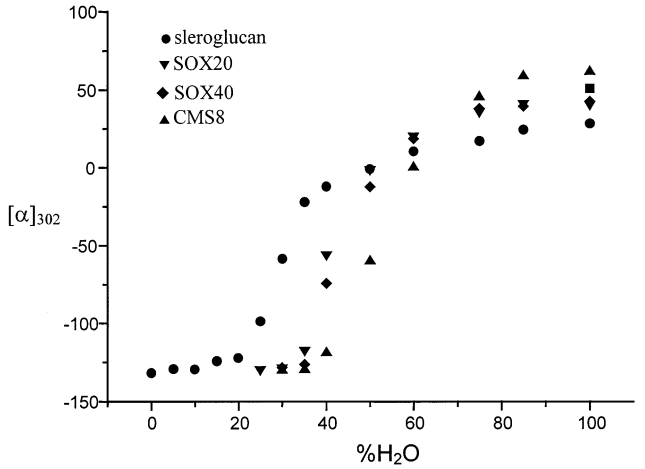


Fig. 5. The optical rotation (in deg/dm dmol) as a function of the solvent composition (Me_2SO-H_2O) at 25 °C for scleroglucan and several derivatives.

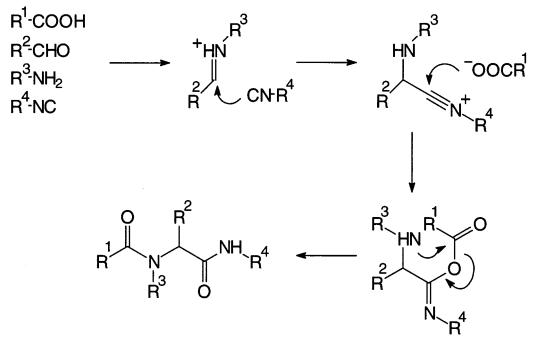
slightly lower percentage of dimethylsulfoxide (50–60%). From these data, it is tempting to conclude that the triple helix in native scleroglucan is more stable than the ordered conformation of the modified scleroglucans. However, it is possible that other factors such as the solubility of the modified scleroglucans in dimethyl sulfoxide may be of importance. Anyhow, these data are in agreement with the data obtained varying the pH and temperature and it is more than likely that the modified scleroglucans with a low degree of modification show some ordered conformation in water.

The nature of the ordered conformation of the lower modified scleroglucans is not clear. Several interactions of these chains may be envisioned, including double and triple helices or even macrocycles. An important difference, as noted above, between the carboxymethylation and the oxidation is that, during the former reaction, scleroglucan is in its disordered coil conformation and during the latter reaction the polysaccharide is probably in its ordered triple-helical conformation since it is performed at pH 9–9.5 (possibly the polysaccharide can maintain this conformation until the degree of oxidation, that is, the repulsion between the charges, becomes too high). Furthermore, the TEMPO-mediated oxidation is selective for the primary alcohols, which are directed outwards from the triple helix [34], whereas the carboxymethylation is probably more or less random in the polymer. The high viscosity of CMS8 below pH 12, which is comparable to that of native scleroglucan, seems to indicate that a large fraction of triple helix may be present. Note also that the viscosities of CMS8 and CMS16 are comparable above pH $\sim$ 12.5, indicating that the molecular weights of the single strands are more or less equal. The possibility that the higher-substituted samples are degraded too much to form an ordered conformation can thus be ruled out, and it seems that any ordered conformation here is unfavourable due to the change in primary structure of the strands.

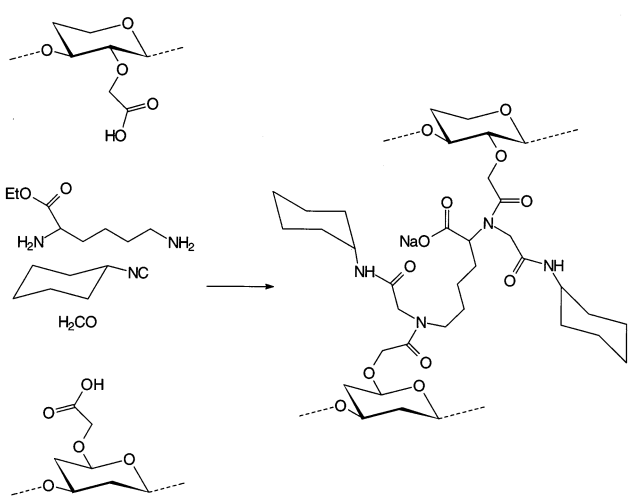
The influence of the charge density on the conformation of scleroglucan is preferentially studied using the TEMPO-mediated oxidation, since it is performed at a lower pH than the carboxymethylation and the degree of oxidation can be very precisely monitored. From Fig. 2 it is seen that a charge density higher than 40% (with respect to the primary alcohols present) yields a disordered conformation. The SOX20 sample may still be in its original triple-helical conformation, as is indicated by its high intrinsic viscosity at 0.1 M NaCl and its stability at high temperatures. It seems that the SOX40 sample is only partly in an ordered conformation, or in another type of ordered conformation than the triple helix, since the viscosities of SOX20 and SOX40 above pH $\sim$ 12.5 are practically identical, indicating a similar molecular weight for the individual strands, whereas the viscosity below pH 12 of SOX20 is very much higher than that of SOX40. However, it is difficult to indicate a certain charge density at which the transition from ordered to disordered occurs because this will also depend on other variables like the concentration of added salt etc.

Synthesis of hydrogels from modified scleroglucan.—Recently, we described [25] the use of the Ugi four-component condensation [35] for the synthesis of several polysaccharide hydrogels. Here we used this condensation for the synthesis of negatively charged scleroglucan hydrogels. The four-component condensation is shown in Scheme 3. Several carboxymethylated scleroglucans were used and the three other components were formaldehyde, cyclohexyl isocyanide and lysine ethylester as the bifunctional coupling

reagent. A possible cross-link is shown in Scheme 4. The advantage of lysine ethylester as the cross-linking reagent was that the ester may be hydrolysed very easily after the reaction, yielding a charged cross-link that improves the ability of the network to take up water. Furthermore, using this reagent the hydrogels were in all cases completely transparent, whereas the use of a less soluble diamine yielded in several cases slightly turbid hydrogels. In Table 4, the influence of the initial concentration of polysaccharide and the theoretical density of cross-links is shown on the swelling behaviour of the gel (entries 1–9). As expected, a higher initial concentration of polysaccharide with the same percentage of cross-links yielded a gel that absorbed less water. The same is true when starting from the same initial concentration of polysaccharide and increasing the degree of cross-linking.



Scheme 3.



Scheme 4.

Table 4
Swelling behaviour of networks formed by the Ugi reaction (entries 1–9) and with DES (entries 10–15) on scleroglucan

Entry	Polysaccharide	C_p (w/V) [a]	Cross-link [b]	Swelling [c]
1	CMS71	1.3	10	750
2	CMS26	1.3	8.5	270
3	CMS22	1.3	8.5	210
4	CMS22	1.3	5.5	350
5	CMS20	1.3	5.5	290
6	CMS20	3.2	5.5	190
7	CMS16	1.3	5.5	250
8	CMS16	1.3	2.5	510
9	CMS16	3.2	5.5	130
10	APS [d]11	2	3.5	130
11	APS16	2	3.5	190
12	APS16	2	7	140
13	APS16	2	10.5	100
14	APS25	2	5	270
15	APS85	4	15	520

[a] Initial concentration of polysaccharide.
[b] Theoretical proportion of cross-links.
[c] Swelling of the hydrogel given as the ratio of the equilibrium weight of the gel swollen in water divided by the dry weight of the gel.
[d] Aminopropyl scleroglucan.

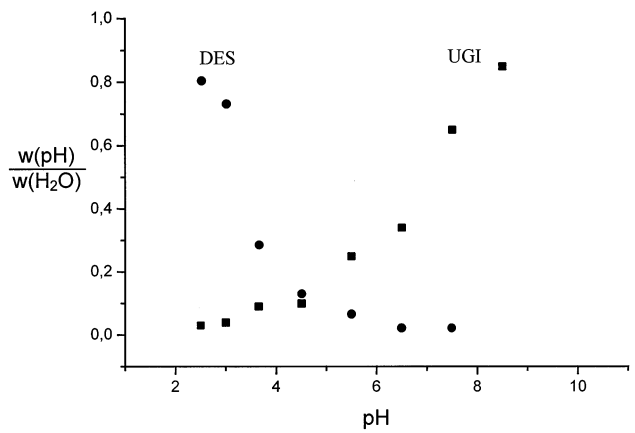
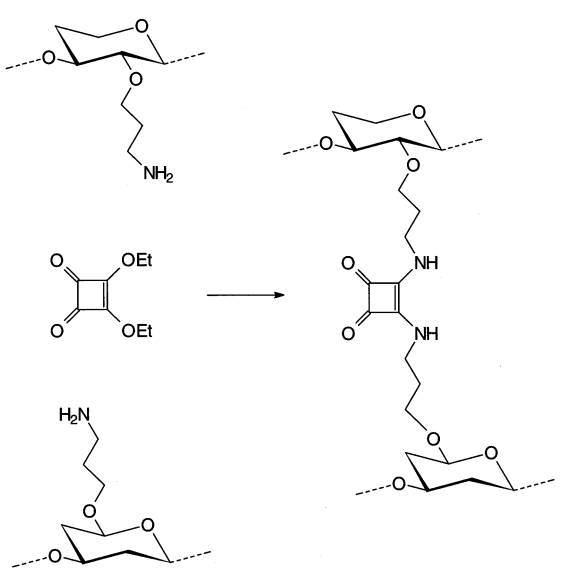


Fig. 6. Swelling of a Ugi-gel ((■), Table 4, entry 3) and a DES-gel ((●), Table 4, entry 13) as given by the weight at a certain pH (w(pH)) divided by the initial weight in water ($w(H_2O)$) vs. the pH at 25 °C.

The influence of pH on the swelling behaviour is shown in Fig. 6. The measurements were simply performed by adding either a small amount of diluted hydrochloric acid or sodium hydroxide to the gel swollen previously in distilled water. The pH and the weight of the gel were measured after 48 h. Clearly, the weight at every pH was smaller

than that in pure water due to screening of the charges by the added salt. Lowering the pH showed an added decrease in swelling due to association of the carboxylic acid.

We previously showed that DES can be used as a coupling reagent for the synthesis of a chitosan network [26]. Here we applied this reaction to various amine-containing scleroglucan derivatives, yielding a network with a structure as shown in Scheme 5. It should be noted that the reaction of DES at ambient temperature with the various derivatives was very rapid at a pH of about 10. This is in contrast to the synthesis of chitosan networks using DES, which had to be performed at a pH of about 5.5 due to the poor solubility of chitosan at higher pH. Obviously, at pH 10 much more free amine, which reacts as a nucleophile, is present in comparison with pH 5.5. In Table 4, the results are summarised (entries 10–15). The influence of the initial concentration of polysaccharide and cross-linker on the swelling capacity of the gel shows a similar trend as seen with the negatively charged hydrogels described above. Also, the increase in swelling capacity upon increasing the initial charge density is clear. In a plot of the relative swelling of the gel as a function of pH (Fig. 6), it is seen that the gel swells more at low pH than at high pH due to protonation of the amine. This behaviour is of course contrary to that observed for the negatively charged hydrogels.



Scheme 5.

4. Conclusions

In this work, we have shown that three simple methods can be used to obtain charged scleroglucan derivatives with various charge densities. Negatively as well as positively charged polyelectrolytes could be obtained, albeit the latter ones only with a moderate degree of substitution due to side reactions. From a study towards the presence of some ordered conformation in the modified scleroglucans, it has become very probable that this is the case for the samples with the lower degrees of substitution. Thus, we found a conformational transition for the CMS8, SOX20 and SOX40 samples as a function of pH, temperature and solvent. All measurements were in agreement with each other and very similar to the conformational transition observed for native scleroglucan under these conditions. Although it cannot be concluded that this transition is one from a triple-helical conformation to a coil for the modified scleroglucan samples, this may well be the case for the SOX20 sample since it was prepared under conditions that are non-destructive for the triple helix and the intrinsic viscosity of the sample is relatively high. The CMS8 sample is interesting since it shows the characteristics of a conformational transition although it was prepared under conditions where scleroglucan is present as a coil. It has to be concluded that some renaturation takes place in this case, which of course is not necessarily in the form of a triple helix.

The modified scleroglucans could be easily transformed into networks that were able to contain a large amount of water. Thus, in a two-step sequence native scleroglucan was transformed in either positively or negatively charged hydrogels. All reactions were performed in aqueous solution and should, in principle, also be applicable to other polysaccharides.

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