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Influence of the Charge Density on the Solution Behaviour of Polycarboxylates Derived from the Polysaccharide Scleroglucan

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SUMMARY

Solution properties of polycarboxylates obtained by periodate oxidation of scleroglucan, with different degrees of oxidation, have been studied as a function of pH. Viscosity, calorimetric, optical and chiro-optical measurements show that the two carboxylated samples with higher degrees of oxidation (S-1·0 and S-0·7) can assume an ordered conformation at low pH. On the contrary the sample characterized by a lower extent of oxidation (S-0·2) shows no conformational changes. Optical measurements suggest that this polymer would be in an ordered helical form in dilute aqueous solution.

INTRODUCTION

Uncharged, regular carbohydrate polymers which, by virtue primarily of particular inter-residue linkages, adopt the so-called hollow helical conformation in the solid state, often dissolve in water, keeping their regular conformation for long stretches of the chains. Well known, relevant examples are represented by curdlan and scleroglucan (or its twin polymer, schizophyllan) which have backbones built up by the repeated, regular β-(1, 3)-linking of D-glcp residues. In the case of scleroglucan, triple helical chains can be rather easily dispersed in water at room temperature (as opposed to the case of curdlan) due essentially to the presence of flexible D-glcp side groups regularly linked β-(1,6) to every third D-glcp residue along the chains. Only by raising the pH to *c.* 12 or by heating beyond *c.* 90°C can one disrupt the helical conformation of scleroglucan present in solution (or, similarly, of schizophyllan) to yield single, randomly-coiled chains.

We considered it important to investigate the perturbing influence that charged groups distributed along the chain might exert on the ability of the β-(1,3)-glucan skeleton to assume an ordered, helical conformation in aqueous media.

One relatively easy means of introducing such groups at the desired level consists of selectively oxidizing the β-(1,6)-D-glcp residues of scleroglucan.

In a previous paper we described the preparation and characterization of a polycarboxylic acid derived from scleroglucan by quantitative oxidation of its sidegroups using aqueous periodate and chlorite ions (Crescenzi *et al.*, 1983). Such a polyelectrolyte (S-1·0) exhibits rather peculiar behaviour in dilute aqueous solution, in particular in its interactions with Ca^{2+} ions (Crescenzi *et al.*, 1983). We wish to report here additional, original evidence on the solution properties of S-1·0 and of a similarly prepared new polycarboxylate from scleroglucan in which only *c.* 72% of the lateral β-(1,6)-D-glcp residues were oxidized (S-0·7).

The influence of protonating the side-chain carboxylate groups of both S-1·0 and S-0·7 samples has been studied by means of chiro-optical, viscometric and calorimetric measurements.

A pH-induced conformational change is clearly and consistently disclosed for S-1·0 and S-0·7 with an onset at a critical, characteristic charge density of the chains. The results are discussed also on the basis of more limited relevant data collected using a polycarboxylate sample prepared from scleroglucan but with a degree of side-chain oxidation limited to *c.* 20% (S-0·2).

2. EXPERIMENTAL

2.1 Preparation, purification and characterization of oxidized samples

A purified scleroglucan sample from Ceca AS, France (Actigum CS-11) has been used for this study. Polycarboxylated polymers (S-1·0, S-0·7 and S-0·2) were obtained by periodate oxidation (in water) of sclero-

TABLE 1
Specific Optical Activity of the Polycarboxylates Derived from Scleroglucan (Na^+ salt: see Section 2)

Sample	$[\alpha]^{25}_{302}$ *(degrees* $cm^2\,g^{-1}$*)* $\times 10^{-1}$		
	H_2O	*0·05* M *NaCl*	*0·2* M *NaCl*
S-1·0	97 ± 1	97 ± 1	97 ± 1
S-0·7	54 ± 2	54 ± 1	–
S-0·2	103 ± 3	–	–

glucan glucopyranosic sidechains (β-(1,6)-linked) followed by oxidation by $NaClO_2$, as described in the previous paper (Crescenzi *et al.*, 1983).

Different extents of oxidation: ~100% (S-1·0), ~72% (S-0·7) and ~20% (S-0·2) were obtained using a molar ratio for IO_4^-/polysaccharide (repeating unit) of ~3·6, ~1 and ~0·25, respectively. An excess of $NaClO_2$ was always used to convert aldehydic groups into carboxylic ones. Solutions containing oxidized samples were dialysed against EDTA and, eventually, against distilled water after addition of NaCl to a final concentration of ~0·5 M.

Potentiometric titrations gave an equivalent weight for the polycarboxylate repeating unit of 360, 482 and 1730 for S-1·0, S-0·7 and S-0·2, respectively.

The values of the specific optical activity at 302 nm, $[\alpha]^{25}_{302}$, for the three samples are shown in Table 1.

Structural characterization of samples was obtained by ^{13}C-n.m.r. spectra at 100 MHz. Figure 1(A) shows the ^{13}C-n.m.r. spectrum for the polyanion S-0·7 at 80°C in D_2O. The assignments were given, in view of their likeness, by analogy with those reported in the previous paper (Crescenzi *et al.*, 1983) for the carboxylated polymer S-1·0. On the contrary the ^{13}C-n.m.r. spectrum of S-0·2, obtained in C_2D_6SO because of the low sample solubility in water (Fig. 1(B)), exhibits features quite similar to those of scleroglucan. In fact, the chemical shifts of the assigned signals are in good agreement with those already reported for the intact natural polysaccharide (Rinaudo & Vincendon, 1982; Crescenzi *et al.*, 1983). Moreover, due to the low percentage of oxidation, carbon resonances relative to the split moiety do not come out significantly different from the noise level.

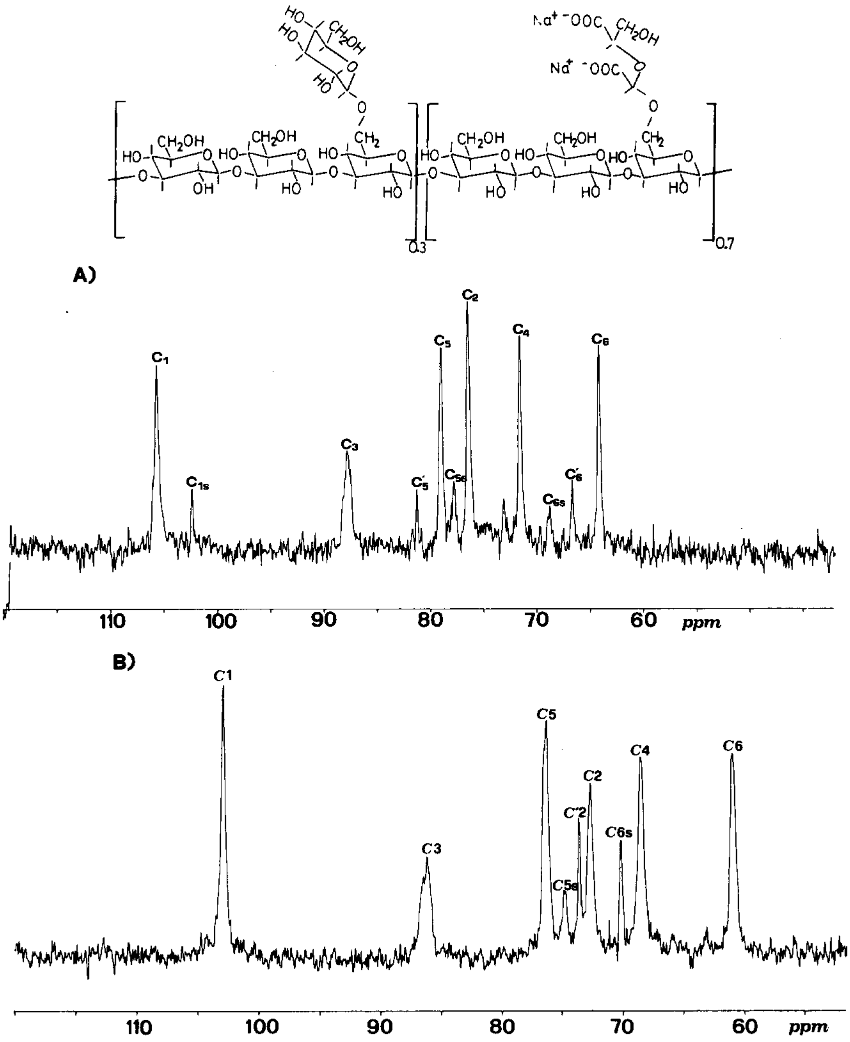

Fig. 1. S-0·7 oxidized sample repeating unit. (A) ^{13}C-n.m.r. spectrum at 100 MHz and 80°C of S-0·7 in D_2O; (B) ^{13}C-n.m.r. spectrum at 100 MHz and 80°C of S-0·2 in C_2D_6SO.

2.2 Other chemicals

Sodium metaperiodate and methylene blue (MB) were Merck analytical grade products. Sodium chlorite was a Fluka technical grade product. MB stock solutions were stored in the dark, and their titres were checked by means of optical density measurements ($\epsilon = 7{\cdot}8 \times 10^4\,\text{M}^{-1}\,\text{cm}^{-1}$ at 664 nm). HCl solutions have been prepared using Merck Titrisol phials.

2.3 Instrumentation

Optical activity measurements were carried out using a Perkin Elmer 241 spectropolarimeter. Readings were taken at 302 nm and 25°C with 1 dm thermostated cells.

Calorimetric measurements of the enthalpies of protonation in dilute aqueous solution were obtained using a LKB-10070 batch microcalorimeter at 25°C (following procedures already described (Crescenzi *et al.*, 1981*a*, *b*)).

Visible spectra at 25°C of solutions containing methylene blue (MB) were recorded with a Cary-219 spectrophotometer.

Circular dichroism (CD) spectra were recorded using a Cary-61 dichrograph.

Viscosity measurements have been made at 25°C using a Cannon–Ubbelhode viscometer with an efflux time of 99·0 for aqueous 0·05 M NaCl.

All polymer solution concentrations were in the range 0·1–0·2% (w/v). Concentrations expressed as eq. dm^{-3} refer to equivalents of ionisable groups per dm^3.

The ^{13}C-n.m.r. spectra were obtained at 80°C with a Brucker WP-100 instrument. The S-0·7 sample (Na^+ salt) was dissolved in D_2O (~5% w/v), while the S-0·2 samples (Na^+ salt) was dissolved in C_2D_6SO at a concentration of about 5% w/v. Experimental conditions were as follows: 90° pulse; 8K acquisition data points; 70K–80K pulses; chemical shifts were measured with reference to internal acetone (31·07 ppm with respect to external sodium-2,2-dimethyl-2-silapentane-5-sulphonate (DSS)). Resolution-enhanced spectra were obtained by exponential multiplication using an LB = 1 parameter.

3. RESULTS AND DISCUSSION

3.1 Viscosity data

A series of viscosity measurements using S-1·0 (Na^+ salt) aqueous solutions at different ionic strengths, *I* (NaCl), and 25°C has yielded the

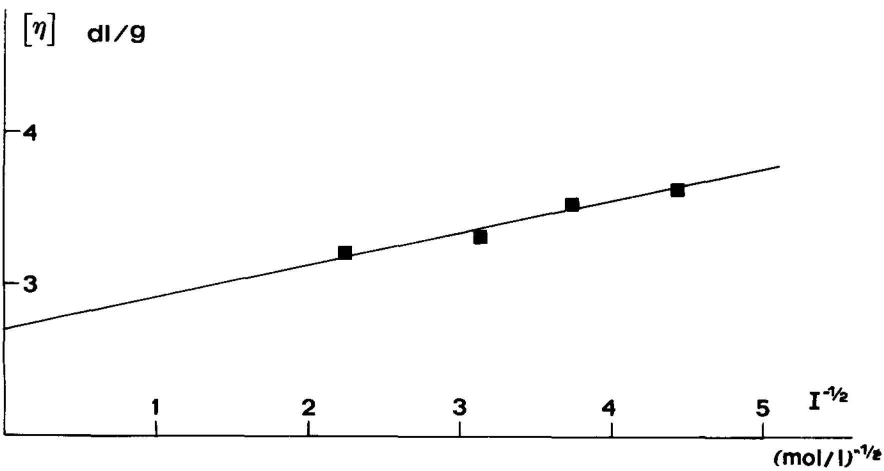

Fig. 2. Dependence of the intrinsic viscosity of S-1·0 on the ionic strength $I^{-1/2}$ (mol dm^3)$^{-1/2}$.

intrinsic viscosity values, [η], which are plotted in Fig. 2 against $I^{-1/2}$ (mol dm^{-3})$^{-1/2}$.

The linear [η] versus $I^{-1/2}$ relationship may be used, employing the Smidsröd & Haug (1971) approach, to derive some information on the hydrodynamic properties of the S-1·0 chains. In particular, it appears from a comparison of the data of Fig. 2 with the data of table II of Smidsröd & Haug (1971) that S-1·0 should be a stiff polymer in aqueous salt solution, with quite expanded chains, as is, for instance, the case of alginates.

In conclusion, raising the ionic strength of S-1·0 solutions (with NaCl) up to $I = 1$ mol dm^{-3} leads only to a moderate, continuous contraction in the average hydrodynamic volume of the macroions.

The behaviour of S-1·0 is different on changing its degree of protonation in 0·05 M NaCl (25°C) as is evident from the viscosity data of Fig. 3.* These results, obtained at a fixed total polymer concentration

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* The degree of protonation, αp, is defined as:

$$\alpha p = \frac{\text{Protonated polysaccharide eq.}}{\text{Total polysaccharide eq.}}$$

The concentration of protonated polysaccharide was calculated after each known addition of HCl taking into account the free [H$^+$] as evaluated with a pH-meter.

by mixing appropriate aliquots of solutions of the polyelectrolyte in the Na^+ and H^+ forms, indicate, in fact, that there is a critical range in the degree of protonation, αp, from about 0·6 to 0·7 where there is a discontinuity in the η_{sp}/c values. Such a trend might reflect a slight increase in average chain dimensions with, possibly, the transition to an even stiffer conformation, typical of the final fully protonated (i.e. uncharged) form.†

On the other hand, the intrinsic viscosity of the fully protonated form of S-1·0 (measured in NaCl at 0·05 M, pH = 2·2 and 25°C) resists 2·85 $dl\,g^{-1}$, that is *lower* than the intrinsic viscosity of the Na^+ salt of S-1·0 at the same ionic strength and temperature ($[\eta] = 3{\cdot}6\ dl\,g^{-1}$).

3.2 Calorimetric data

The influence of protonation on the solution behaviour of S-1·0 and S-0·7 was further explored by means of calorimetric experiments (25°C). The results given in Fig. 4 show unambiguously that, for both carboxylates, there is a critical chain charge-density below which the integral enthalpy of protonation becomes negative. This strongly suggests, in conjunction with similar evidence for different synthetic and natural polyelectrolytes (Crescenzi *et al.*, 1981*a*,*b*, 1983), the occurrence of an (exothermic) conformational transition. The phenomenon takes place for S-1·0 in the αp range in which an anomaly in the viscosity was found (Fig. 3).

For S-0·7 (for which the calorimetric data in H_2O are quite reproducible, as opposed to the viscosity data in 0·05 M NaCl) the onset of the transition would occur at around $\alpha p = 0{\cdot}5$ (Fig. 4). It may be concluded, recalling that if ξ_0 is the charge-density parameter of S-0·7 (Na^+) then for S-1·0 (Na^+) one has $\xi_0/0{\cdot}7$, that both polyelectrolytes exhibit distinct anomalies at nearly the *same* formal, reduced charge density (i.e. $0{\cdot}5\xi_0$ and $0{\cdot}35/0{\cdot}7\xi_0$).

It appears, in consequence, reasonable to propose that at such charge density the tendency of the regular β-(1,3)-D-glcp backbone to assume an ordered, helical conformation overwhelms coulombic repulsions among residual ionized carboxylate sidegroups along the chains.

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† With S-0·7 qualitatively similar results have also been obtained: in this case, however, the viscosity data were not as reproducible as for S-1·0 suggesting a tendency to chain aggregation consequent upon protonation for S-0·7.

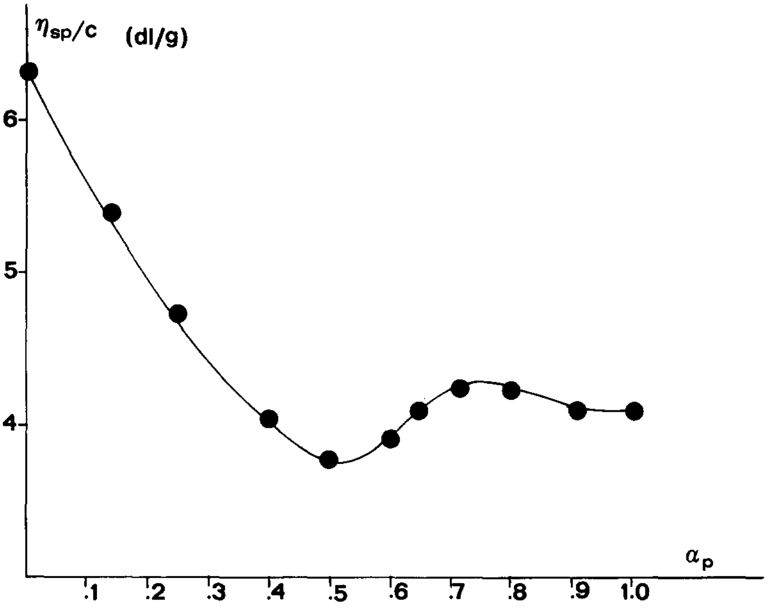

Fig. 3. Dependence of the reduced specific viscosity of S-1·0 on αp in 0·05 M NaCl. (Polymer concentration = 0·2% (w/v).)

The roughly approximate figure for the enthalpy of transition would then be −0·8 kcal/eq. for both these polyelectrolytes, according to Fig. 4 (Crescenzi *et al.*, 1983).

3.3 Chiro-optical data

The results of optical activity measurements illustrated in Fig. 5 qualitatively but clearly confirm the suggestions discussed in Sections 3.1 and 3.2.

It is noteworthy that the $[\alpha]^{25}_{302}$ values first decrease on increasing αp and then sharply rise to attain plateau values which are nearly three times as large as those recorded at the minima.

Also the CD spectra recorded in the 200–250 nm region show that the ellipticity associated essentially with the carboxyl chromophores first decreases and then rises with increasing protonation of S-1·0 and S-0·7 in water.

In addition, it is interesting to consider the data obtained by studying the absorption spectra and the CD, in the visible range, of aqueous

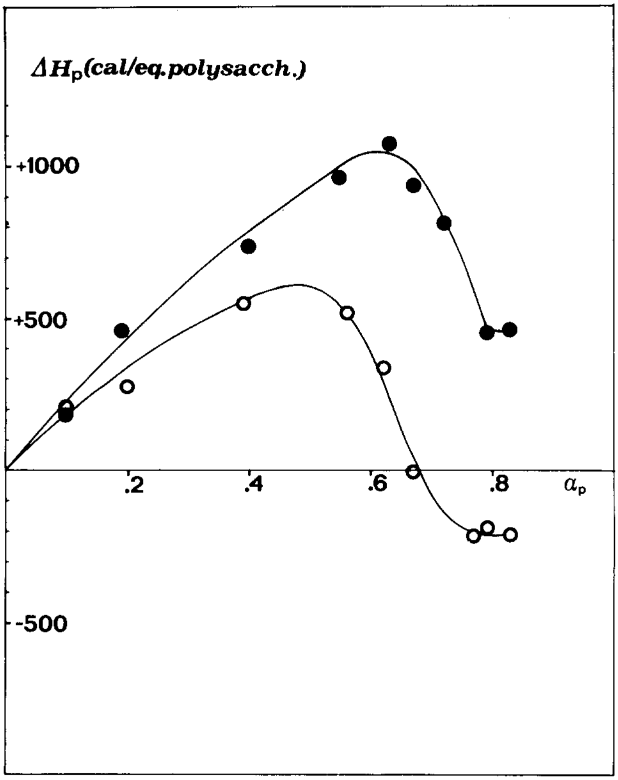

Fig. 4. Enthalpy of protonation at 25°C of: (●) S-1·0, polymer concentration = $2{\cdot}5 \times 10^{-3}$ eq. dm^{-3}; (○) S-0·7, polymer concentration = $2{\cdot}1 \times 10^{-3}$ eq. dm^{-3}.

solutions of S-1·0, S-0·7 and scleroglucan, each containing MB at a polymer/dye equivalent concentration ratio of *c*. 400 (Fig. 6).

Dye binding by S-1·0 and S-0·7 (Na^+ salts) is rather extensive but there is no detectable extrinsic optical activity associated with bound MB molecules. Protonation of the polyelectrolytes, however, brings about the development of a CD band centred at *c*. 580 nm (i.e. at a wavelength where MB bound aggregates absorb) despite the distinct reduction in the total amount of bound dye molecules in both cases (as expected and demonstrated by the optical density data of Fig. 6).

In the case of scleroglucan, dye molecules are also bound onto the polymer chains (to an obviously lesser degree than for the polyanions) and exhibit extrinsic ellipticity always at around 580 nm.

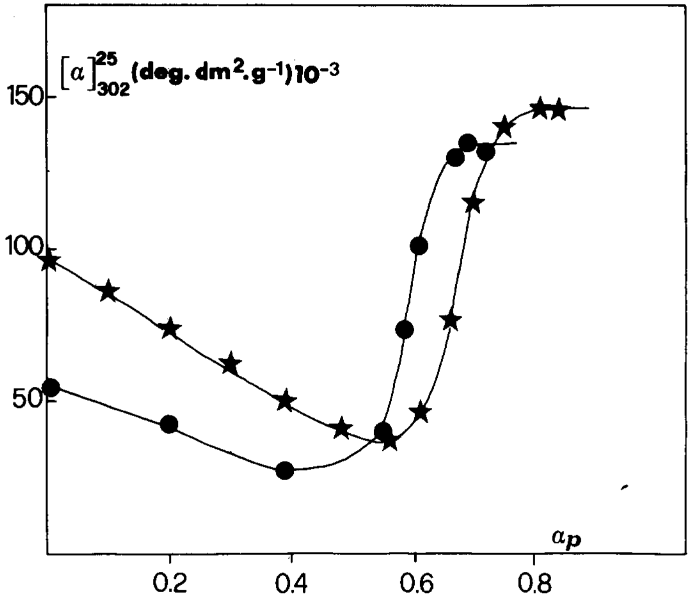

Fig. 5. Dependence of the specific optical activity of S-1·0 (★) and S-0·7 (●) at 302 nm and 25°C on the degree of protonation. (Polymer concentration = 4·1 × 10^{-3} eq. dm^{-3}.)

When we consider that scleroglucan in water would be in an ordered helical conformation (Yanaki *et al.*, 1981), the close similarity between the CD bands of the dye bound to the three polymers (Fig. 6) in their ordered conformational states, suggests that these states might be similar if not identical.

In reality, scleroglucan is reported to assume, in aqueous solution, a stable, triple-helical conformation but we cannot conclude, on the basis of the data collected so far, whether this is also the state for our polycarboxylate samples (S-1·0 and S-0·7). Work is in progress to clarify this important point.

In any event, CD shown by MB (Fig. 6) should be traced to a binding (with stacking) of dye molecules onto the polymer *backbones*. This would have points in common with what has been observed by others studying the interactions between different dyes and a variety of uncharged polysaccharides in aqueous media (Wood, 1980).

Finally, it is relevant to consider a few results obtained studying the S-0·2 polymer (see Section 2). The latter dissolves in water upon gentle

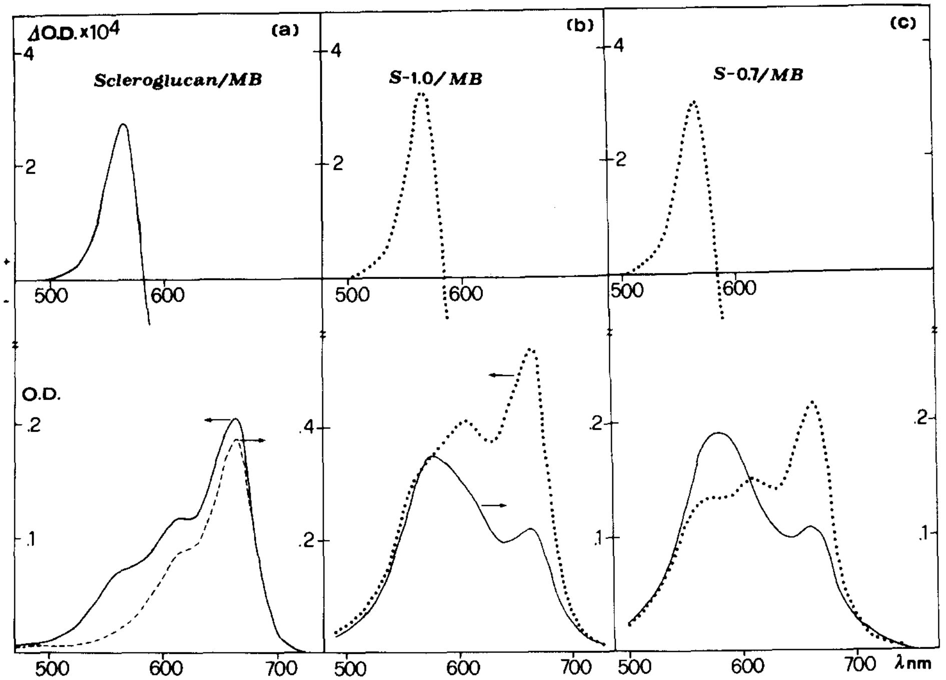

Fig. 6. (a) *Bottom*: (– – –) visible spectrum of MB; (——) visible spectrum of MB in the presence of scleroglucan. *Top*: CD spectrum of MB in the presence of scleroglucan. (Polymer concentration = 2·9 × 10^{-3} mono. mol dm^{-3}; dye concentration = 6·2 × 10^{-6} M.) (b) *Bottom*: (——) visible spectrum of MB in the presence of S-1·0 (polymer concentration = 2·5 × 10^{-3} eq. dm^{-3}, dye concentration = 6·2 × 10^{-6} M); (· · ·) visible spectrum of MB in the presence of S-1·0 and H^+ ions (excess added) (polymer concentration = 2·5 × 10^{-3} eq. dm^3, dye concentration = 1·25 × 10^{-5} M). *Top*: CD spectrum of MB in the presence of S-1·0 and H^+ ions (excess added) (polymer concentration = 2·5 × 10^{-3} eq. dm^{-3}, dye concentration = 1·25 × 10^{-5} M). (c) *Bottom*: (——) visible spectrum of MB in the presence of S-0·7, (· · ·) visible spectrum of MB in the presence of S-0·7 and H^+ ions at the equivalent concentration ratio H^+/polysaccharide = 1·2 (αp = 0·8). *Top*: CD spectrum of MB in the presence of S-0·7 and H^+ ions at the equivalent concentration ratio H^+/polysaccharide = 1·2 (αp = 0·73). (Polymer concentration = 2·5 × 10^{-3} eq. dm^{-3}; dye concentration = 6·2 × 10^{-6} M.)

heating and gives clear, stable solutions at concentrations not higher than 6 × 10^{-4}–1·2 × 10^{-3} eq. dm^{-3}. Additions of HCl or of $Ca(NO_3)_2$ solutions to an aqueous 6 × 10^{-4} eq. dm^{-3} solution of S-0·2 (Na^+ salt) in water at 25°C *do not* change the $[\alpha]^{25}_{302}$ reading ($[\alpha]^{25}_{302}$ = 103) as opposed to what was found for S-1·0 and S-0·7 (see figures 5 and 3 of Crescenzi *et al.*, 1983).

Moreover, the visible CD spectrum of a S-0·2/MB solution (polymer concentration = 6×10^{-4} eq. dm^{-3}, MB = $6{\cdot}2 \times 10^{-6}$ M) shows the presence of extrinsic optical activity of bound molecules, like scleroglucan.

On the basis of the preceding discussion these findings clearly suggest that the S-0·2 polymer (Na^+ salt) would be in an ordered helical conformation in dilute aqueous solution. This is in agreement with what has been found with the S-1·0 and S-0·7 samples, for which a reduction of charge-density along the chains (e.g. by protonation) is necessary to let the β-(1,3)-D-glcp backbone assume its low-energy helical form. In fact, in the S-0·2 polymer (Na^+ salt) a charge-density parameter lower than the critical one ($0{\cdot}5\xi_0$; see Section 3.2) may be observed.

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