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Ionic strength and temperature dependence of oxidized scleroglucan solution properties: optical activity and viscosity data

Tommasina Coviello[a,b], Mariella Dentini[a], Vittorio Crescenzi[a] & Antonio Vincenti[a,c]

[a]*Department of Chemistry, La Sapienza University, 00185 Rome, Italy*
[b]*Present address: Department of Chemistry and Technology of Biologically Active Compounds, La Sapienza University, Rome, Italy*
[c]*Present address: Fidia Advanced Biopolymers (F.A.B.), c/o C.N.R.S.M., Mesagne (Br), Italy*

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The physicochemical properties of oxidized scleroglucan have been investigated in aqueous solution as a function of ionic strength and/or temperature by means of polarimetry and viscosimetry techniques. For low salt concentrations (0·2 M NaCl) a normal polyelectrolyte behaviour was detected. Increasing the ionic strength up to 5·0 M NaCl a variation of chain structure would occur. Peculiar to the system was the temperature role which resulted in an irreversible annealing whose effect became stronger as the thermal cycles were repeated: a similar annealing process was also shown in the presence of salt (NaCl).

INTRODUCTION

Scleroglucan is a microbial non-ionic polysaccharide which has been extensively studied for its peculiar solution properties, notably rheological properties.

Scleroglucan chains in aqueous media feature a triple helical conformation of remarkable stability (Norisuye *et al.*, 1980; Norisuye, 1985; Yanaki *et al.*, *1980;* Yanaki *et al.*, 1981; Yanaki & Norisuye, 1983; Enomoto, 1984). In addition, because of its primary structure exhibiting a backbone built up by (1,3) linked β-D-glc*p* units with single glc*p* side chains linked β-(1,6) to every third residue in the main chain, the polysaccharide lends itself to simple derivatization by means of selective, controlled periodate oxidation of the side chains (Hofreiter *et al.*, 1957; Crescenzi *et al.*, 1983, 1988; Gamini *et al.*, 1984).

The ensuing aldehyde groups can be reacted with compounds—either monomeric or polymeric—bearing amino groups (reductive amination) thus obtaining novel, linear or cross linked polysaccharide derivatives (Crescenzi *et al.*, 1995), or can be oxidized to carboxylate groups thus yielding polyelectrolytes of variable, controlled charge density (Crescenzi *et al.*, 1983; Gamini *et al.*, 1984). The latter exhibit interesting conformation dependent solution properties (Crescenzi *et al.*, 1983, 1988; Gamini *et al.*, 1984). Both scleroglucan and its oxidized derivative have been found suitable for the formulation of sustained drug release forms (Alhaique *et al.*, 1985, 1986, 1993; Romanelli *et al.*, 1993).

In accord with our interest in scleroglucan derived polycarboxylates (hereafter collectively indicated as 'sclerox') we have studied details of the ionic strength (NaCl) and temperature dependence of the chiroptical and viscosity properties of a sclerox sample (100% oxidized scleroglucan, sodium salt form: Fig. 1) in dilute aqueous solution.

EXPERIMENTAL

Oxidized scleroglucan (sclerox) samples were prepared according to procedures described elsewhere (Crescenzi *et al.*, 1983; Gamini *et al.*, 1984). Optical activity measurements were performed with a Perkin-Elmer 241 polarimeter using a 10 cm pathlength, the temperature being controlled by means of a Lauda circulating water bath. The values of optical activity were calculated without taking account of the moisture content of the polysaccharide samples and, therefore, might differ slightly from those reported earlier (Crescenzi *et al.*, 1983; Gamini *et al.*, 1984).

Viscosity measurements for the Na^+ salt of sclerox were performed in the temperature range 20–70°C using a Schott–Geraete automatic viscometer equipped with a

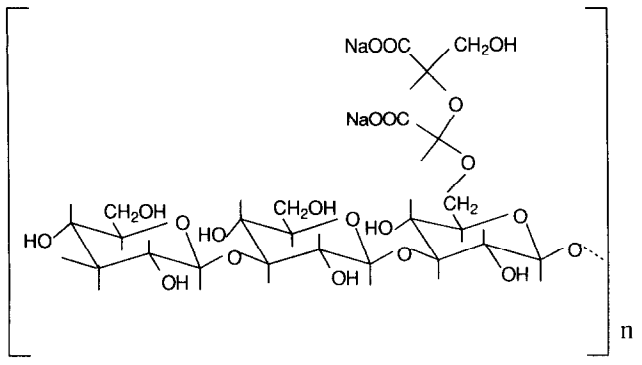


Fig. 1. Repeating unit of oxidized scleroglucan (sclerox).

water thermostat. A range of ionic strengths was investigated; these were controlled by varying the level of NaCl added. The freshly prepared sclerox samples and the salt solutions had been filtered 4 times with Millipore filters of 8·0 and 0·22 μm average pore size, respectively.

RESULTS

Isothermal data

As shown in Fig. 2, the optical activity of sclerox changes markedly and in a peculiar fashion with increasing ionic strength, I, reaching a maximum between 1 and 2 M NaCl (25°C).

For the same range of ionic strength, the intrinsic viscosity data reported in Fig. 3a and b clearly demonstrate that, after an initial decrease due essentially to the electrostatic screening of sclerox fixed charges by added Na^+ counterions (a normal polyelectrolyte effect: see insert of Fig. 3b), the $[\eta]$ values assume an upward trend.

The phenomenon appears more discernible by the plot shown in Fig. 3c. A reasonable deduction is that by increasing the ionic strength two regimes are encountered: the first for NaCl concentrations between 0·01 and 0·20 M approximately, and the second above 0·20 M.

In the first regime sclerox macroions apparently behave 'normally' and appear to comply with expectations based on approximate polyelectrolyte theories

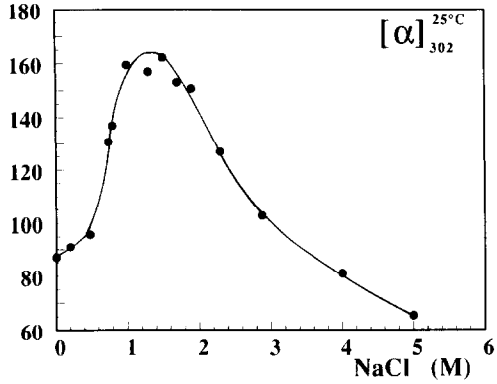
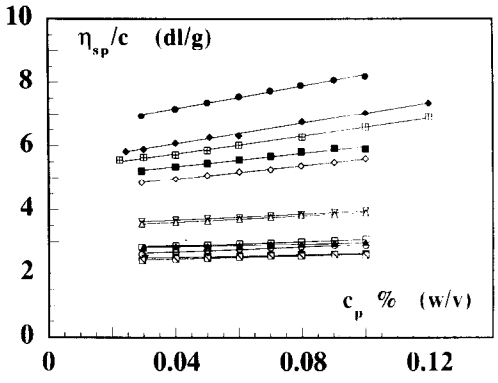
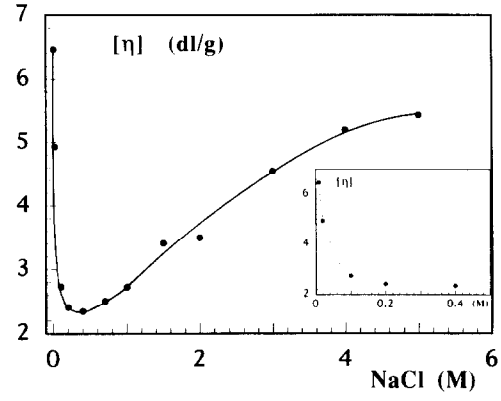


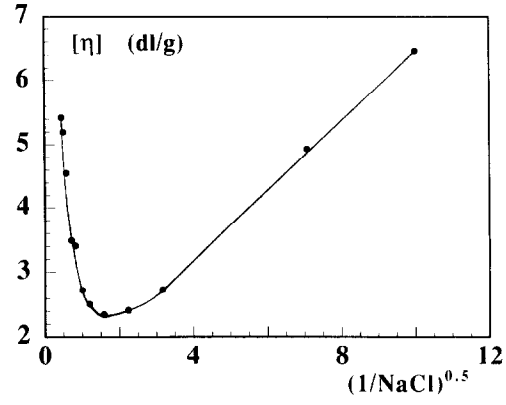
Fig. 2. Optical activity dependence (λ = 302 nm, 25°C) on salt (NaCl) concentration of sclerox aqueous solutions. Polymer concentration c_p = 0·25% (w/v).



(a)



(b)



(c)

Fig. 3. (a) Reduced specific viscosity of aqueous sclerox solutions at different NaCl concentrations at 25°C. The I values (M) are: (●) 0·01; (■) 0·02; (▲) 0·10; (▼) 0·20; (⊡) 0·40; (○) 0·70; (□) 1·00; (△) 1·50; (▽) 2·00; (◇) 3·00; (⊞) 4·00; (◆) 5·00. (b) Dependence of sclerox intrinsic viscosity on ionic strength I (NaCl) at 25°C. The insert shows the magnified first part of the curve. (c) Variation of sclerox intrinsic viscosity at 25°C with $I^{-0.5}$.

Table 1.

Polymer	*B*
Polyphosphate[a]	0·44
NaPCPP[b]	0·26
Sclerox in NaOH[c]	0·255
Polyacrylate[a]	0·23
Carboxymethylamilose[a]	0·20
Sclerox in NaCl (this work)	0·14
Gellan in 0·03 Me_4NCl at 45°C[d]	0·085
Oxidized guar[e]	0·067
Carboxymethylcellulose (DS = 1)[a]	0·065
Alginate (copolymer blocks)[a]	0·065
Hyaluronic acid[a]	0·065
Alginate (high content in mannuronic acid)	0·040
Alginate (high content in guluronic acid)[a]	0·031
Rhizobium trifolii EPS in NaCl[f]	0·030
Sodium pectinate (D.E. = 0·27)[a]	0·15
Rhizobium trifolii EPS in NaCl (ordered)[f]	0·0077
DNA[a]	0·0055

NaPCPP, poly[bis(carboxylatephenoxy)phosphazene].
[a]Smidsrod & Haug (1971).
[b]Masci (1994).
[c]Coviello *et al.* (1995).
[d]Crescenzi *et al.* (1987*b*).
[e]Dentini & Crescenzi (1986).
[f]Crescenzi *et al.*, (1987*a*).

(Smidsrod & Haug, 1971; Smidsrod *et al.*, 1980). For example, the value of the so-called 'viscosity parameter, *B* (Smidsrod *et al.*, 1980) results by interpolation of the linear, low ionic strength portion of the plot of Fig. 3c equal to 0·14. If this value can be compared with known *B* values for different polyelectrolytes (Table 1), then the sclerox chain stiffness would be 'moderate'.

The second viscosity regime would, on the contrary, reflect a notable stiffening of sclerox chains which in 4·0–5·0 M NaCl exhibit an intrinsic viscosity nearly three times higher than in 0·3 M NaCl (Fig. 3c).

On the basis of the data shown in Figs 2 and 3 we suggest that initial additions of salt to dilute sclerox solutions efficiently shield coulombic repulsion on the macroions but only affect charged side chains' mobility and local conformation: this would have 'normal' consequences on the viscosity (Fig. 3) although a notable change in optical activity (Fig. 2) is revealed. At high ionic strengths a conformational change involving the sclerox backbone would take place resulting in stretched chains whose rigidity would steadily increase up to the maximum NaCl concentration (5 M) used.

It should be noted that in the experiments discussed above all readings were not influenced by time effects, both the optical activity and the viscosity of sclerox solutions assuming immediately the values reported in Figs 2 and 3 which were stable over prolonged periods.

It is also noteworthy that in all cases the values of the Huggins viscosity constant were between 0·3 and 0·6 thus providing evidence, although indirect, of the absence of aggregation phenomena.

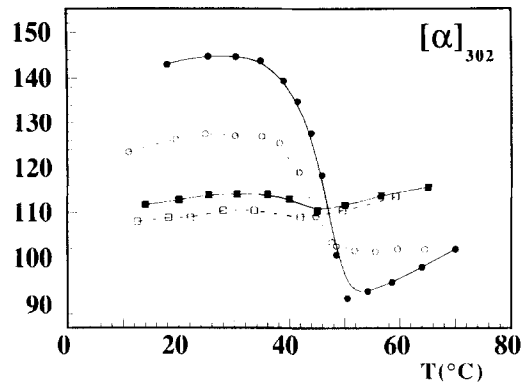


Fig. 4. Optical activity dependence (λ = 302 nm) on temperature of sclerox solution in 1·5 M NaCl. First cycle: (●) heating; (○) cooling. Fifth cycle: (■) heating; (□) cooling. Polymer concentration c_p = 0·25% (w/v).

Temperature dependence of sclerox optical activity

The temperature dependence of sclerox optical activity in 1·5 M NaCl is illustrated in Fig. 4. The results exhibit a number of peculiar features.

In the first thermal cycle there is a clearly anomalous, sigmoidal trend on heating: on cooling the anomaly is still evident but the initial optical activity readings are not reproduced. The phenomenon would not be traceable simply to hysteresis in as much as the 20°C optical activity ($[\alpha]_{302}$ = 125) at the end of the first cycle does not recover (even after 2 weeks) the original value characteristic of the 'never heated' sample ($[\alpha]_{302}$ = 144).

Additional thermal cycles (Figs 4, 5) involve a dramatic change in the shapes of the curves until the optical activity values become almost independent of temperature and the heating and cooling data finally almost coincide.

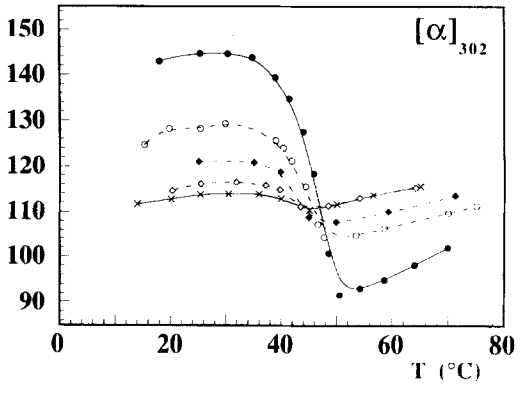


Fig. 5. Optical activity dependence (λ = 302 nm) on temperature of sclerox solution in 1·5 M NaCl. Heating curves for five subsequent cycles: 1° (●); 2° (○); 3° (◆); 4° (◇); 5° (×). Polymer concentration c_p = 0·25% (w/v).

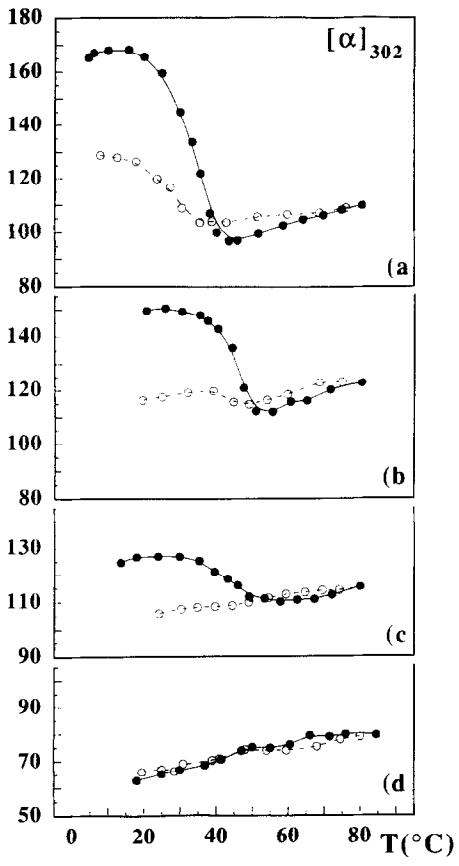


Fig. 6. Optical activity dependence ($\lambda = 302$ nm) of sclerox solutions for different NaCl concentrations as a function of temperature. Salt concentration (M): (a) 1·0; (b) 1·9; (c)2·3; (d) 5·0. (●) heating; (○) cooling. Polymer concentration $c_p = 0{\cdot}25\%$ (w/v).

Data collected working at different NaCl concentrations are reported in Fig. 6: in all cases, only a single thermal cycle was performed. It is seen that both anomaly and irreversibility are drastically reduced and eventually eliminated with increasing ionic strength.

From the optical activity–temperature profiles a set of 'melting' temperatures, T_M, were estimated at the respective inflection points: these T_M values are reported in the 'phase diagram' (Fig. 7).

Polyelectrolyte theory (Manning, 1972, 1978) predicts a single, linear relationship between the logarithm of the ionic strength and the inverse of the melting temperatures if the latter are connected to a single temperature induced conformational transition involving a difference in charge density along the macroions. In our case (Fig. 7), two straight lines crossing at I = 1·5 M were obtained.

Temperature dependence of sclerox viscosity

The temperature dependence of sclerox reduced viscosity (polymer concentration 0·14% w/V; 1·5 M NaCl) is illustrated in Fig. 8.

Two different thermal treatments have been applied: in the first case, the solution was heated slowly (8 h), kept overnight at 70°C, and then cooled down at the same rate (8 h) while in the second the entire thermal cycle was performed non-stop in 8 h (using a freshly prepared solution).

The main interesting feature stemming from Fig. 8 is that the reduced viscosity data collected on cooling are much higher than those recorded on heating: this is particularly evident for the 'slow' cycle. Working at the highest ionic strength (5·0 M NaCl) this phenomenon is virtually cancelled (Fig. 9).

DISCUSSION

In our opinion, the rather complex array of data presented above may be tentatively interpreted as follows:

(1) For NaCl concentrations lower than 0·2 M approximately the intrinsic viscosity of sclerox dilute aqueous solutions at 25°C changes in a simple fashion

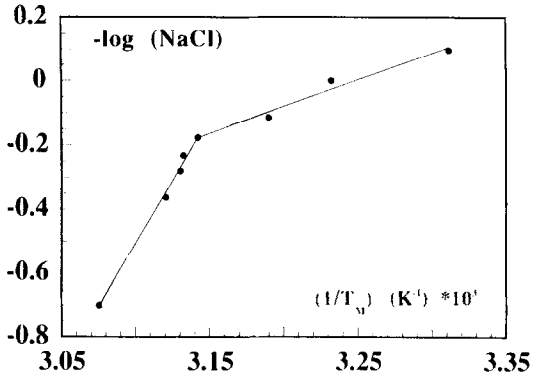


Fig. 7. Correlation between T_M, the temperature at inflection point of heating curves of sclerox solutions and ionic strength I.

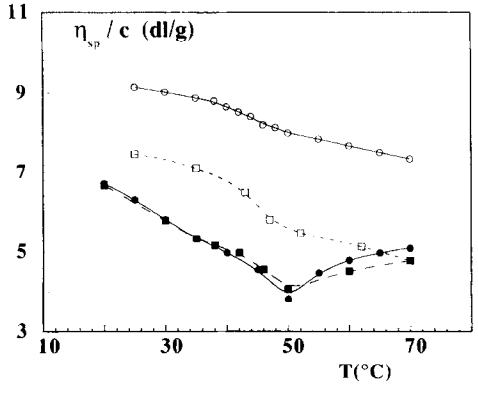


Fig. 8. Reduced specific viscosity dependence on temperature of two sclerox solutions in 1·5 M NaCl. Slow cycle: (●) heating; cooling (○). Fast cycle: (■) heating; (□)cooling. Polymer concentration $c_p = 0{\cdot}14\%$ (w/v).

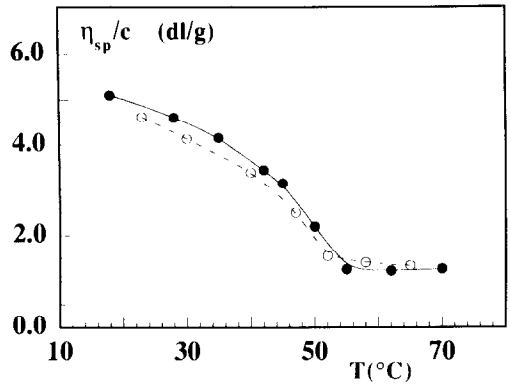


Fig. 9. Reduced specific viscosity dependence on temperature of sclerox solution in 5·0 M NaCl (fast cycle). (●) heating; (○) cooling. Polymer concentration $c_p = 0{\cdot}11\%$ (w/v).

with ionic strength and yields a value for the 'viscosity parameter' B suggesting moderate chain stiffness (Fig. 3*c*).

(2) For higher NaCl concentrations, up to 1·5 M approximately, sclerox chains would undergo relatively modest changes in shape (i.e. changes resulting mainly from fixed charges screening by added counterions) and would experience minor, probably local changes in conformation. This would be reflected by the steeper portion of the plot shown of Fig. 3*c*.

(3) Addition of NaCl beyond a critical amount (approximately 0·5 M) and repeated thermal cycles result in what we may call irreversible 'annealing' effects on the sclerox chains conformation in dilute aqueous solution.

On the basis of our viscosity data, the phenomenon, reflected in the less steep portion of the plot of Fig. 3*b*, would essentially consist of the stabilization of rather elongated and stiff chain conformations. The latter would attain maximum stability in 5·0 M NaCl after treatment at 50°C (Fig. 9).

In a previous paper (Crescenzi *et al.*, 1988) the hypothesis was advanced that sclerox chains in dilute aqueous solution would preserve, at least partially, vestiges of a triple stranded state reminiscent of that typical of scleroglucan.

Data reported here, particularly the results summarized in item 3 above, might then be interpreted as a progressive increase in the triple stranded character of sclerox chains, the attainment of which is favoured by high NaCl concentrations and facilitated by thermal annealing.

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