Eur Biophys J (1987) 15:185–191

European Biophysics Journal

Application of conductivity studies and polyelectrolyte theory to the conformation and order-disorder transition of xanthan polysaccharide

S. A. Jones[1], **D. M. Goodall**[1]*, **A. N. Cutler**[2], **and I. T. Norton**[2]

[1] Department of Chemistry, University of York, York YO1 5DD, UK
[2] Unilever Research, Colworth Laboratory, Sharnbrook, Bedford MK44 1LQ, UK

Received October 22, 1986/Accepted in revised form April 29, 1987

Abstract. The conductivity of xanthan (extracellular polysaccharide from *Xanthomonas campestris*) in the potassium salt form has been studied over the temperature range 5–80 °C spanning the order-disorder conformational transition. In salt-free solution data analysis using Manning's polyelectrolyte-conductivity theory gives a charge spacing, b, of 0.58 ± 0.04 nm for the low temperature ordered form, consistent with a single rather than a double helix ($b = 0.58$ and 0.29 nm respectively). In solutions with 0.01 M added KBr the increase in counterion condensation on conformational ordering is found from conductivity studies to be $-\Delta\xi^{-1} = 0.20 \pm 0.02$, in good agreement with the value 0.20 ± 0.02 using polyelectrolyte-equilibrium theory for the variation of transition-midpoint temperature with added salt determined from optical-rotation data.

Key words: Xanthan, conductivity, polyelectrolyte, conformation, polysaccharide

Introduction

Xanthan, the extracellular bacterial polysaccharide from the plant pathogen *Xanthomonas campestris*, is a polyanion with the pentasaccharide repeating structure shown in Fig. 1. The linear 1,4-linked β-D-glucose backbone is identical to that in cellulose, and a charged trisaccharide side chain is attached to every second glucose unit. There has been much interest in the biophysical properties of xanthan, which has wide technological uses because the polymer forms high-viscosity solutions which show pseudo-plastic sheer thinning behaviour and are resistant to thermal and chemical degradation (Sandford and Baird 1983).

There has been some controversy about the solid-state structure of xanthan and the nature of the solution conformations, particularly in regard to whether chain dimerization occurs or not. X-ray diffraction analysis of oriented fibres is consistent with either lateral packing of single helices (Moorhouse et al. 1977) or co-axial packing into a double helix (Okuyama et al. 1980). In solution the polymer undergoes a thermally induced conformational transition. The ordered form is stabilised by decreasing the temperature or increasing salt concentration. Hydrodynamic measurements have been interpreted as supporting both the single-stranded model for the ordered form (Rinaudo and Milas 1978; Muller et al. 1984, 1986) and the alternative double helix model (Holzwarth 1978; Whitcomb and Masako 1978; Sato et al. 1984). A doubling of $\bar{M}_w$ when comparing xanthan in the disordering solvent cadoxan and in 0.1 M NaCl at 25 °C supports the double helix model (Sato et al. 1984). Solution thermodynamics (cation activity; salt-concentration dependence of the transition-midpoint temperature T_m) gives polyelectrolyte charge parameters which are consistent with a single-helix ordered form (Rinaudo and Milas 1978; Milas and Rinaudo 1979; Norton et al. 1984; Lambert et al. 1985) with limited aggregation (Paoletti et al. 1983). Kinetic measurements using stopped-flow optical rotation (Norton et al. 1984) have shown that conformational ordering is an intramolecular process, consistent with single-helix formation, which is followed by secondary aggregation on a long time scale. Photon correlation spectroscopy reveals side-by-side aggregation of rod-like chains (Southwick et al. 1982; Morris et al. 1983), which are formed over a period of days in salt-free solution (Southwick et al. 1980).

In the present study we have investigated the conductivity of solutions of xanthan in the specific potassium ion form, and show that the results enable clear distinction to be made between the various conformational models. There has been a previous brief report of activity-coefficient measurement using conductivity (Rinaudo and Milas 1978), and we now give detailed

---

* To whom offprint requests should be sent

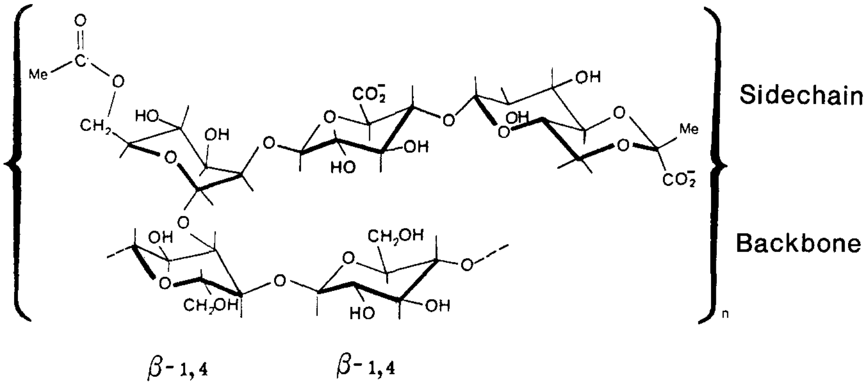

Fig. 1. Pentasaccharide repeating structure of xanthan. The proportion of pyruvate and acetate substituents present are respectively 70% and 100% in the sample used in this work

data for xanthan and analyse this using recent theoretical developments in the polyelectrolyte theory of electrophoretic mobility and conductance (Manning 1975, 1981). Our results provide further evidence that conformational ordering is an intramolecular process to give a single-helix species.

Experimental

Materials

A 0.5% aqueous solution of xanthan (Kelco Keltrol F, food grade) was prepared by autoclaving at 120 °C for 10 min, then clarified of cell debris by filtration (0.45 μm Millipore) and ion exchanged into the potassium-salt form (Amberlite I.R. 120 analytical grade resin). The sample eluted from the column was dialysed against deionised water for 48 h and freeze dried. Portions of the solid were assayed by elemental analysis (Butterworth Laboratories Ltd.). The pyruvate and acetate methyl content was determined by ^{1}H NMR (Morris et al. 1977) using a 1% solution in D_2O and $(CH_3)_2SO$ as a peak area reference. Values for percentages of substitution were: pyruvate, 70 ± 10%, acetate, 100 ± 10%. Calculated and experimental percentage analyses for a potassium xanthan sample with 70% pyruvation (average relative molar mass per residue = 982) and 11.2% water content are, with the calculated values in brackets: C, 37.4 (37.4); H, 5.4 (5.6); N, 0.0 (< 0.2); K, 5.2 ± 0.4 (3 determinations) (5.9); Ca, 0.13 (0.0); (O, 51.7, (51.0)). The absence of nitrogen shows that the xanthan was free from protein contamination. The total equivalent content of metal ions, 5.4 ± 0.4%, is within experimental error equal to the value of 5.9 ± 0.2% expected for a sample with 70% pyruvation, given the NMR assay limits of ± 10% in absolute pyruvate levels. Charge balance for the carboxylate groups on the polymer is provided 95% by K^+, 5% by Ca^{2+}.

Xanthan solutions were prepared by dissolving a known mass of the freeze-dried material in deionized water or KBr solution.

Methods

Conductivities were determined using a standardized cell (Philips PW 9512/60) and conductivity meter (Pye 9527). Thermostating was controlled by a water bath and cooler (Julabo F10-VC) programmed by a microcomputer (Apple IIe), and conductivity recorded digitally at 5 °C temperature intervals during heating and cooling cycles. Typically three runs were made for each polymer concentration and conductivity values found to be reproducible within 2%. The conductivity was found to be independent of the bridge frequency (80 Hz or 4 kHz) and a measurement of the DC resistance of the solution gave the same value.

Optical-rotation studies were carried out at 546 and 365 nm by polarimetry (Perkin Elmer 141 and 241), using thermostated 10 cm cells.

Theory

The equivalent conductivity per unit charge of the polyanion, λ_p, was calculated using theory developed for a polyion consisting of a linear array of discrete charges (Manning 1975, 1981)[1]. For a polyion with associated univalent counterions in salt-free solution, the limiting expression for λ_p in dilute solution is

$$\lambda_o/\text{mol}^{-1}\,\text{cm}^2\,\Omega^{-1} = \frac{279\,A\,|\ln(\kappa b)|}{1 + 43.2\,A\,(\lambda_1^0)^{-1}\,|\ln(\kappa b)|}. \qquad (1)$$

In excess-salt conditions with a 1:1 small electrolyte

$$\lambda_p/\text{mol}^{-1}\,\text{cm}^2\,\Omega^{-1} = \frac{322\,A\,|\ln(\kappa b)|}{1 + 54\,A\,(\lambda_1^{-1} + \lambda_2^{-1})\,|\ln(\kappa b)|}, \qquad (2)$$

where λ_1^0 is the limiting equivalent conductivity of the cationic counterion, λ_1 and λ_2 are cation and anion equivalent conductivities in the salt solution, b is the average charge separation in the polyion, and

---

[1] Throughout the paper CGS units are used for consistency with Manning's papers and other treatments of polyelectrolyte effects

Table 1. Conductivity of xanthan in salt-free solution calculated for random coil, single helix, and double helix conformations. Polymer concentration = 0.25% w/v ($C_p = 3.3 \times 10^{-3}\,M$)

Conformation	b/nm	Temperature [°C]	ξ	λ_p/mol^{-1} cm^2 Ω^{-1}	K/μS cm^{-1}
Random coil	0.64	20	1.11	45.1	287
		60	1.17	90.9	527
Single helix	0.58	20	1.22	47.4	266
		60	1.29	95.5	487
Double helix	0.29	20	2.44	63.1	152
		60	2.58	126.9	279

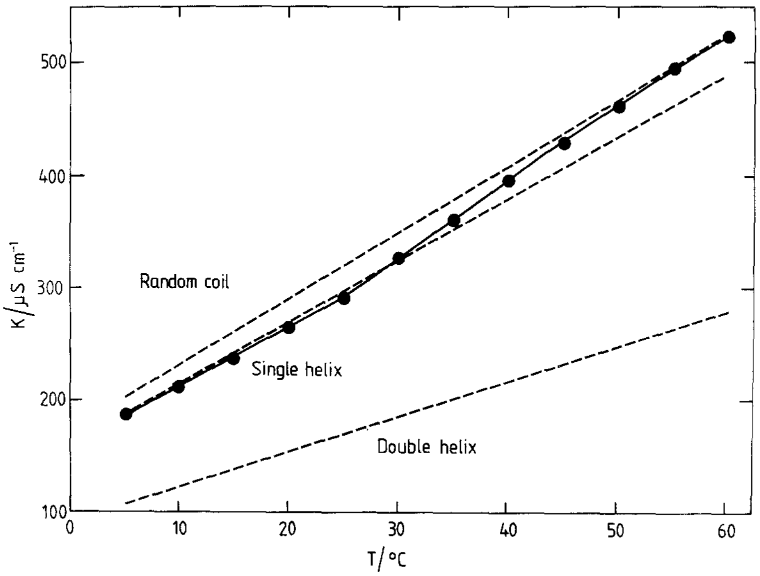

Fig. 2. Specific conductivity as a function of temperature for xanthan in salt-free solution. Polymer concentration = 0.25%w/v ($C_p = 3.3 \times 10^{-3}\,M$). Theoretical curves (---) are for random coil, single helix and double helix conformations

$A = \varepsilon kT/3\pi\eta q$. ε is the solvent dielectric constant, k Boltzmann's constant, T the absolute temperature, η the solvent viscosity, and q the electronic charge. In Eqs. (1) and (2) κ^{-1} is the Debye screening length, given by

$$\kappa^2 = (4\pi q^2/\varepsilon kT)\,10^{-3}\,L \sum_k C_k z_k^2\,, \qquad (3)$$

$$\sum C_k z_k^2 = 2C_1 + \xi^{-1} C_p \quad \text{(excess salt)}\,,$$

$$\sum C_k z_k^2 = \xi^{-1} C_p \quad \text{(salt free)}\,.$$

C_1 is the concentration of added salt and C_p the stoichiometric concentration of charged groups on the polyion. z_k is the valence, which for all ions considered here is unity, and L is Avogadro's constant. ξ is the linear charge density of the polyion, which is inversely proportional to b.

$$\xi = q^2/\varepsilon kTb\,. \qquad (4)$$

These expressions relate to a polyion with univalent charged groups where $\xi > 1$, for which condensation of the univalent counterions occurs to leave a concentration $\xi^{-1} C_p$ of free counterions in solution in addition to the counterions from the added salt.

The specific conductivity of the salt free solution, K, is (Manning 1975)

$$K = 0.866 \times 10^{-3}\,\xi^{-1}\,C_p(\lambda_1^0 + \lambda_p)\,. \qquad (5)$$

The specific conductivity of the polyelectrolyte after subtracting out the contribution of the added salt, K_s, from the observed specific conductivity K is (Manning 1981)

$$K_p = 10^{-3}\,\xi^{-1}\,C_p[\{\lambda_1 - (\lambda_2/6)\} + \lambda_p]\,. \qquad (6)$$

Results and discussion

The specific conductivity of 0.25% w/v xanthan in salt-free solution is shown as a function of temperature over the range 25 — 60 °C in Fig. 2, together with theoretical values for the random-coil, single-helix and double-helix conformations of xanthan as a polyanion with potassium counterions.

Model calculations are given in Table 1 at 20 °C and 60 °C. Manning's polyelectrolyte theory (Eqs. (1) and (5)) used in these calculations was developed for a single counterion. Temperature dependent values of λ_1^0

for the potassium ion (Robinson and Stokes 1959) were combined with those for η and ε from standard tables (Weast 1982). The potassium ion provides charge balance to 95% of the carboxylate groups on the polyanion in our xanthan sample. The remaining 5% of sites are neutralized by Ca^{2+}, which at this K^+/Ca^{2+} ratio is known to be firmly bound (Lambert et al. 1985). In calculating the equivalent conductivity of the polyelectrolyte allowance has been made for site bound calcium ions by increasing the charge spacing, b, in proportion to the degree of site binding (Manning 1981). Values of b for the single and double helix (Table 1) were calculated from X-ray fibre diffraction data (Moorhouse et al. 1977). With five pentasaccharide residues in the helix pitch of 4.7 nm, and the average number of carboxylate groups per residue not involved in site binding equal to 1.7×0.95, it follows that $b = 4.7/(5 \times 1.7 \times 0.95) = 0.58$ nm for the single helix and 0.29 nm for the alternative double helical ordered form. A fully extended chain geometry with projected pentasaccharide residue length of 1.03 nm was assumed for the disordered form (Rinaudo and Milas 1978; Paoletti et al. 1983; Lambert et al. 1985), this value being taken from the axial repeat determined for the cellulose backbone in β-D-cellobiose (Sundararajan and Marchessault 1972). The charge spacing in the disordered form of our xanthan sample is calculated to be $b = 1.03/(1.7 \times 0.95) = 0.64$ nm.

Figure 2 shows that the best fit at the highest temperature studied (60 °C) is provided by a conformation with specific conductivity for a fully extended random coil, and at low temperature (< 25 °C) by a single helix. The double-helix model is seen to be inappropriate. The charge spacing is taken to be invariant with temperature in constructing the theoretical curves of Fig. 2.

In Figure 3, b is treated as the variable parameter in Eqs. (1), (3), (4) and (5) to fit Eq. (5) to experimental data. At 60 °C the results from all three salt-free solutions of xanthan (0.05, 0.10 and 0.25%) are fitted with a charge spacing $b = 0.64$ nm, identical to the value for the fully extended random coil. b decreases gradually with temperature for 0.05 and 0.10% xanthan. For 0.25% xanthan there is a sigmoidal change in b with temperature, consistent with a disorder-order transition, and the charge spacing at or below 20 °C is equal to the value of 0.58 nm calculated for the single helix. In determining the charge spacing it should be noted that the standard deviation in the mean value of the potassium ion content of the polymer, and C_p, results in a standard deviation of ± 0.04 on any absolute value of b calculated using Eq. (5) or (6). Changes in b, for instance the amplitude of the disorder-order transition, are free from this uncertainty.

The order-disorder transition has a transition midpoint $T_m = 33 \pm 1$ °C in salt-free solution of 0.25% w/v xanthan, and the change in charge spacing for the transitions at T_m is $\Delta b = 0.05 \pm 0.01$. Corresponding to this change in b, the increase in counterion condensation on conformational ordering is readily calculated from Eq. (4) to be $-\Delta\xi^{-1} = 0.06 \pm 0.02$ per charged group on the polyanion.

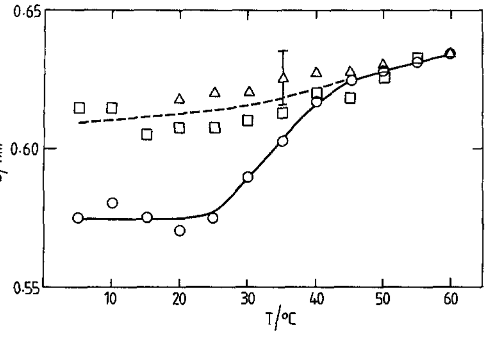

Fig. 3. Variation of charge spacing, b, with temperature determined by fitting observed conductivity data for xanthan in salt-free solution to Manning's theory. Polymer concentration 0.25% (○), 0.10% (□), 0.05% (△). Error bar shows effect on b of typical 2% standard deviation on mean conductivity value

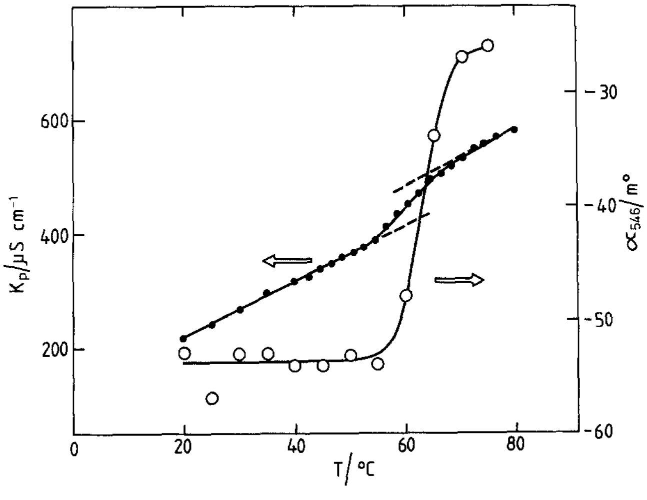

Fig. 4. Temperature variation of polyelectrolyte specific conductivity and optical rotation for xanthan in 0.01 M KBr. Xanthan concentration = 0.25% w/v. O.R. cell pathlength = 10 cm, $\lambda = 546$ nm

Figure 4 shows the polyelectrolyte specific conductivity, K_p, and optical rotation for 0.25% w/v xanthan in 0.01 M KBr. Addition of salt stabilises the ordered form, and both techniques for monitoring the transition gave the same value of $T_m = 62 \pm 1$ °C. The change in conductivity during the conformational transition is seen to be more pronounced in the solution with added salt than in salt free solution. Experimental data was fitted with b as the variable parameter using Eq. (6), giving $b = 0.55 \pm 0.04$ nm for the ordered form below T_m, and $-\Delta\xi^{-1} = 0.20 \pm 0.02$ for the increase in counterion condensation on conformational ordering. This is three times greater than the value found in salt-free solution.

The experimental data of Fig. 2 was determined for solutions autoclaved at 120 °C for 5 min to ensure destruction of any xanthan microgels (Morris et al. 1983).

Specific conductivities for salt-free solutions with and without autoclaving were compared, and no significant differences were found. Initially clear solutions left standing for several days were observed to exhibit scattering, consistent with the slow build-up of aggregates (Southwick et al. 1980). We may conclude that the salt-free solutions considered in the conductivity study did not contain any aggregates or microgels.

Optical rotation

We have seen that the transition-midpoint temperature determined by conductivity agrees with that found from optical rotation (Fig. 4). A study of the variation of T_m with concentration of added KBr was carried out to check whether the theoretical treatment of polyelectrolyte equilibria (Manning 1978; Record et al. 1978) would give an account of the behaviour of xanthan consistent with the results of the polyelectrolyte conductivity work.

Curves of optical rotation as a function of temperature and concentration of added KBr are presented in Fig. 5, and melting temperatures are given in Table 2. The melting temperature is expected to correlate with counterion activity, a_{M^+}, according to Eq. (7)

$$\mathrm{d}T_m/d \ln a_{\mathrm{M}^+} = -f \Delta\xi^{-1} RT_m^2/2\Delta H_c \tag{7}$$

(Record et al. 1978). R is the gas constant, ΔH_c the calorimetric enthalpy change per charged unit in the disorder-order transition, $-\Delta\xi^{-1}$ the increase in counterion condensation on conformational ordering, and $f = [1 + d \ln \gamma_{\mathrm{M}^+}/d \ln a_{\mathrm{M}^+}]$ where γ_{M^+} is the counterion activity coefficient. When terms in Eq. (7) other than T_m and a_{M^+} are invariant to changes in these variables the equation may be integrated to

$$1/T_m = (f \Delta\xi^{-1} R/2\Delta H_c) \ln a_{\mathrm{M}^+} + W, \tag{8}$$

where W is a constant. Equation (8) shows that the reciprocal of T_m should vary linearly with the logarithm of the counterion activity.

The activity coefficient of the potassium counterion, γ_{M^+} was evaluated as the product of contributions $\gamma_{\pm}^{\mathrm{PM}}$ for polyion-mobile ion interactions (Manning 1969) and $\gamma_{\pm}^{\mathrm{MM}}$ for mobile ion-mobile ion interactions (Wells 1973; Iwasa and Kwak 1976). The counterion activity is

$$a_{\mathrm{M}^+} = \left(\frac{C_{\mathrm{M}^+}}{C^{\ominus}}\right) \gamma_{\mathrm{M}^+} = \left(\frac{C_1 + C_p}{C^{\ominus}}\right) \gamma_{\pm}^{\mathrm{PM}} \gamma_{\pm}^{\mathrm{MM}}. \tag{9}$$

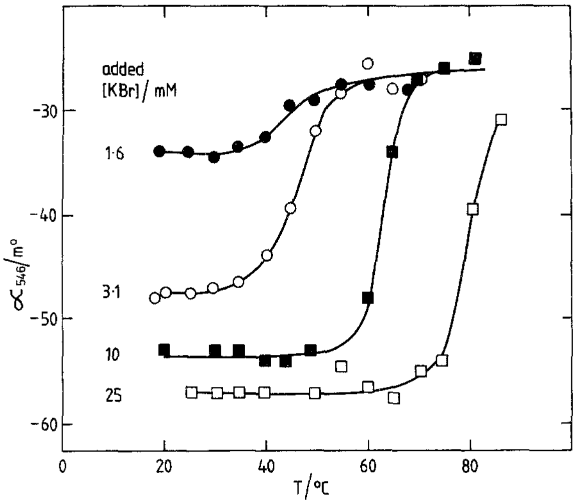

Fig. 5. Optical rotation as a function of temperature and concentration of added KBr. Xanthan concentration = 0.25% w/v

Table 2. Transition midpoint temperature for the order-disorder transition of xanthan as a function of the concentration of added KBr. Polymer concentration = 0.25% w/v (C_p = 3.3 mM). Activity coefficients calculated as described in text

C_1/mM	T_{M}/K	$\gamma_{\pm}^{\mathrm{MM}}$	$\gamma_{\pm}^{\mathrm{PM}}$	γ_{M^+}
25	353	0.80	0.95	0.76
12.5	339	0.86	0.91	0.79
10	335	0.87	0.90	0.78
6.25	325	0.89	0.86	0.76
3.13	319	0.91	0.78	0.71

In Eq. (9), the standard concentration $C^{\ominus}$ is 1 M. $\gamma_{\pm}^{\mathrm{MM}}$ values in Table 2 were calculated from Debye-Huckel theory (Robinson and Stokes 1959) and $\gamma_{\pm}^{\mathrm{PM}}$ using Eq. (45) of Manning's 1969 paper with $\bar{\xi}^{-1}$ in place of ξ^{-1}. $\bar{\xi}^{-1}$ is the average of the linear charge densities of ordered and disordered polyions, and is the quantity relevant at T_m. It was evaluated using Eq. (4) from the conductometric measurements on xanthan with and without added KBr to be 0.84 ± 0.01.

Data in Table 2 gave a straight line plot of $1/T_m$ versus $\ln a_{\mathrm{M}^+}$ (Eq. (8)) with a gradient of $-(2.0 \pm 0.2) \times 10^{-4}\,\mathrm{K}^{-1}$. This is in good agreement with previous measurements for xanthan in Na^+ and K^+ salt solutions: $-(1.7 \pm 0.1) \times 10^{-4}\,\mathrm{K}^{-1}$ (Norton et al. 1984) and $-(2.1 \pm 0.1) \times 10^{-4}\,\mathrm{K}^{-1}$ (Milas and Rinaudo 1979; Paoletti et al. 1983). Various extrathermodynamic assumptions and approximations in activity calculations (Manning 1969, 1978; Kwak 1973; Iwasa and Kwak 1976; Wells 1973; Record 1978) have led to variants of Eqs. (7) – (9) being used in the interpretation of salt effects on polyelectrolyte conformational transitions. Norton et al. (1984) used C_{M^+} and Milas and Rinaudo (1979) $C_1 + \phi\, C_p$ in place of a_{M^+} in

Eq. (8), with ϕ the osmotic coefficient of xanthan in pure polyelectrolyte solution. It should be noted that the exact form of the expression for a_{M^+} is found not to have a significant effect on the gradient of $1/T_M$ versus $\ln a_{M^+}$, with the alternative expressions giving values within the range of our result of $-(2.0 \pm 0.2) \times 10^{-4} K^{-1}$.

The increase in counterion condensation on conformational ordering, $-\Delta\xi^{-1}$, may be evaluated using Eq. (8). The calorimetric enthalpy change per residue has previously been determined by differential scanning calorimetry (Norton et al. 1984) as 7.1 kJ mol^{-1}, and when divided by the number of charges per residue, 1.7, this gives $\Delta H_c = 4.2$ kJ mol^{-1}. Using the data in Table 2, f was calculated to be 1.0 ± 0.1 over the concentration range studied. Thus

$$\begin{aligned} -\Delta\xi^{-1} &= \{(2.0 \pm 0.1) \times 10^{-4} \times 2 \times 4.2 \times 10^3\}/ \\ &\quad \{(1.0 \pm 0.1) \times 8.31\} \\ &= 0.20 \pm 0.02\,. \end{aligned}$$

This figure is in excellent agreement with $-\Delta\xi^{-1} = 0.20 \pm 0.02$ determined in our conductometric study for xanthan in 0.01 M KBr, a concentration of added salt in the middle of the range used for evaluating $-\Delta\xi^{-1}$ from the equilibrium study. This shows that polyelectrolyte theories of conductivity and equilibria are self consistent when applied simultaneously to the polysaccharide xanthan.

It is evident from Fig. 5 that the amplitude of the optical rotation change in the disorder-order transition decreases strikingly as the concentration of added KBr decreases. Figure 6 shows the dependence of the normalised transition amplitude ($\Delta\alpha/\Delta\alpha$(max)) on the concentration of free counterions at T_M, $[M^+] = C_1 + \xi^{-1} C_p$. Half-maximum amplitude occurs at $[M^+] = 6$ mM. Lambert et al. (1985) have previously noted that the conformational transition of xanthan monitored by optical rotation at a fixed temperature, 25 °C, attains half maximum amplitude for added $[Na^+] = 4$ mM (equivalent to total $[M^+] = 5$ mM).

Several considerations should be borne in mind in considering the results of present and previous work. In particular, approximations in the development of linear polyelectrolyte theory have been shown to lead to an unrealistic discontinuity in the salt-dependence of counterion condensation, in contrast to recent theoretical treatments using non-linear theory (Davis and Russel 1986). The change in effective charge density with ionic strength was shown to be greatest in the range around $\kappa a = 0.1$, where a is the polyelectrolyte cylinder radius. For xanthan in 1 : 1 electrolyte solutions this range is 1 − 10 mM. This could qualitatively account for the increase with salt concentration of the amplitudes of both the optical-rotation transition, and the change of counterion condensation on conformational ordering. Our results suggest that the principal effect is on the disordered conformation of xanthan, since the linear charge density and charge spacing of the ordered form derived from conductivity measurements do not vary with salt concentration, and accord with values for a single-helix conformation. The persistence length and electrostatic diameter of the disordered form of xanthan has been shown to be strongly dependent on the ionic strength, with the maximum distance between charged groups of the side chain and the backbone (Fig. 1) reached at ~ 10^{-3} M salt (Muller et al. 1986). Limited aggregation of xanthan promoted by increasing the salt concentration could lead to a stabilisation of the ordered helical structure and higher counterion condensation, through an increase in the linear charge density on aggregation (Paoletti et al. 1983). However, there is no significant evidence for aggregation in the present work using a xanthan sample with 70% pyruvation, and a recent study of a xanthan with 100% pyruvation showed that the material remained single-stranded in the salinity range up to 1 M NaCl (Muller et al. 1986).

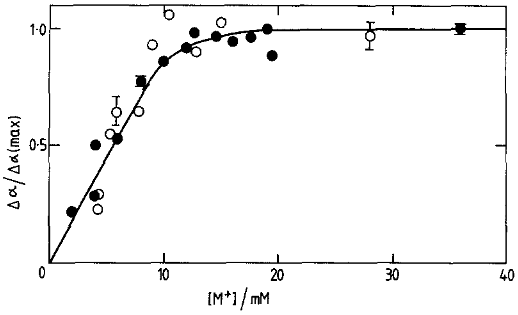

Fig. 6. Variation of optical rotation transition amplitude, normalized to limiting value at high salt concentration, with counterion concentration. (○) Added KBr, xanthan concentration = 0.25% w/v, $\lambda = 546$ nm. (●) Added KCl, xanthan concentration = 0.3%, $\lambda = 365$ nm

Conclusions

This is the first detailed study of the anionic polysaccharide xanthan using conductivity measurements and polyelectrolyte theory of electrophoretic mobility and conductance (Manning 1975, 1981). Data obtained in salt-free solution of the potassium-salt form of the polymer over a range of temperatures and polymer concentrations has been analysed with the polyelectrolyte charge spacing, b, as variable. At 60 °C all data accords with a disordered conformation having b equal to the value of a fully extended random coil. For 0.25% w/v xanthan there is evidence for a conformational transition on decreasing T, and below 20 °C

$b = 0.58$ nm, which is identical to the value calculated from X-ray fibre-diffraction data assuming a single-helix model.

In solutions with added salt the order-disorder transition was monitored by both optical rotation and conductivity. For 0.25% w/v xanthan in 0.01 *M* KBr both techniques gave the same value of the transition midpoint temperature. The increase in counterion condensation on conformational ordering, $-\Delta\xi^{-1} = 0.20 \pm 0.02$ from conductivity studies, is in excellent agreement with $-\Delta\xi^{-1} = 0.20 \pm 0.02$ using polyelectrolyte-equilibrium theory (Record et al. 1978) to analyse O.R. data for the variation of T_M with cation activity. The variation of $-\Delta\xi^{-1}$ and the O.R. transition amplitudes with concentration of KBr may reflect changes in the charge density of the polymer, and the conformation of the disordered form.

Acknowledgement. We should like to thank the AFRC for a research grant, and the SERC for a research studentship (CASE award) to S.A.J.

References

Davis RM, Russel WB (1986) On the theory of dilute polyelectrolyte solutions: extensions, refinements, and experimental tests. J Polymer Sci (Polym Phys Ed) 24:511–533

Holzwarth G (1978) Molecular weight of xanthan polysaccharide. Carbohydrate Res 66:173–186

Iwasa K, Kwak JCT (1976) On the correction term for interactions between small ions in the interpretation of activity data in polyelectrolyte-simple electrolyte mixtures. J Phys Chem 80:215–216

Kwak JCT (1973) Mean activity coefficients for the simple electrolyte in aqueous mixtures of polyelectrolyte and simple electrolyte. The system sodium polystyrenesulfonate-sodium chloride. J Phys Chem 77:2790–2793

Lambert F, Milas M, Rinaudo M (1985) Sodium and calcium counterion activity in the presence of xanthan polysaccharide. Int J Biol Macromol 7:49–52

Manning GS (1969) Limiting law and counterion condensation in polyelectrolyte solutions I. Colligative properties. J Chem Phys 51:924–933

Manning GS (1975) A limiting law for the conductance of the rod model of a salt-free polyelectrolyte solution. J Phys Chem 79:262–265

Manning GS (1978) The molecular theory of polyelectrolyte solutions with applications to the electrostatic properties of polynucleotides. Qu Rev Biophys 11:179–246

Manning GS (1981) Limiting laws and counterion condensation in polyelectrolyte solutions. 7. Electrophoretic mobility and conductance. J Phys Chem 85:1506–1515

Milas M, Rinaudo M (1979) Conformational investigation of the bacterial polysaccharide xanthan. Carbohydrate Res 76:189–196

Moorhouse R, Walkinshaw MD, Arnott S (1977) Xanthan gum molecular conformation and interactions. In: Sandford PA, Lankin A (eds) Extracellular microbial polysaccharides. ACS Symp Series 45:90–102

Morris ER, Rees DA, Young G, Walkinshaw MD, Darke A (1977) Order-disorder transition for a bacterial polysaccharide in solution – a role for polysaccharide conformation in recognition between xanthomonas pathogen and its plant host. J Mol Biol 110:1–16

Morris VJ, Franklin D, I'Anson K (1983) Rheology and microstructure of dispersions and solutions of the microbial polysaccharide from *Xanthomonas campestris* (xanthan gum). Carbohydrate Res 121:13–30

Muller G, Lecourtier J, Chauveteau G, Allain C (1984) Conformation of xanthan in an ordered structure. Makromol Chem Rapid Commun 5:203–208

Muller G, Anrhourrache M, Lecourtier J, Chauveteau G (1986) Salt dependence of the conformation of a single-stranded xanthan. Int J Biol Macromol 8:167–172

Norton IT, Goodall DM, Frangou SA, Morris ER, Rees DA (1984) Mechanism and dynamics of conformational ordering in xanthan polysaccharide. J Mol Biol 175:371–394

Okuyama K, Arnott S, Moorhouse R, Walkinshaw MD, Atkins EDT, Arnott S, Wolf-Ullish Ch (1980) Fiber diffraction studies of bacterial polysaccharides. In: French AD, Gardner KH (eds) Fiber diffraction methods. ACS Symp Series 141:411–427

Paoletti S, Cesaro A, Delben F (1983) Thermally induced conformational transition of xanthan polysaccharide. Carbohydrate Res 123:173–178

Record MT, Anderson CF, Lohman TM (1978) Thermodynamic analysis of ion effects on the binding and conformational equilibria of proteins and nucleic acids: the role of ion association or release, screening, and ion effects on water activity. Qu Rev Biophys 11:103–178

Rinaudo M, Milas M (1978) Polyelectrolyte behaviour of a bacterial polysaccharide from *Xanthomonas campestris*. Biopolymers 17:2663–2678

Robinson RA, Stokes RA (1959) Electrolyte solutions. Butterworths, London

Sandford PA, Baird J (1983) Industrial utilization of polysaccharides. In: Aspinall GO (ed) The polysaccharides, vol 2. Academic Press, New York, pp 412–490

Sato T, Norisuye T, Fujita H (1984) Double stranded helix of xanthan. Macromolecules 17:2696–2700; Polymer J 16:341–350

Southwick JG, Lee H, Jamieson AM, Blackwell J (1980) Self association of xanthan in aqueous solvent systems. Carbohydrate Res 84:287–295

Southwick JG, Jamieson AM, Blackwell J (1982) Conformation of xanthan dissolved in aqueous urea and sodium chloride solutions. Carbohydrate Res 99:117–127

Sundararajan PR, Marchessault RH (1972) Conformational terminology for crystalline cellulose. Can J Chem 50:792–794

Weast RC (ed) (1982) CRC handbook of chemistry and physics, 63rd edn. CRC Press, Boca Raton, Fla

Wells JD (1973) Thermodynamics of polyelectrolyte solutions. An empirical extension of the Manning theory to finite salt concentrations. Biopolymers 12:223–227

Whitcomb PJ, Macosko CW (1978) Rheology of xanthan gum. J Rheol 22:493–505