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POLYSACCHARIDE SHAPES AND THEIR INTERACTIONS - SOME RECENT ADVANCES

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Abstract - Recent improvements in methods for the investigation of ordered polysaccharide states in the hydrated environment have led to a number of new insights. For example, the spectroscopic origin of the conformation sensitivity of the optical rotation of polysaccharides now seems to be better understood, opening up wider possibilities for application. Fast reaction kinetic studies with stopped-flow polarimetry have been helpful for determination of the number of strands in the activated complex between ordered and disordered states, and point to two-stranded conformations (most likely double helices) for agarose, kappa-carrageenan and iota-carrageenan, and a single-stranded conformation (most likely with stabilizing interactions between the main chain and side chains) for xanthan.

Site-binding of cations can be important for the stability of ordered conformations of polysaccharide polyelectrolytes and the counter-ion selectivity in a number of such instances, seems to be explicable in terms of sandwiching of arrays of cations; this may involve Group II cations between two-fold buckled ribbons in polyguluronate and polygalacturonate (i.e. alginate and pectin respectively), or Group I cations between carrageenan double helices.

Ordered states are usually stabilized by interchain interaction, and the pairwise association of chains can often be recognised as a distinct event even when larger aggregates form eventually.

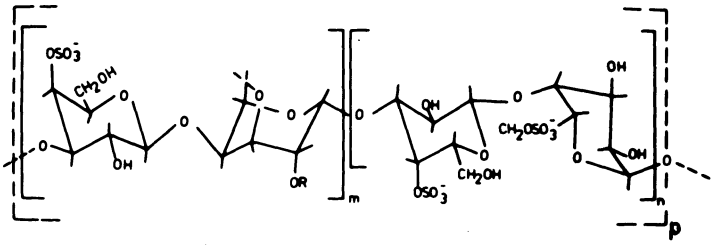
INTRODUCTION

It has been known for ten to fifteen years that polysaccharide chains can adopt a variety of ordered shapes in the condensed phase, such as the various types of ribbons and helices including double and triple helices (Ref. 1 & 2). The methods of X-ray fibre diffraction analysis are now so well advanced that such structures can sometimes be determined with near-atomic resolution. Some of these ordered forms can carry over to the hydrated environment of solutions and gels and this lecture will describe recent advances with systems of this type which show order-disorder transitions in aqueous environments. This will illustrate the advanced methodology now available and suggest general principles of conformational behaviour that begin to emerge. As with other large biological molecules, the investigation of ordered polysaccharide conformations in solution must rely heavily on the details of reference conformations determined for the condensed phase by X-ray diffraction analysis. Our strategy is usually to devise experiments to test or confirm the possibility that such conformations persist into solution. In addition, however, solution methods can sometimes resolve ambiguities which remain after diffraction analysis - especially when, owing to poor crystallinity, the quality of the diffraction data has limited the scope of the powerful methods of modern interpretation.

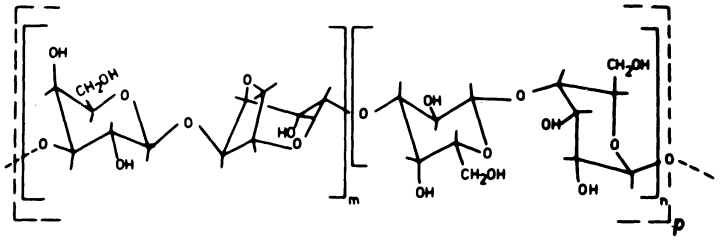
CATION BINDING AND DOMAIN STRUCTURE OF CARRAGEENAN GELS

Our studies of order-disorder transitions in polysaccharide systems began with certain polysaccharides from marine red algae, namely carrageenans and agarose, for which double helical models had been derived from X-ray diffraction analysis on oriented fibres (Ref. 3, 4 & 5). The sol-gel interconversion (Ref. 6 & 7), or more conveniently for studies of mechanism the behaviour over a similar temperature range of segments of shorter chain length which cannot form a continuous network (Ref. 8), showed physical changes of a form that indicated a cooperative conformation change. There is now much evidence (Ref. 2) that this represents an interconversion between the coil and double helix. Another research group (Ref. 9) has recently suggested that it is possible under certain conditions to observe order-disorder

behaviour for carrageenans without change in molecular weight which would point to the existence of an additional type of ordered conformation. The ionic conditions differ from ours and our own comparative investigation is still in progress.



CARRAGEENAN I(a) I(b)



AGAROSE II(a) II(b)

Fig. 1. Formulae of carrageenan (above) and agarose (below) showing sequences which are diastereomeric except that carrageenan is sulphated. R = SO_3^- for iota carrageenan; R = H for kappa carrageenan. Each polysaccharide is made up from 3,6-anhydride-containing sequences (I(a), II(a)) which are helix-forming, and α-D-galactose-containing sequences which are helix-breaking.

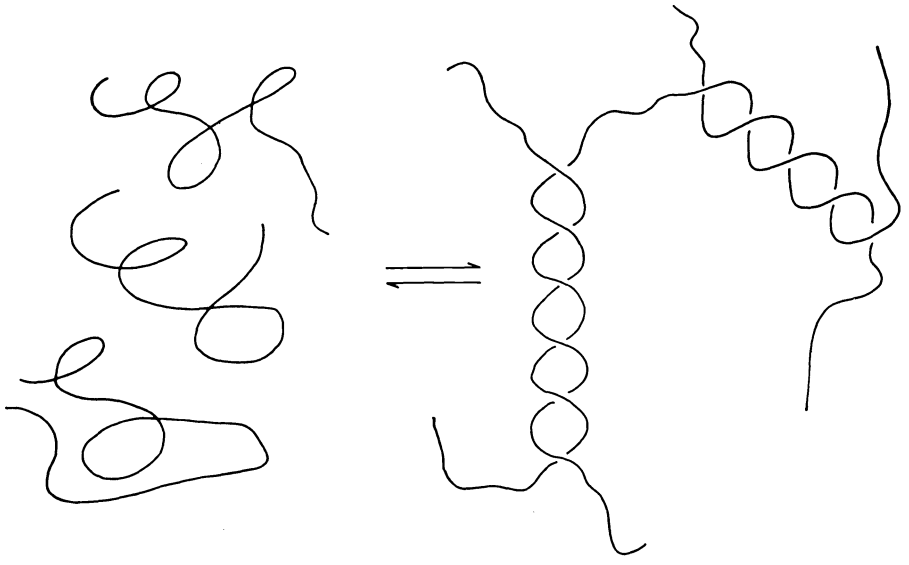


Fig. 2. Simple model (1969) for the cross-linking of carrageenan chains in gel formation.

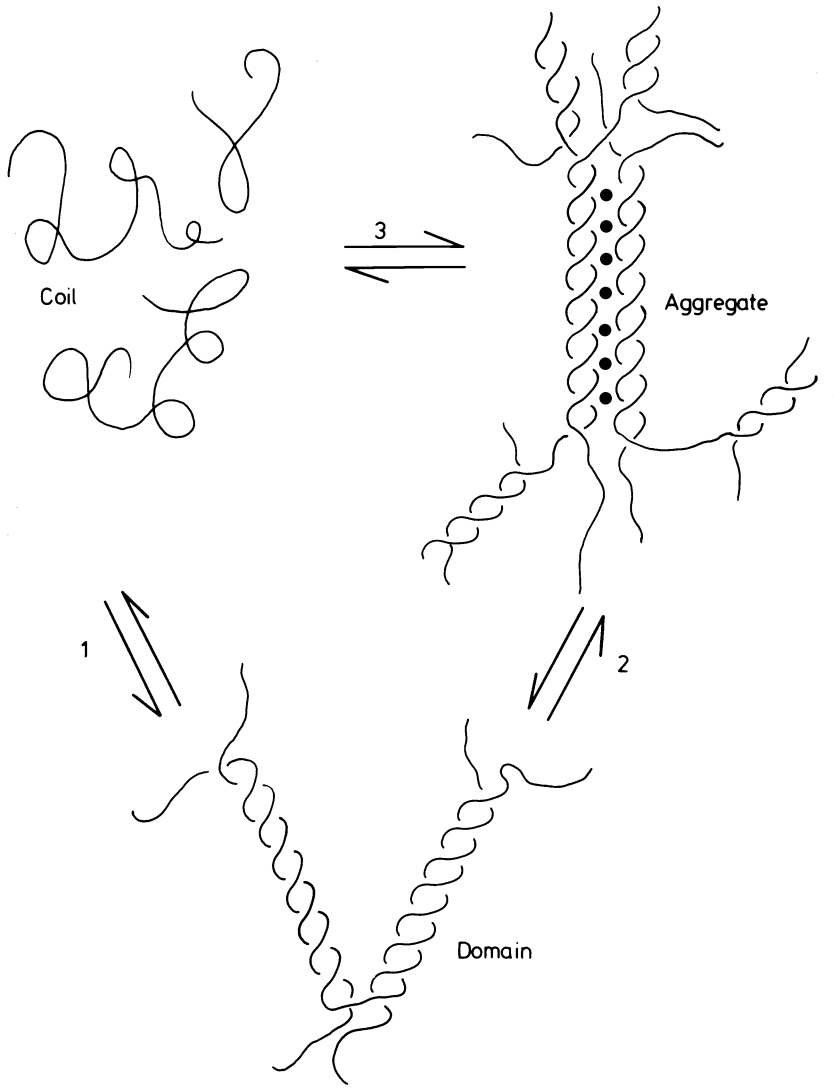


Fig. 3. The domain model of carrageenan gelation. 1: The primary mode of interchain association for ι-carrageenan is the coil-domain transition which is promoted by cooling and reversed on heating. In an ionic environment which maintains isolation of the individual helices (e.g. Me_4N^+) the domain is the stable ordered state. 2: Below the helix melting point, and in the presence of cations (●) which promote gelation (e.g. K^+, Rb^+, Cs^+, NH_4^+, or high concentrations of Na^+), further association occurs by the domain-aggregate transition. Only bound counterions relevant to the model are shown. 3: In the presence of gel-promoting cations the coil-aggregate transition can occur directly at a higher temperature than the coil-domain transition.

TABLE 1 Comparison of observed and calculated optical rotations for carrageenan and agarose transitions

	$[\alpha]_D$ Observed	Calculated
Iota carrageenan double helix	+64°	+63°
Iota carrageenan random coil	+38°	+34°
Agarose double helix	-44°	-43°
Agarose random coil	-28°	-22°

Agarose has a covalent structure which is analogous but diastereomeric (see Fig. 1) with carrageenan. Its ordered conformation is also a double helix but with each strand has a left-handed rather than right-handed sense (Ref. 5). The pitch is shorter and the inter-chain contacts are different in detail.

In native carrageenans and agars, the regular repeating sequence of sugar residues (Fig. 1) is interrupted by a proportion of other sequences believed to have a helix-breaking influence (Ref. 6). A plausible mechanism for gel formation can then be proposed (Ref. 7), in which the regular alternating sequences associate in double helix formation to form the network crosslinks, and the helix-breaking sequences represent spacers to cause each chain to associate with more than one partner as is necessary to form a three dimensional network (Fig. 2).

Such a model does indeed account successfully for many aspects of gel behaviour including the variation in gel properties with covalent structure (Ref. 7). However, it has been known for many years that gelation of carrageenans is promoted much more strongly by the larger Group I cations (Ref. 10 & 11). In particular, Cs^+, Rb^+, K^+, NH_4^+ promote gelation of kappa-carrageenan whereas Na^+ and Li^+ do not. To investigate the origin of this selectivity we studied the influence of these cations and of tetramethylammonium on helix formation and on chain-chain association as measured by light scattering measurements, both for short non-gelling carrageenan segments of regular sequence and also for the native polymers (Ref. 12). For iota carrageenan we found that helix formation as monitored by optical rotation or by change in segmental mobility reflected in n.m.r. relaxation parameters, could occur to completion in the presence of all cations - but in contrast the extent of chain association that occurred in parallel was very cation-dependent. Iota carrageenan segments having regular residue sequence show a precise doubling of molecular weight with the tetramethylammonium cation as expected for a simple coil-to-double helix transition. With K^+ which, unlike tetramethylammonium does promote gelation of the native polymer, larger molecular weight changes are observed to 6-8 times the starting value to show that aggregation here proceeds beyond the double helix. With native iota carrageenan as distinct from the segments derived from it, the corresponding molecular weight change is approximately tenfold with tetramethylammonium showing that association through double helical junction zones is limited to small clusters or "domains"; with K^+ the molecular weight increases to infinity as shown by the formation of a rubbery gel. All this leads to an elaboration of the simplified "junction zone" model of polysaccharide gel structure; as previously, it is proposed that chains are linked through double helices terminated by irregularities in covalent structure but is is now clear that this step leads only to soluble domains unless further association by cation-mediated helix-helix aggregation occurs to develop a cohesive network. This elaborated model is outlined schematically in Fig. 3. It is possible that similar domain mechanisms might apply to other biopolymer gels.

The concept is important for a number of reasons, especially that the mechanical rupture of such structures clearly does not require the breakage of covalent bonds as might be the case for a network cross-linked entirely by isolated double helices. For the domain structure, it is also possible that network formation need not involve such severe topological problems as would be the case for the original model.

OPTICAL ACTIVITY AND POLYSACCHARIDE CONFORMATION

Monochromatic optical rotation has been invaluable throughout studies of order-disorder transitions in polysaccharides, as an empirical indicator of the conformational state and to show cooperative character where this exists (Ref. 5, 6 & 8). It has also been useful as a measure of the degree of conformational change for thermodynamic (Ref. 13 & 14) and kinetic (Ref. 15 & 16) analysis. We derived a relationship between optical activity and the conformation angles in carbohydrate chains (Ref. 17 & 18) which enabled the sign and magnitude of the optical rotation shift to be predicted for both carrageenan (Ref. 19) and agarose (Ref. 5) transitions - see Table 1 - thus providing important evidence that the double helical forms exist in solutions and gels as well as in the solid state.

Even though optical activity has proved so useful and sensitive as an indicator for the conformation of polysaccharides, the measurements have been open to empirical interpretations only, because the active electronic transitions normally occur at lower wavelengths than can be reached with conventional spectropolarimeters. However, several vacuum c.d. instruments have now been built which penetrate further into the ultraviolet and we have collaborated with Dr. E.S. Stevens in the application of the new possibilities to some of our systems. For example, for agarose it is now possible (Ref. 20) to detect a circular dichroism band near 180 nm which alters with the order-disorder transition (Fig. 4). This band is sensitive to the same conformational parameters as monochromatic optical rotation because the amplitude changes (see Fig. 4) parallel the measurements reported earlier by optical rotation (Ref. 5). However, the optical rotation shift is <u>negative</u> while the amplitude of the band changes in a <u>positive</u> sense. This points to other

changes in the circular dichroism spectrum, even further into the ultraviolet, which have an overriding influence.

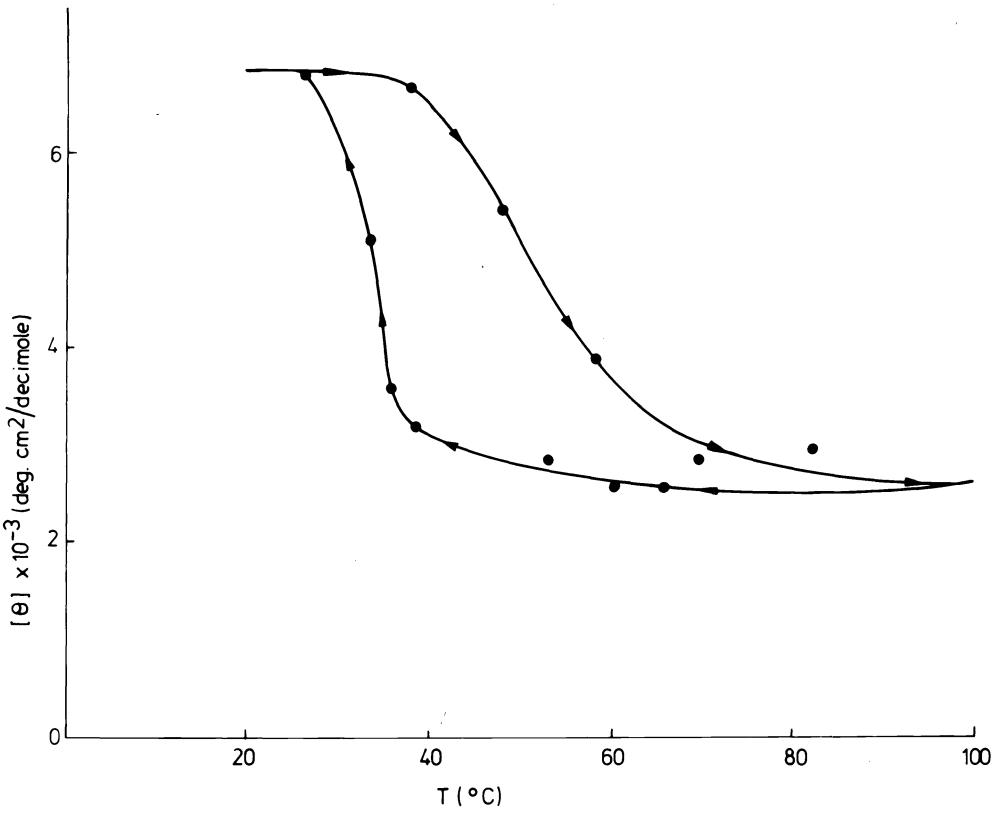


Fig. 4. Temperature course of conformation changes during agarose gelation and liquifaction as monitored by the intensity of the c.d. band at 180 nm.

The use of dry agarose films rather than solutions and gels allows still greater penetration into the ultraviolet, and we then see a second and larger band at around 152 nm which is of opposite sign (Fig. 5). This could not be observed directly in the aqueous systems because of difficulties in optical transmission.

Since the optical rotatory dispersion curve (even that part observable experimentally in the region above 200 nm) is the summation of individual dispersion curves associated with each optically active electronic transition including those that are inaccessible, we were able to use the measured dispersion envelope to obtain more information about the origin of the conformationally sensitive change in optical activity. The mathematical relationship (Ref. 21) between the form of the dispersion curve and the forms of the circular dichroism bands associated with the individual transitions is a precise one and is known as the Kronig-Kramers transform. Using this to calculate the o.r.d. contribution from the c.d. that could be observed directly, we were then able to determine by subtraction the residual o.r.d. envelope representing all other transitions in the molecule. As shown in Fig. 6, for the random coil (sol) state, as an example, this envelope was mathematically consistent with a single c.d. band of the same position and width as the low wavelength band in the dried films (Fig. 5). A good fit could also be obtained for the helix (gel) state on the same assumption that this same low wavelength band was the only additional contributor to the envelope; moreover, not only were the position and width consistent as before with the dried films, but the fitted amplitude directly observed for the band near 152 nm also matched that in the film. Thus this combination of direct experimental observation and mathematical analysis leads to the compelling and satisfying conclusion that we can completely explain the conformationally sensitive optical activity of agarose in terms of two circular dichroism bands at around 180 and 152 nm. This recognition of the spectroscopic origin allows the empirical relationship between the monochromatic optical rotation and the conformational angles to be used with more confidence because we can now check (by vacuum circular dichroism measurements coupled with the mathematical

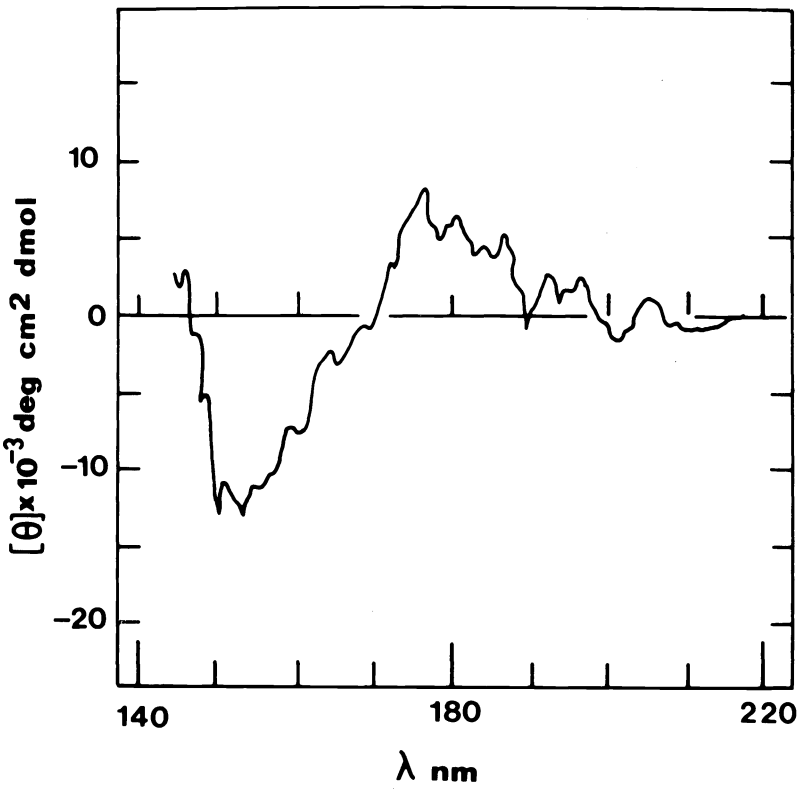


Fig. 5. Vacuum ultraviolet circular dichroism spectrum of an agarose solid film.

TABLE 2 Comparison of observed and calculated optical rotations for amylose solutions.

	$[\alpha]_D$ Observed	$[\alpha]_D$ Calculated (Note a)
V-amylose : Left handed		$+157^\circ$
: Right handed		$+104^\circ$
B-amylose : Left handed		$+190^\circ$
(double helical) : Right handed		$+74^\circ$
Aqueous butanol solution (5%)	$+157 \pm 5^\circ$	
Aqueous dimethyl sulphoxide solution (2.5%)	$+160 \pm 5^\circ$	
Palmitate complex in solution	$+104 \pm 5^\circ$	
Palmitoyl CoA complex in solution	$+82 \pm 5^\circ$	
Aqueous solution or gel	$+195 \pm 5^\circ$	

Note a: Calculations were based on conformation angles of trial structures for B-amylose kindly supplied by Dr. A. Sarko to fit unit cell dimensions (see Ref. 24 & 25) and, for V-amylose, from the trial structures of Ref. 26.

analysis) that a particular system is spectroscopically homologous with others in which the relationship has been shown to apply. In addition, more fundamental approaches to prediction of optical activity should now be possible by analysing the factors which determine rotational strengths of the two relevant bands.

Recent work in our Laboratory has shown that similar considerations apply to other polysaccharides. Especially revealing and exciting results have been obtained recently on the solution properties of amylose (Ref. 23). As shown in Table 2, measurements and calculations similar to those on agarose have confirmed that the optical activity of B amylose is indeed consistent with a double helical model as has been proposed from X-ray diffraction analysis (Ref. 24 & 25) but favour a left-handed rather than a right-handed model.

For V amylose, the optical rotation changes suggest that the handedness of the helix depends on the nature of the included species. For dimethyl sulphoxide and n-butanol, as well as iodine (Ref. 27), the helix is apparently left-handed, as indeed has been concluded from X-ray diffraction analysis (Ref. 28). However, for long chain fatty acid derivatives such as palmitate our preliminary results suggest the surprising conclusion that the V amylose helix has here the right-handed form.

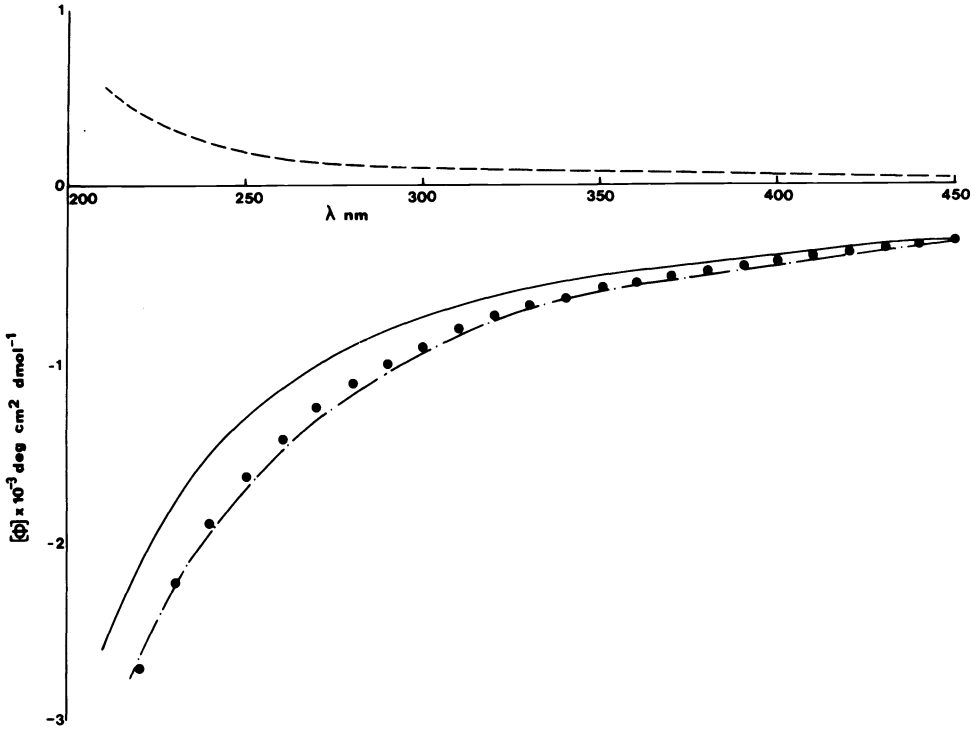


Fig. 6. Relationships between o.r.d. and c.d. spectra for agarose in the random coil state. Solid curve: measured o.r.d. spectrum. Broken curve: contribution to this, calculated from the observed c.d. band near 180 nm. Dash-dot curve: residual when the above two curves are subtracted. Filled circles: fit obtained on the assumption that the residual is to be explained entirely in terms of a c.d. band at 152 nm.

STEREOCHEMICAL HOMOLOGIES FOR POLYURONATES

A so-called "egg-box" model (Ref. 29) has been proposed for the interactions of poly-L-guluronate segments with Ca^{2+} ions in alginate gelation (Fig. 7). This model is based upon the physicochemical properties of alginate gels (Ref. 7) and the cation binding properties (Ref. 30), together with the conformations derived X-ray fibre diffraction analysis (Ref. 31 & 32) and evidence on the cooperativity (Ref. 33) and stoichiometry (Ref. 34) of the interactions in Ca^{2+}-induced gelation. The primary event is a

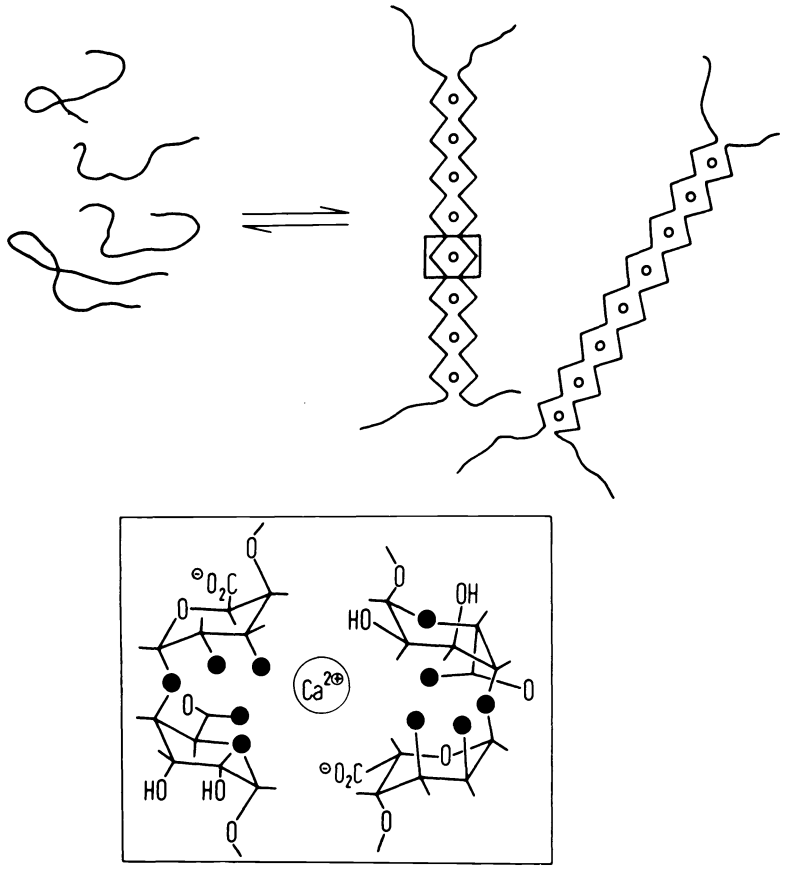


Fig. 7. Schematic representation of the egg-box mechanism for alginate gelation. Upper: Conversion of random coils (left) to buckled ribbons which pack to contain arrays of Ca^{2+} ions. Lower: Proposed stereochemistry of Ca^{2+} ion complexation, with the oxygen atoms involved in the coordination sphere shown as filled circles.

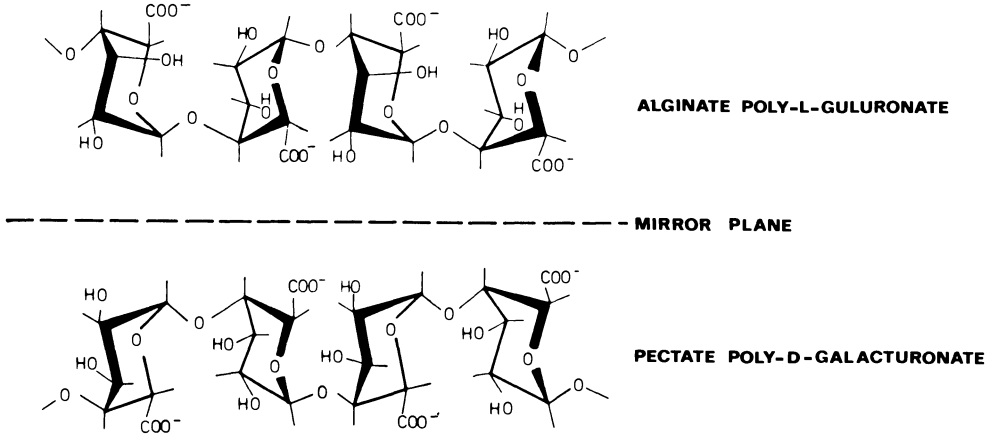


Fig. 8. Stereochemical relationship between polyuronate chains.

pairwise association of the polyguluronate chains which, owing to their diaxial glycosidic linkages, have a buckled form which accommodates Ca^{2+} ions in interstices when they pack, providing close ion-pair association with the carboxylate anion and efficient coordination by other electronegative oxygens. This intimate and specific ionic interaction gives rise to a profound change in the $\underline{n} \rightarrow \pi^*$ c.d. band of the carboxylate (Ref. 29 & 34).

Until very recently the details of the geometry of the interactions in the Ca^{2+}-induced gelation of polygalacturonate derivatives (pectins) has, however, been less clear. The close stereochemical relationship between polygalacturonate and polyguluronate might lead to the expectation that their chain conformations would be similar, including the conformations involved in the interactions with Ca^{2+} - the structural formulae are exact mirror images, apart from the configuration at C(3) (Fig. 8). However, X-ray diffraction studies show very different situations for polyguluronate and polygalacturonate derivatives. It seems the polyguluronate chains are always two-fold buckled ribbons as indeed in the egg-box model (Ref. 32) but the polygalacturonate forms studied in any detail so far have corresponded to three-fold twisted ribbons which clearly require to pack in some other way (Ref. 35).

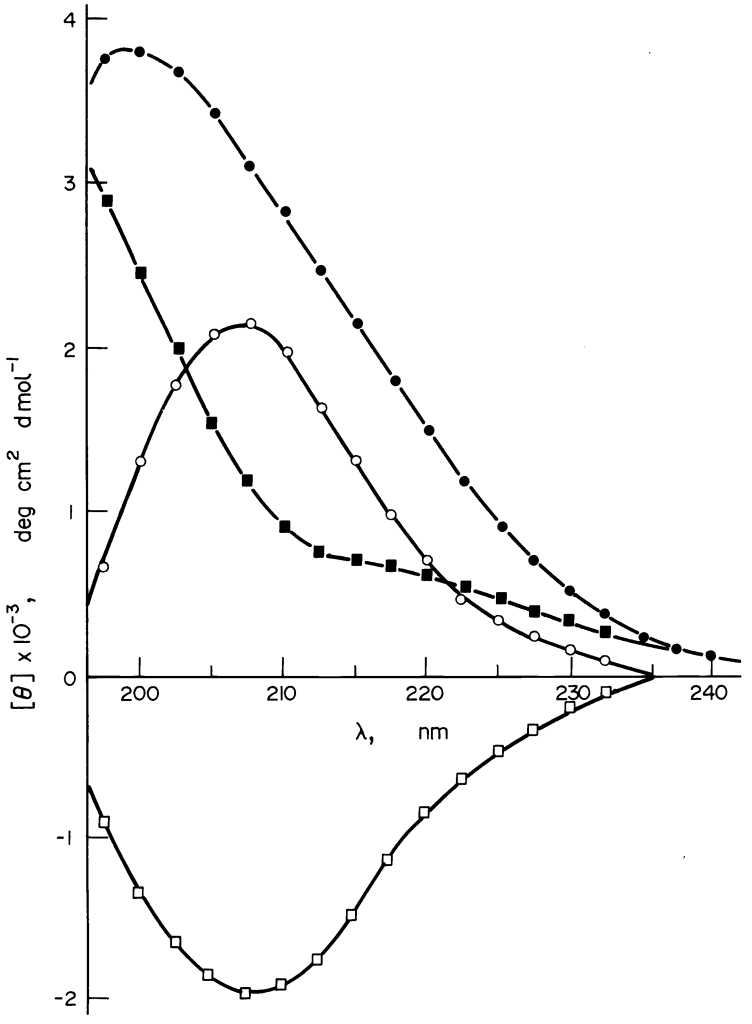


Fig. 9. C.d. spectra and changes with the Ca^{2+}-induced gelation of polygalacturonate compared with polyguluronate. Filled circles, polygalacturonate solution; filled squares, polygalacturonate gelled with Ca^{2+}; open squares, difference between the above two spectra; open circles, corresponding difference spectrum for polyguluronate.

To investigate this, we have exploited the very characteristic perturbation of the $n \rightarrow \pi^*$ transition in the carboxylate c.d. which no doubt reports on the stereochemical environment of the carboxylate and especially its relative orientation with respect to the cation (Ref. 29 & 34). If polygalacturonate were to form egg-box structures which were stereochemically similar to those of polyguluronate, then we might expect related changes in c.d.; otherwise the changes would be fundamentally different. The results (Fig. 9) showed a marked change in the c.d. of sodium polygalacturonate (filled circles) with diffusion of Ca^{2+} ions to form the gel; the amplitude became less positive and a new inflection developed around 210 nm (filled squares). The difference curve was a smooth gaussian band centred at 210 nm (open squares), almost exactly the mirror image of the corresponding difference curve for polyguluronate (open circles). We conclude (Ref. 36) that the Ca^{2+} carboxylate relationships in polygalacturonate and polyguluronate approximate closely to mirror image situations and that calcium polygalacturonate probably forms an egg-box structure analogous to polyguluronate.

While this conclusion seems entirely reasonable from comparison of structural formulae (Fig. 8), it leaves the important question of the relationship of these results to the X-ray diffraction evidence that the conformations of polyguluronate and polygalacturonate can be quite different in the solid state. A further examination of c.d. behaviour provides an answer to this puzzle also. When calcium polyguluronate gels are dried down to solid films and the

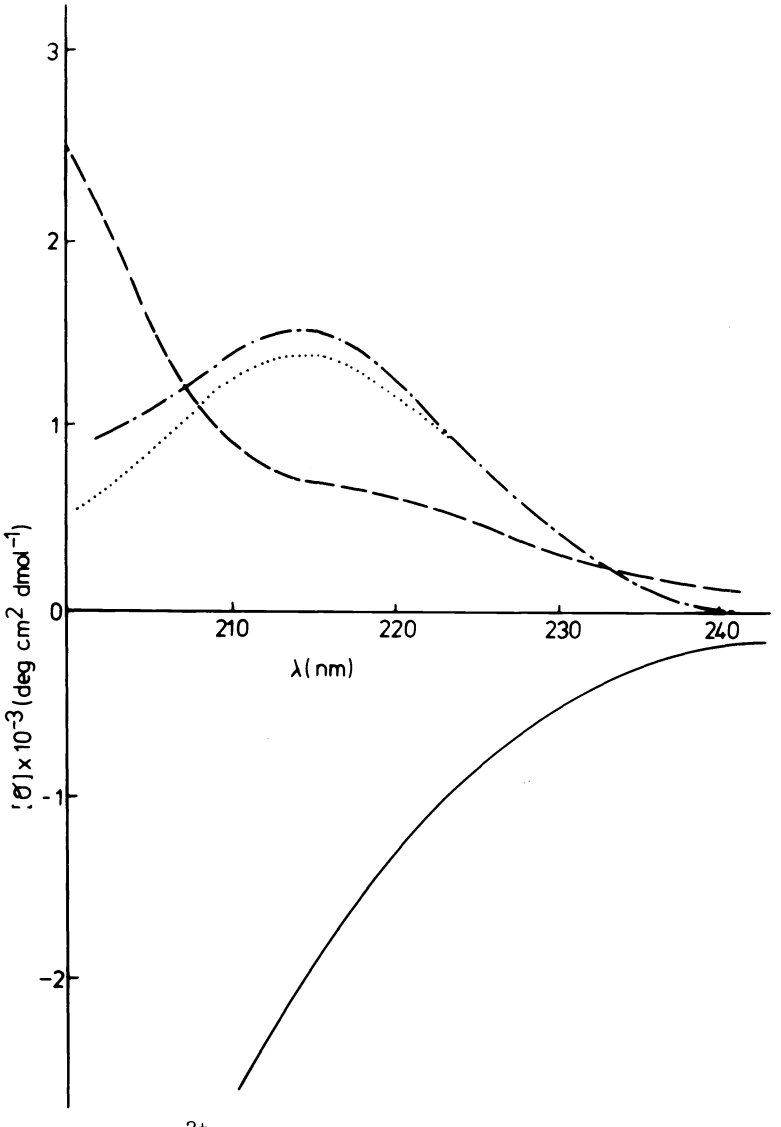


Fig. 10. C.d. of Ca^{2+} polyguluronate in gel form (dotted line) and film form (dash-dotted line); compared with c.d. of Ca^{2+} polygalacturonate in gel form (broken line) and film form (solid line).

spectrum is recorded again, there is little or no c.d. change (Ref. 14), as expected if as we believe (Ref. 29 & 32) the egg-box structure is undisturbed by removal of water. In contrast, however, a dramatic change in c.d. is found to accompany the similar drying of calcium polygalacturonate gels (Fig. 10). We conclude that, unlike the polyguluronate egg-box, the polygalacturonate egg-box does not survive removal of water under these conditions, presumably because more favourable possibilities for chain packing exist in larger aggregates in the dry state. This is the first known example of an ordered hydrated polysaccharide conformation which fails to correspond to a common form in the condensed phase. It illustrates the value of independent investigations into solution conformations rather than relying on uncritical extrapolation from the solid state. The conclusion is however sufficiently unusual and surprising to call for independent confirmation. We have developed other experimental methods for investigating the strandedness and symmetry of ordered polysaccharide states in solution, based upon competitive inhibition of network formation and the stoichiometry of Ca^{2+} binding when in competition with a swamping excess of univalent cations. By both criteria (Ref. 36 & 37) we have confirmed that the Ca^{2+}-mediated association of polygalacturonate chains proceeds by pairwise association of two-fold chains.

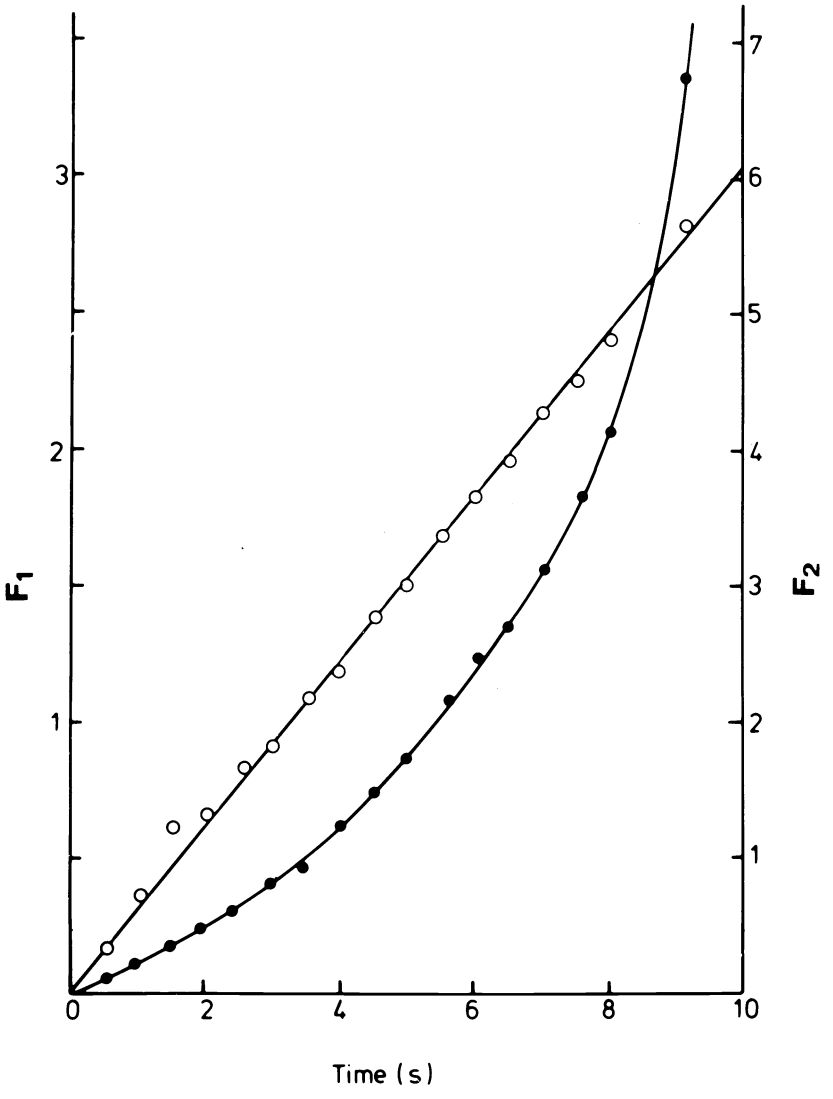


Fig. 11. Dynamics of the disorder-order transition of xanthan (338.2 K; 0.5% w/v xanthan; 0.5 M KCl) analysed in terms of first order (open circles) and second order (filled circles) kinetics.

CONFORMATIONAL ORDERING OF A BRANCHED POLYSACCHARIDE

The extracellular polysaccharide (xanthan) from the gram negative bacterium Xanthomonas campestris has evoked much interest over the past ten years because of its unusual and useful solution properties which have been shown to be related to an ordered solution conformation (Ref. 38 & 39). The geometry of this ordered conformation has however been difficult to establish beyond the fact that it is a five-fold helix of some type, because it has proved difficult to obtain sufficiently crystalline samples for unambiguous X-ray diffraction analysis. It is, however, possible to narrow down the alternatives to a choice between a single helix model and a double helix model (Ref. 40). The covalent structure has a branched sequence in which trisaccharide side chains attach to alternate residues of a β-1,4 glucan backbone (Ref. 41 & 42). In either of the likely conformations, the side chains are likely to fold down to align with the backbone and presumably contribute through favourable non-bonded interactions to the stability of the ordered state in solution.

Recent results from our solution studies in collaboration with the University of York, now seem to narrow down substantially the range of possibilities that is tenable for the ordered conformation. One of our approaches is through measurements of the dynamics of the disorder-order transition by a polarimetric stopped flow technique using a rapid increase in ionic strength (salt jump) to induce the transition to the ordered conformation. Separate solutions of potassium chloride and of xanthan in deionised water were simultaneously discharged into a chamber through a high-speed mixer; when flow ceased, the rate of change of optical rotation was measured to monitor the conformational state. The results were analysed in terms of alternative reaction schemes:

$$\text{Coil} \xrightarrow{k_1} \text{Helix}$$

$$2\ \text{Coil} \xrightarrow{k_2} \text{Double Helix}$$

i.e. in terms of first order and second order rate equations respectively. At all temperatures our results are in good agreement with the first order reaction scheme as shown by the linear plot in Fig. 11, whereas a curve rather than a linear relationship was obtained when results were plotted according to the second order equation.

We conclude that first order kinetics are obeyed rather than second order, and therefore that the activated complex for the disorder to order transition is a single chain rather than a two-chain species. This would rule out the possibility of an intermolecular double helix, especially since the application of the same method to iota carrageenan (Ref. 15), kappa carrageenan (Ref. 16) and an agarose derivative (Ref. 43) by the same method showed second order kinetics consistent with the established double helical models for these polysaccharides. A likely model for xanthan would therefore be a single-stranded helix stabilized by interactions between side-chain and backbone, although we cannot on the basis of the present evidence alone exclude the possibility of an anti-parallel two-stranded intramolecular structure formed by chain folding.

Further evidence that the conformation transition for xanthan is an intramolecular event is obtained when the molecular weight is monitored by small-angle light scattering as the transition proceeds (Fig. 12; Ref. 44). There is no evidence for any change in molecular weight over the temperature range of the transition itself, although some aggregation does seem to be consequent upon the transition.

CONCLUSIONS

Recent advances in the characterisation of ordered polysaccharide shapes in solutions and gels, both in our Laboratory and elsewhere, are leading to some general conclusions, which are illustrated by the examples given in this lecture.

1. The diversity of sequence types of polysaccharides gives rise to a diversity of conformation types including ribbons and helices of different contours and strandedness. However, some gel-forming polysaccharides of different conformation types show a similar tendency to chain pairing as the first event associated with the conformation transition, even if this is followed by further aggregation. Examples in this lecture have been the chain pairing in the carrageenan and agarose double helices and in the polyguluronate and polygalacturonate egg-boxes, and the side chain-main chain associations that are inferred for xanthan.

2. For polysaccharide polyelectrolytes, gel formation may proceed with counterion fixation which can show high selectivity, for example for the larger Group I cations by carrageenans, or between Ca^{2+} and Sr^{2+} by polyguluronate and polygalacturonate. In these examples the tight binding is of regular arrays of cations in sandwich-like

structures, whether the polysaccharide chains are in double helical carrageenans or the buckled polyguluronate and polygalacturonate ribbons.

3. Methodology for the investigation of polysaccharide shapes in solution and gels has now developed to the point where we can sometimes resolve issues that are difficult for X-ray diffraction analysis. For example, circular dichroism spectroscopy has shown conformational relationships between ordered conformations of polyuronates, and the recent advances in instrumentation have given the ability to measure optically active transitions which are sensitive to backbone conformation and can indeed be related to conformational parameters. The use of stopped-flow polarimetry has also proved its value for investigation of the number of strands which come together in the activated complex on the pathway to the ordered state.

Acknowledgement - I especially thank Dr. E.R. Morris for his collaboration in the work from the Colworth Laboratory presented here.

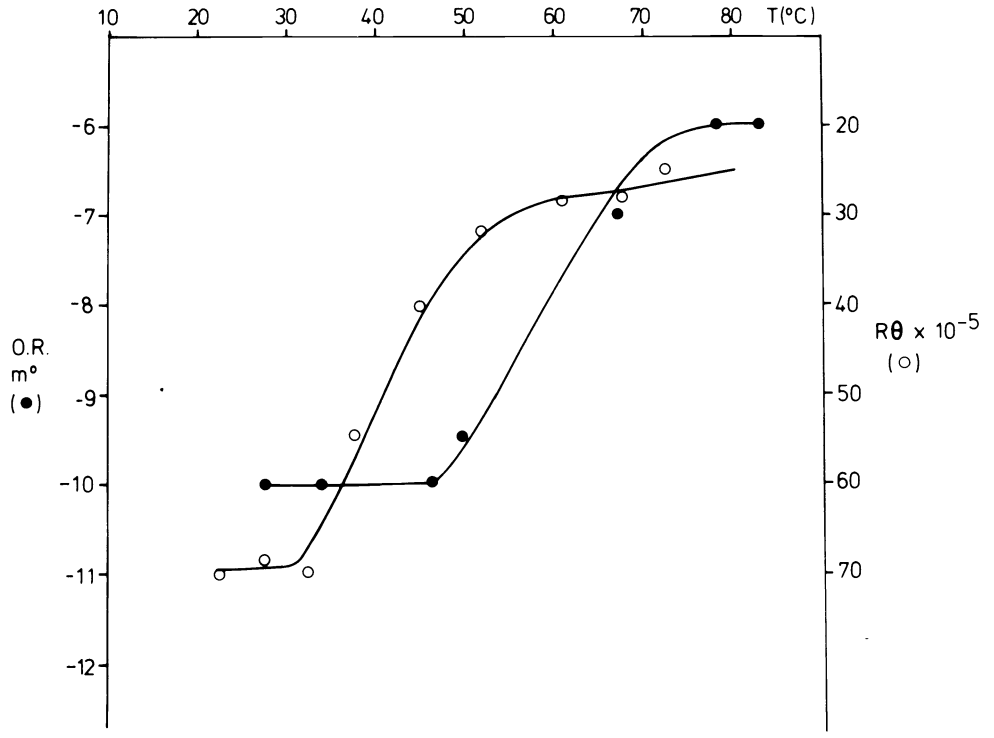


Fig. 12. Changes with temperature in optical rotation (filled circles, O.R.) and in the Rayleigh ratio (open circles, Rθ), on the same xanthan solution (0.1% w/v) in distilled water. Similar results were obtained on the heating and cooling paths. The parameter Rθ was measured on the Chromatix KM-6 low angle laser light scattering photometer at 633 nm and has an approximately inverse proportionality to molecular weight. The absolute molecular weight at 25°C is $(1.9 \pm 0.1) \times 10^6$.

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