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INVESTIGATIONS OF THE N.M.R. RELAXATION OF AQUEOUS GELS OF THE CARRAGEENAN FAMILY AND OF THE EFFECT OF IONIC CONTENT AND CHARACTER

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ABSTRACT

Measurements of the temperature dependences of the spin–spin relaxations of the water protons have been made in gels of kappa- and iota-carrageenan and of mixtures thereof as functions of gel concentration, and ionic content and character. A maximum and subsequent minimum are observed in the temperature dependence of the spin–spin relaxation time T_2, with the minimum generally being related to the onset of gel melting. The temperature and the depth of the T_2 minimum are enhanced by a decrease in the sulphate content of the polysaccharide and an increase in ionic content, with differential cationic and anionic effects being observed. A model is applied in which it is assumed that, in the temperature region between the T_2 maximum and minimum, the relaxation is dominated by an increasingly effective contribution by a highly immobile proton species associated with the polysaccharide. The determination of the relaxation of the water protons by the mobility and rate of exchange of this immobile species allows the water relaxation to act as a sensitive probe of the polysaccharide dynamics and aggregation.

INTRODUCTION

The family of gels formed from agarose, kappa-carrageenan, and ioto-carrageenan provides an apparently simple series for investigation, as a common mechanism of gelation has been proposed[1–3]. This mechanism invokes the formation of junction zones and superjunctions involving helical structures and aggregates of helices. The idealised covalent structures of the repeating units of the carrageenan series studied[4] are shown in Fig. 1, each of which contains sequences in which residues of 3-linked β-D-galactose alternate with residues of 4-linked 3,6-

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anhydro-α-D-galactose The level of sulphation decreases from iota-carrageenan (two sulphate groups per disaccharide residue) to kappa-carrageenan (one sulphate group per disaccharide unit) to furcelleran (only 40% of the 3-linked residues are sulphated). A direct extension to a zero content of sulphate does not occur.

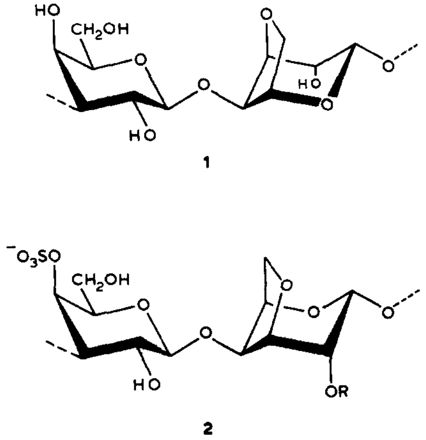

Fig 1 Idealised repeat structures **1**, agarose, **2**, iota- (R = SO_3^-) and kappa-carrageenan (R = H)

The presence of charged sulphate groups should have a significant effect on many of the observable properties, influencing the solubility and response of the polysaccharides to changes in water structure[5,6] and the association or aggregation of the polysaccharide chains to form the gel[1], the kinetics of the gelation[7] and the ageing of the network, the rheological properties[8,9], and the stability towards freeze–thaw damage and syneresis

The occurrence of double helices at low temperatures has been most clearly established for iota-carrageenan[1,10,11], but it is believed that they occur throughout the entire series[1,12]. The structures of the disaccharide repeating-units shown in Fig. 1 are idealised, with interruptions by non-anhydro residues occurring along the chain[4,13]. As a consequence, a given polysaccharide molecule will contain many helical regions giving rise to the possibility of the establishment of an extensive three-dimensional network[1]. The formation of helices has been shown to be coupled to gel formation, with the transition from helix to random coil, and gel to sol, being cooperative and temperature dependent[1,7,11-14].

It is well established that aggregation occurs to different extents in the different systems, ranging from little association beyond double-helix formation in iota-carrageenan[1,6], to ~10 chains on average in each aggregate in kappa-carrageenan[1,14], to the order of tens to even hundreds of chains for agarose[15-17]. The water-proton spin–spin relaxation has been shown to be very sensitive to the mobility and aggregation of the polysaccharide[18-20].

We have now extended pulse n m.r. measurements of the water T_2 to the carrageenan series in order to examine the models and concepts developed previously and to explore the effect of electrolytes upon the carrageenans and their aggregation. The effects of the sulphate content, the nature of the counterion and the added ions on the motions and conformations of the carrageenans have been studied.

EXPERIMENTAL

Samples of kappa- and iota-carrageenan were supplied by C. Bellion (CECA, SA France). The iota-carrageenan was derived from *E. spinosum* batch SE 2280/G, and the kappa-carrageenan from *E. cottonii* batch B 5466.

Specific salt forms of the carrageenans were prepared by dialysing against three changes of deionised water in order to remove any excess salt, followed by exchange[14] on Amberlite IR-120 resin, followed by freeze-drying. The purity of the samples was confirmed by n.m.r. spectroscopy, and the ionic content and type were determined by an elemental analysis (Butterworth Micro-analytical Co. Ltd.). The samples employed had a purity of >95% whether in the carrageenan or the salt form.

Samples for n.m.r. measurements were prepared by dissolving the required amount of freeze-dried material in deionised water, dialysing the solution to the required ionic activity, and diluting to the required concentration. Absolute concentrations were determined by polarimetry[14]. In order to ensure the consistent production of homogeneous samples, they were heated to well above the gel melting temperature and cooled before study This procedure was carried out normally within 1 day

Freeze–thaw cycles, storage at sub-zero temperatures, and subsequent heating to 80° disrupted the gel network, resulting in the development of multi-exponential decays For this reason, data were recorded only for fresh samples which resulted in the observed relaxations being single-component exponentials.

Spin–spin relaxation measurements were made using a Carr–Purcell–Gill–Meiboom pulse sequence and a high-power pulse spectrometer operating at 16 MHz. The amplitude of one thousand echoes in the sequence was recorded, stored in a signal averager, and averaged over several echo trains. As only the echo amplitudes were recorded and the initial free induction decay was ignored, signals from the host polysaccharide were not detected. The relaxation times were derived using a least-squares fitting procedure based upon Newton–Raphson methods. The temperatures of the samples were controlled by using a thermocouple to regulate the heating of a sample gas-flow system to 0.1–0 5°

DISCUSSION

Model to describe agarose. — In the earlier n.m.r. study, the gelation properties of agarose were examined by recording the spin–spin relaxation times (T_2) of the aqueous solvent and the macromolecular solute[18]. Four species of proton were identified in the gels by their different effects on the observed relaxation behaviour. Species A and B comprised protons of water molecules which were, respectively, effectively free and bound, the bound molecules possessing modified motional properties as a consequence of their interactions with the macromolecule. Species C comprised a small number of extremely motionally restricted protons derived either from one or two water molecules per disaccharide unit, or from the hydroxyl groups of the polysaccharide. Species D consisted of the non-exchangeable protons of the macromolecules. On the n.m.r. time-scale, there was rapid exchange between the protons or water molecules of species A and B, whereas there was a transition from slow to fast exchange between species C and the other two species, over the temperature range corresponding to the gel–sol transition. This change in exchange rate for species C was manifested in the distinctive occurrence of a maximum and subsequent minimum in the temperature dependence of T_2 (Fig. 2).

The general equation *1* was used to describe the observed relaxation behaviour of the water in agarose.

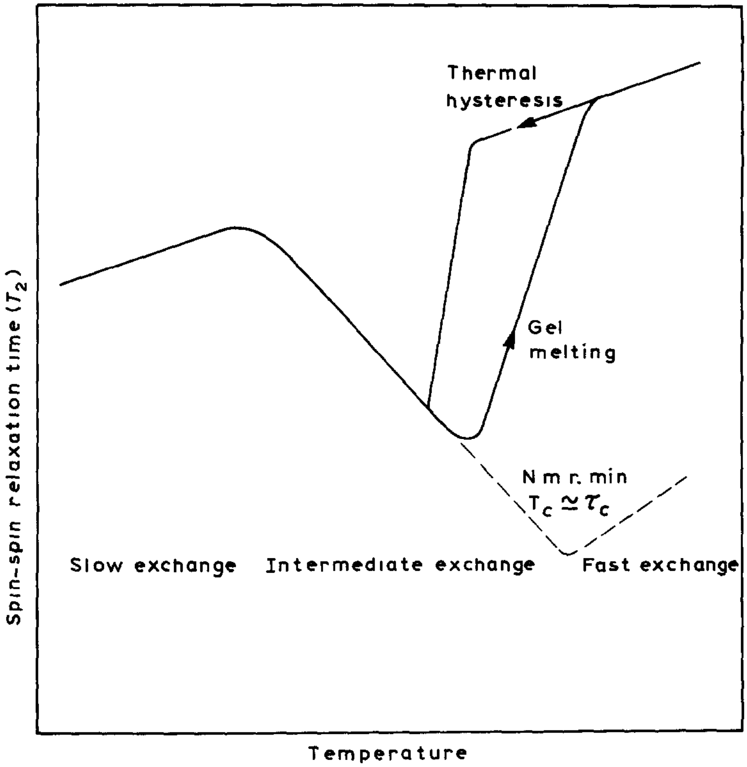

Fig 2 Schematic representation of the effect of exchange rate and gel melting on the temperature dependence of the proton spin–spin relaxation time (T_2) of the water

$$\frac{1}{T} = \frac{P_A}{T_A} + \frac{P_B}{T_B} + \frac{P_C}{T_C + \tau_C} \tag{1}$$

In Eq. *1*, $P_A + P_B + P_C = 1$, T_i is the relaxation time of the ith species, and τ_C is the lifetime of species C. At low temperatures when the lifetime of species C is long compared to its relaxation time (T_C), C will be in slow exchange and so does not contribute significantly to the observed relaxation data. Hence, in the slow-exchange region, equation *1* reduces to equation *2*.

$$\frac{1}{T} = \frac{P_A}{T_A} + \frac{P_B}{T_B} \tag{2}$$

At higher temperatures, when there is intermediate exchange, equation *1* will apply. It can be seen from inspection of equation *1* and Fig. 2 that the minimum in T_2 for the gel would occur when $T_C = \tau_C$. However, the gel-to-sol transition can occur before this condition is fulfilled and this causes the minimum to occur at a lower temperature. This arises because the phase transition reduces the magnitude of the term $P_C/(T_C + \tau_C)$ by either decreasing P_C and/or increasing T_C, until finally the intrinsic relaxation rates of species C and B become comparable and equation *1* reduces to the simple fast-exchange model, *i.e.*, equation *2*.

Change in sulphate content. — Fig. 3 displays the temperature dependence of the proton relaxation of a series of gels where the content of sulphate is decreased progressively from iota- to kappa-carrageenan, but where the concentration of the polysaccharide is maintained at 5% and the ionic activity maintained constant by dialysis against 0.2M KCl. The presence of the polysaccharide markedly reduces the single-component spin–spin relaxation times (T_2) from the dialysate values. Whilst less pronounced than in the agarose samples discussed previously, the effect of exchange with the species C is again manifested for all of the gel samples studied by the occurrence of a maximum and subsequent minimum, or by a point of inflection, in the graphs of T_2 against temperature. However, it is apparent that the effect is more marked in the samples having a lower content of sulphate. The gel-sol-gel cycles (Fig. 3) show thermal hysteresis in the recorded T_2 values. This feature is more pronounced in the samples having a low content of sulphate, with the largest hysteresis being observed for agarose[19,20].

A close correlation between the temperature of the T_2 minimum and the transition mid-point for molecular ordering was observed in this study and on studies of other samples where the carrageenan and ionic contents are varied[20]. This result is very different to that for agarose[18] where such a close correlation between gel melting and T_2 minimum was not observed, but an apparent true exchange minimum occurred 40–50° before the molecular melting. This finding suggests that, for carrageenan, the breakdown of the gel network eliminates the C phase and, hence, causes a sharp increase in relaxation time preventing the observation of the true n.m.r. exchange minimum. This view is further supported by the

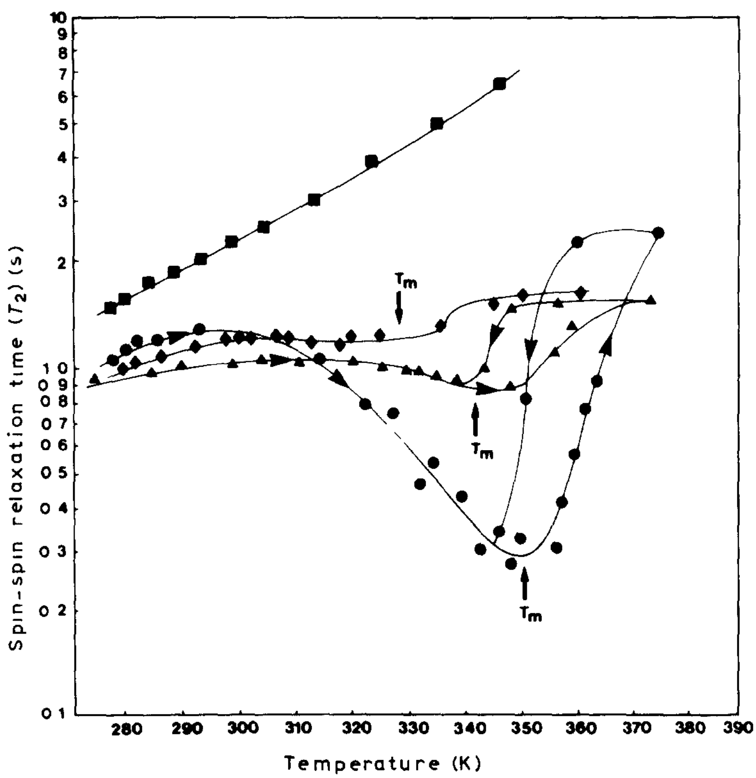

Fig 3 The effect of temperature on the spin–spin relaxation time (T_2) of the water for 5% (w/w) samples in 0 2M KCl iota-carrageenen (◆), kappa-carrageenan (●), an equiweight mixture of iota- and kappa-carrageenan (▲), and 0 2M KCl dialysate (■) The transition mid-points obtained from polarimetry are shown for comparison

observation that the sol relaxation rates appear to be extrapolations of the low-temperature gel measurements which, because of slow exchange with species C, involve only species A and B (Fig. 3). The enhancement of the sol and the low-temperature gel relaxation rates compared to those recorded for the dialysate indicate that species B is the determining influence. The observation that the sol-phase relaxation can be represented as an extrapolation of the low-temperature gel relaxation also implies that the intrinsic relaxation of species B is largely independent of the dynamic state of the macromolecule.

From the data shown in Fig. 3, it is concluded that the effect of an increase in the content of sulphate is a marked reduction in the contribution of species C to the observed relaxation, attributed to a decrease in P_C or a reduced relaxation rate for species C corresponding to an increased mobility. This conclusion is consistent with an increase in aggregation in the order iota-carrageenan < kappa-carrageenan < agarose.

Change in polysaccharide concentration — The data represented in Fig 4 illustrate the increase in relaxation rate with increasing concentration of kappa-carrageenan, and Fig. 5 shows the equivalent effect for the mixed gels. As with the agarose gels, the depth of the T_2 minimum is greater the higher the gel concentration, but, unlike the earlier agarose study, the temperature of the T_2 minimum exhibits a slight concentration dependence, increasing by 5° for a four-fold increase in polymer concentration in a manner consistent with the observed increase in the temperature of gel melting.

These same features should be emphasised if gels of higher concentration are prepared. However, difficulties were experienced in the consistent preparation of gels with uniformly homogeneous high concentrations and, hence, in obtaining consistent relaxation data.

Whereas the number and range of concentrations studied were limited, it appears that, at all temperatures investigated, there was a linear relationship between the observed relaxation rate and the concentration of carrageenan. However, as the evidence of exchange with species C becomes apparent, extrapolation to zero concentration gives values of relaxation rate in excess of the dialysate values.

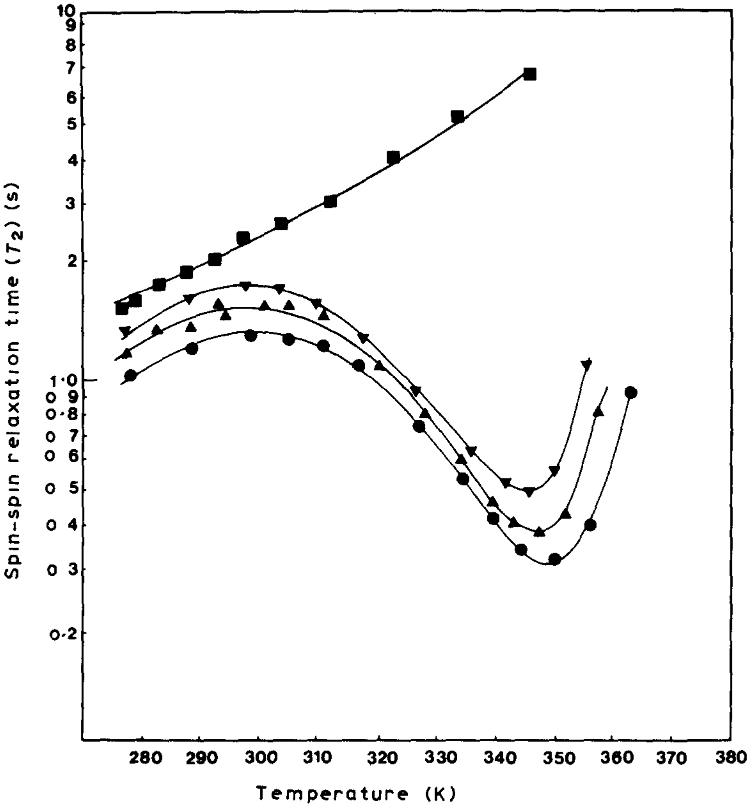

Fig 4 The effect of concentration of polysaccharide on the spin–spin relaxation time (T_2) of the water for kappa-carrageenan in 0 2M KCl 5 (●), 2 5 (▲), and 1 25% w/w (▼), and 0 2M KCl dialysate (■)

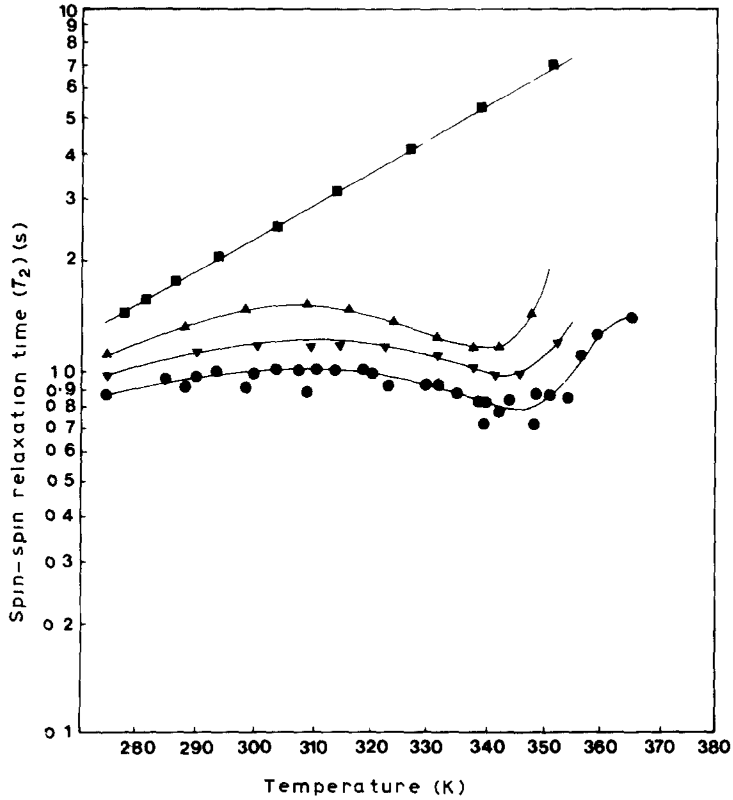

Fig 5 The effect of concentration of polysaccharide on the spin–spin relaxation time (T_2) of the water for an equiweight mixture of iota- and kappa-carrageenan in 0 2M KCl 5 (●), 3 75 (▼), and 2 5% w/w (▲), and 0 2M KCl dialysate (■)

The anomalous intercept might be explained by adapting equation *1* to allow the value of P_C, the fractional population of species C, to assume a value $\alpha c - \beta(c - c_0)$ when the concentration (c) exceeds some critical value of c_0 in the order of 1% or less. This critical concentration may be related to the critical concentration of polysaccharide required for gelation and thus be related to, and correlated with, rheological measurements of this parameter. Alternatively, a similar concentration dependence of the exchange rate $1/\tau_C$, allowing τ_C to increase with increasing concentration of polysaccharide, might be invoked. The measurements reported here are inadequate for the purposes of deciding which, if either, of these two proposals is correct, and supplementation is required

Change in ionic strength. — In addition to the dependence of the observed water-proton spin–spin relaxation upon the content of sulphate, a dependence upon the ionic content was also observed (Fig. 6). The effect of an increase in ionic activity, illustrated for KCl, is a marked increase in the depth and the temperature of the T_2 minimum. An increased presence of cations should decrease the inter-

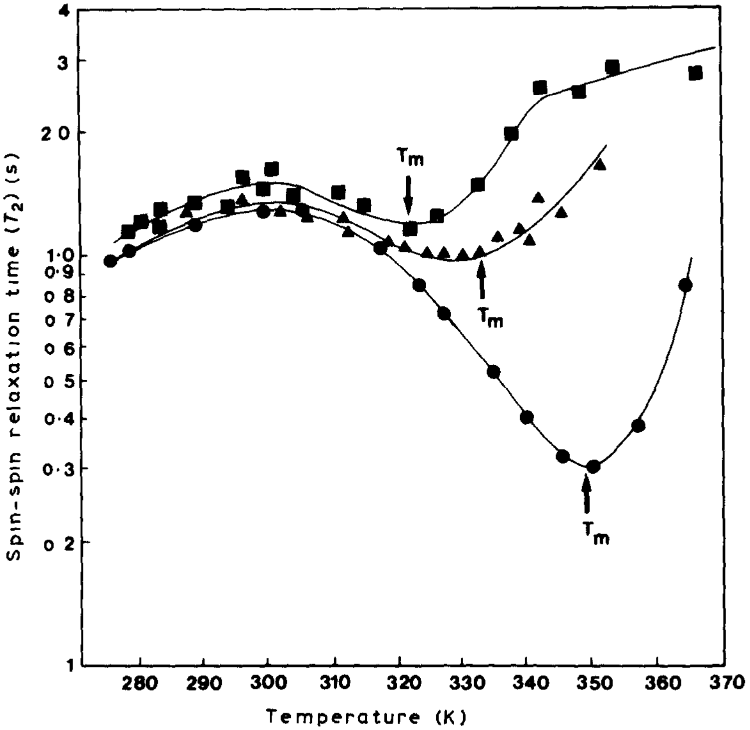

Fig 6 The effect of concentration of KCl on the spin–spin relaxation time (T_2) of the water for 5% w/w kappa-carrageenan 0.2 (●), 0.1 (▲), and 0.05M (■) The transition mid-points obtained from polarimetry are shown for comparison

chain electrostatic repulsion of sulphate groups, thus permitting a greater aggregation of the polysaccharide. This effect should inhibit chain mobility, thereby increasing the relaxation rate of protons of species C. Depending upon the spatial positions of the protons of species C–for example, inclusion within the aggregates–there may be inhibition of exchange between species C and the other more mobile protons, resulting in an increase in τ_C.

The effects of these two changes, namely, a decrease in T_C or an increase in τ_C, upon the observed relaxation would be different. As indicated in Fig. 7, a decrease in τ_C should increase the depth and temperature of the true T_2 minimum where $\tau_C = T_C$. The intermediate exchange region, where T_2 decreases with increasing temperature, would be largely unchanged Alternatively, if τ_C were to be increased, the onset of the intermediate exchange would be delayed, and whereas the temperature of the T_2 minimum would be increased, its depth would be decreased. For the systems examined here, it is believed that the sharp increase in T_2 on increasing the temperature is produced by the melting of the gel before the condition $\tau_C = T_C$ for the observation of the n.m.r. T_2 minimum is achieved. Therefore, the effects of an increase in T_{2_C} are not expected to be very marked. Thus, as

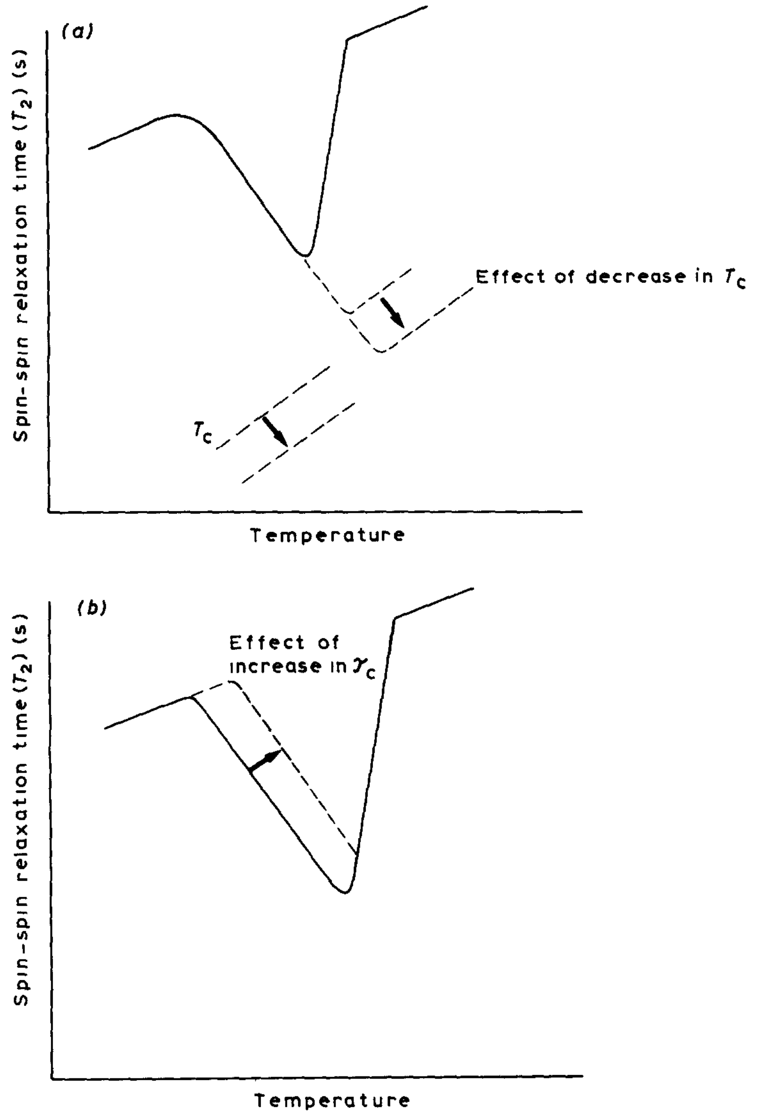

Fig 7 Schematic representation of how either (*a*) a decrease in the relaxation time T_C of species C or (*b*) an increase in the lifetime τ_C of species C would change the temperature profile of the observed spin–spin relaxation time (T_2) of the water.

a result of the intervention of the complicating factor of a gel melting, resolution between the alternative proposals is not possible and neither set of predictions is completely consistent with observation, which corresponds to an increase in the depth and temperature of the T_2 minimum with decreasing sulphate and increasing ionic content. This is attributed to a delayed onset of gel melting.

However, these effects are accompanied by an enhancement of the relaxation

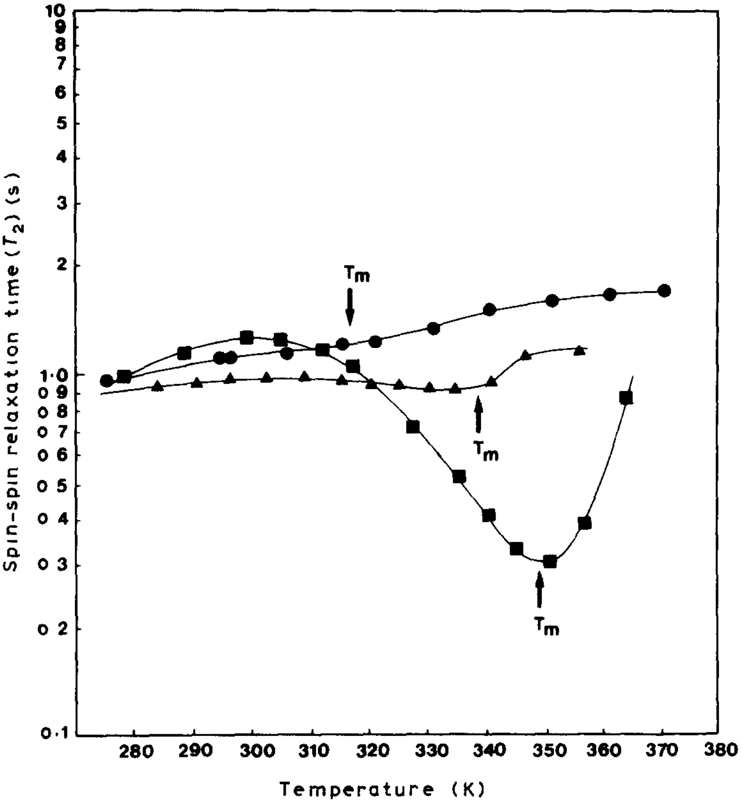

Fig. 8 The effect of concentration of KCl on the spin–spin relaxation time (T_2) of the water for 5% w/w iota-carrageenan in 0.4 (▲) and 0 15M salt (●), and 5% w/w kappa-carrageenan in 0.2M salt (■) The transition mid-points obtained from polarimetry are shown for comparison

rate in the intermediate exchange region corresponding to an increase in the $P_C(T_C + \tau_C)^{-1}$ contribution inconsistent with an increase in the value of τ_C. An increase in P_C is the most probable cause, but independent evidence for the occurrence of such changes is not available at present.

In practice, the T_2 minimum is not generally apparent in the iota-carrageenan samples. However, when the ionic content is increased from 0.15 to 0.4M KCl, a T_2 minimum is apparent in the relaxation data for iota-carrageenan. Fig 8 indicates that the higher concentration of salt results in a greater contribution of species C as a consequence of increased aggregation.

Effect of different ionic species. — The gelation properties of both iota- and kappa-carrageenan are dependent upon the amount and character of the added cations. In addition, the temperature and/or critical concentration for the gelation of kappa-carrageenan depends upon the type of anion, with the effect following the lyotropic series. Reflecting these differences in aggregation properties, the n.m.r. relaxation displays a dependence upon the cationic species as shown in Fig. 9

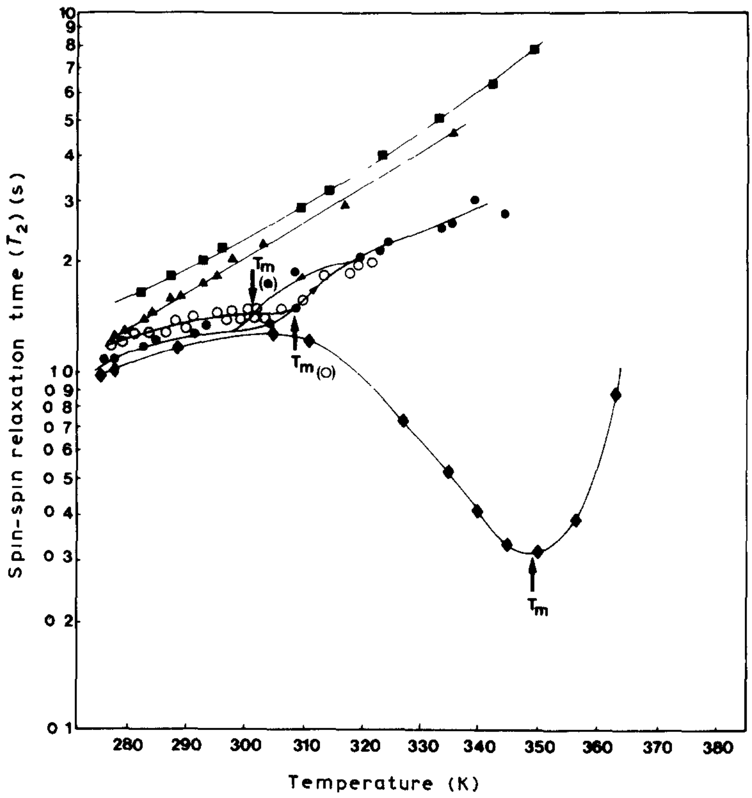

Fig 9 The effect of anion species on the spin–spin relaxation time (T_2) of the water for 5% w/w kappa-carrageenan in 0 2M TMABr (O), $(TMA)_2SO_4$ (●), and TMACl (▲), 0 2M TMACl dialysate (■), and 5% w/w kappa-carrageenan in 0 2M KCl (◆) The transition mid-points obtained from polarimetry are shown for comparison

Tetramethylammonium (TMA) chloride is less efficient in promoting the aggregation of kappa-carrageenan and is markedly less effective in inducing a T_2 minimum than is potassium chloride, although polarimetric studies indicate that the carrageenan is in the helical state, and clear gels were produced.

The effects of different anions on the T_2 minimum of kappa-carrageenan with the TMA cation are also shown in Fig. 9. These data show that, in the presence of $(TMA)_2SO_4$, a small but real T_2 minimum is observed together with a small thermal hysteresis between the heating and cooling scans. In the presence of TMACl or TMABr, there is little evidence for a T_2 minimum, and heating and cooling curves are overlaid. This finding clearly demonstrates that some aggregation occurs with the sulphate anion, but less ordering beyond helical formation occurs with bromide or chloride.

These results demonstrate the sensitivity of the n m.r technique to the aggregation in polysaccharides, with the results obtained here being totally consistent with, and complementary to, those previously reported[5]

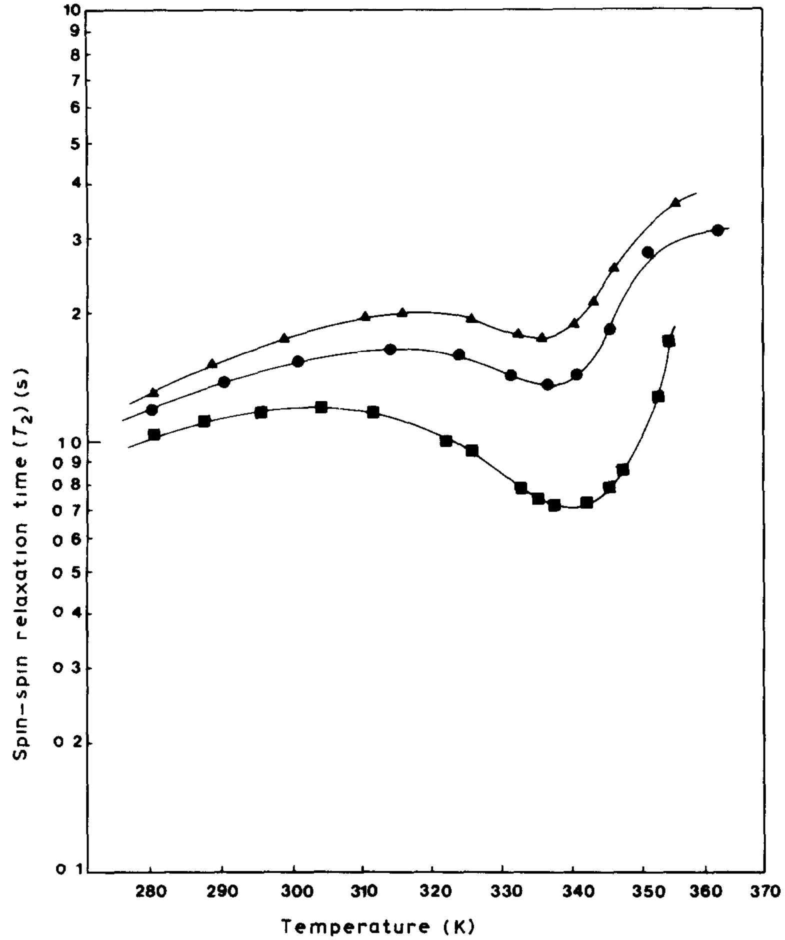

Fig 10 The effect of concentration of polysaccharide on the spin–spin relaxation time (T_2) of the water for kappa-carrageenan in 0 2M KBr. 5 (■), 2 5 (●), and 1 25% w/w (▲)

In order to demonstrate that the anionic effects are not confined to systems which show little aggregation, measurements of TMA cations were made for 1.25–5% concentrations of carrageenan in 0.2M KBr. These results are shown in Fig. 10, a comparison of which with Fig. 4 reveals that an anionic dependence occurs even in the presence of a cation that strongly promotes the aggregation of the kappa-carrageenan. Thus, the results obtained demonstrate the importance of the water-structuring properties of anions and thus of the solvent quality for the moderately charged kappa-carrageenan.

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