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Gelation of Gellan Gum

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SUMMARY

The microbial polysaccharide, gellan gum, was studied in aqueous solution and in the gel state by osmometry, viscometry, light scattering, polarimetry and NMR and by measurements of gel strength and cation-exchange selectivity. In solutions of the tetramethylammonium (TMA) salt of the polymer, increasing concentrations of TMACl induced contraction, ordering, and association of the chains, as revealed by viscometry and changes in optical rotation. Chain ordering occurred at ionic strengths (I) between 0·005 and 0·05 (M), and light scattering indicated an elongated chain structure in 0·025 M TMACl. Gelation was dependent upon both ionic strength and the identity of the cation. For monovalent cations at $I = 0{\cdot}1$ (M), gel strength increased in the order:

$$TMA^+ < Li^+ < Na^+ < K^+ < Cs^+ < H^+$$

For divalent cations the series was:

$$Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+} < Zn^{2+} < Cu^{2+} < Pb^{2+}$$

The lack of specificity among the alkaline-earth cations distinguished gellan gum markedly from other uronic-acid containing polysaccharides like alginate and pectin. Studies of cation-exchange equilibria likewise showed no selectivity within the alkali-metal or the alkaline-earth metal cations, but for all the divalent ions the affinity series:

$$Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+} < Zn^{2+} < Cu^{2+} < Pb^{2+}$$

was the same as the series for increasing gel strength.

Measurements of optical rotation and NMR spectroscopy indicated that chain association and gelation were related phenomena. The results are interpreted as indicating that gelation occurs in two steps, namely, chain ordering and chain association.

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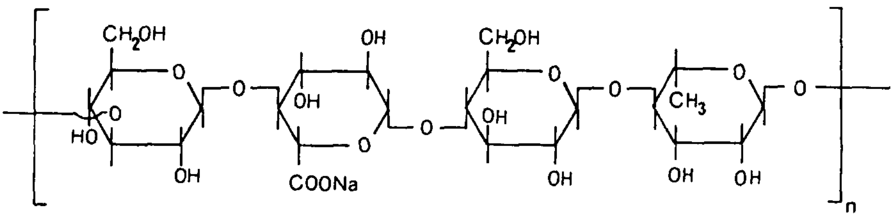
INTRODUCTION

Gellan gum is the trade name of a deacetylated extracellular polysaccharide produced by Kelco Division, Merck and Co., from the bacteria *Pseudomonas elodea*. The acetylated polysaccharide (containing approximately one *O*-acetyl group for every eight sugar units) has been referred to as polysaccharide S-60 in some US patents (Kang *et al.*, 1982; Kang & Veeder, 1982, 1983) and as PS-60 by the Lindberg school, which determined its chemical structure (Jansson *et al.*, 1983). This structure, which has also been determined by O'Neill *et al.* (1983), has a tetrasaccharide repeating unit consisting of two β-D-glucose, one β-D-glucuronic acid and one α-L-rhamnose residue, linked together as shown in Fig. 1.



→3)-β-D-Glc*p*-(1→4)-β-D-Glc*p*A-(1→4)-β-D-Glc*p*-(1→4)-α-L-Rha*p*-(1→

Fig. 1. The repeating unit in gellan gum.

Both the acetylated and the deacetylated polysaccharide form thermoreversible gels (Kang *et al.*, 1982; Kang & Veeder, 1982, 1983), varying in mechanical properties from soft and elastic for the acetylated form to hard and brittle for the fully deacetylated polysaccharide. In fact, a wide range of gel types from very elastic to very brittle can be prepared, depending on the degree of deacetylation (Kang & Veeder, 1982), although only the fully deacetylated form is commercially available. It is also reported that gelation occurs with both monovalent and divalent cations, but it seems that monovalent cations, e.g. potassium ions, cause setting of the gels at a lower temperature than divalent ones, e.g. calcium ions (Kang & Veeder, 1982). There are also indications that, among divalent cations, magnesium and calcium ions have almost the same gelling effect (Carrol *et al.*, 1982; Moorhouse *et al.*, 1981). This is in sharp contrast with the behaviour of alginate, which does not gel in its magnesium form. The gels are characterized by very pronounced hysteresis, the difference between melting and setting temperatures being 45–60°C, as it is in agar (Kang & Veeder, 1982).

The fully deacetylated PS-60 gellan gum, with its simple, well-defined primary structure, is a particularly interesting model substance for detailed studies of the gelation mechanism of a carbohydrate polyelectrolyte (Smidsrød, 1984). The structural irregularities (often statistical) of other gel-forming polysaccharides, such as alginate, carrageenan, agar, pectin and galactomannans, which have hampered both the understanding and comparison of experimental results, appear to be absent from gellan gum. This should make it much easier to study molecular gelation mechanisms, both qualitatively and quantitatively.

We now present some observations on the effect of varying the ionic conditions of the system. It was important to have the gellan gum in an ionic form that was readily soluble in water at room temperature, and the tetramethylammonium (TMA) salt met this condition. Preliminary characterization of the polysaccharide chain in solution was thereby facilitated, and evidence for the occurrence of a conformational transition was obtained from measurements of intrinsic viscosity, optical rotation (OR) and NMR (Smidsrød, 1984). A combination of gel-strength measurements, ion-exchange equilibrium studies, and some OR and NMR measurements then provided an overview of the effect of varying some basic parameters like concentration of polysaccharide, ionic type and ionic strength and temperature. The results are compared with the behaviour of gel systems of the alginate and carrageenan type.

MATERIALS AND METHODS

Analysis of ionic composition of gellan gum

The sample studied was Gelrite gellan gum EX-4856, obtained from the Kelco Company, San Diego, USA. The polymer (100 mg) in water (5 ml) was dialysed four times against 0·2 M nitric acid. The dialysates were combined and analysed for K^+, Na^+, Ca^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+} and Sr^{2+} in a Perkin Elmer Model 560 atomic absorption spectrometer. A fifth dialysate was checked for metal-ion content with negative results.

Preparation of TMA — gellan gum

The filtered commercial sample (2·5 g in 1 l water) was passed through a column (2 cm × 50 cm) of cation-exchange resin (Dowex 50 W× 8, TMA form). To remove excess TMA ions, the effluent was concentrated to 400 ml by evaporation under diminished pressure, and dialysed against distilled water at room temperature. The measured pH of 4·2 was then adjusted to 6·8 with TMA-hydroxide, and the solution was freeze-dried.

NMR

JEOL FX-100 and Bruker WM-400 NMR spectrometers were used as described earlier for alginate (Grasdalen *et al.*, 1981; Grasdalen, 1982). The samples (14 mg (ml $D_2O)^{-1}$ for ^1H and 85 mg (ml $D_2O)^{-1}$ for ^{13}C) were run at 90°C. One sample (100 mg (ml $D_2O)^{-1}$) was subjected to ^1H-NMR analysis at a series of temperatures from 95°C down to 24°C. Some ^{133}Cs-NMR spectra of TMA–gellan gum (1·5–3·5 mg (ml $D_2O)^{-1}$) in the presence of CsCl (5–25 mM) were recorded at 19 MHz and 24°C on the JOEL FX-100.

Light scattering

Static light-scattering measurements were performed at 23°C with a laser beam ($\lambda_0 = 514{\cdot}5$ nm) spectrometer as described previously (Strand *et al.*, 1982). The concentration of polymer was varied from 0·3 g l^{-1} to 0·9 g l^{-1}. The solutions were clarified by centrifugation for 2 h at 200 000 *g*, followed by filtration through a 0·4 μm Unipore filter directly into the light-scattering cell. The cell was then inspected for dust by using a He–Ne laser beam in conjunction with a microscope. There were always some dust particles left in the sample, which produced the main uncertainty in the result.

For the refractive-index increment of the solution, dn/dc, the value 0·15 ml g^{-1} was assumed, as in an earlier study of alginate (Dingsøyr & Smidsrød, 1977).

Osmometry

The number-average molecular weight was determined by osmometric measurements at 25°C in a Knauer membrane osmometer, Type 01.00, using a cellulose acetate Sartorius membrane, Type 11736. The concentration of polymer was varied from 1 g l^{-1} to 10 g l^{-1}.

Viscometry

Viscosities were measured at 20°C in a Cannon-Ubbelohde viscometer having a flow time for water of 206·5 s. Relative viscosities were determined for four or five different polymer concentrations, from which the intrinsic viscosity, $[\eta] = \lim_{c \to 0} (\eta_{sp}/c)$, was extrapolated. At low ionic strengths, where TMA^+ ions from the polymer contributed to the ionic strength, the dilution was made iso-ionically, assuming full dissociation of the TMA^+ ions from the polymer salt. No correction to c was made for the presence of 8–10% (w/w) water in the polymer sample.

Optical rotation

Measurements were performed at 589 nm, in a Perkin Elmer Model 241 polarimeter equipped with jacketed cells, through which water was circulated from a thermostatically controlled bath. The hot polymer solutions were poured into the cell (diam. 0·3 cm, length 10 cm), previously warmed to 80°C. All subsequent measurements were carried out by cooling or by a cooling–heating cycle which normally lasted for 8 h. The specific optical rotation, $[\alpha]_D$, was calculated without correcting for the water content of the sample, and without making any correction for different weights of the counterions.

Preparation of gels

The TMA form, or the salt form of the commercial polymer was dissolved by heating to 90°C. The solution of desired concentration was poured into perspex cylinders with a height of $L_0 = 15$ mm and an inner cross-sectional area of $A = 154$ mm^2, covered with dialysis tubing at one end. The other end was rapidly closed with another piece of dialysis tubing, and the cylinders (inner volume, 2·3 ml) were dropped into 50 ml of the desired ionic buffer of room temperature (22 ± 2°C). The buffer was changed three times a day for 2 days, after which time atomic absorption analysis showed that cation-exchange was complete to more than 98%. This was the case for both salt forms of the polymer. Gel-strength measurements (see below) showed that starting with the TMA form or the commercial salt form gave identical results. In most cases the commercial salt form was used for preparation of gels.

Gel-strength measurements

These were generally performed as described earlier (Smidsrød *et al.*, 1972). After dialysis the gels were removed from the perspex tubing, weighed and the height and diameter measured. This standard procedure had been developed for calcium alginate gels, for which severe shrinkage could occur. In the present case neither significant shrinkage nor swelling was apparent. Compression of the gels was studied at room temperature (22 ± 2°C), in an Instron TTK Universal Testing Machine, at a compression rate of 0·2 cm min^{-1}. The applied force, F, at 1 mm compression $(L_0 - L)$ did not deviate significantly from the position of the initial tangent at 1 mm deformation in the force — deformation diagram (see Fig. 4), and could be used to calculate the modulus:

$$E = \frac{F \cdot L_0}{(L_0 - L) \cdot A} \cdot \frac{W^2}{W_0^2}$$

where W_0 and W are the weights of the gel before and after dialysis. The last correction was included because the gel strength increases with the square of the polymer concentration (see Fig. 8). All measurements were carried out in duplicate. The standard deviation in the modulus E was $\pm 7\%$.

Ion-exchange equilibrium measurements

These were generally performed as described previously for other polyelectrolytes (Smidsrød & Haug, 1968*a*). Duplicate samples of polymer (20 mg) were suspended in water (2 ml) in dialysis bags, and dialysed four times against 50 ml portions of 0·2 M solutions of mixed salts. These solutions contained six different ratios, C_{Na^+}/C_{K^+}, of the monovalent cations Na^+ and K^+, or five different ratios, $C_{Me^{2+}}/C_{Mg^{2+}}$, of divalent cations, where Me^{2+} was Ca^{2+}, Sr^{2+}, Zn^{2+}, Cu^{2+} or Pb^{2+}. The bags were then dialysed four times against ion-free water (50 ml) to remove excess ions, and then four times against aqueous nitric acid (0·2 N; 20 ml). The pooled, acidic dialysates were then analysed for cation content by atomic absorption spectroscopy. A fifth acid dialysate was always checked for metal-ion content, and included in the estimate of ion concentration, if necessary. The mole-fractions of counterions bound by the polymer, X_{Me^+} or $X_{Me^{2+}}$ thus obtained were used to calculate selectivity coefficients from the formulae:

$$K^{K^+}_{Na^+} = \frac{X_{K^+} \cdot C_{Na^+}}{X_{Na^+} \cdot C_{K^+}}$$

or

$$K^{Me^{2+}}_{Mg^{2+}} = \frac{X_{Me^{2+}}}{X_{Mg^{2+}}} \cdot \frac{C_{Mg^{2+}}}{C_{Me^{2+}}}$$

RESULTS

Chemical characterization

The ionic composition of the commercial sample of gellan gum, determined by atomic absorption spectroscopy, is shown in Table 1. It is seen from the Table that the polyelectrolyte is a mixed salt with K^+, Na^+, Ca^+ and Mg^{2+} as the main counterions. By comparing the total ionic content with that expected from the chemical structure, an excess amount of salt of 0·21 meq g^{-1} was found giving an equivalent weight of 610 compared to the theoretical value of about 700.

TABLE 1

Metal-ion Content (meq g^{-1}) of Commercial Sample of Gellan Gum

K^+	Na^+	Ca^{2+}	Mg^{2+}	Cu^{2+}	Zn^{2+}	Pb^{2+}	Sr^{2+}
0·671	0·41	0·32	0·21	0·016	0·008	<0·001	<0·001

Total ionic content:	1·64 meq g^{-1}
Theoretical from chemical structure:	1·43 meq g^{-1}
Excess salt:	0·21 meq g^{-1}
Found eq weight:	610
Theoretical eq weight:	~700

The TMA salt, prepared as described in the experimental section, was found to give the highest solubility at room temperature, and was used when a soluble system was required. Its chemical composition was controlled by running some ^1H- and ^{13}C-NMR spectra in D_2O. The spectra agreed almost completely with those reported by Jansson *et al.* (1983) for a sample of deacetylated gum. Our ^1H-NMR spectrum indicated signals for four anomeric protons at $\delta = 5{\cdot}16$ (1H α-L-Rhap, not resolved), 4·73 (1H Glcp, $J_{12} = 7{\cdot}5$ Hz) and 4·56 (1H Glcp + 1H GlcpA, $J_{12} \approx 7{\cdot}3$ Hz). Jansson *et al.* (1983) observed the composite doublet at $\delta = 4{\cdot}70$ instead of at 4·56, presumably because a partly protonated glucuronic acid residue was in their sample. All the peaks could be accounted for by the chemical structure shown in Fig. 1, and no signals indicating impurities or acetyl groups were visible.

Physical characterization in solution

The TMA salt of the gum was readily soluble in salt-free water, but addition of TMA-chloride to high concentrations resulted in precipitation or gel formation, depending on the concentration of salt and gum. A concentration of TMACl of 0·025 M was chosen for light-scattering experiments, with the results shown in Table 2. Osmometry of the same sample was attempted but, due to a combination of high molecular

TABLE 2

Macromolecular Parameters of Two Samples of Gellan Gum

	Light scattering of non-degraded TMA gellan gum in 0·025 M *TMACl:*	
$\bar{M}_w$	$450\,000 \pm 60\,000$ Dalton	
A_2	$(3{\cdot}0 \pm 0{\cdot}2) \times 10^{-3}$ mol cm^{-3} g^{-2}	
$(\bar{R}_G)_z$	100 ± 20 nm	
$[\eta]$	28 dl g^{-1}	
	Osmometry of degraded sample in:	
	0·025 M TMACl	0·05 M TMACl
$\bar{M}_n$	39 000 Dalton	39 000 Dalton
A_2	$6{\cdot}4 \times 10^{-3}$ mol cm^{-3} g^{-2}	$2{\cdot}2 \times 10^{-3}$ mol cm^{-3} g^{-2}
$[\eta]$	4·9 dl g^{-1}	5·3 dl g^{-1}

weight and high second virial coefficient, A_2, a reliable estimate of the molecular weight was not obtained. Osmometry was therefore performed on a sample slightly degraded by ultrasonication. To get better estimates of the molecular weight, two different ionic strengths (0·025 and 0·05 M TMACl) were used. The results are given in Fig. 2 and Table 2. In Table 2 the corresponding intrinsic viscosities, $[\eta]$, are also given for both ultrasonicated and non-degraded samples. The intrinsic viscosities and radius of gyration are higher than for κ-carrageenan (Smidsrød & Grasdalen, 1984*b*) and alginate (Smidsrød & Haug, 1968*b*) of the same molecular weight, but lower than corresponding values for xanthan (Sato *et al.*, 1984*a*, *b*). The A_2 values are high and comparable in magnitude to the other polyelectrolyte systems. From these preliminary data it may be inferred that TMA-gellan molecules in low ionic strengths of TMACl behave as highly elongated, non-aggregating chains. Whether the high elongation is due to a stiff, single-chain backbone in a disordered conformation, ordered single chains or a multiply-stranded structure cannot be decided from the present data.

To get a better understanding of the chain rigidity, the intrinsic viscosities at different ionic strengths were measured and used to calculate the rigidity parameter, *B*, defined by Smidsrød & Haug (1971). This method uses the slope of a linear $[\eta] - 1/\sqrt{I}$ plot, where I is the ionic strength, as a measure of inherent chain flexibility. Such a plot is shown for TMA-gellan gum in TMACl in Fig. 3. The plot is clearly non-linear,

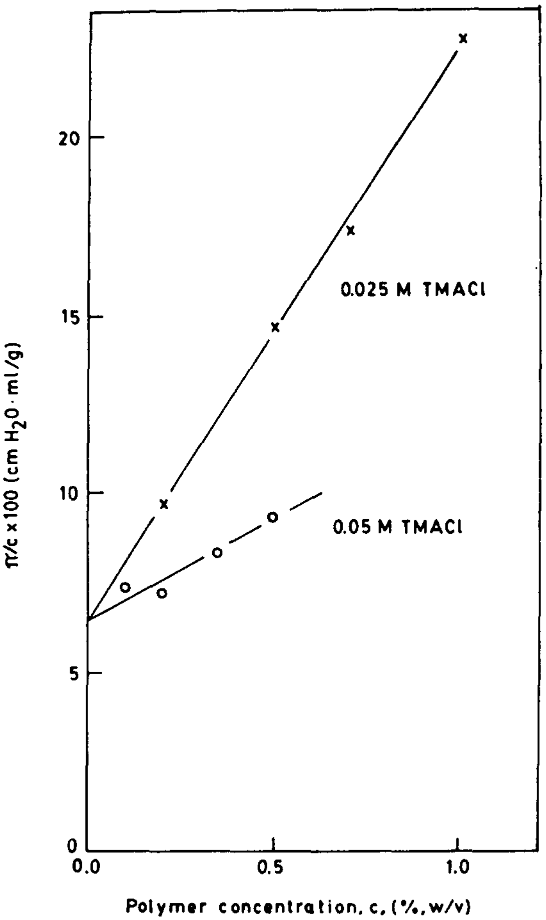


Fig. 2. Osmometry of an ultrasonicated sample of TMA-gellan gum in the presence of TMACl. ×, 0·025 M TMACl; o, 0·05 M TMACl.

and very similar to $[\eta] - I/\sqrt{I}$ plots for iota — (Smidsrød *et al.*, 1980) and kappa-carrageenan (Smidsrød & Grasdalen, 1984*b*), which show a conformational transition from a disordered to an ordered chain state with increasing ionic strength. The polymer behaves differently in three different ranges of ionic strength, I, II and III (Fig. 3). In zone I, ranging from $I = 0{\cdot}00125$ to $I = 0{\cdot}005$, the intrinsic viscosity decreases with increasing ionic strength as it does for many other polyelectrolytes undergoing electrostatic contraction without conformational transition (Smidsrød & Haug, 1971). In zone II, ranging from $I = 0{\cdot}005$ to $I = 0{\cdot}05$, the intrinsic viscosity increases by a factor of about three, indicating

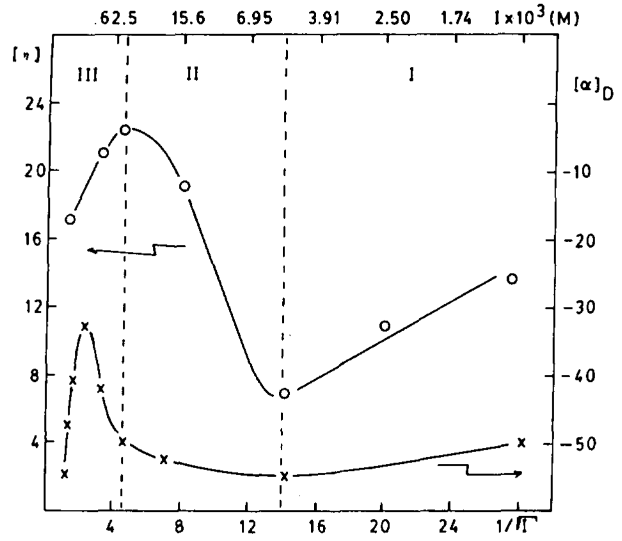


Fig. 3. Intrinsic viscosity $[\eta]$ and specific optical rotation $[\alpha]_D$ at 20°C, for 0·1% (w/v) gellan gum against $1/\sqrt{I}$, where the ionic strength I was adjusted by addition of TMACl. ○, viscosity data, left axis; ×, specific optical rotation, right axis.

some conformational ordering of single chains or a limited number of chains. In zone III, ranging from $I = 0{\cdot}05$ to infinity, a slight decrease in the intrinsic viscosity with increasing ionic strength is again observed.

At the highest ionic strengths, some time-dependent irregularities in the viscosity measurements were observed, especially at higher polymer concentrations. Also, visible turbidity, indicative of aggregation, was occasionally seen, and there was invariably a reduction in light transmittance in the polarimeter with increasing ionic strength.

It is clear that a full molecular understanding of the complex $[\eta] - 1/\sqrt{I}$ relationship in Fig. 3 requires much experimentation with different experimental techniques. Such work is currently in progress in this and another laboratory (Crescenzi *et al.*, 1986). We remark here only that, according to the explanation given above, the light scattering and viscometry data in Table 2 were obtained from ordered chains, which may explain the observed, highly elongated chain structure. The maximum in optical rotation occurred at somewhat higher ionic strength than the maximum in intrinsic viscosity. Whether this indicates some further conformational ordering of the chains when the intrinsic viscosity has started to fall, and possibly some aggregation, is not clear. The inference is nonetheless strong that the changes in intrinsic viscosity and optical rotation have the same molecular origin. On the basis of the tentative molecular explanations given above, zones I, II and III are hereinafter referred to as the chain-contracting zone, the chain-ordering zone and the chain-association zone, respectively.

Physical characterization in the gel state

An effort was made to compare the effect of different monovalent and divalent cations on the gelling of the gum. An easily obtainable quantity for characterization of the gel strength was required for this. Figure 4 shows a force-deformation plot, obtained in the Instron testing machine with a 1·5% (w/v) gellan gum gel dialysed to equilibrium against 0·1 M KCl. The plot is very similar to those obtained earlier for calcium alginate gels (Smidsrød *et al.*, 1972) with an initial tangent suitable for calculation of the modulus (see the Materials and Methods section), and with an abruptly decreased force at high deformations, indicative of gel-breaking.

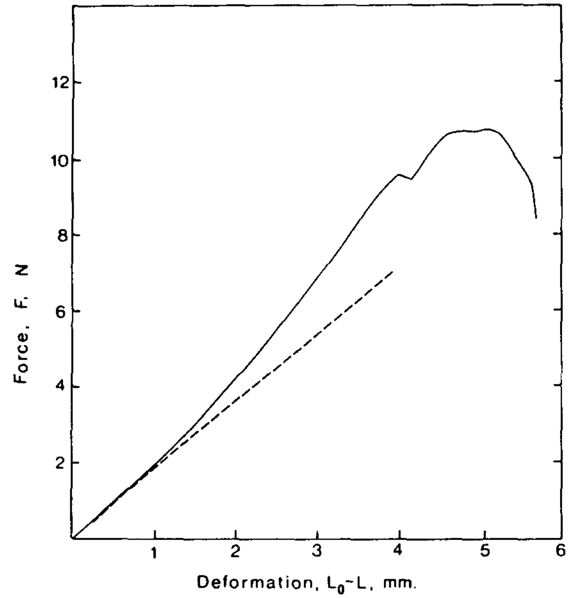


Fig. 4. Force deformation diagram of 1·5% (w/v) gellan gum gel in 0·1 M KCl. L_0 = 14·9 mm, A = 153 mm^2.

Since the ionic strength so markedly influences the properties of the polymer in solution, some gel-strength measurements were carried out with 1·5% (w/v) TMA polymer, dialysed extensively against different concentrations of KCl. The results (Fig. 5) show a large effect of ionic strength on gel strength. The curve is especially steep around 0·1 M, and this ionic strength was therefore chosen as a standard for comparison of different salts, when investigating specific ionic effects.

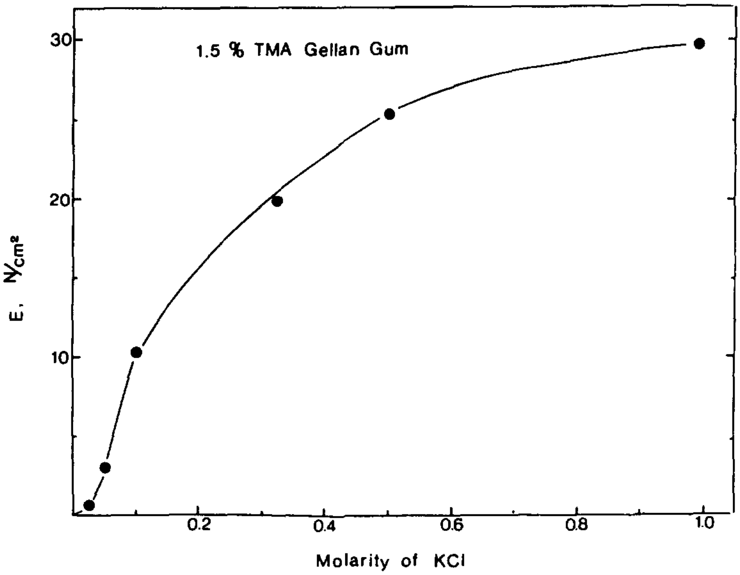


Fig. 5. Modulus of rigidity, *E*, against the molarity of KCl for 1·5% (w/v) gellan gum. Temperature 22 ± 2°C.

Figures 6 and 7 show the results of gel-strength measurements at $I = 0{\cdot}1$ for different polymer concentrations in the presence of a number of monovalent and divalent salts and of HCl. For comparison, data obtained earlier on agarose (Smidsrød, 1972) are included, and also data for gellan gum with no addition of salts. It is evident that gelation is associated with considerable specific ion effects. At a polymer concentration of 2% (w/v), TMACl yielded a gel with a strength two orders of magnitude lower than the most potent gel-former, HCl. With HCl, the gel strengths are generally one order of magnitude higher than for agarose. The other monovalent cations induce gels whose strengths increase with atomic number ($Li^+ < Na^+ < K^+ < Cs^+$). The differences among the divalent salts are not so marked. Within the alkaline-earth metal ions (Mg^{2+}, Ca^{2+}, Sr^{+2}, Ba^{2+}), the gel strengths are hardly distinguishable. It is also noteworthy that the alkaline-earth and the most

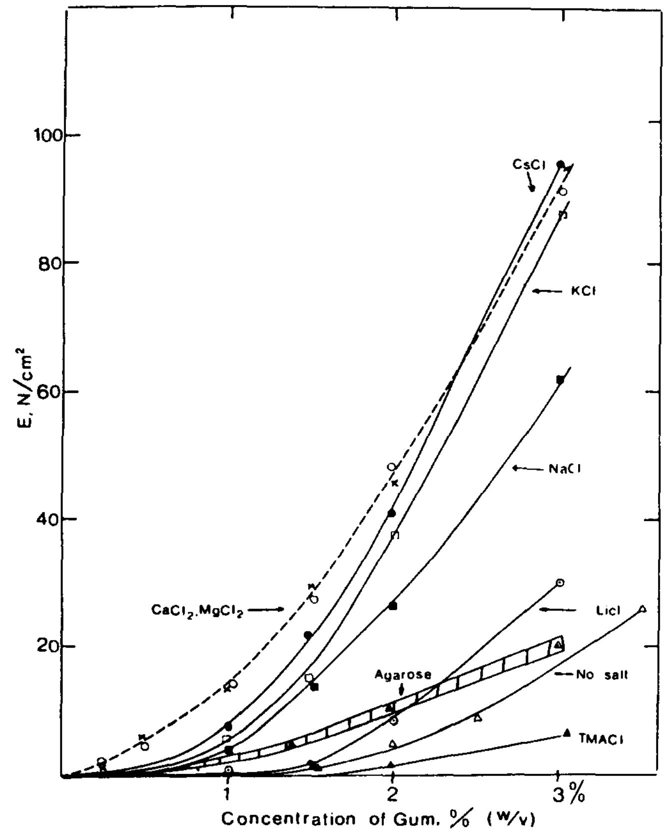


Fig. 6. Modulus of rigidity, *E*, against polymer concentration, *c*, for gellan gum and agarose. Temperature 22 ± 2°C. ▥, Agarose; △, native gum, no salt addition; ▲, 0·1 M TMACl; ⊙, 0·1 M LiCl; ■, 0·1 M NaCl; □, 0·1 M KCl; ●, 0·1 M CsCl; ○, 0·033 M $MgCl_2$; ×, 0·033 M $CaCl_2$.

potent alkali-metal ion, Cs^+, give much the same gel strength. These two observations distinguish gellan gum very clearly from the polyuronide gel systems (Smidsrød, 1972).

The transition elements are somewhat more potent gel-formers than the alkaline-earth metal ions, with increased strength in the order $Zn^{2+} < Cu^{2+} < Pb^{2+}$. Also the minimum polymer concentration giving measurable gels varies widely among the salts. For example, the onset of gelation, which occurs at about 0·2% (w/v) of polymer with CsCl, occurs at about 2% (w/v) polymer with TMACl. It was also observed that the setting temperature for the gels varied considerably in the presence of different salts.

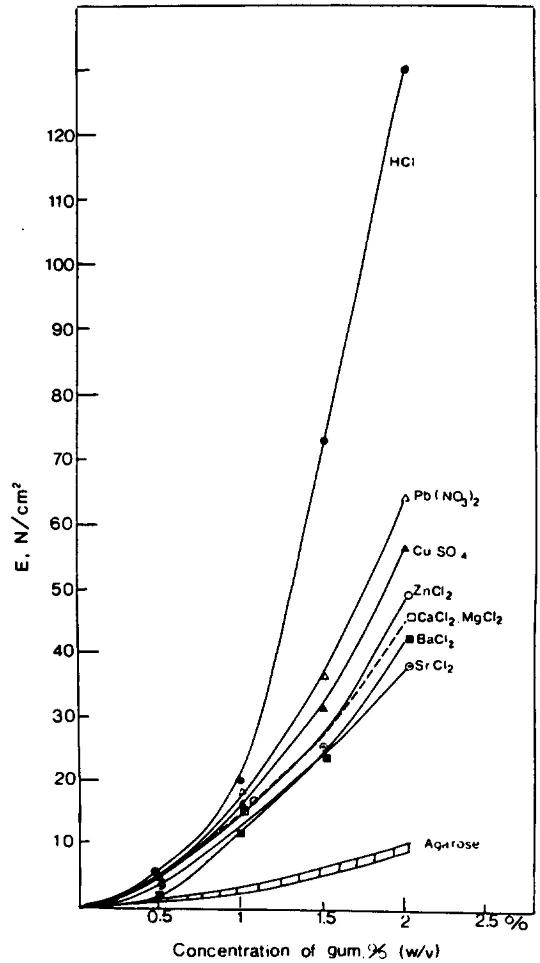


Fig. 7. Modulus of rigidity, *E*, against polymer concentration, *c*, for gellan gum and agarose. Temperature 22 ± 2°C. ▥, Agarose; □———□, $CaCl_2$, $MgCl_2$; ⊙, 0·033 M $SrCl_2$; ■, 0·033 M $BaCl_2$; ○, 0·033 M $ZnCl_2$; ▲, 0·025 M $CuSO_4$; △, 0·033 M $Pb(NO_3)_2$; ●, 0·1 M HCl.

All the curves in Figs 6 and 7 show generally an upward curvature, and in Fig. 8 some of the data are plotted against the square of the polymer concentration, c^2. Apart from some deviation at the lowest concentrations it is seen that the modulus increases quite linearly with c^2 much in the same way as for a lot of other polysaccharide gel systems (Smidsrød & Grasdalen, 1984*a*) in the same concentration range.

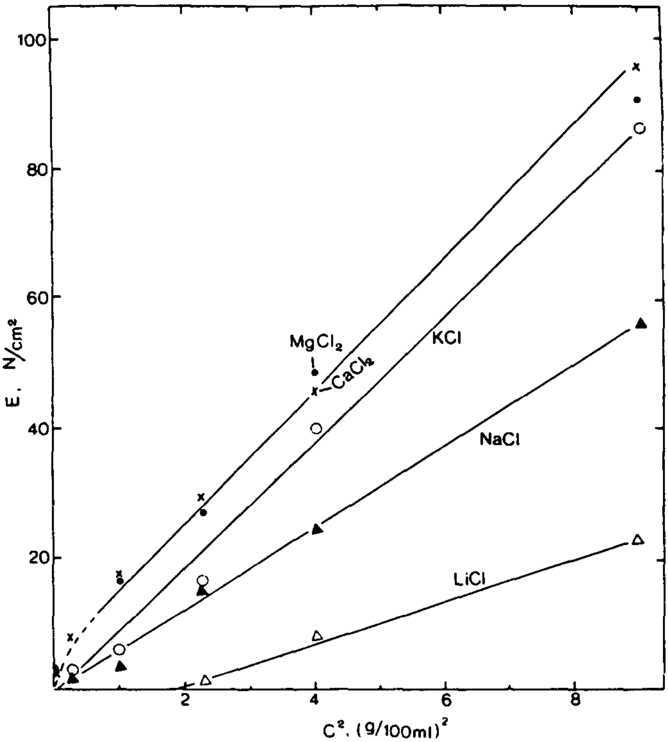


Fig. 8. Modulus of rigidity, *E*, against the square of the polymer concentration, c^2, for gellan gum at $I = 0{\cdot}1$ (M). Data from Fig. 5. △, LiCl; ▲, NaCl; ○, KCl; ×, $CaCl_2$; ●, $MgCl_2$.

Ion-exchange equilibra

With alginate and κ-carrageenan, a correlation has been found between the affinity of the polymer for different metal ions, and gelation (Smidsrød & Grasdalen, 1984*a*). Since Na^+ and K^+ gave gels of different strengths with gellan gum, selectivity coefficients were measured for the Na^+–K^+ exchange reaction. The results (Fig. 9) revealed no sign of selectivity.

In κ-carrageenan, a specific site-binding of Cs and K ions could be detected by Cs NMR (Grasdalen & Smidsrød, 1981). In one experiment, Cs-NMR spectra were run of aqueous CsCl, both in the absence and presence of TMA-gellan gum, also in the gel state. Only a very small down-field shift, $\delta \approx 0{\cdot}3$ ppm, and a moderate excess broadening, $\Delta\nu_{1/2\text{ex.}} \approx 3$ Hz, of the Cs-NMR line were observed in the gel state, indi-

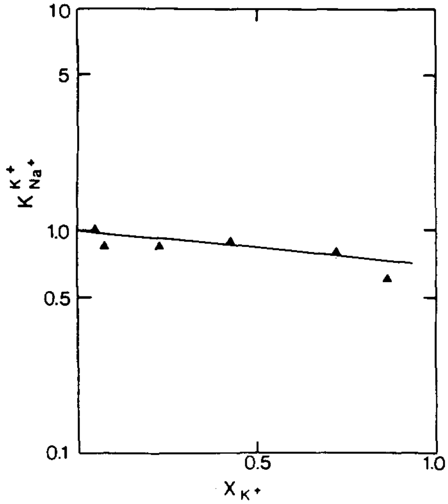


Fig. 9. Selectivity coefficients, $K_{Na^+}^{K^+}$ against the molfraction X_{K^+} of potassium counterions.

cating a lack of specific site-binding of the potent, gel-forming Cs^+ ion. These two experiments taken together suggest a lack of correlation between ion-binding and gelation among the alkali-metal ions in the present case, and no further ion-exchange studies were performed with the monovalent ions.

Figure 10 shows the selectivity coefficients for the Mg^{2+}–Ca^{2+}, Mg^{2+}–Sr^{2+}, Mg^{2+}–Zn^{2+}, Mg^{2+}–Cu^{2+} and Mg^{2+}–Pb^{2+} exchange reactions. Although selectivity in the binding reaction is observed, the differences are very small compared with data for polyuronides such as alginate and pectate (Smidsrød, 1972), the complete lack of selectivity within the alkaline earth group being especially noticeable. Such ion-exchange behaviour is typical for carboxyl-containing polyelectrolytes without the possibility for binding by chelation (Haug & Smidsrød, 1970). The series of relative binding strengths deduced from the data, $Pb^{2+} > Cu^{2+} > Zn^{2+} > Sr^{2+}$, Ca^{2+}, Mg^{2+} is, however, identical with the series found from gel strength measurements. Among the divalent ions, a correlation between ion binding and gel formation therefore exists.

Chain association and chain ordering

An effort was made to correlate the chain association phenomena observed in the presence of TMACl (zone III in Fig. 3) with the gelling behaviour of gellan gum. Since 0·1 M CsCl should induce a gel in 0·3%

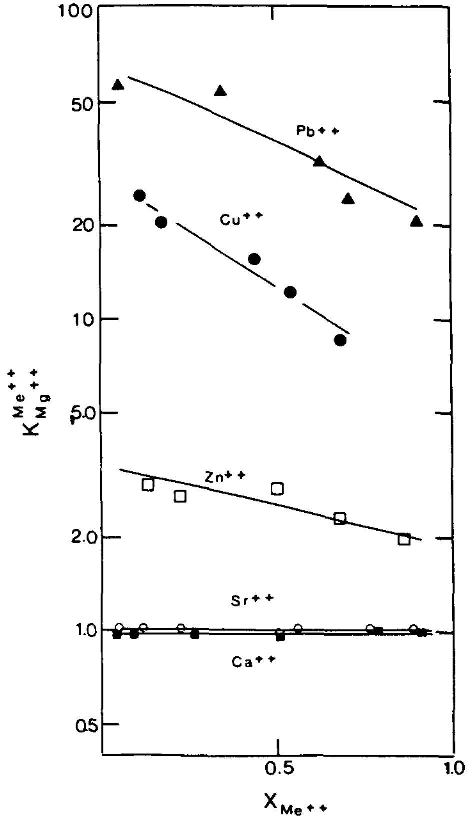


Fig. 10. Selectivity coefficients, $K^{Me^{2+}}_{Mg^{2+}}$, against the molfraction $X_{Me^{2+}}$ of different metal ions in equilibrium exchange with magnesium ions. ■, $Me^{2+} = Ca^{2+}$; ○, $Me^{2+} = Sr^{2+}$; □, $Me^{2+} = Zn^{2+}$; ●, $Me^{2+} = Cu^{2+}$; ▲, $Me^{2+} = Pb^{2+}$.

(w/v) gellan gum upon cooling, in contrast to 0·1 M TMACl (Fig. 6), two such solutions were cooled in the polarimeter from 80°C to 7°C, and the changes in optical rotation were recorded. The results are shown in Fig. 11. (In this Figure, the direction of the axis for the specific optical rotation is opposite from that in Fig. 3.)

In CsCl, there is an increased negative value of the specific optical rotation $[\alpha]_D$, characteristic for chain association as in zone III of Fig. 3, starting at about 50°C. At the lowest temperature a marked change in $[\alpha]_D$ has occurred, and reduced light transmittance in the polarimeter indicated chain association. In TMACl, a slightly reduced negative value of $[\alpha]_D$ is observed, starting at about 40°C. This change, which is indicative of chain ordering as in zone II of Fig. 3, is reversed at about 20°C, suggesting chain association as in zone III. Although these changes are

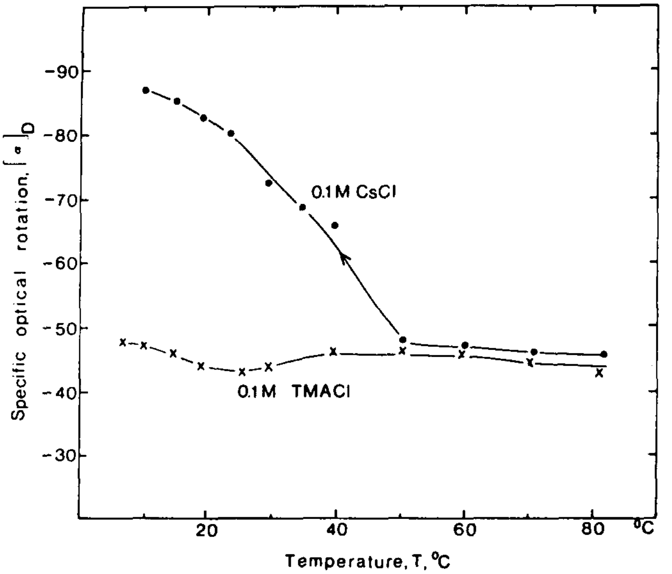


Fig. 11. Specific optical rotation $[\alpha]_D$, obtained by cooling of 0·3% (w/v) TMA gellan gum with different salts added. ×, 0·1 M TMACl; ●, 0·1 M CsCl.

small, they are highly reproducible, reversible and of a magnitude exactly to be expected from the data in Fig. 3. It seems therefore that chain association predominates with Cs^+ ions whereas both chain ordering and chain association can be observed with TMA^+ ions.

Since both phenomena must be dependent upon ionic strength, an experiment with lower concentrations of polymer and CsCl was carried out, with the results shown in Fig. 12. The lower polymer concentration made it possible to measure the cooling–heating cycle as indicated in the Figure. In 0·05 M CsCl, marked changes in specific rotation, in the direction indicative of chain association, and with hysteresis, as is usually associated with such reactions, were observed. In 0·01 M CsCl some signs of a change in $[\alpha]_D$, in the opposite direction (indicative of chain ordering) is observed. Additional experiments are planned to test the significance of this observation. In 0·05 M CsCl, any observable signs of chain ordering are completely masked by the direct onset of chain association.

Since chain association should, in principle, be dependent on polymer concentration, at least at very low polymer concentrations, experiments with different polymer concentrations in 0·05 M CsCl were carried out. The onset of chain association, as observed by a sharp increase in the

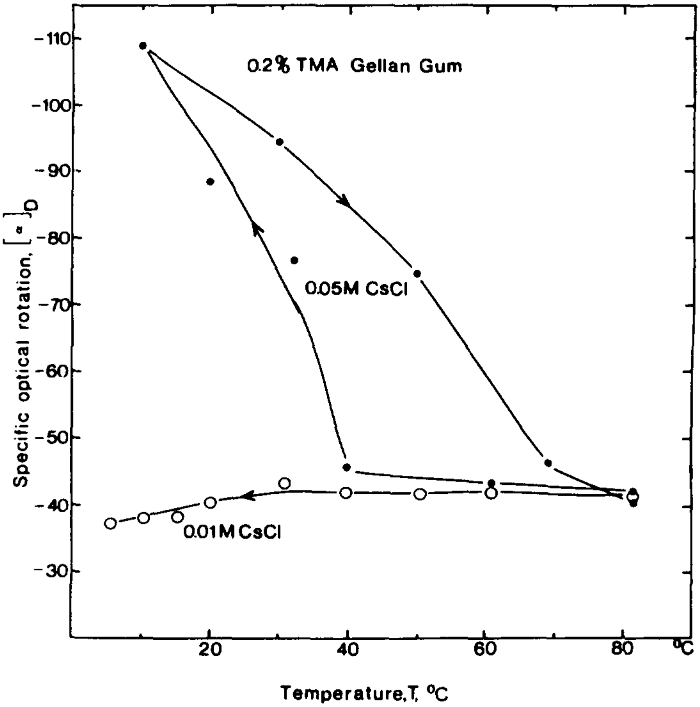


Fig. 12. Specific optical rotation, $[\alpha]_D$, obtained in a cooling–heating cycle of 0·2% (w/v) TMA-gellan gum with addition of different amounts of CsCl. ○, 0·01 M; ●, 0·05 M.

negative value of $[\alpha]_D$, was recorded in each case. The results (Fig. 13) confirm that the reaction is bi- or multi-molecular.

It is reasonable to assume that chain ordering and chain association occur simultaneously under conditions favouring gelation, but this would not be observable in every case because the two phenomena cause $[\alpha]_D$ changes of opposite sign. Chain stiffness induced by ordering and/or association is easily observed as a broadening of peaks or as a reduced peak intensity in NMR spectra. In Fig. 14, relative peak intensities and $[\alpha]_D$ values are given for 1% (w/v) gellan gum in the native salt state and without addition of salt. The ionic strength is not so well defined in this case, but obviously high enough to give both chain ordering and chain association upon cooling. The slightly reduced negative value of $[\alpha]_D$ at 35°C, compared to those at higher temperatures, before the marked increase at 30°C occurs, may suggest in itself some chain ordering prior to chain association in this case. That marked chain ordering and chain association are overall effects of the cooling is, however, also clearly

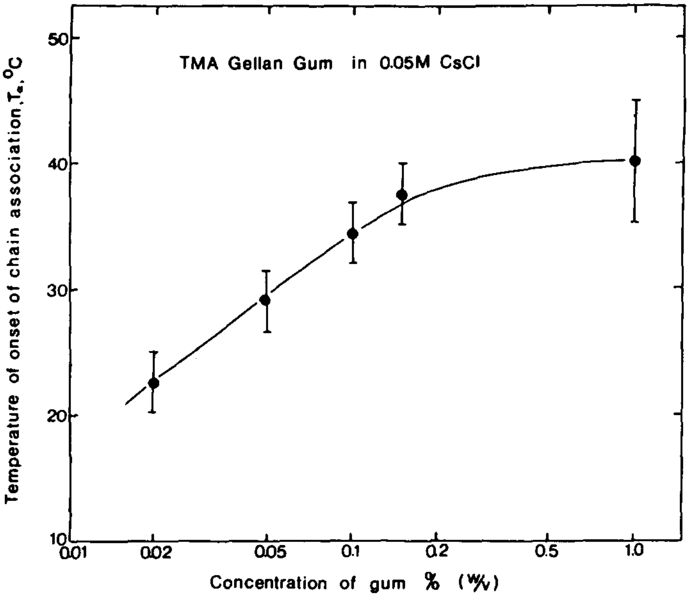


Fig. 13. Temperature of onset of chain association determined by optical rotation, T_α, against concentration of gum for TMA gellan gum with 0·05 M CsCl added. (Because of low energy transmittance in the polarimeter due to increased turbidity, the errors in these measurements were quite large as indicated by the error bars.)

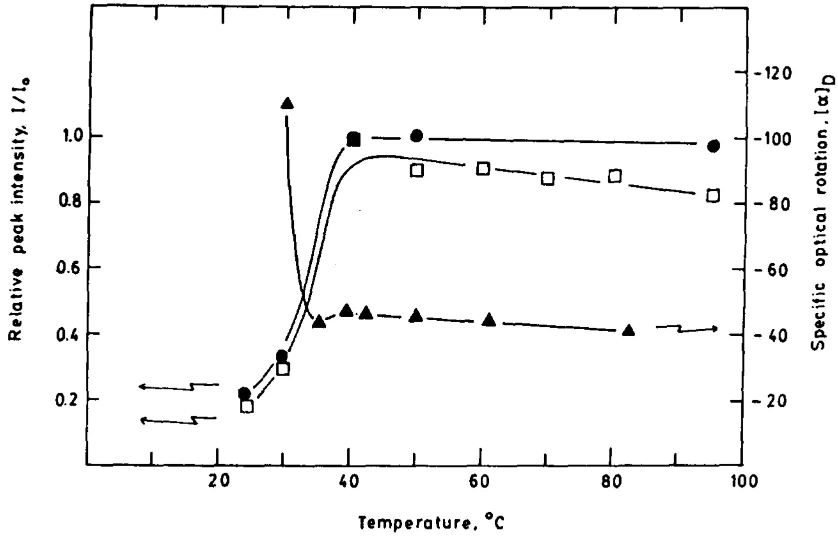


Fig. 14. Relative NMR peak intensities, I/I_0, and specific optical rotation, $[\alpha]_D$, against temperature by cooling of 1% gellan gum in the native salt form and with no addition of salt. ▲, $[\alpha]_D$; □, I/I_0 for anomer proton of rhamnose; ●, I/I_0 for methyl proton of rhamnose.

evident from the markedly reduced peak intensities of the methyl and the anomeric protons of rhamnose.

In conclusion, the data in this section indicate that gelation is associated with both chain ordering and chain association in much the same way as for gels of the carrageenan type, although the bulk of evidence in favour of this idea is much greater in the case of carrageenan.

DISCUSSION

Even a first level, qualitative understanding of a polyelectrolyte gelling system is hard to achieve. It requires a detailed knowledge of the fundamental properties of individual chains, their conformation and topology in networks, hydration of individual chains and networks, the effects of electrolytes upon *intra-* and *inter*-chain forces, and also a basic understanding of the properties of water and how they are modified by electrolytes. The somewhat scattered set of observations presented in this work may, therefore, serve more to promote further thinking and experimentation than real understanding of the gelling process, and we shall discuss them only briefly with main reference to the electrolyte effects.

We assume a two-step model for gelation which has been presented earlier as the simplest possible way of explaining the gelation of κ-carrageenan (Smidsrød & Grasdalen, 1982). It assumes *intra*-molecular chain ordering and chain association to be two different processes which occur simultaneously in the presence of gel-promoting cations, but which can be demonstrated separately under carefully chosen conditions.

The data for TMA-gellan gum in TMACl suggest that *intra-* molecular ordering of isolated polymer chains may occur in a narrow range of low TMACl concentrations. The high viscosities, the high radius of gyration and the high second virial coefficients all support this view, although more experimentation is needed to investigate possibilities for ordering between a small number of chains, e.g. dimer formation. This chain ordering is promoted by screening of the charges along the chain, and the low ionic strength needed for this shielding is only to be expected, since the distance between the carboxylic negative charges on gellan gum is quite large. Since the large TMA^+ ions, which interact only weakly with polyelectrolyte chains, are able to induce chain ordering, there is no evidence here for specific ion effects in chain ordering, but this should be looked for in future works.

The chain association, causing gelation at sufficiently high polymer concentrations, is strongly dependent upon the ionic strength (Figs 5 and 12), but is additionally highly cation specific (Figs 6, 7 and 11). With a gel-promoting cation like Cs^+ the data in Figs 11–14 suggest that chain association may occur at lower ionic strengths and at higher temperatures than the chain ordering induced by TMA. In such cases it is reasonable to believe that the chain ordering is promoted by a side-by-side chain-association reaction, causing the two processes to occur simultaneously.

The cation-specific chain association must clearly be correlated with the cation-specific gelation process. The reason for the cation specificity is, however, not fully understood. Among the monovalent cations no selectivity was observed in K^+–Na^+ exchange, and no evidence was obtained for site binding of Cs^+ ions. For the alkali-metal cations the specificity in chain association and junction formation may be attributed to the degree of hydration of the different ions, the highly hydrated Li^+ ions causing less dehydration and less possibility for *inter*-chain contacts than the much less hydrated Cs^+ ion. It is also possible that the packing of the chains in junction zones requires small hydrated ions for geometrical reasons. This idea would explain why the large TMA^+ ions have such a weak gel-promoting effect. The pronounced gel-promotion effect of protons could be caused either by the complete discharging of the chain ($pH = 1$ in 0·1 M HCl in Fig. 7 compared to a pK_a of glucuronic acid of about 3·2 (Haug, 1964)), allowing for strong *inter*-chain contacts, or it could be associated with special geometry of hydrogen bonding effects favouring junction formation.

Among the divalent cations, gelation and ion-binding are correlated; lack of selectivity among the alkaline-earth metal ions and selectivity among the transition metal ions being characteristic features. In the latter case, increased gel strength may be explained by an increased discharging of the chains. The effects on gel strength are relatively small compared to polyuronate gels (Smidsrød, 1972, 1973), however, and there is no evidence for chelation effects, either *intra*- or *inter*-molecularly.

A further discussion of specific cation effects in junction formation would require knowledge of the topology of the junction zones. Such knowledge is lacking, at present, but it is hoped that the present overview of the effects will serve as a basis for more detailed studies.

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