Carbohydrate Polymers 3 (1983) 129–141

Rheological Studies of Specific Cation Forms of Kappa Carrageenan Gels

V. J. Morris & G. R. Chilvers

ARC Food Research Institute, Colney Lane, Norwich NR4 7UA, UK

(Received: 17 June 1982)

SUMMARY

Measurements have been made of the shear modulus of calcium, potassium and sodium kappa carrageenate gels as a function of polysaccharide concentration and temperature. Under the experimental conditions used the efficiency of the cations in gelling the polysaccharide has been found to be $Ca^{2+} > K^+ > Na^+$*. The relative gelling efficiencies of the cations is attributed to their extent of hydration which controls the solubility of the salt form of the polysaccharide. Gelation is attributed to 'microcrystallite' formation at localised sites on adjacent polysaccharide chains. The sharp decrease of the shear modulus on heating is attributed to localised melting of these ordered regions.*

INTRODUCTION

Carrageenan is a collective name for a family of sulphated D-galactans characterised by an alternating β(1,3), α(1,4) structure (Percival & McDowell, 1967). Structural regularity is marred by the presence of 1 → 3 linked α-D-galactose residues in the unbridged conformation which have been designated as kink points (Anderson *et al.*, 1973). Although there will be variations in chemical structure both regionally within polymers and between macromolecules from a given extract, samples from *Eucheuma spinosum*, *Eucheuma cottonii* and *Gigartina acicularis* approach the idealised structures called iota, kappa and lambda carrageenan (Smith & Cook, 1953; Smith *et al.*, 1955; Anderson & Rees, 1965; Anderson *et al.*, 1973; Reid, 1978).

These biopolymers are used in the food industry as gelling and thickening agents for modifying the texture of food products. Their ability to form thermo-reversible gels depends on the carrageenan type and the nature and quantity of cations associated with the half ester sulphate groups (Smith & Cook, 1953; Bayley, 1955; Smith *et al.*, 1955; Pernas *et al.*, 1967; Reid, 1978; Morris *et al.*, 1980). Cations are exploited in the commercial application of these materials (Guisley, 1968). In particular it is widely accepted that iota carrageenan is specifically gelled by calcium ions whereas potassium ions are specific for gelling kappa carrageenan (Glicksman, 1969). However, the role of cations at the molecular levels is still poorly understood.

Gelation has been attributed to double helix formation involving the regular sequences between kink points on two adjacent chains (Rees, 1972). Evidence for double helix formation arose mainly from X-ray diffraction studies on dried orientated fibres (Anderson *et al.*, 1969; Rees, 1972; Arnott *et al.*, 1974*b*). Evidence for the existence of these structures in the hydrated state has come mainly from optical activity and n.m.r. studies (McKinnon *et al.*, 1969; Rees *et al.*, 1969; Bryce *et al.*, 1974). The interpretation of optical rotations and n.m.r. data has recently been questioned by Smidsrod *et al.* (1980) who claim to find no definitive evidence for inter-molecular helix formation in carrageenan samples. X-ray diffraction studies on orientated fibres of the related polysaccharide agarose (Arnott *et al.*, 1974*a*) have also been attributed to double helical structures. However, more recent X-ray diffraction studies (Foord, 1980) suggest that an equally acceptable structural model may be the existence of parallel arrays of stacked single helical structures. The existence of such fibrillar crystals has been suggested by Atkins *et al.* (1977) and such structures have been observed by electron microscopy in beaded Sepharose 4B (Amsterdam *et al.*, 1975). This latter model has been applied in the interpretation of recent light scattering studies of the gelation of agarose (Letherby & Young, 1981).

Models for the gelation of carrageenans have tended to neglect the effect of cations and concentrate on the molecular changes related to junction zone formation (Reid, 1978). In general the action of cations has been attributed to controlling the solubility of the polysaccharide and hence its tendency to precipitate or crystallise from solution (Percival & McDowell, 1967). Ion-selective bridges have been proposed as an integral part of gelation (Bayley, 1955; Smidsrod *et al.*, 1980; Grasdalen & Smidsrod, 1981). The Rees' model has been extended to include cation effects by proposing specific cation induced aggregation of double helices (Morris *et al.*, 1980).

Clearly, despite the importance of cation effects in the gelation of carrageenans there is only a limited understanding of their role on gelation. The work reported here is aimed at improving this understanding. The three cations commonly associated with the half ester sulphate groups of commercial samples of kappa carrageenan are calcium, potassium and sodium. Thus we have prepared pure samples of sodium, potassium and calcium kappa carrageenate and studied the rheological properties of the gels formed. The present studies are intended to compliment previous investigations of cation effects in iota carrageenan (Morris & Belton, 1980, 1981; Morris & Chilvers, 1981).

MATERIALS AND METHODS

Samples of kappa carrageenan, extracted from *Eucheuma cottonii*, were purchased from the Sigma Chemical Company Limited. Pure cation forms were prepared by ion exchange. Difficulties were encountered in removing potassium from the original sample by ion exchange at room temperature. To obtain acceptably pure cation forms the following procedure was employed. A commercial ion exchange resin (Dowex 50W-X8) was converted to the required cation form. A non-gelling solution of kappa carrageenan was prepared and heated to 90°C. The resin was added and the mixture stirred at 90°C for approximately 10 min. The solution was then siphoned through a glass sinter to remove the resin, cooled to room temperature and freeze dried. Polysaccharide concentrations were determined by freeze drying and vacuum drying samples. Sulphate concentrations were evaluated as described by Dodgson (1961) and Dodgson & Price (1962). Cation concentrations were determined using atomic absorption (AA) spectroscopy.

Rheological studies involved measuring the shear modulus (G_R) by modifications of the methods described by Saunders & Ward (1954) and Scott-Blair & Burnett (1957). Values of (G_R) may be obtained from the relation (Sherman, 1970)

$$G_R = \frac{\Delta P R_t^4}{8 L R_c^2 \Delta h_g} \tag{1}$$

where R_t and L are the radius and length of the gel sample, R_c the radius of the capillary tube and Δh_g the displacement of the monitor fluid in the capillary arising due to the excess pressure ΔP, relative to atmospheric pressure, applied to the gel. ΔP and Δh_g are related to the maximum stress (p_m) and maximum strain (ϵ_m) by the equation:

$$p_m = \frac{\Delta P R_t}{2L}, \quad \epsilon_m = \frac{4 R_c^2 \Delta h_g}{R_t^3} \tag{2}$$

The design of the apparatus is modular. This aids gel preparation, the efficient use of the apparatus and cleaning of the components. The gel containers may be removed and stoppered for preparation of gel samples. To ensure that no slippage occurs at the interface between the gel and the container, the inner surfaces of the tubes have been coated with ground glass granules. Each tube is then calibrated to determine an 'effective radius' R_t. Calibration involves comparative studies on gelatin gels using coated and uncoated tubes. For the uncoated tubes G_R may be determined absolutely using eqn. (1).

Gels were prepared by dissolving the polysaccharide in double distilled deionised water at 95°C. Wetting the powdered sample with ethanol was found to aid dispersion. Samples were then poured hot into the stoppered containers and allowed to cool to room temperature. Rheological measurements were made 48 h after preparation. All studies were performed with the apparatus enclosed in a temperature controlled water jacket.

RESULTS

Table 1 shows the cation and sulphate contents of the three cation forms of kappa carrageenan used in the present studies. The sulphate content is typical of kappa carrageenan. Figure 1 shows plots of p_m–ϵ_m for the sodium, potassium and calcium forms of kappa carrageenan at various temperatures. The p_m–ϵ_m plots were found to be linear for all

TABLE 1

Cation and Sulphate Contents of Three Forms of Kappa Carrageenan

Material ion	*Sodium form (%)*	*Potassium form (%)*	*Calcium form (%)*
Sodium	4·24	<0·09	<0·38
Potassium	<0·44	6·45	<0·12
Calcium	<0·10	<0·03	3·75
Sulphate	14·2	14·2	14·2

cation forms, at all biopolymer concentrations and all temperatures employed in the present studies. The deformation measured was the instantaneous response to the applied stress and no creep was observed. For the temperature dependent studies the temperature was raised in small increments and the gels allowed to equilibrate for 10 min at each temperature.

Values of G_R were calculated using eqn. (1) and a least-squares-fit to the p_m–ϵ_m plots. Figure 2 shows the dependence of G_R on biopolymer concentration (c) for the three cation forms at room temperature. Figure 3 shows the dependence of G_R on temperature for the three cation forms at selected biopolymer concentrations.

To ensure that the differences illustrated in Fig. 2 resulted from the cation type and were not artefacts of sample preparation, the following experiments were performed. A sample of potassium kappa carrageenate was gelled and G_R determined. This sample was ion exchanged into the calcium salt and G_R measured after gelation. The sample was then reconverted into the potassium salt, gelled and G_R again measured. By this method we were able to demonstrate that at a given biopolymer concentration conversion from the potassium salt to the calcium salt increased G_R and reconversion into the potassium salt decreased G_R.

DISCUSSION

The objective of the present studies was to investigate the role of the cations Ca^{2+}, K^+ and Na^+ on the gelation of kappa carrageenan. The ion contents of the pure samples prepared are specified in Table 1. In

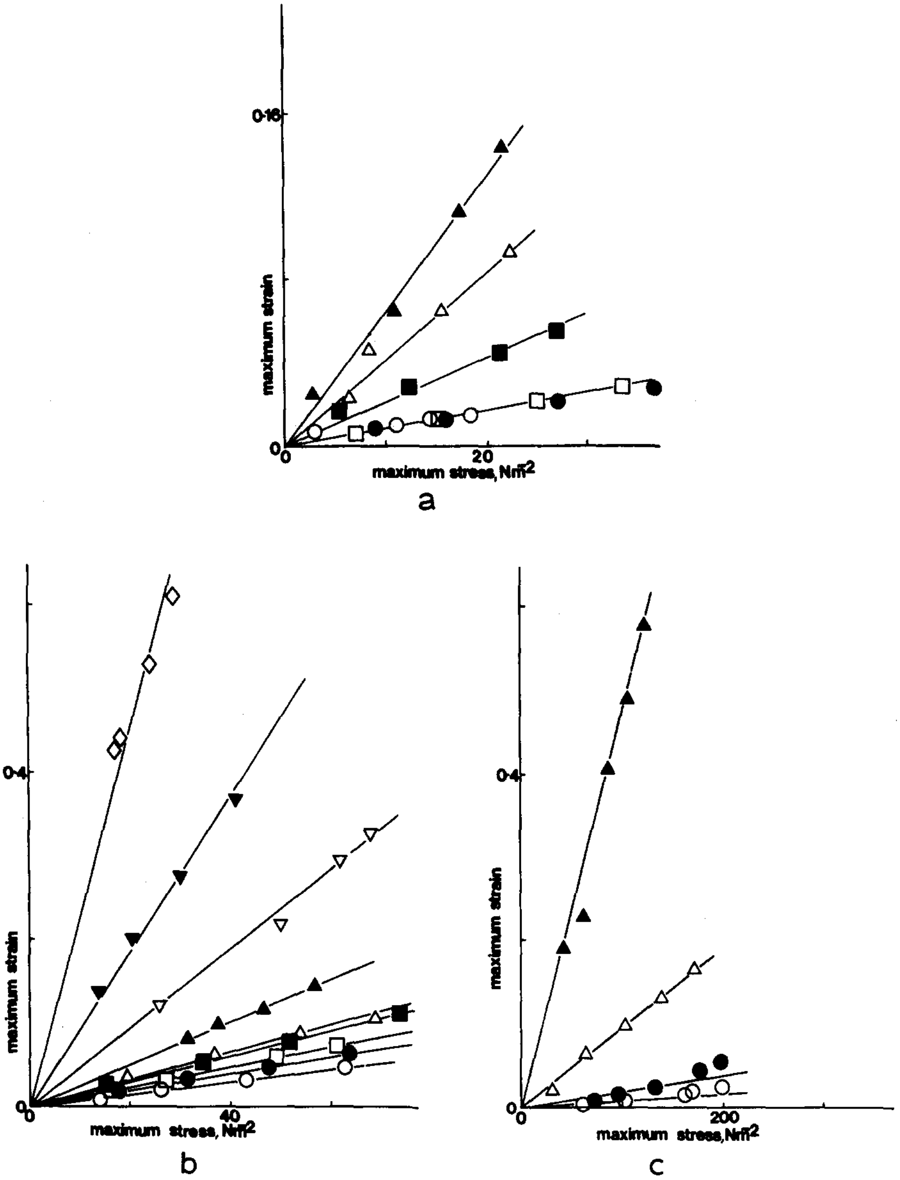

Fig. 1. Plots of maximum stress (p_m) versus maximum strain (ϵ_m) for kappa carrageenate gels at various temperatures: (a) 4·8% sodium form ○, 13·5°C; ●, 15°C; □, 17°C; ■, 21°C; △, 22·5°C; ▲, 24°C. (b) 1·5% potassium form ○, 17°C; ●, 20·5°C; □, 23°C; ■, 26°C; △, 30°C; ▲, 33°C; ▽, 35°C; ▼, 37°C; ◇, 39°C. (c) 2·1% calcium form ○, 20°C; ●, 25°C; △, 31°C; ▲, 36°C.

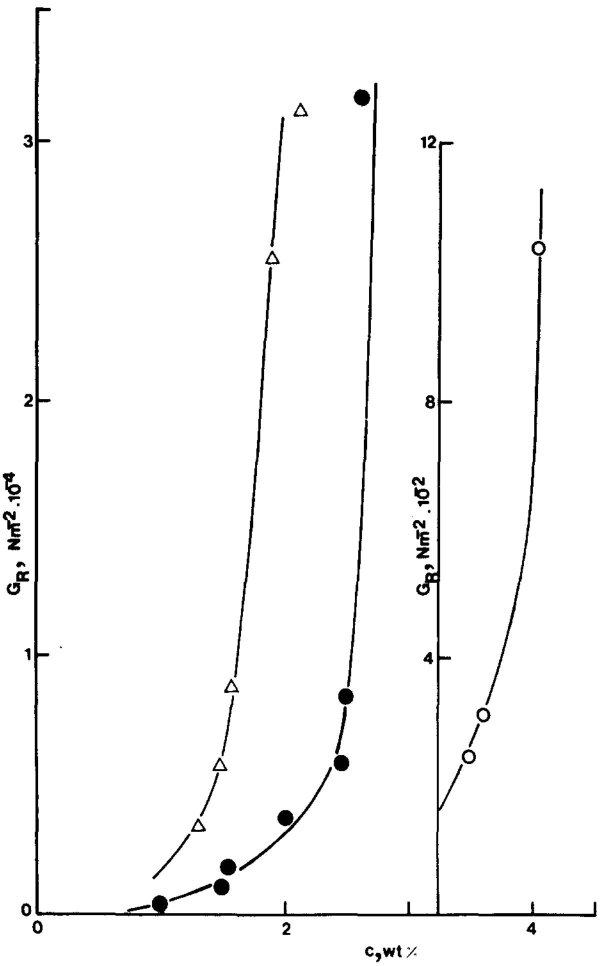

Fig. 2. Plots of shear modulus (G_R) versus biopolymer concentration (c) for kappa carrageenate gels ○ – sodium form, ● – potassium form and △ – calcium form. Concentrations are expressed as percentage mass of polymer per total mass of gel.

investigating the rheological properties we have concentrated on studies of deformation under shear which is dominated by the polymeric network rather than compressional tests which will be dominated by the solvent in weak low concentration polysaccharide gels. G_R has been found to depend on both the cation type and polysaccharide concentration (Fig. 2). At a given polysaccharide concentration G_R varies as

$Ca^{2+} > K^+ > Na^+$. If G_R is taken as an index of the density of junction zones within the gel then the present data does not support the widely held belief that potassium is a specific cation for gelling kappa carrageenan (Glicksman, 1969). The present data mirror those obtained previously for iota carrageenan (Morris & Belton, 1980, 1981; Morris & Chilvers, 1981). However, for a given cation type and given biopolymer concentration G_R is consistently larger for kappa carrageenan gels than for iota carrageenan gels. If G_R is taken as an indicator of the density of junction zones per unit volume then the data indicate an increase in junction zone density with increasing concentration. The concentration dependence differs for each ion form and does not appear to be describable by simple power law dependences. To relate rheological studies to molecular structure it is necessary to assume a molecular model for the gel. Current opinion suggests that the gels may be pictured as a random network consisting of anisometric rigid junction zones linked by 'free polymer'. If the large deformations observed are attributed to configurational changes of the polymer linkages then provided such linkages are sufficiently long, simple entropic models used to describe deformations of rubbers may be applicable and we have (Treloar, 1973)

$$G_R = \frac{c\mathrm{R}T}{M_n} \tag{3}$$

where R is the gas constant, T the absolute temperature and M_n the mean molecular weight of a linkage. Equation (3) can be extended to account for the nature of the junction zones (Flory, 1953) and the effects of loose polymer ends (Flory, 1944). Entanglements act as defects but are difficult to quantify. Figure 4 shows plots of the calculated M_n values as a function of polysaccharide concentration. In Fig. 3 the effect of heating the gels on M_n is shown. Assuming a value of 384 for the dissaccharide repeat unit the average number of repeat units per linkage, N, may be determined. Values of N are shown in Fig. 4. We note that for the majority of gels N is too small to justify a gaussian description.

The failure of eqn. (3) may lie in the basic assumption of a purely entropic effect for a gel in which the linkages are very short, or in the assumption of a uniform random network for the gel. Detailed quantitative analysis of rheological data therefore requires improvements in the theoretical description of such systems.

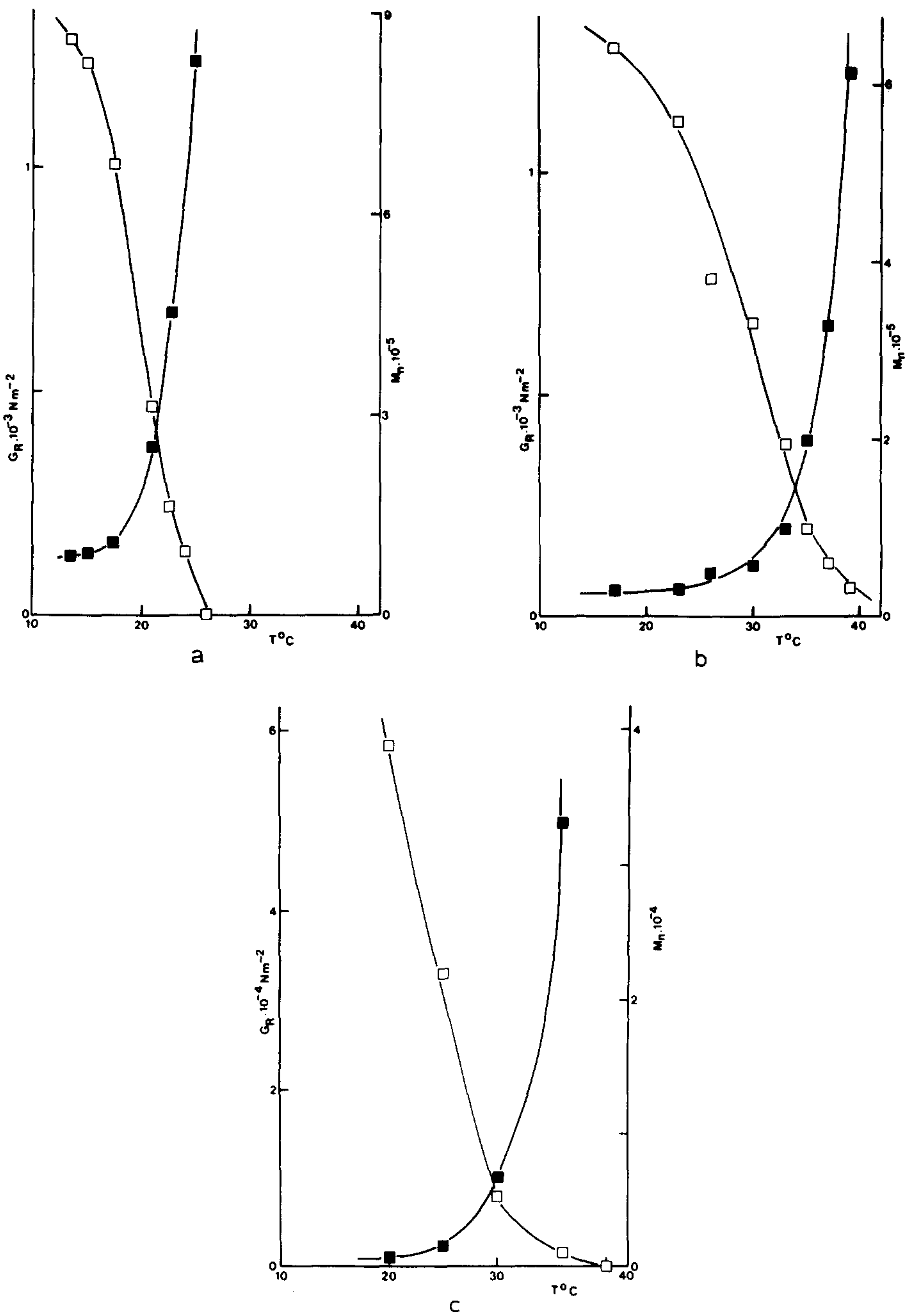

Fig. 3. Temperature dependence of G_R (—□—) for kappa carrageenate gels: (a) 4·8% sodium form; (b) 1·5% potassium form; (c) 2·1% calcium form. The calculated average molecular weight (M_n) per linkage between junction zones is shown on the graphs (—■—).

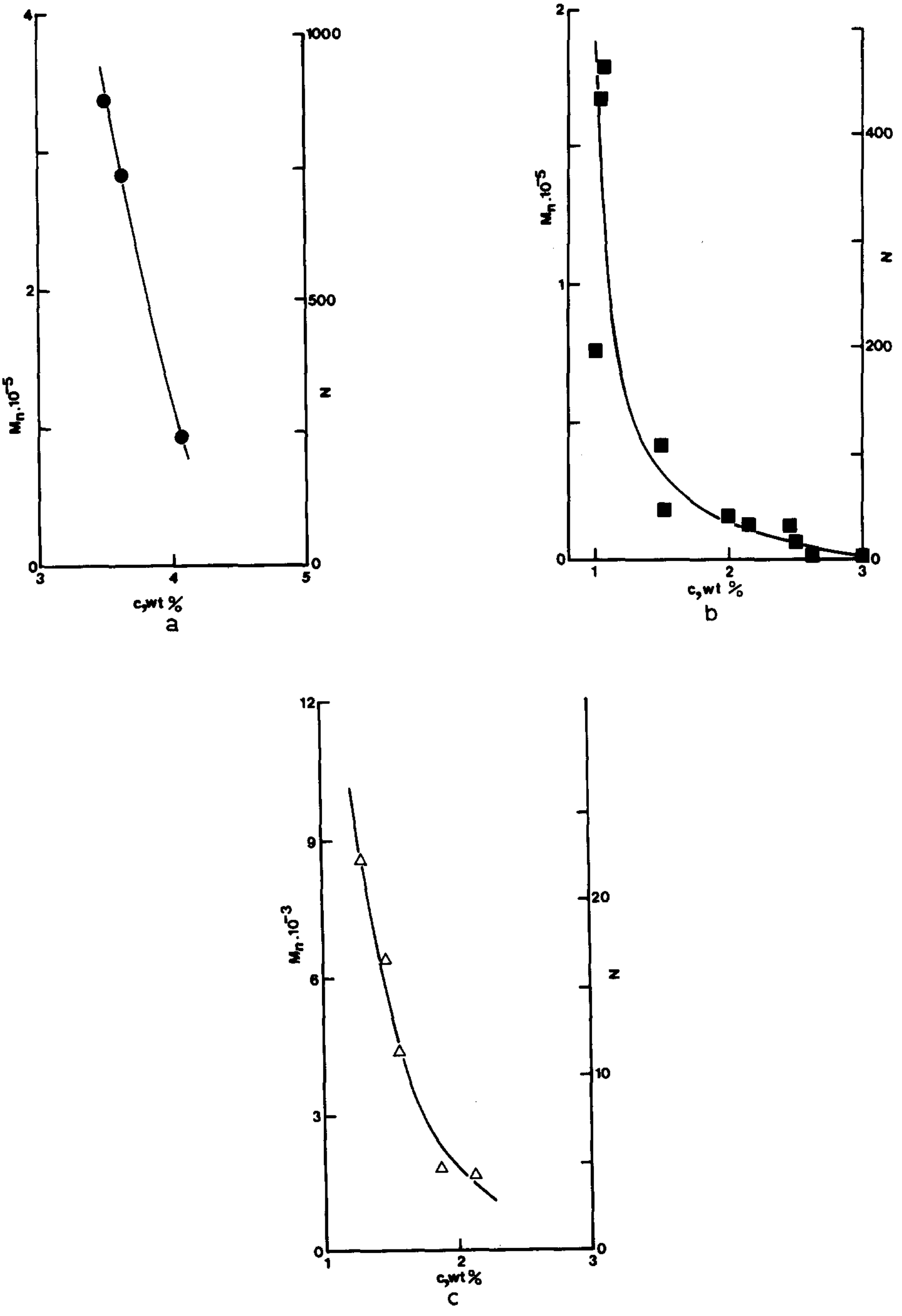

Fig. 4. Plots of the calculated values of M_n and N against biopolymer concentration (c) for: (a) sodium; (b) potassium; (c) calcium kappa carrageenate gels.

Qualitatively the data in Fig. 3 seems to imply a 'melting' of the gel network. The broader temperature range of melting than that observed for iota carrageenan gels (Morris & Belton, 1981; Morris & Chilvers, 1981) seems to imply a broader range of sizes or of extent of ordering of the polymer within the junction zones. Recent investigations (Morris *et al.*, 1980) have proposed a Hoffmeister type series ($Rb^+ > Cs^+ > K^+ > Na^+ > Li^+$) for the effectiveness of monovalent cations in gelling carrageenans. The cations (Cs^+, Rb^+, K^+) have been dubbed gel promoting cations and the cations (Na^+, Li^+) are considered to be non-gelling cations (Morris *et al.*, 1980). Recent n.m.r. studies of ion motion in gels have been taken to suggest that Cs^+ and K^+ are more strongly bound than Na^+ or Li^+ in kappa carrageenan gels. Further the binding occurs on gelation suggesting immobilisation of cations at the junction zones within the gel (Grasdalen & Smidsrod, 1981). The simplest explanation for the dependence of G_R on cation type for kappa carrageenan and for iota carrageenan (Morris & Belton, 1980, 1981; Morris & Chilvers, 1981) consistent with n.m.r. data (Morris & Belton, 1980, 1981; Grasdalen & Smidsrod, 1981) is to suggest that gelation involves localised ordering or crystallisation of the polysaccharide salt forms on cooling. The solubility of the polysaccharides is considered to be reduced on cooling promoting phase separation and alignment of the polysaccharide chains into microcrystalline like structures. Complete crystallisation would be inhibited by the 'kinks' in the chains and polymer entanglements. The relative efficiency of cations for gelling the polymer may then be linked to their ease of hydration (Nightingale, 1966; Hinton & Amis, 1971; Horne, 1972; Arakawa, 1980). Following the terminology of Samoilov (1969), cations whose Jones–Dole B coefficient of viscosity is negative are structure disordering and show positive hydration whereas cations with positive coefficients are termed structure ordering and show negative hydration. It is perhaps significant in the present context that Cs^+ and K^+ have positive B coefficients whereas Na^+ and Li^+ have negative B coefficients. Further, the extent of sulphation will affect the hydrophilic character of the polyanion controlling its solubility. Hence for a given cation type and concentration at a given polysaccharide concentration kappa carrageenan should show a greater tendency to phase separate than iota carrageenan.

The role of cations in controlling gelation of polyelectrolytes is complex and in such studies it is essential to specify the total ion content and not simply the concentration of added electrolyte. Counterions will screen charges on the polyanions due to direct ion binding, counterion condensation at the surface of the polyanion and through the width of the electrical double layer. These factors will not only affect intermolecular interactions but will control the conformation of the polyanion through controlling intramolecular charge–charge repulsions.

CONCLUSIONS

Rheological studies on pure cation forms of kappa carrageenan suggest that the efficiency of the cations in gelling the biopolymers under the present experimental conditions are $Ca^{2+} > K^+ > Na^+$. These results do not support the widely accepted belief that kappa carrageenan is specifically gelled by potassium ions. Gelation is attributed to phase separation at low temperature leading to localised crystallisation of short regions of the aligned polymer chains. The ease of gelation is related to the solubility of the salt form of the polysaccharide and hence the extent of hydration of the cations and the polyanions.

REFERENCES

Amsterdam, A., Er-El, Z. & Shaltiel, S. (1975). *Archs. Biochem. Biophys.* **171**, 673.

Anderson, N. S., Campbell, J. W., Hardin, M. M., Rees, D. A. & Samuel, J. W. B. (1969). *J. Mol. Biol.* **45**, 85.

Anderson, N. S., Dolan, T. C. S. & Rees, D. A. (1973). *J. Chem. Soc., Perkin Trans. I.* 2173.

Anderson, N. S. & Rees, D. A. (1965). *J. Chem. Soc.* 5880.

Arakawa, K. (1980). In *Water and metal cations in biological systems*, eds B. Pullman and K. J. Yagi, Tokyo, Japan Sci. Soc. Press, 13.

Arnott, S., Fulmer, A., Scott, W. E., Dea, I. C. M., Moorhouse, R. & Rees, D. A. (1974*a*). *J. Mol. Biol.* **90**, 269.

Arnott, S., Scott, W. F., Rees, D. A. & McNab, C. G. A. (1974*b*). *J. Mol. Biol.* **90**, 253.

Atkins, E. D. T., Isaac, D. H., Keller, A. & Myasaka, K. (1977). *J. Polym. Sci., Polymer Phys. Ed.* **15**, 211.

Bayley, S. T. (1955). *Biochem. Biophys. Acta* **17**, 194.

Bryce, T. A., McKinnon, A. A., Morris, E. R., Rees, D. A. & Thom, D. (1974). *Faraday Discussions Chem. Soc.* **57**, 221.

Dodgson, K. S. (1961). *Biochem. J.* **78**, 312.

Dodgson, K. S. & Price, R. G. (1962). *Biochem. J.* **84**, 106.

Flory, P. J. (1944). *Chem. Rev.* **35**, 51.

Flory, P. J. (1953). In *Principles of polymer chemistry*, Cornell Univ. Press, Chap. 11.

Foord, S. A. (1980). *Model building methods and X-ray diffraction studies of polysaccharides*, Ph.D. Thesis, University of Bristol.

Glicksman, M. (1969). In *Gum technology in the food industry*, New York, Academic Press, Chap. 8.

Grasdalen, H. & Smidsrod, O. (1981). *Macrmolecules* **14**, 229.

Guisley, K. B. (1968). *Encyclopaedia of chemical technology*, **1**, 763, New York, J. Wiley.

Hinton, J. F. & Amis, E. S. (1971). *Chem. Rev.* **71**, 627.

Horne, R. A. (1972). *Water and aqueous solutions*, New York, Wiley-Interscience.

Letherby, M. R. & Young, D. A. (1981). *J. Chem. Soc. Faraday Trans.* I, **77**, 1953.

McKinnon, A. A., Rees, D. A. & Williamson, F. B. (1969). *J. Chem. Soc. Chem. Commun.* 701.

Morris, E. R., Rees, D. A. & Robinson, G. (1980). *J. Mol. Biol.* **138**, 349.

Morris, V. J. & Belton, P. S. (1980). *J. Chem. Soc. Chem. Commun.* 983.

Morris, V. J. & Belton, P. S. (1981). *Proceedings of the conference on 'Gums and stabilisers for the food industry', Wrexham (1980)*, London, Plenum Press (in press).

Morris, V. J. & Chilvers, G. R. (1981). *J. Sci. Food Agric.* **32**, 1235.

Nightingale, E. R. Jr. (1966). In *Chemical physics of ionic solutions*, eds B. E. Conway and R. G. Barradas, New York, J. Wiley, 87.

Percival, E. & McDowell, R. H. (1967). *Chemistry and enzymology of marine algal polysaccharides*, London, Academic Press, Chap. 6, 127.

Pernas, A. J., Smidsrod, O., Larsen, B. & Haug, A. (1967). *Acta Chem. Scand.* **21**, 98.

Rees, D. A. (1972). *Chem. Ind.* 630.

Rees, D. A., Steele, I. W. & Williamson, F. B. (1969). *J. Polymer Sci.* **C29**, 261.

Reid, D. S. (1978). In *Ions in macromolecular and biological systems*, eds D. H. Everett and B. Vincent, Bristol.

Samoilov, O. Ya. (1969). In *Water in biological systems*, ed. L. P. Kayushin, New York, Consultant Bureau, Vol. 1.

Saunders, P. R. & Ward, A. G. (1954). *Proc. 2nd Intern. Congr. Rheol.*, London, Butterworths, 284.

Scott-Blair, G. W. & Burnett, J. (1957). *Lab. Pract.* **6**, 570.

Sherman, P. (1970). *Industrial rheology*, London, Academic Press, 67.

Smidsrod, O., Andersen, I. L., Grasdalen, H., Larsen, B. & Painter, T. (1980). *Carbohydrate Res.* **80**, C11.

Smith, D. B. & Cook, W. H. (1953). *Archs. Biochem. Biophys.* **45**, 232.

Smith, D. B., Cook, W. H. & Neal, J. L. (1955). *Archs. Biochem. Biophys.* **53**, 192.

Treloar, L. R. G. (1973). *The physics of rubber elasticity*, 3rd edn., Oxford, Clarendon Press.