Carbohydrate Polymers **13** (1990) 257–271

Interactions between Agarose and κ-Carrageenans in Aqueous Solutions

J. Zhang* & C. Rochas

Centre de Recheches sur les Macromolécules Végétales, Cermav-CNRS BP 53 X, 38041 Grenoble cedex, France

(Received 10 March 1989; revised version received 24 August 1989; accepted 10 September 1989)

ABSTRACT

Blends of agarose and carrageenan were investigated at different temperatures by optical rotation, calorimetry, and mechanical tests. It was shown that the conformational change of either of the two polymers is independent of the other. The contribution of the polymers to the elastic modulus of the binary gel are additive. The temperature dependence of the elastic modulus of the carrageenan gels shows that energy is stored on deformation as a change in enthalpy.

INTRODUCTION

Agarose and carrageenan are widely used as gelling agents and stabilisers in the food industry. They are extracted from red seaweed. The properties of the individual substances have been extensively studied by many authors as reviewed by Clark and Ross-Murphy (1987) but, so far we have not found any reports on the behaviour of blends of agarose and carrageenan. No information exists on possible intermolecular association or synergistic interactions for these binary gels and we do not know if the mixed gel consists of interpenetrating networks, phase-separated networks or coupled networks according to the nomenclature of Cairns *et al.* (1987). These points will be addressed in this paper.

*On leave from: Department of Polymer Materials, East China, University of Chemical Technology, Shanghai 200237, People's Republic of China.

Carbohydrate Polymers 0144-8617/90/$03.50 —

MATERIALS

κ-Carrageenan, extracted from *Eucheuma cottonii* was provided by MRS (Baupte, France), and was utilised without further purification. The weight average molecular weight was 690 000 (Rochas *et al.*, 1989). Agarose was donated by the FMC corporation. The sulphate content was lower than 0·35% and the weight average molecular weight was 139 000 (Rochas & Lahaye, 1989).

Measurements

Optical rotation measurements were carried out with a Perkin Elmer 241 polarimeter at a wavelength of 365 nm using 100-mm thermostated cells. The integration time was 2·0 s and the temperature scan rate was 25°C/h.

Mechanical properties were measured with a 4301 Instron machine. The sample of gel was prepared in a cylindrical tube. The solution was heated in boiling water and then poured into the tube for casting. After cooling at room temperature the cylinder was cut into slices of 17·3 mm diameter and 17 mm length and immersed for one night in a large excess of the solvent used to form the gel. As described earlier (Rochas & Landry, 1988) the rate of compression used was 25 mm/min for all measurements. Three parameters were measured: the yield stress F_m, the elastic modulus E and the strain at break $\Delta H/H$. For measurements of the elastic modulus the degree of compression did not exceed 3%. To determine E and F_m at different temperatures the sample was placed in a thermostated oil bath. When determining the temperature dependence of the elastic modulus the temperature was stabilised for 5 min before measurement.

In addition, preliminary dynamic experiments were made on a Carrimed CS 50. For both polymers and for the mixture the loss modulus G'' is smaller than the dynamic shear modulus G' and there is no dependent of G' and G'' on the frequency (as shown by Eliot and Ganz (1975) for carrageenan and by Morris (1984) for agarose). Consequently it appears that these gels are almost fully elastic. In addition, it was also shown that the relation $E = 3G'$ was valid, and thus it is possible to assume that the gels were isotropic.

Calorimetric experiments were performed with a Calvet calorimeter as described by Rochas and Rinaudo (1982).

RESULTS AND DISCUSSION

Conformational change of agarose and carrageenan and their blends

In solutions of biological polymers the conformation can often be changed by alteration of the salt concentration or the temperature. The conformation transition for agarose and κ-carrageenan does not occur at the same temperature (Fig. 1). The optical rotation temperature dependences were recorded for solutions of κ-carrageenans, agarose and for their blends in 0·1 M KCl (Fig. 2). The results show that the values of the individual solution are additive in the blend, hence

$$\alpha_B(C_A + C_K) = \alpha_K(C_K) + \alpha_A(C_A) \quad (1)$$

where α is the optical rotation and C is the concentration. Subscripts B, K and A refer to the blend, κ-carrageenan and agarose respectively.

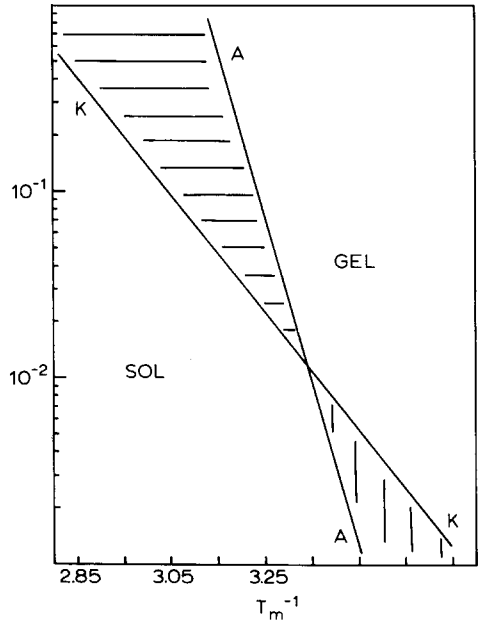

Fig. 1. Reciprocal temperature (T^{-1}) of the conformational change (cooling process) versus the potassium (eq/litre) concentration for (K) κ-carrageenan (Rochas & Rinaudo, 1980), and (A) concentration for agarose (Rochas, 1987). The area with horizontal dash lines represents a domain where κ-carrageenan is in helical conformation, whereas agarose is in coil conformation. The area with vertical dash lines represents a domain where κ-carrageenan is in coil conformation but agarose is in helical conformation.

Thus the optical rotations of κ-carrageenan and agarose are not changed on mixing. It follows that κ-carrageenan and agarose have the same conformation as single components, and in the blend at low concentrations. Consequently, helical dimers composed of one agarose chain and one carrageenan chain are not formed.

A similar phenomenon occurs if the ordering of κ-carrageenan starts after the ordering of agarose. It is possible to change the temperature of the conformational change of κ-carrageenan using NaCl instead of KCl. Identical melting or gelling temperatures are obtained for the blend (Fig. 3) or for the pure solution of carrageenan and agarose (Rochas & Rinaudo, 1980; Rochas, 1987). Optical rotation is only able to monitor the conformational ordering or the gelation of moderately concentrated solutions of agarose or carrageenan. Due to birefringence and absorption, highly concentrated solutions of these polymers cannot be investigated by this method. The transition of carrageenan (Rochas & Rinaudo, 1982) and agarose (Rochas, 1987) can be followed at higher concentrations using calorimetry. The results using this technique show that the values of the individual solutions are additive in the blend (Fig. 4). Thus,

$$\Delta H_B(C_A + C_K) = \Delta H_K(C_K) + \Delta H_A(C_A) \quad (2)$$

where ΔH is the enthalpy of the conformational transition.

Once again, as for optical rotation, it is found that the transition of agarose and κ-carrageenans is identical for the individual components and for the blend. In both cases the transitions are observed at the same

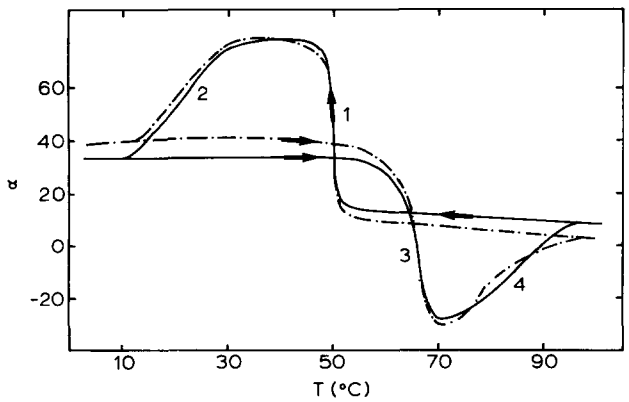

Fig. 2. Optical rotation temperature dependence of a solution of κ-carrageenan plus the optical rotation temperature dependence of a solution of agarose (——). Optical rotation temperature dependence and the blend (·—·—). In both cases C agarose = 1 g/litre, C carrageenan = 0·7 g/litre, 0·1 M KCl. (1) Ordering of carrageenan, (2) ordering of agarose, (3) disordering of carrageenan, (4) disordering of agarose.

temperature and the enthalpies are also the same (Fig. 4). Consequently for both the dilute blend ($C = 0{\cdot}17\%$) and the concentrated blend ($C = 1{\cdot}5\%$) the conformational transition of one polysaccharide in the blend is independent of the other.

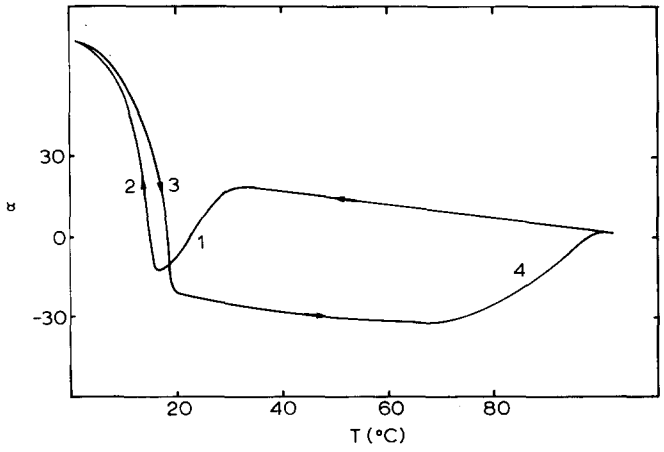

Fig. 3. Optical rotation temperature dependence of a blend agarose–κ-carrageenan. *C* agarose = 1·6 g/litre, *C* carrageenan = 0·7 g/litre, 0·1 M NaCl. (1) Ordering of agarose, (2) ordering of carrageenan, (3) disordering of carrageenan, (4) disordering of agarose.

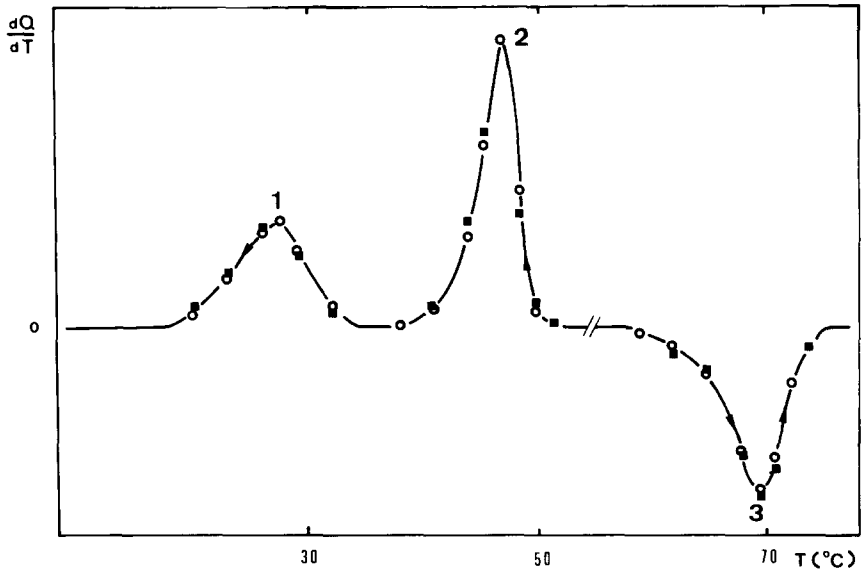

Fig. 4. Derivative of the heat (dQ/dT) arbitrary units, versus the temperature for pure solution of polymer (○) and for the blend (■). In both cases *C* agarose = 10 g/litre, *C* carrageenan = 10 g/litre, 0·1 M KNO_3. (1) Ordering of agarose, (2) ordering of carrageenan, (3) disordering of carrageenan.

At high temperatures in the coil state no phase separation is observed. In addition, the blends have no tendency to form two phases during gelation. Though there is not direct evidence of this fact it seems reasonable to assume that the solutions of the two polymers are miscible, whatever the conformations of the polysaccharides. The hypothesis of immiscibility seems to be unrealistic. If two phases exist, a phase containing concentrated carrageenan and a phase containing concentrated agarose would be formed. The polymer concentration in each phase would be higher than the average concentration taking into account the total volume. This local increase in concentration should be detected by optical rotation or calorimetry because the results from both techniques are concentration dependent for agarose (Rochas, 1987). We have shown (Figs 3 and 4) that there is no local change of concentration, consequently this blend cannot be considered to be formed by phase separated networks.

In addition, according to the nomenclature of Cairns *et al.* (1987), this is not a coupled network (agarose binding to carrageenan) because optical rotation and calorimetry show no interaction between the two polymers. Consequently this binary gel is formed by interpenetrating networks when the two polymers are ordered. If the conformation of one of the two polymers is disordered and the other ordered, the binary polysaccharide gel structure is a single polymer network containing the second polymer as a sol within the gel.

Elastic modulus temperature dependence

It is found that the optical rotation temperature dependence is similar to the elastic modulus temperature dependence for κ-carrageenan (Fig. 5). For temperatures higher than the melting temperature, the elastic modulus decreases slightly if the temperature increases. When the temperature is close to the temperature of the conformational transition, the elastic modulus decreases drastically. The hysteresis observed for the temperature dependence of the elastic modulus is not similar to that observed for the optical rotation. With the mechanical test used it is not possible to follow the elastic modulus up to complete melting due to the destruction of the gel. This limits the domain of temperatures investigated. Before melting, the activation energy Ea can be calculated from the relationship $E = E_0 \exp(-Ea/RT)$. E is the elastic modulus and E_0 a constant. For the investigated concentration, the energy found is $Ea = 10 \pm 0{\cdot}3$ kJ/mol (Table 1). The modulus temperature dependence is negative as found for alginate (Andresen & Smidsrød, 1977). This shows

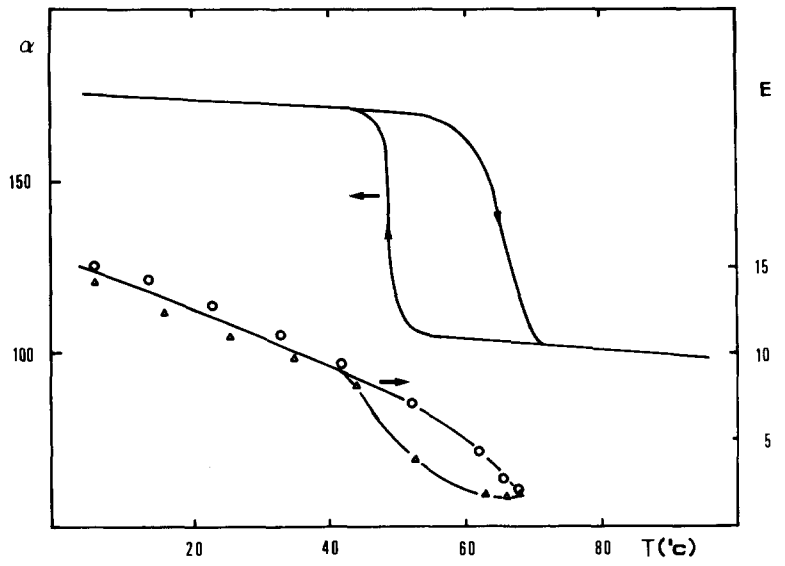

Fig. 5. Optical temperature dependence, $C = 0{\cdot}7$ g/litre, and elastic modulus (10^5 dyne/cm^2) temperature dependence of κ-carrageenan ($C = 20$ g/litre), (○) decreasing temperature, (△) increasing temperature, 0·1 M KCl.

TABLE 1
Activation Energies of κ-Carrageenan Gels

C *(g/litre)*	*E* *(kJ/mol)*
10	9·8
15	10·0
15	10·3
20	9·7
30	10·5
30	9·7

that the elasticity of this gel is not entropic and consequently that the behaviour is not rubber-like.

In contrast, the temperature dependence of the modulus for agarose is different. Before the conformational ordering, the modulus slightly increases with increasing temperature up to 40°C (Fig. 6), as previously observed by Nishinari *et al.* (1984). This dependence could be attributed to the entropic nature of the elastic gel. However, agarose gels have been shown to be non-rubber-like (Pines & Prins, 1973). The increase in

modulus with temperature may correspond to an increase in the degree of aggregation of helical molecules. Between 40°C and 60°C the elastic modulus decreases as the temperature increases. Above 60°C the modulus decreases drastically and this variation is clearly correlated with the optical rotation change. We do not observe the same hysteresis for the modulus temperature dependence and the optical rotation temperature dependence, because as for carrageenan the increase in temperature is stopped before the complete melting of the gel. This is why different temperature dependences of the modulus can be obtained for a cooling process (Fig. 6). For carrageenan and agarose the sharp decrease in the

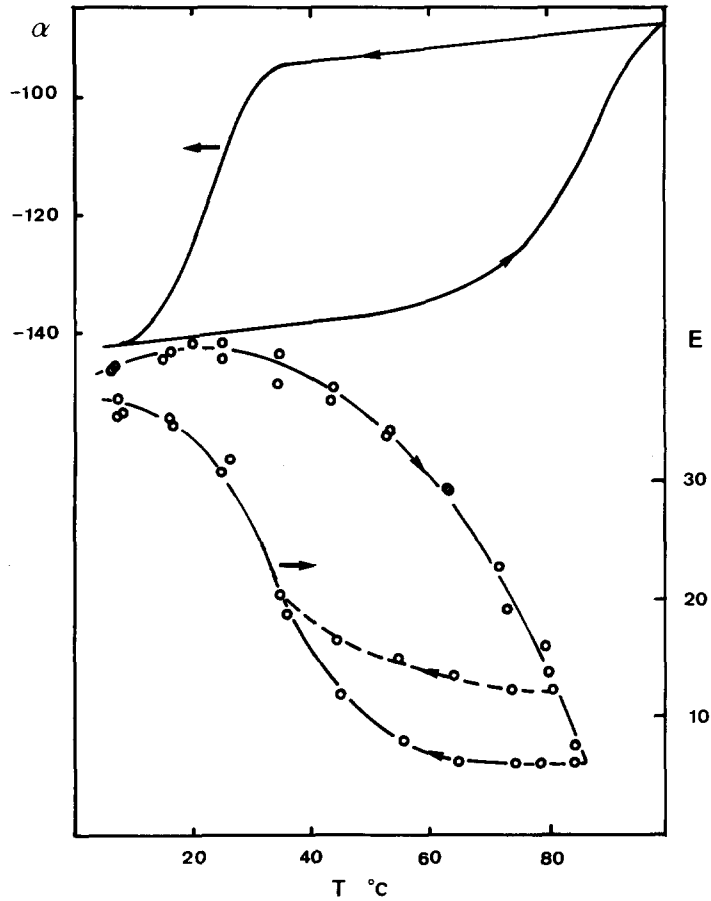

Fig. 6. Optical temperature dependence ($C = 1{\cdot}0$ g/litre) and elastic modulus (10^5 dyne/cm^2) temperature dependence of agarose ($C = 20$ g/litre), 0·1 M KCl. Two different temperature cycles are shown for the modulus temperature dependences. Cycle 1 is obtained after heating at 81°C, and cycle 2 is obtained after heating at 85°C.

modulus with increasing temperature is closely related to the conformational change, hence it is possible to assume that the helix content (ordered form) of the gel controls the magnitude of the modulus. However, it can be seen that for agarose a large decrease of the modulus is obtained before the beginning of the conformational change (Fig. 5). Consequently, the temperature has two different effects. One is to change the number of junction zones in the network by altering the helix content. This occurs over the temperature range for the melting of the ordered form. The second effect of temperature is probably to change the stability of the junction zones.

In the blend the same phenomena are shown (Fig. 7). Different steps can be recognised and attributed to the transition of agarose and carrageenan. The modulus of the blend can be represented by the following relationship:

$$E_B(C_A + C_K) = E_A(C_A) + E_K(C_K) \tag{3}$$

where E_B is the calculated modulus of a blend at concentration C_A of agarose and C_K of κ-carrageenan, E_A and E_K are the experimental modulus of an agarose gel at the concentration C_A and a κ-carrageenan gel at the concentration C_K respectively. The calculated value of the blend modulus is very close the experimental one (Fig. 7), though always

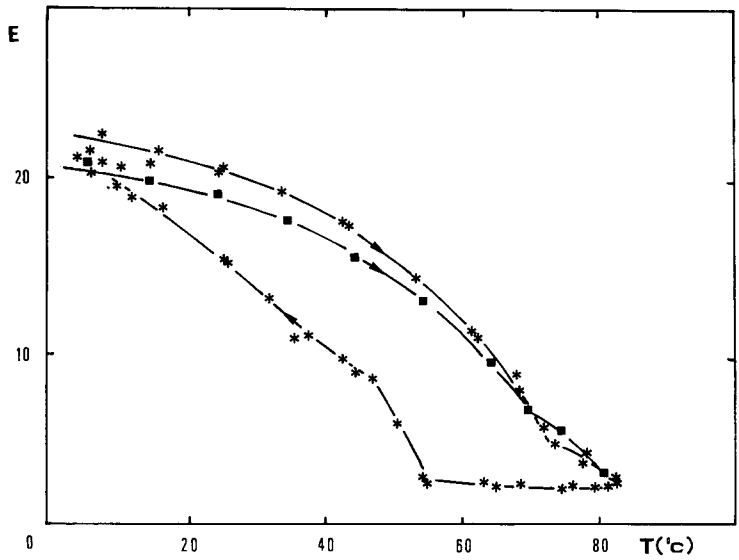

Fig. 7. Modulus (10^5 dyne/cm^2) temperature dependence of a blend agarose–κ-carrageenan *C* agarose = 15 g/litre, C carrageenan = 15 g/litre, 0·1 M KCl. (✱) Experimental points, (■) calculated points.

slightly lower than it. This difference decreases with increasing temperature. The elastic modulus data is thus consistent with optical rotation (Fig. 2) or calorimetry (Fig. 4) and shows that there are no strong interactions between the network of agarose and the network of κ-carrageenan at every temperature in the concentration range investigated.

The dependence of the ultimate properties (yield stress) gives less information (Fig. 8). We can see only a decrease in the yield stress with increasing temperature for the individual gel or for the gel blend. It is not possible to calculate correctly the yield stress for the blend from the values for the individual components because the stress–strain curve is not the same for the two polymers and for the blend. Consequently, we do not know if the difference observed between a calculated yield stress value and the experimental one is due to non-identical deformation in the two cases or to possible interactions shown by the yield stress, and

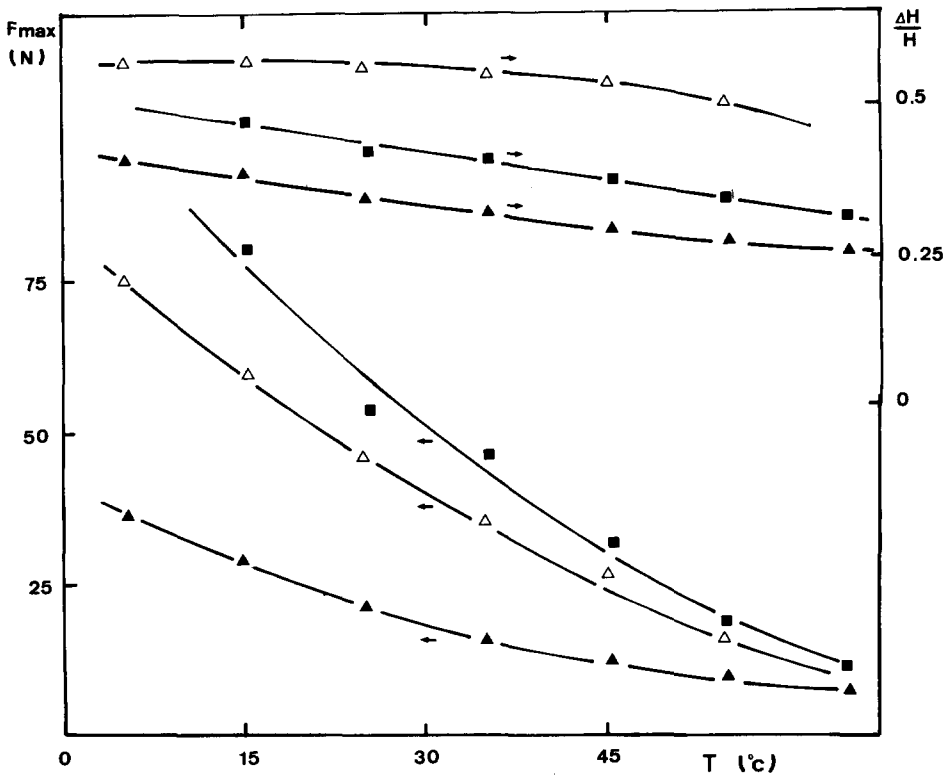

Fig. 8. Yield stress (F_{max}) and deformation ($\Delta H/H$) temperature dependence. (▲) Agarose, (△) κ-carrageenan, (■) blend agarose–carrageenan. *C* agarose = 15 g/litre, *C* carrageenan = 15 g/litre.

not by the modulus as it is observed for the mixtures of carrageenans (Landry & Rochas, 1987, Rochas *et al.*, 1989). According to Flory (1953) it is possible to obtain the Gibbs equation for a closed system without chemical reaction in the form

$$F=(\delta U/\delta l)_{T,V}+T(\delta F/\delta T)_{V,l} \tag{4}$$

where F is the elastic force, U is the internal energy of the system and δl the variation in height during deformation; T, V and l are the temperature, the volume and the height respectively. According to Andresen and Smidsrød (1977) it is possible to write

$$(\delta F/\delta T)_{V,l}=-(\delta S/\delta l)_{T,V}=(\delta F/\delta T)_{\tau} \tag{5}$$

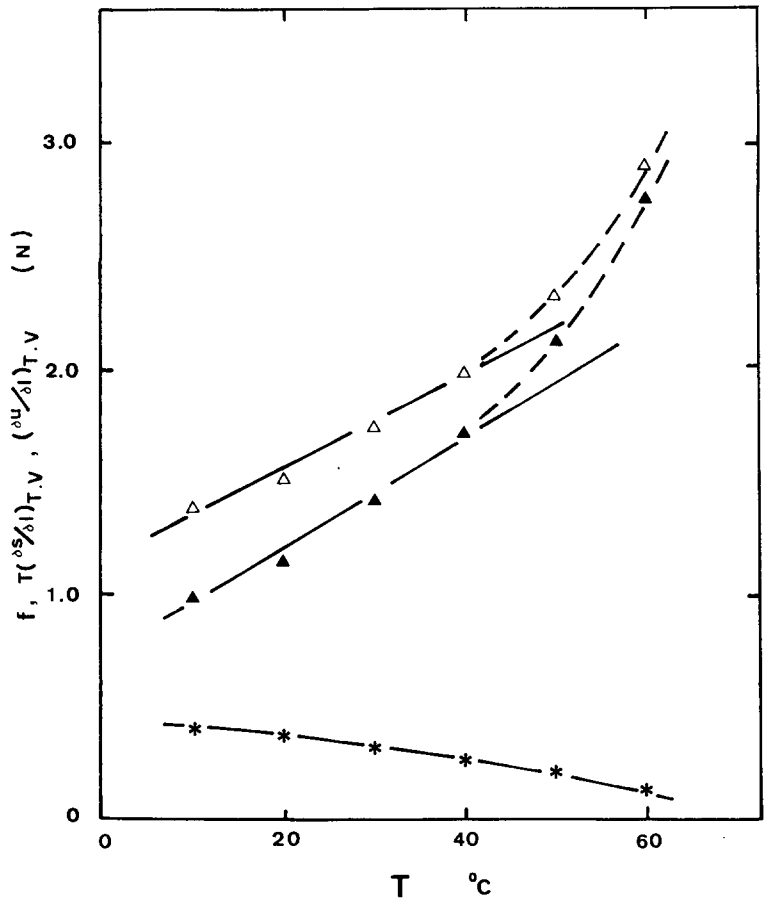

Fig. 9. (✱) Force, (▲) changes in entropy and (△) internal energy during compression (2%) of carrageenan gel for increasing temperature, C = 15 g/litre, 0·1 M KCl.

where r is the ratio between the length $l-\delta l$ at an applied elastic force and the initial height l, and S is the entropy.

F and $(\delta F/\delta T)$ can be obtained by experiments and the results for agarose, carrageenan and a blend are shown in Figs 8–10. For an ideal elastomer $(\delta U/\delta l)_{T,V}=0$, its modulus will increase with increasing temperature — this is the entropic elasticity. With the exception of the temperature range 0–20°C for agarose we can see that the change in entropy and internal energy during the compression of a carrageenan gel is similar to the change observed for the alginate gels (Andresen & Smidsrød, 1977). In both cases this change increases with increasing temperature. The fact that $(\delta U/\delta l)_{T,V}$ is larger than zero clearly establishes that agarose and carrageenan networks cannot be described

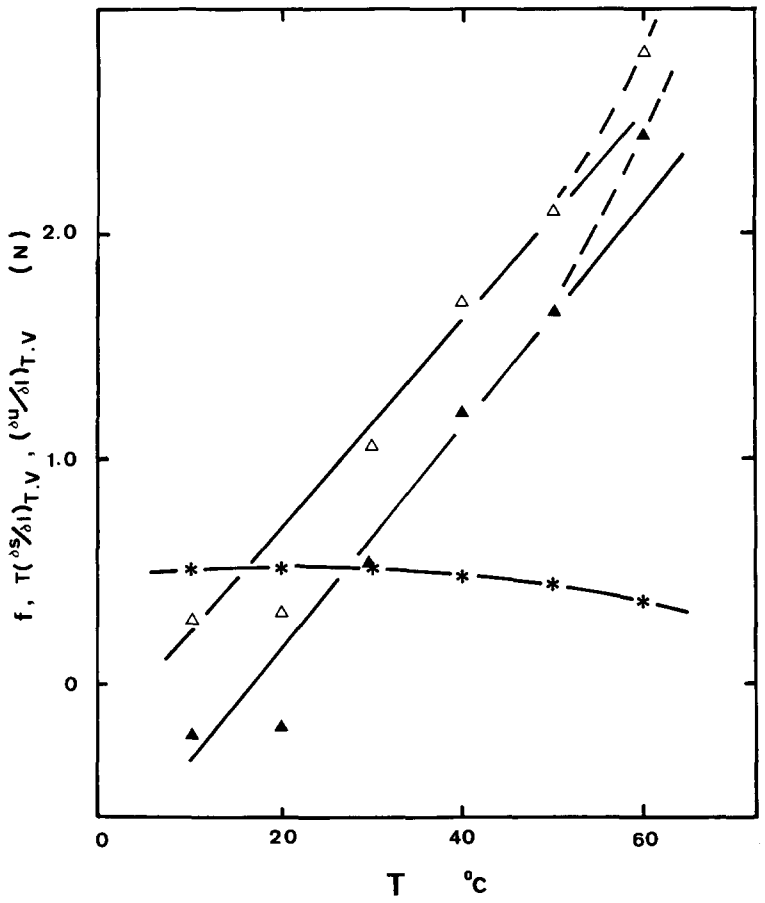

Fig. 10. (✱) Force, (▲) changes in entropy and (△) internal energy during compression (2%) of agarose gel for increasing temperature, $C=15$ g/litre, 0·1 M KCl.

by rubber elasticity theory. For agarose in the range 0–20°C, a possible reorganisation of the network can explain the negative value of the entropic term $T(\delta S/\delta l)$. In addition, the fact that the modulus temperature dependence is in contradiction to the theory of rubber elasticity can be explained by the stiffness of the polysaccharide network chains.

When two networks exist in a blend (agarose–κ-carrageenan) the entropy and the internal energy of each component are additive (Fig. 11). The calculated values are similar to the experimental ones but the agreement is better at lower temperatures than at higher temperatures. This is presumably because eqn (5) is valid for a closed system without

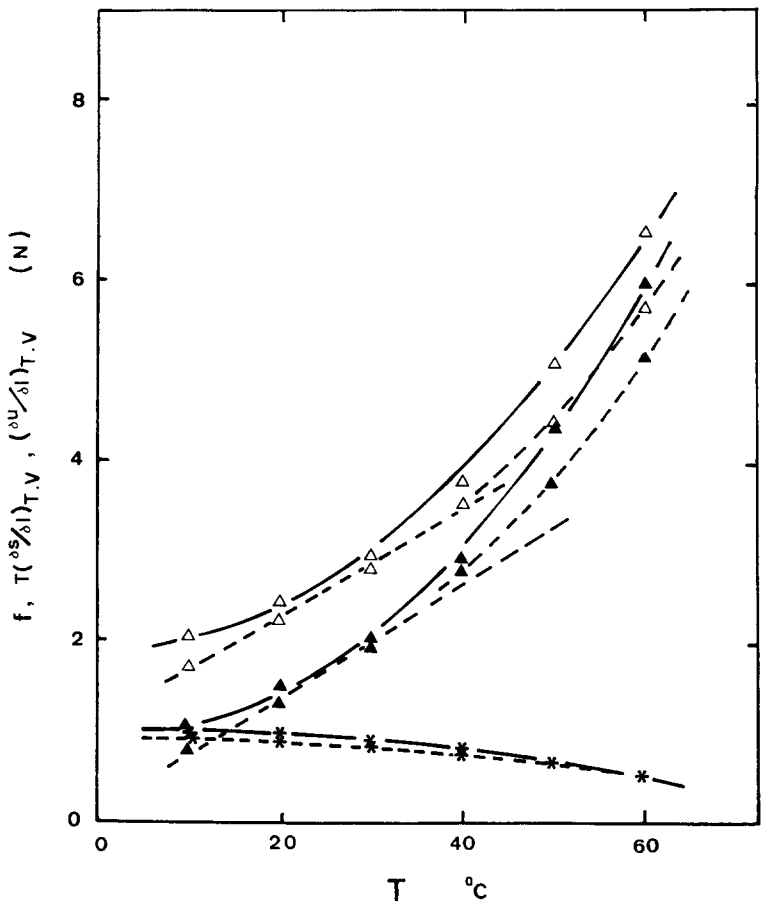

Fig. 11. (✱) Force, (▲) changes in entropy and (△) internal energy during compression (2%) of a blend carrageenan for increasing temperature, (15 g/litre) — agarose (15 g/litre) gel, 0·1 M KCl.

chemical reaction but in our case when the temperature is raised the reaction helix → coil occurs.

CONCLUSION

In the κ-carrageenan–agarose blend investigated, the networks of the individual polymers are conserved and consequently the blend is composed of interpenetrating networks interacting only by mutual entanglements. The properties of the blend are well predicted by the additivity of the properties of the individual gels, whatever the temperature.

The contribution of one polysaccharide to the elastic modulus of the binary gel agarose–κ-carrageenan is independent of the conformations (helical–gel or coil–sol) of the other polymer.

ACKNOWLEDGEMENT

J. Zhang expresses his gratitude to the Institut Franco-Chinois, Lyon, France for financial support.

REFERENCES

Andresen, I. L. & Smidsrød, O. (1977). Temperature dependence of the elastic properties of alginate gels. *Carbohydr. Res.*, **58**, 271–9.

Cairns, P., Miles, M. J., Morris, V. J. & Brownxy, G. J. (1987). X-ray fibre-diffraction studies of synergistic, binary polysaccharides. *Carbohydr. Res.*, **160**, 411–23.

Clark, A. H. & Ross-Murphy, S. B. (1987). Structural and mechanical properties of biopolymers gels. *Advances in Polymer Science*, **83**, 57–92.

Eliot, J. H. & Ganz, A. J. (1975). Gel characterization with the Weissenberg rheogoniometer: application to carrageenan gels. *J. Food Sci.*, **40**, 394–8.

Flory, P. J. (1953). *Rubber Elasticity in Principles of Polymer Chemistry*, ed. P. J. Flory. Cornell University Press, Ithaca and London, pp. 432–94.

Landry, S. & Rochas, C. (1987). Mechanical properties of mixed gels of carrageenan. *Food Hydrocolloids*, **1**, 591.

Morris, E. R. (1984). Rheology of hydrocolloids. In *Gums and Stabilisers for the Food Industries 2*, ed. G. O. Phillips, D. J. Wedlock & P. A. Williams. Pergamon Press, Oxford, pp. 57–98.

Nishinari, K., Watase, M. & Ogino, K. (1984). On the temperature dependence of the elasticity of agarose gels. *Makromol. Chem.*, **185**, 2663–8.

Pines, E. & Prins, W. (1973). Structure–property relations of thermoreversible macromolecule hydrogels. *Macromolecules*, **6**, 888–95.

Rochas, C. (1987). Calorimetry of galactans. *Food Hydrocolloids*, **1**, 215–25.
Rochas, C. & Lahaye, M. (1989). Average molecular weight and molecular weight distribution of agarose and agarose-type polysaccharides. *Carbohydr. Polym.*, **10**, 289–98.
Rochas, C. & Landry, S. (1988). Rheological characterization of kappa carrageenans gels. In *Gums and Stabilisers for the Food Industries, 4*, ed. G. O. Phillips, D. J. Wedlock & P. A. Williams. IRL Press, Oxford and Washington, pp. 445–51.
Rochas, C. & Rinaudo, M. (1980). Activity coefficient of counterions and conformations in kappa-carrageenans systems. *Biopolym.*, **19**, 1675–87.
Rochas, C. & Rinaudo, M. (1982). Calorimetric determination of the conformational transition of kappa carrageenan. *Carbohydr. Res.*, **105**, 227–36.
Rochas, C., Rinaudo, M., Landry, S. (1989). Relation between the molecular structure and mechanical properties of carrageenan gels. *Carbohydr. Polym.*, **10**, 115–27.