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Studies of the Gel Formation of κ-Carrageenan Above the Coil-Helix Transition Temperature Range

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SUMMARY

The temperatures of gel formation and coil-helix conformational transition of κ-carrageenan have been compared. For a 1·5% solution of the potassium salt of κ-carrageenan the gelation temperature is higher than the coil-helix transition temperature range when the KCl concentration exceeds 3-4 × 10^{-2} mole litre^{-1}. The gelation temperature, T_g, and the midpoint temperature of the coil-helix conformational transition, $T_{1/2}^{c-h}$, are linearly increasing functions of the logarithm of the concentration of potassium ions, and

$$\delta T_g/\delta(\lg[K^+]) > \delta(T_{1/2}^{c-h})/\delta(\lg[K^+])$$

Creep compliance studies on a 1·5% gel of κ-carrageenan in 0·11 M *KCl have shown that the standard breakdown enthalpy of gel network cross-links at temperatures above the coil-helix transition range is about 12 kJ mole^{-1}. Upon helix formation this value increases 20 times. The relaxation behaviour of the κ-carrageenan gel in the high-temperature region is typical of thermoreversible gels. The network of such gels is generally made up of associates of macromolecules.*

INTRODUCTION

Two models for the formation of gels of ι- and κ-carrageenan have been advanced. According to the 'domain' model developed by Morris

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et al. (1980*b*), the formation of double helical junction zones between macromolecules results in the formation of soluble clusters ('domains') which combine to form a continuous network through interaction of the helical segments of macromolecules belonging to different domains. This interaction involves the participation of ions which promote gel formation. These authors believe that the domain model is applicable to the thermoreversible gelation of a number of polysaccharides and also gelatin. It conforms to the ideas developed over many years by Zsigmondi (1905, 1914), Menz (1909), Bachmann (1912), Arisz (1915), Krishnamurti (1929, 1930), Katti (1952), Beyer (1954), Boedtker & Doty (1954), Engel (1961), and others regarding the mechanism of gel formation of gelatin, agar–agar, and other polymers.

An alternative scheme for gel formation of carrageenans, suggested by Smidsrød *et al.* (1980), involves *intramolecular* ordering of macromolecules and their combination into a continuous network through selective ionic cross-links between the segments of different chains. In this model a co-axial double helix is not formed.

In both models the coil to helix conformational transition of carrageenan macromolecules is considered as the primary process leading to gel formation. The cations favouring gelation, e.g. potassium ions, promote this conformational transition (Morris *et al.*, 1980*a*) and participate in the formation of contacts between the ordered segments of macromolecules.

However, the studies conducted in our laboratory have revealed that structure formation in κ-carrageenan solutions containing potassium ions can proceed at temperatures higher than the coil–helix transition temperature range. Grinberg *et al.* (1980) have shown that associates are formed in dilute κ-carrageenan solutions in the presence of potassium ions. These associates have similar dimensions at 75 and 19°C, that is above the coil–helix transition temperature range and close to the lower boundary of the range. Plashchina *et al.* (1980) have also ascertained that the melting temperature of a 1·5% κ-carrageenan gel (sodium salt of κ-carrageenan in the presence of a 3-fold equivalent excess of KCl) exceeds the complete helix-unwinding temperature by 13°C. This clearly indicates that a helical conformation is not the prerequisite for the *existence* of the κ-carrageenan gel network. Nevertheless it is not clear if the formation of a helix is a necessary condition for the *formation* of such a network. For this reason we have compared in this communication the temperatures at which a gel network appears in a κ-carrageenan solution and at which the coil–helix transition for this polysaccharide takes place. In addition, the rheological properties of the gels of κ-carrageenan have been studied in a temperature range where the junction zones in the network cannot consist of contacts between helical segments of macromolecules.

MATERIALS AND METHODS

A commercial sample (type 1, No. C1013) of carrageenan manufactured by the Sigma Chemical Co., USA, was used in this study. κ-Carrageenan was isolated, purified and characterized as described by Grinberg *et al.* (1980).

The samples were prepared by dissolving lyophilized κ-carrageenan in twice-distilled water at 80°C. KCl solution was added to the hot solution of the polysaccharide at a rate of 0·8 ml min^{-1} while stirring.

Optical rotation was measured with a Jouan–Roussel polarimeter (France) at 436 nm to an accuracy of 5×10^{-4} degrees. The measurements were taken in a thermostated silica cell (wall thickness, 1·5 mm). The optical path length was 0·01 dm. To decrease the probability of the development of internal stresses in the gel samples, the cell was not filled to the brim. The gels were checked for birefringence using a polarizing microscope NU-2 (Karl Zeiss, Jena, GDR). In no case was any birefringence detected.

In the polarimetric studies the temperature was varied at a rate of 0·3°C min^{-1}. Measurements were taken to ± 0·5°C with a thermocouple by placing its hot junction in the polarimeter cuvette.

The gel formation temperature, T_g, was determined with the rotational viscometer VPN-2 produced in the Special Design Bureau of the A. V. Topchiyev Institute of Petrochemical Synthesis, USSR Academy of Sciences. This viscometer permits deformation to be measured at a constant stress (Vasilyev *et al.*, 1977). This allows samples of gel to be tested at stresses below the rupture point.

The κ-carrageenan solution was kept in the viscometer cell for 10 min at 80°C, the temperature was then lowered at a rate of 0·3°C min^{-1}. A shear stress of 8·5 Pa was periodically applied to the sample and the viscosity was measured. The gel formation point was taken as the temperature at which $\delta\eta/\delta T \to \infty$, i.e. when a yield stress appears. To avoid drying of the sample, a thin layer of silicone oil was applied on the melt surface in the annular gap of the viscometer. The cell temperature was recorded with a thermocouple to ± 0·5°C.

The fusion temperature of the gels, T_f, was determined (1) using the rotational viscometer in a manner similar to that used for determining T_g, but heating rather than cooling, and (2) by observing how the melt is displaced by a heavier fluid (perfluorodimethylcyclohexane, $\rho_4^{20} = 1{\cdot}78\ \text{g cm}^{-3}$) predeposited on the gel sample (Braudo *et al.*, 1973). In both cases the temperature was increased at a rate of 0·3°C min^{-1}. The discrepancy between the values of T_f determined by these methods did not exceed 0·4°C.

The procedure for measuring the creep compliance of gels in uniaxial compression has been described by Plashchina *et al.* (1983). Measurements were made in the linear viscoelastic region.

To prepare gel samples, the melt was poured into acrylic plastic moulds coated on the inside with silicone oil. The filled moulds were kept at room temperature for 2 h. After gelation the samples were taken out of the moulds, immersed in silicone oil and kept first at 8°C for 24 h and then at room temperature for 1 h.

To obtain the equilibrium elastic deformation, the gel samples were kept at 66°C for 1 h and then at the temperature of the experiment until the residual deformation attained a constant value.

RESULTS AND DISCUSSION

Gel formation and conformational transition in κ-carrageenan solutions

Results

Figure 1 shows the temperature dependences of the specific optical rotation and the viscosity of 1·5% solutions of sodium and potassium salts of κ-carrageenan containing a 3-fold equivalent excess of KCl. For both systems the gelation temperature, T_g, is higher than the midpoint temperature of the coil-helix conformational transition, $T_{1/2}^{c-h}$, by about 10°C. The difference between the melting temperature, T_f, and the midpoint temperature of the helix-coil conformational transition is even greater: 21 and 15°C respectively for the sodium and potassium salt of κ-carrageenan.

The effect of potassium ion concentration on the gelation temperature and the coil-helix transition temperature of the potassium salt of

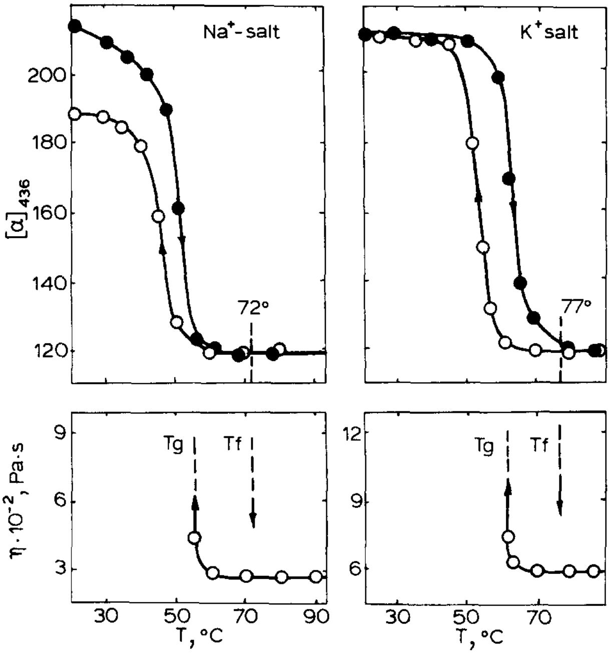

Fig. 1. Temperature dependence of the specific optical rotation and viscosity of κ-carrageenan solutions in the presence of 0·11 M KCl. o, cooling; ●, heating. Polymer concentration: Na⁺-salt of κ-carrageenan, $3{\cdot}7 \times 10^{-2}$ g-equiv. litre^{-1} (1·5%); K⁺-salt of κ-carrageenan, $3{\cdot}5 \times 10^{-2}$ g-equiv. litre^{-1} (1·5%).

κ-carrageenan in KCl solutions is shown in Fig. 2. The concentration of polysaccharide was kept constant but that of the potassium ions was changed by varying the concentration of KCl. In this case, gel formation takes place at temperatures higher than the conformational transition temperature range throughout the range of potassium ion concentrations investigated.

Figure 3 shows the dependence of the gelation temperature and the midpoint temperature of the coil–helix conformational transition of the potassium salt of κ-carrageenan in KCl solutions as a function of the logarithm of the concentration of potassium ions. Both temperatures rise linearly with the increase in the logarithm of potassium ion concentration. In this case,

$$\delta T_g/\delta(\lg[K^+]) > \delta T_{1/2}^{c-h}/\delta(\lg[K^+])$$

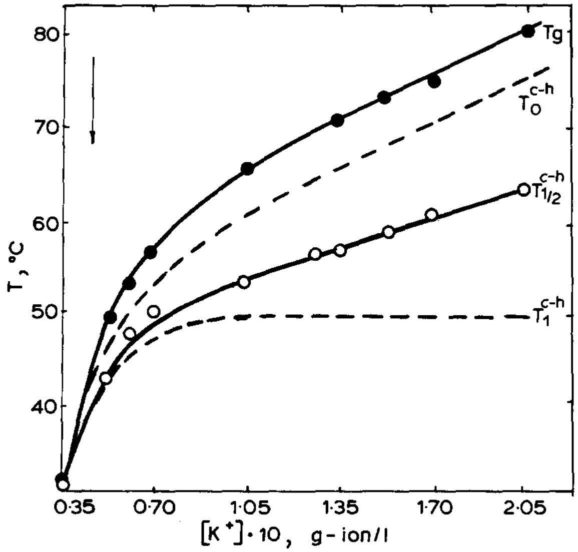

Fig. 2. Gelation temperature (T_g) and temperatures of the beginning (T_0^{c-h}), midpoint ($T_{1/2}^{c-h}$), and end (T_1^{c-h}) of the coil-helix transition of K$^+$-salt of κ-carrageenan in KCl solution vs the concentration of potassium ions. Polymer concentration, 3·5 x 10^{-2} g-equiv. litre^{-1} (1·5%); cooling rate, 0·3°C min^{-1}.

The slopes of the curves and the coordinates of the intersection point are given in Table 1. This point corresponds to the solution which does not contain added KCl, i.e. to the origin of the coordinates in Fig. 2.

The potassium ion concentration can be varied not only by changing the concentration of KCl but also by varying the concentration of the potassium salt of κ-carrageenan. Figure 4 shows the dependence of gelation temperature and the midpoint temperature of the coil-helix transition on the logarithm of the potassium ion concentration for aqueous solutions of the potassium salt of κ-carrageenan. Here also, both dependences are linear and again (see Table 1)

$$\delta T_g/\delta(\lg[K^+]) > \delta T_{1/2}^{c-h}/\delta(\lg[K^+])$$

To the left of the point of intersection of the curves, gelation takes place when most of the macromolecule is in the helical conformation,

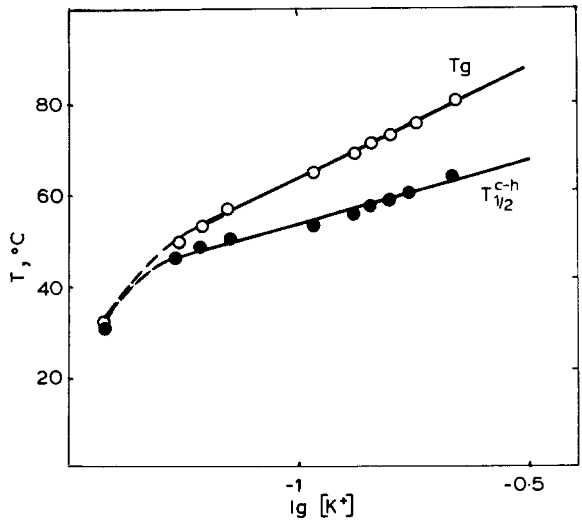

Fig. 3. Dependence of gelation temperature (○) and midpoint temperature of coil-helix transition (●) of K^+-salt of κ-carrageenan in KCl solution on the logarithm of total concentration of potassium ions. Polymer concentration, $3{\cdot}5 \times 10^{-2}$ g-equiv. $litre^{-1}$ (1·5%); Cooling rate, 0·3°C min^{-1}.

i.e. the process proceeds in accordance with the traditional concepts of the mechanism for carrageenan gel formation.

Figure 5 shows the dependences of T_g and $T_{1/2}^{c\text{-}h}$ on the logarithm of potassium ion concentration for sodium salt solutions of κ-carrageenan, containing different amounts of KCl. The nature of these dependences is analogous to those considered above. The values of slopes and the coordinates of the points of intersection of the curves are given in Table 1.

A comparison of the coordinates of the intersection points of the dependences $T_g = f([K^+])$ and $T_{1/2}^{c\text{-}h} = f([K^+])$ (Table 1) reveals that these points lie in a comparatively narrow concentration range for potassium ions and temperature range.

Unlike T_g, $T_{1/2}^{c\text{-}h}$ is independent of the concentration of the polysaccharide and is determined only by the concentration of potassium ions (Fig. 5). This is more distinctly evident from Fig. 6 where the data obtained for a rather large range of concentrations for the sodium salt of κ-carrageenan in KCl solutions are shown.

TABLE 1
Characteristics of Gelation and Coil-Helix Transition Processes of κ-carrageenan

System	*Polysaccharide concentration (%)*	$\delta T_{1/2}^{c\text{-}h}/\delta(\lg c_{A^+})$, (°C)	$\delta T_g/\delta(\lg c_{A^+})$, (°C)	*Coordinates of the intersection point of plots* $T_{1/2}^{c\text{-}h} = f(c_{A^+})$, *and* $T_g = f(c_{A^+})$		β	$\beta\xi_0$	$-\Delta h_g$, kJ (g-equiv.)$^{-1}$	T_g *for which* Δh_g *is calculated, (K)*
				$c_{A^+} \times 10^2$, mole litre^{-1}	T, (K)				
K-salt of κ-carrageenan + KCl	1·50	36·1 ±0·1	50·6 ±0·1	4·0	303·2	0·763	1·370	4·5	318·2
Na-salt of κ-carrageenan + KCl	1·25	25·8 ±0·1	44·0 ±0·2	4·0	302·2	0·756	1·358	5·4	320·2
	2·50	25·8 ±0·1	35·6 ±0·3	3·0	300·7	0·753	1·352	6·9	321·2
K-salt of κ-carrageenan in water	0·25–3·00	23·6 ±0·1	70·2 ±0·1	3·8	302·2	–	–	–	–

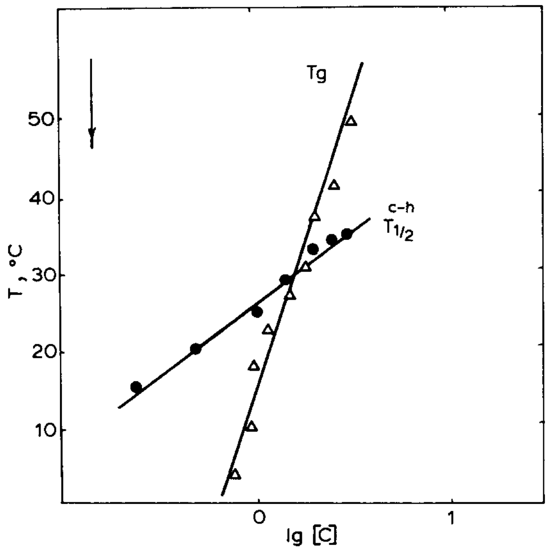

Fig. 4. Dependence of the gelation temperature (△) and midpoint temperature of the coil–helix transition (●) of the K$^+$-salt of κ-carrageenan in water on the logarithm of concentration of the polymer. Cooling rate, 0·3°C min^{-1}.

Discussion

The lack of dependence of $T_{1/2}^{c\text{-}h}$ on the concentration of polysaccharide can be explained in two ways:

1. The conformational transition is a monomolecular process. This viewpoint has been developed by Smidsrød and his colleagues (1980).
2. Above the temperature range of the conformational transition in κ-carrageenan solutions there exist regions of strong correlations of chain segments (associates) which act as nuclei for helix formation. The data reported by Grinberg *et al.* (1980) support this interpretation. These authors have demonstrated the existence of associates of macromolecules of κ-carrageenan above the temperature range of the coil–helix conformational transition.

The discussion of the dependence of T_g and $T_{1/2}^{c\text{-}h}$ of κ-carrageenan on potassium ion concentration is based on the theory of the coil–helix transition for DNA, developed by Manning (1972). The approach

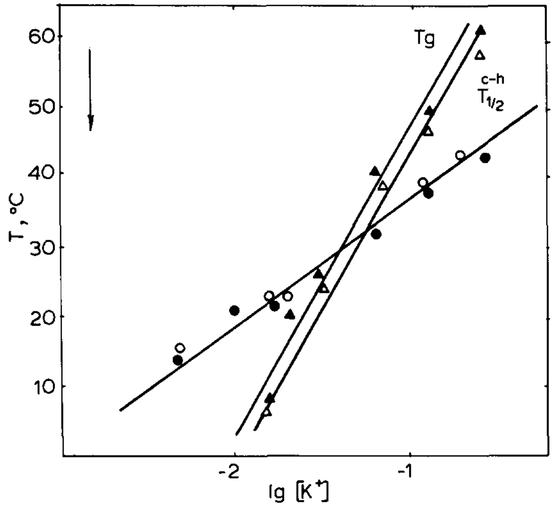

Fig. 5. Dependence of gelation temperature (△,▲) and midpoint temperature of coil-helix transition (○,●) of the Na^+-salt of κ-carrageenan in KCl solutions on the logarithm of concentration of potassium ions. Polymer concentration: (△,○), $3{\cdot}1 \times 10^{-2}$ g-equiv. litre^{-1} (1·25%); (▲,●), $6{\cdot}2 \times 10^{-2}$ g-equiv. litre^{-1} (2·50%); Cooling rate, 0·3°C min^{-1}.

advanced in this work can be readily extended to helix formation of macromolecules having a linear charge density $\xi < 1$ in the coil conformation.

The dependence of the polyanion transition temperature on cation concentration is determined by:

1. Non-coulombic bonding of cations by polyanions or by their condensation on a polyanion if the linear charge density increases to values more than 1;
2. The change in the shielding of charges due to the change in linear charge density or in the number of charged polyanion groups.

For a linear polyanion (in the presence of an excess of low molecular weight salt) the contribution of these factors to the Gibbs free energy of any transition is proportional to the logarithm of the concentration (or more precisely the activity) of cations.

If the transition is accompanied by an increase in the degree of binding of the cations (caused by non-coulombic binding as well as

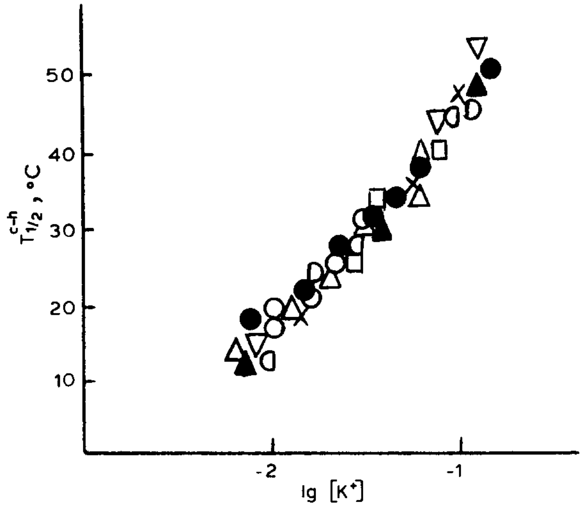

Fig. 6. Dependence of midpoint temperature of the coil-helix transition of the Na^+-salt of κ-carrageenan in KCl solution on the logarithm of the concentration of potassium ions. Polymer concentration (%): ●, 0·06; ○, 0·125; △, 0·25; ▲, 0·5; x, 0·75; □, 1·0; ᗡ, 1·25; D, 1·5; △, 1·8.

condensation), the dependence of the Gibbs free energy change (for 1 g-equiv. of the polymer dissacharide unit) on the concentration of the cation is expressed as

$$-\Delta G = \text{const} + 2{\cdot}3\,\eta RT(1 \pm 0{\cdot}5)\,\lg c_{A^+} \qquad (1)$$

where η denotes the variation in the concentration of the charged groups of the polyanion, and c_{A^+} stands for the molar concentration of cation.

The first term of this equation, which depends on c_{A^+}, is determined by the cation and polyanion mixing entropy because the increase in c_{A^+} contributes to the formation of a form with a higher degree of cation bonding. The second term is determined by charge shielding: an increase in the concentration of low molecular weight salt stabilizes a state with higher charge density. Since the first term is twice the second in absolute value, the Gibbs free energy always decreases with increasing cation concentration.

The dependence of the transition temperature, T_i, on c_{A^+} is expressed as

$$\frac{\delta T_i}{\delta(\lg c_{A^+})} = 2{\cdot}3(1 \pm 0{\cdot}5)\,\eta R\,T_i^2(\Delta h_i)^{-1} \qquad (2)$$

where Δh_i is the enthalpy change of the transition.

The dependence of the transition temperature on $\lg c_{A^+}$ will be linear, provided $T_i^2(\Delta h_i)^{-1} = \text{const.}$

Let us see how c_{A^+} depends on the change in the Gibbs free energy of the κ-carrageenan solution upon gelation at a temperature above the coil–helix transition temperature range, and also upon helix formation of macromolecules in the gel phase.

Gel formation at high temperatures is not accompanied by an appreciable change in the conformation of κ-carrageenan macromolecules. Grinberg *et al.* (1980) have suggested that associates of macromolecules in a random coil conformation are formed due to intermolecular ionic-coordination bonds involving potassium ions. We believe that similar interactions are responsible for the formation of the κ-carrageenan gel network. The discussion of the sol–gel transition is based on the assumption that in the sol state non-coulombic binding of counterions does not take place and the sol–gel transition involves both the formation of associates of macromolecules of κ-carrageenan and their linking-up into a gel network.

The increase in the concentration of potassium ions should shift the sol–gel transition equilibrium towards a state with a higher degree of potassium ion binding, hence promoting gel formation. At the same time, if the conformation of κ-carrageenan remains unaltered, the binding of potassium ions is accompanied by a decrease in the linear charge density. This is the reason why the shielding of charges causes the absolute value of ΔG for the sol–gel transition to decrease as c_{A^+} is increased.

For a single chain of κ-carrageenan in the helical conformation, the helical symmetry order, n, is 3, and the pitch, h, equals 2·43 nm (Anderson *et al.*, 1969). Thus, in the absence of non-coulombic binding of cations the linear charge density parameter $\xi = 0{\cdot}90\text{–}0{\cdot}94$ in the temperature range from 314 to 343 K. In the random coil conformation the average distance between the charged groups in a macromolecule is more than in the helical conformation, and $\xi < 0{\cdot}9$. Thus, it may be assumed that in the sol state the degree of binding of potassium ions is equal to zero and the concentration of charged polyanion groups equals the total number of ionizable groups. If β is the effective value of the fraction of free ionizable groups in the gel state*, we express the dependence of the gel point, T_g, on c_{A^+} as follows:

$$\frac{\delta T_g}{\delta(\log c_{A^+})} = -1\cdot15(1-\beta)R\,T_g^2(\Delta h_g)^{-1} \tag{3}$$

where Δh_g is the sol–gel transition enthalpy change.

In the double helix conformation the linear charge density parameter for κ-carrageenan, without taking into account the binding of ions during the sol–gel transition is given by $\xi_0 = 1\cdot80$ at 314·2 K. When ion binding is considered, $\xi = \beta\xi_0$. In Table 1 it shows that $\beta\xi_0 > 1$. This is the reason why the coil–double helix transition is accompanied by (1), the condensation of counterions and (2), an increase in the linear charge density. Both these factors determine the contribution of cation concentration to the Gibbs free energy of the coil–helix transition and stabilize the helical conformation.

The dependence of the midpoint temperature $T_{1/2}^{\text{c-h}}$, of the coil–helix transition on c_{A^+} is given by the equation

$$\frac{\delta T_{1/2}^{\text{c-h}}}{\delta(\lg c_{A^+})} = 3\cdot45\ [(\beta\xi_0)^{-1} - \beta]\,R\,(T_{1/2}^{\text{c-h}})^2\,(\Delta h_{\text{c-h}})^{-1} \tag{4}$$

where $\Delta h_{\text{c-h}}$ is the coil–helix transition enthalpy change.

Equations (3) and (4) contain three unknowns: β, Δh_g, and $\Delta h_{\text{c-h}}$. Having experimentally determined one of these quantities for the given value of c_{A^+} one can calculate the other two.

Braudo *et al.* (1984) have calculated the standard enthalpy of the helix–coil transition, using data from calorimetric and thermorheological studies and by assuming the helix–coil transition to be strictly cooperative. At 314·2 K $\Delta h^0_{\text{h-c}} = 5\cdot2$ kJ(g-equiv.)$^{-1}$. This assumption results in some underestimation of $h^0_{\text{h-c}}$. β and Δh_g however depend very weakly on $\Delta h_{\text{c-h}}$.

An initial value of $\Delta h_{\text{c-h}}$ of $-5\cdot2$ kJ (g-equiv.)$^{-1}$ was taken for the computation of β and Δh_g. As this value of $\Delta h_{\text{c-h}}$ was calculated without taking into account the enthalpy change that occurs during

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* Since, according to the law of mass action, the extent to which a cation is bound by a polyanion depends on c_{A^+}, β cannot be constant. Obviously, calculations yield an effective value of β appropriate for the given range of c_{A^+}.

cross-linking above the helix–coil transition range (Braudo *et al.*, 1984), it was refined by iteration.

The values of β and Δh_g computed for the potassium and sodium salts of κ-carrageenan in the presence of KCl are listed in Table 1. These parameters relate to systems for which $T_{1/2}^{c-h} = 314{\cdot}2$ K.

It should be noted that eqn (4) used in calculating β and Δh_g was obtained by making the following two assumptions:

1. The method for calculating the degree of condensation of counterions, developed by Manning for the so-called limit-diluted solutions, i.e. for solutions where there are no polymer–polymer interactions, is applicable to gel-forming systems;
2. The coil–helix transition does not result in additional non-coulombic binding of cations.

When the second assumption does not hold, the quantity computed using eqn (4) is influenced by cation binding at both stages of the structure-formation process. The fraction of free ionizable groups in the gel phase at temperatures higher than the coil–helix transition range will be underestimated and the absolute values of Δh_g will be overestimated by the calculation. Most likely, however, Δh_g is comparable with Δh_{c-h}. The values in Table 1 can be compared with the experimentally determined enthalpy of melting of a 1·84% gel of the potassium salt of κ-carrageenan in 0·1 M KCl of 9·4 kJ (g-equiv.)$^{-1}$ (Morris *et al.*, 1980*b*).

Rheological properties and the structure of κ-carrageenan gels

A question arises as to whether the coil–helix transition affects the structure of the cross-links of a κ-carrageenan gel network or whether this structure remains unchanged after its formation. Information on the structure of network cross-links can be obtained from the data on the creep of gels at different temperatures (Braudo *et al.*, 1984).

Figure 7 illustrates the temperature dependence of Young's modulus (calculated 3 h after the application of the stress) of a 1·5% gel of the sodium salt of κ-carrageenan containing a 3-fold equivalent excess of KCl. Also shown in Fig. 7 is a curve showing the variation in specific optical rotation of this gel upon heating. It is seen that the helix–coil conformational transition is accompanied by a decrease in Young's modulus. The completion of the conformational transition causes a change in the nature of the dependence of Young's modulus on tempera-

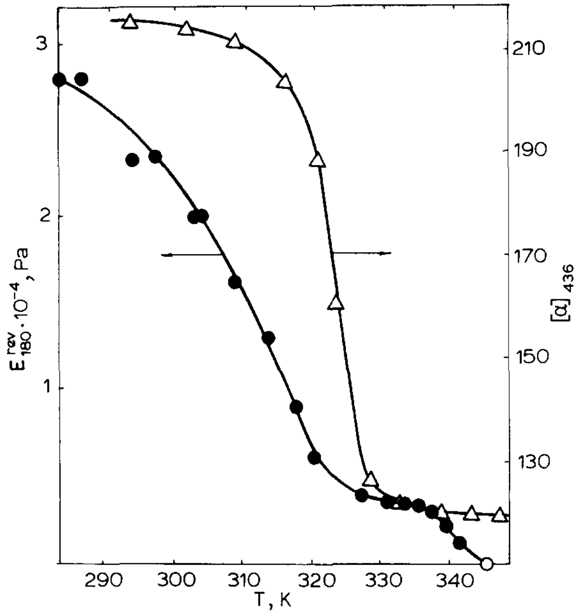

Fig. 7. Temperature dependence of Young's modulus of the gel of Na^+-salt of κ-carrageenan in 0·11 M KCl (●); temperature dependence of the specific optical rotation of the same gel (△). Polymer concentration, 3·7 × 10^{-2} g-equiv. $litre^{-1}$ (1·5%).

ture: the curve first goes parallel to the abscissa axis and then only goes down near the melting point. The change in the nature of the dependence of the elastic modulus on temperature may be due to the change in the nature of cross-links in the gel network due to a conformational transition in the κ-carrageenan macromolecules.

A De-Guzmán–Arrhenius plot of the temperature dependence of the largest Newtonian viscosity of a 1·5% gel of κ-carrageenan in 0·11 M KCl is shown in Fig. 8. Data for temperatures below 50°C have been taken from Plashchina *et al.* (1980).

The plot can be divided into three regions:

I ($T \leqslant 41$°C). In this region the helical conformation of macromolecules is stable;

II (41°C $< T \leqslant$ 54°C). This is the helix-coil conformational transition region;

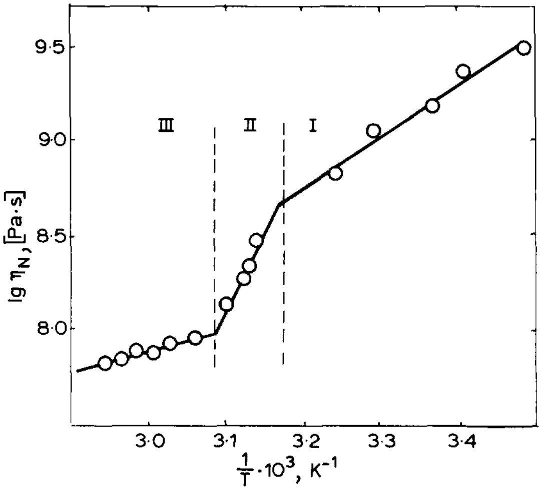

Fig. 8. Temperature dependence of the largest Newtonian viscosity of the gel of the Na$^+$-salt of κ-carrageenan in 0·11 M KCl. Polymer concentration, $3{\cdot}7 \times 10^{-2}$ g-equiv. litre^{-1} (1·5%).

III (54°C$<T\leqslant$66°C). In this region the gel network of κ-carrageenan is formed by macromolecules that have lost their helical conformation.

The activation energies for viscous flow, E_η^G, are listed in Table 2.

The master curve obtained by time–temperature superposition for the reversible component of the compliance in region III is shown in Fig. 9. Also shown in this Figure are the temperature dependences of the reduction parameters a_T and b_T. The activation energy of relaxation, E_{a_T}, and the standard breakdown enthalpy of the gel network cross-links, $\Delta H_{b_T}^0$, are summarized in Table 2.

The standard breakdown enthalpy of gel network cross-links can also be computed by the equation

$$\Delta H_1^{\ddagger} = E_\eta^G - E_\eta^S$$

where $E_\eta^S = 15{\cdot}0$ kJ mole^{-1} is the activation energy for the viscous flow of solvent (Braudo *et al.*, 1984). The values of $\Delta H_1^{\ddagger}$ are given in Table 2.

Table 2 also lists standard breakdown enthalpy of gel network cross-links, calculated by the equation of Ferry-Eldridge (Eldridge & Ferry, 1954),

TABLE 2

Thermorheological Characteristics of κ-carrageenan (1·5% Solution of the Na-salt of κ-carrageenan Containing 0·11 M KCl)

Temperature range	*Activation energy of relaxation, kJ mole*$^{-1}$ E_{a_T}	*Activation energy of viscous flow, kJ mole*$^{-1}$ E_η^G	*Standard breakdown enthalpy of network cross-links, kJ mole*$^{-1}$		
			$\Delta H_1^\ddagger$	$\Delta H_{b_\mathrm{T}}^0$	ΔH_f^0
I[a]	87 ± 2	51 ± 2	36 ± 2	27 ± 4	–
II[a]	–	250 ± 2	235 ± 2	–	–
III	96 ± 2	26 ± 1	11 ± 1	12 ± 2	38 ± 0·5[a,b]

[a] Data reproduced from Plashchina *et al.* (1980).

[b] This value relates to 0·25–1·5% concentration of κ-carrageenan in 0·11 M KCl.

$$\Delta H_\mathrm{f}^0 = -R\frac{\delta(\ln c)}{\delta T_\mathrm{f}}$$

where T_f is the melting temperature of the gel. This value for the standard enthalpy has been reproduced from Plashchina *et al.* (1980).

The difference between the parameters ΔH_{b_T} and $\Delta H_1^\ddagger$, on the one hand, and ΔH_f^0, on the other, is that the first two characterize the properties of a gel at one concentration over the temperature range used for the thermorheological studies, while the latter pertains to gels of different concentrations and characterizes the standard breakdown enthalpy of the most stable network cross-links which are preserved until melting. As seen from Table 2, $\Delta H_\mathrm{f}^0 > (\Delta H_{b_\mathrm{T}}^0)_\mathrm{III} \approx (\Delta H_1^\ddagger)_\mathrm{III}$. Here, the subscript III refers to the high-temperature region (Fig. 8).

The data in Table 2 reveals that the standard breakdown enthalpy of the κ-carrageenan gel network cross-links depends strongly on the conformational state of the macromolecules. At high temperatures, when the gel network is formed from non-helical macromolecules, the standard breakdown enthalpy of the network cross-links is relatively small. The appearance of the coil–helix conformational transition upon cooling the κ-carrageenan gel is accompanied by a 20-fold increase in the standard breakdown enthalpy of the gel network cross-links. This is indicative of a change in their structure.

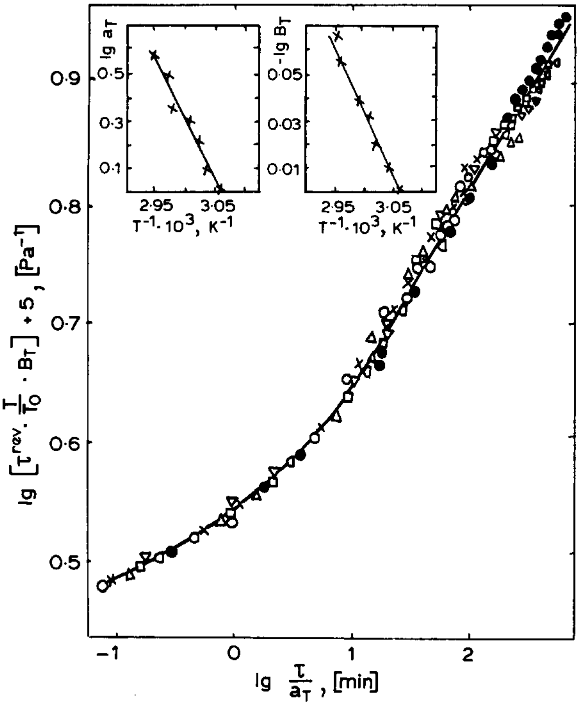

Fig. 9. Master curve for the reversible component of the compliance for the gel of the Na^+-salt of κ-carrageenan in 0·11 M KCl. Polymer concentration, $3 \cdot 7 \times 10^{-2}$ g-equiv. litre^{-1} (1·5%). Reference temperature, T_0, 327·2 K; ○, 327·2 K; x, 329·2 K; △, 331·2 K; □, 333·2 K; ▽, 335·2 K; ◁, 337·2 K; ●, 339·2 K.

The nature of the high cooperativity of the gel network cross-link breakdown process in the conformational transition range of κ-carrageenan is discussed by Plashchina *et al.* (1980) and Braudo *et al.* (1984). Here it should be mentioned that the helical segments of macromolecules form part of the cross-links of the gel network. The breakdown of the cross-links in region II is accompanied by a helix-unwinding of the segments, and the standard breakdown enthalpy characterizes not only the interaction in the cross-links between the helical segments, but also the internal structure of the segments.

Upon further lowering the temperature (transition into region I) the network cross-link breakdown process is deprived of the cooperative nature and standard breakdown enthalpy characterizes the interaction proper between helical segments in the network cross-links.

In regions I and III the activation energy of the relaxation of the κ-carrageenan gel has similar values. This conforms to the rupture of several secondary bonds.

In region III, as in region I, the activation energy of relaxation, E_{a_T}, exceeds the standard breakdown enthalpy of the gel network cross-links. Based on this, Braudo *et al.* (1984) have suggested that the slow relaxation processes recorded in the experiments on creep proceed in the structural elements of the gel network, enclosed between its cross-links.

The network in thermoreversible gels is, as a rule, formed by associates of macromolecules (Rogovina & Slonimsky, 1974). The high local viscosity in these associates determines the type of relaxation behaviour typical for the initial part of the transition zone from the glassy to the high elastic state (Braudo *et al.*, 1984). The curve for the reversible deformation relaxation of κ-carrageenan gel at temperatures above the helix–coil transition range has a similar shape (see Fig. 9). This suggests that in this case the gel network is also formed from the associates of macromolecules. As mentioned above, the existence of such associates in dilute solutions is shown by Grinberg *et al.* (1980).

REFERENCES

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

Arisz, L. (1915). *Kolloidchem. Beih.* **7**, 1.

Bachmann, W. (1912). *Z. anorg. Chem.* **73**, 124.

Beyer, G. (1954). *J. Phys. Chem.* **58**, 1050.

Boedtker, H. & Doty, P. (1954). *J. Phys. Chem.* **58**, 968.

Braudo, E. E., Isjumov, D. B. & Tolstoguzov, V. B. (1973). *Nahrung* **17**, 773.

Braudo, E. E., Plashchina, I. G. & Tolstoguzov, V. B. (1984). *Carbohyd. Polym.* **4**, 23.

Eldridge, J. E. & Ferry, J. D. (1954). *J. Phys. Chem.* **58**, 992.

Engel, J. (1961). *Arch. Biochem. Biophys.* **91**, 150.

Grinberg, N. V., Plashchina, I. G., Braudo, E. E. & Tolstoguzov, V. B. (1980). *Coll. Polym. Sci.* **258**, 1038.

Katti, P. K. (1952). *J. Chem. Phys.* **20**, 1980.

Krishnamurti, K. (1929). *Nature* **124**, 690.

Krishnamurti, K. (1930). *Proc. Roy. Soc.* **129**, 490.

Manning, G. S. (1972). *Biopolymers* **11**, 937.

Menz, W. (1909). *Z. Phys. Chem.* **66**, 129.

Morris, E. R., Rees, D. A., Norton, I. T. & Goodall, D. M. (1980*a*). *Carbohyd. Res.* **80**, 317.

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

Plashchina, I. G., Grinberg, N. V., Braudo, E. E. & Tolstoguzov, V. B. (1980). *Coll. Polym. Sci.* **258**, 939.

Plashchina, I. G., Gotlieb, A. M., Braudo, E. E. & Tolstoguzov, V. B. (1983). *Coll. Polym. Sci.* **261**, 672.

Rogovina, L. Z. & Slonimsky, G. L. (1974). *Uspehy Himii* **43**, 1102.

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

Vasilyev, V. G., Kozlov, O. A., Konstantinov, A. A., Krasheninnikov, S. K., Rogovina, L. Z. & Slonimsky, G. L. (1977). *Kolloidn. Zhurn.* **39**, 938.

Zsigmondi, R. (1905). *Zur Erkentnis der Kolloide. Ueber irreversible Hydrosole und Ultramikroscopie*, Fischer Verlag, Jena.

Zsigmondi, R. (1914). *Colloids and the Ultramicroscope*, Wiley-Chapman and Hall, New York and London.