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Structure Formation in Sodium κ-Carrageenan Solutions

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ABSTRACT

The methods of elastic light scattering and polarimetry were used to estimate the molecular weight, second virial coefficient and radius of gyration of the dissolved particles as well as the conformational state of the sodium κ-carrageenan macromolecules in a solution of 0·1 м *NaCl with respect to temperature. The process of aggregation of the macromolecules on cooling the diluted solutions was found to occur in two stages. The first stage consists of the association of disordered macromolecules, involving a two-fold decrease in the refractive index increment. The second, low-temperature stage of aggregation is the association of helical macromolecules. The data obtained do not contradict the ideas of the formation of double helices during the κ-carrageenan conformational transition.*

INTRODUCTION

It was shown in previous publications (Grinberg *et al.*, 1980; Plashchina *et al.*, 1985, 1986) that aggregation of macromolecules of κ-carrageenan and its gelation occur above the temperature range of the helix–coil transition, provided that the concentration of the potassium ions in the system exceeds a certain characteristic value (*c.* 4×10^{-2} mol litre^{-1}). Based on relaxation data at small strains, it was assumed that, irrespective of the conformational state of the macromolecules, the structural units of the κ-carrageenan gel are represented by macromolecular aggregates (Braudo *et al.*, 1984; Plashchina *et al.*, 1986). The purpose of the work reported in this paper is to study the temperature dependence of the structure formation processes in dilute κ-carrageenan solutions.

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By using elastic light scattering and polarimetry for this purpose information may be obtained about the temperature dependence of the molecular weight of the dissolved particles, their affinity for the solvent and the conformational state of the κ-carrageenan macromolecules. It was the authors' hope that by comparing the temperature behaviour of aggregation and the helix–coil conformational transition it would be possible to determine the contribution of the transition to the formation of the gel network structural units.

The study was made on a sodium salt of κ-carrageenan. This was chosen because although sodium ions have a promoting effect on gelation this is far less pronounced than that of potassium ions (Morris & Norton, 1983). This provided an opportunity to distinguish between the individual stages of structure formation.

MATERIALS AND METHODS

The κ-fraction of carrageenan was isolated from a commercial sample obtained from Sigma (USA, Type 1, No. C1013) by a two-fold reprecipitation with potassium chloride from its aqueous solution and subsequent purification by electrodialysis and ion-exchange chromatography as described elsewhere (Grinberg *et al.*, 1980).

Preparation of solutions

The sodium κ-carrageenan was dissolved in 0·005 M EDTA at room temperature. Solutions of *c.* 0·3% (v/v) concentration were dialysed for three days against 0·1 M NaCl, pH 7·0. κ-Carrageenan concentration was determined by the phenol–sulphuric acid method (Dubois *et al.*, 1956).

The solutions were purified by centrifugation for 4 h at $1{\cdot}4 \times 10^5$ g. A set of 5–6 samples was prepared by dilution of the starting solution with the dialysate. To remove dust the solutions were filtrated under pressure into dust-free cuvettes through ultrafilters with a pore size of 0·45 μm (Selectron, FRG) at 80°C.

Optical rotation measurements

The specific optical rotation of the sodium κ-carrageenan solutions in 0·1 M NaCl was measured on a Jouan Roussel polarimeter at 436 nm in a thermostatted cuvette. The optical path length was 0·05 dm. The temperature was varied at a rate of 1·5°C min^{-1}, both on cooling and heating. The solution was stored at each measurement temperature for

20 min. The temperature in the polarimeter cuvette was recorded by use of a thermocouple to within ± 0·3°C.

Refractive index increment determinations

The refractive index increment $(\partial n/\partial c_2)$ was determined on a Shimadzu differential refractometer at 436 nm over the concentration range 0·1–0·8%. The temperature was varied in the same manner as in the polarimetric measurements. The accuracy of $(\partial n/\partial c_2)$ measurements at each temperature was within $(\pm 0{\cdot}007$ to $\pm 0{\cdot}01) \times 10^{-3}$ m^3 kg^{-1} at a confidence level of 0·95.

Light scattering measurements

Light scattering measurements were made with an FPS-3M photoelectric nephelometer (Central Design Bureau for Unique Apparatus, USSR Academy of Sciences) with nonpolarised light at 436 nm. The Rayleigh constant of dust-free benzene used as a standard was $R_{u90} = 47{\cdot}3 \times 10^{-8}$ m^{-1} (Eskin, 1974). Concentrations were in the range 0·05–0·3%. The rate of temperature variation was the same as that used in the polarimetric experiments. The sample was stored at each measurement temperature for 5 min with subsequent measurement of the angular light scattering intensity dependence between 30 and 150°. The sample was exposed to each temperature for a total of 20 min. Such time–temperature conditions permitted reproducible results to be obtained.

Treatment of the light scattering data

Zimm plots, i.e.

$$\frac{Kc}{\Delta R_\theta} = f\left(\sin^2\frac{\theta}{2} + kc\right)$$

at small angles were observed to become non-linear which can be attributed to the contribution of a small fraction of very large particles (Huglin, 1972; Kratochvil, 1982). In the case of biopolymer solutions this effect is normally caused by microgel particles.

The data were analysed by use of a two-component model where one component is represented by microgel particles and the other by macromolecules or their small aggregates, which will be termed 'dissolved particles'. According to this model, the molecular weight of the mixture $(\bar{M}_w)$ can be expressed as follows:

$$\bar{M}_w = \bar{M}_{w1} \cdot m_1 + \bar{M}_{w2} \cdot m_2 \tag{1}$$

where $\bar{M}_{w1}$ and $\bar{M}_{w2}$ are the weight-average molecular masses of the microgel and dissolved particles, and m_1 and m_2 are the weight fractions of each type of the particles, respectively. The solvent was ignored, i.e. $m_1 + m_2 = 1$.

In the present work two procedures for treating the experimental data, viz. the Zimm and Guinier methods, were used. The joint use of these methods makes it possible to estimate the mass and size of the two types of particles and enhance the reliability of the data obtained from analysis at small angles (Glöckner *et al.*, 1975; Francuskiewicz & Glöckner, 1979; Dautzenberg, 1982). Data treatment by use of the two methods is illustrated in Figs 1 and 2.

With the Zimm method the $\bar{M}_{w2} \cdot m_2$ value was calculated from an intercept obtained by extrapolation from the large angle region to the axis corresponding to zero concentration. The second virial coefficient was determined by the standard method (Huglin, 1972), the extrapolation to zero angle also being made from the large angle region. The radius of gyration of the dissolved particles using the Zimm method was not estimated because of the large error involved (Kratochvil, 1982).

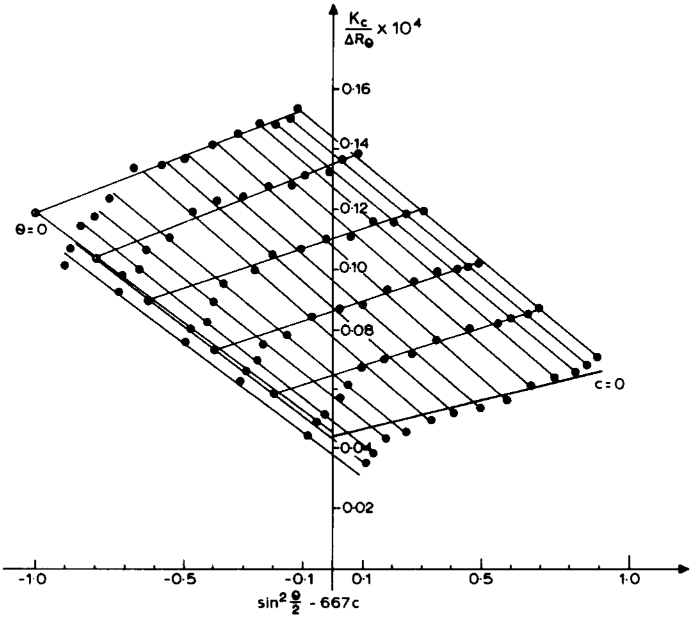

Fig. 1. Characteristic Zimm diagram for the sodium κ-carrageenan solutions in 0·1 M NaCl, obtained in the cooling mode; $T = 30°C$.

To facilitate extrapolation during the construction of the Zimm diagrams (Fig. 1) negative values of k (the proportionality factor for c_2) were used as recommended by van Wijk & Staverman (1966).

With the Guinier method the $\bar{M}_{w2} \cdot m_2$ value was calculated from the intercept obtained by extrapolation from large scattering angles ($\theta > 60°$) to the axis corresponding to zero concentration, whereas the radius of gyration of the dissolved particles, $\langle R_g^2 \rangle_{z2}^{1/2}$, was obtained from the slope at the same range of scattering angles. The range of scattering angles for

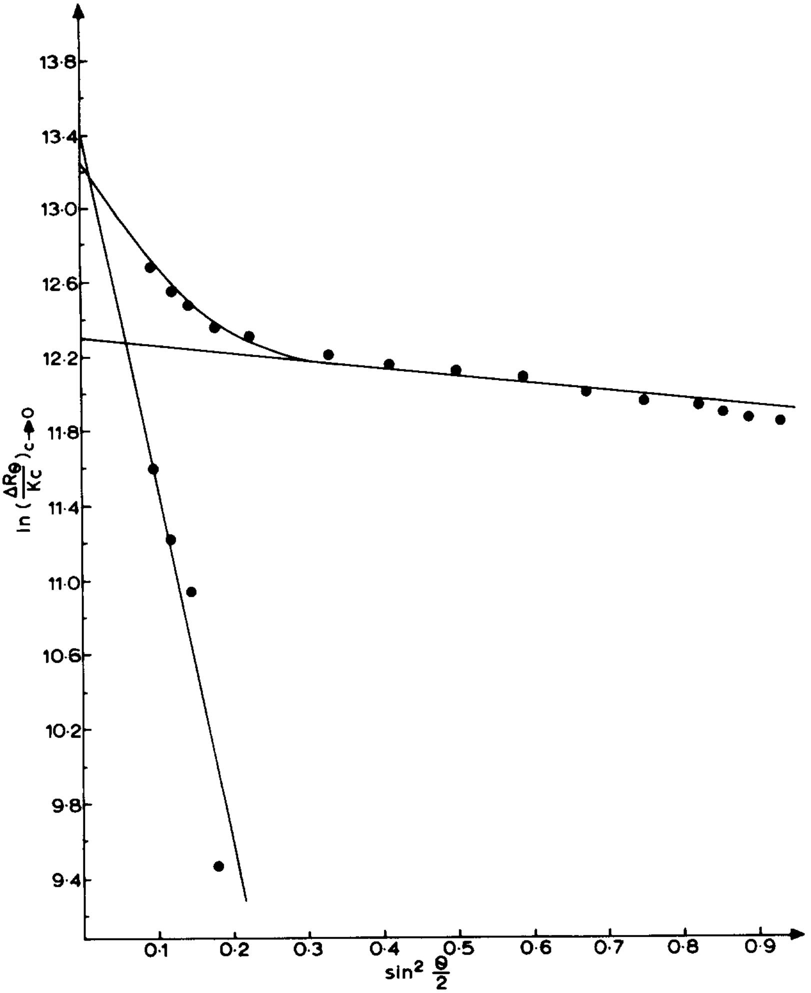

Fig. 2. Characteristic Guinier diagram for the sodium κ-carrageenan solutions in 0·1 M NaCl, obtained in the cooling mode; $T = 30°C$.

extrapolation was selected according to a criterion proposed by Glöckner *et al.* (1975).

The m_1 value was calculated using the equation (Francuskiewicz & Glöckner, 1979):

$$m_1 = 1 - \frac{(m_2.\bar{M}_{w2})/K'_2.(\langle \bar{R}_g^2 \rangle_{z2}^{1/(1+\varepsilon_2)})}{(m_2.\bar{M}_{w2})/K'_2.(\langle \bar{R}_g^2 \rangle_{z2}^{1/(1+\varepsilon_2)}) + (m_1 \bar{M}_{w1})/K'_1.(\langle \bar{R}_g^2 \rangle_{z1}^{1/(1+\varepsilon_1)})} \qquad (2)$$

where $\varepsilon_i = (2a_i - 1)/3$, the a_i being the exponent in the Mark–Howink equation and

$$K'_i = \frac{\bar{M}_{wi}}{\langle \bar{R}_g^2 \rangle_{zi}^{1+\varepsilon_i}}$$

The remaining parameters are as defined above. Following Lange (1970) it was assumed that $K'_1 = K'_2$. The accepted value of 0·5 was given to a_1 (Francuskiewicz & Glöckner, 1979). The value of a_2 of 0·86 at 20°C reported by Vreeman *et al.* (1980) was used. Accordingly, the m_1 value was calculated for the same temperature. The resulting m_1 value was found to be *c.* 10^{-2}. For further calculations m_2 was taken to be *c.* 1.

RESULTS

The temperature dependence of the characteristics of the dissolved particles of the κ-carrageenan sodium salt in 0·1 M NaCl, obtained on cooling the solutions, are reported in Fig. 3. A temperature decrease from 80°C to 60°C results in poorer polymer affinity for the solvent, which is shown by a smaller value of the second virial coefficient A_2 (Fig. 3(d)). The poorer affinity for the solvent causes aggregation of the κ-carrageenan macromolecules. As this takes place, its molecular weight (Fig. 3(a)) and radius of gyration (Fig. 3(b)) increase approximately threefold. At the same time, as can be seen from the dependence of the specific optical rotation on temperature (Fig. 3(c)), no cooperative conformational coil–helix transition of the κ-carrageenan macromolecules in this region is observed. Thus the first stage of the aggregation process occurs under conditions when the macromolecules have an unordered conformation.

The appearance of multiple bonds during aggregation is possibly shown by a sharp change in the refractive index increment in this temperature range (Fig. 3(e)). The decrease in the $(\partial n/\partial c_2)$ value is caused by either a change in the polarisability of the components or their partial specific volumes.

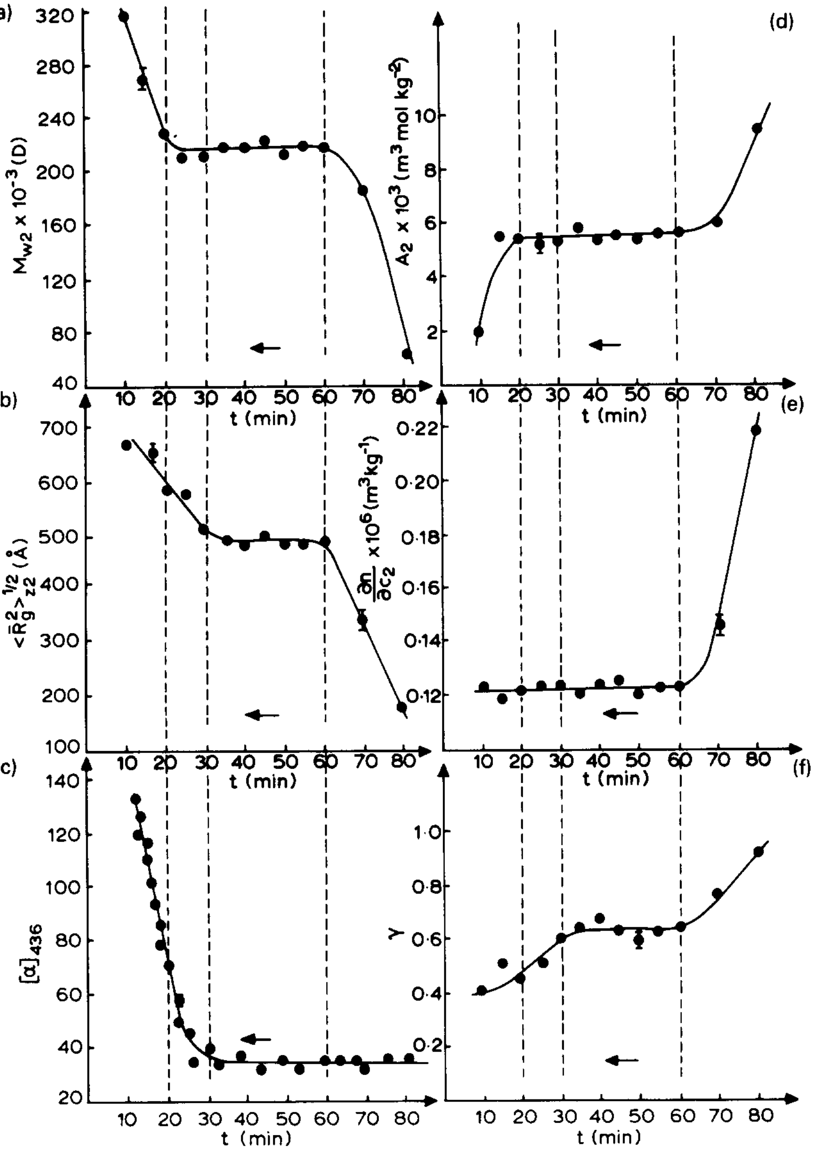

Fig. 3. Temperature dependence of the characteristics of the dissolved sodium κ-carrageenan particles in 0·1 M NaCl. Cooling rate 1·5°C min^{-1}. (a) molecular weight ($\bar{M}_{w2}$); (b) radius of gyration $\langle \bar{R}_g^2 \rangle_{z2}^{1/2}$; (c) specific optical rotation at 436 nm; (d) second virial coefficient (A_2); (e) refractive index increment; (f) ratio of the radius of the equivalent solid sphere to that of gyration of the dissolved particles (γ).

With a further decrease in temperature from 60°C to 30°C no aggregation takes place. Beginning with a temperature of *c.* 25°C one can observe helix-formation of the macromolecules, which is shown by the polarimetric data (Fig. 3(c)). This process is accompanied by an increase in the radius of gyration (Fig. 3(b)). In this case the constancy of the molecular weight points to the fact that the conformational coil–helix transition occurs inside the aggregates. Further cooling of the solution brings about a decrease in A_2 and an increase in the molecular weight. In other words, at a low temperature there will be aggregation of the helical macromolecules.

The aggregation of the κ-carrageenan macromolecules in the unordered conformation also involves a decrease in the γ value (the ratio of the radius of the equivalent solid sphere to that of gyration of the dissolved particles (Fig. 3(f)). The γ value was calculated using the following equation (Tanford, 1961):

$$\gamma = \left(\frac{3}{16\pi N_A}\right)^{1/3} \frac{A_2^{1/3} \cdot M_{w2}^{2/3}}{\langle \bar{R}_g^2 \rangle_{z2}^{1/2}}$$

where A_2 is the experimental value of the second virial coefficient. It would be more correct to use the quantity A_2^{EV}, i.e. a component of A_2 corresponding to the contribution of the excluded volume effect. This quantity is the difference between A_2 and A_2^C, where A_2^C is the electrostatic component of A_2. The method of calculating A_2^C is described elsewhere (Plashchina *et al.*, 1985). The calculation performed for 25°C indicates that the electrostatic component contribution to A_2 is negligible ($A_2^C = 0{\cdot}1 \times 10^{-3}\ \mathrm{m^3\ mol\ kg^{-2}}$).

At 80°C the value of γ approximates to the theoretical one for the isolated molecules of flexible-chain polymers in a good solvent (Berry & Casassa, 1970). Decreasing γ in the high-temperature region suggests a stronger attraction between the dissolved particles. The conformational coil–helix transition also involves decreasing γ (Fig. 3(f)). This may be due both to an enhanced permeability of the coils under the effect of the helical segments and to a stronger attraction between the dissolved particles.

Studies were also made of the disintegration of the structural units of sodium κ-carrageenan in 0·1 M NaCl on heating the solutions using the same time–temperature conditions as in the case of the structure formation studies (Fig. 4). The polarimetric data (Fig. 4(c)) show that the low-temperature aggregation stabilises the helical conformation with the result that the conformational coil–helix transition becomes less cooperative and shifts towards higher temperatures. The helix unwind-

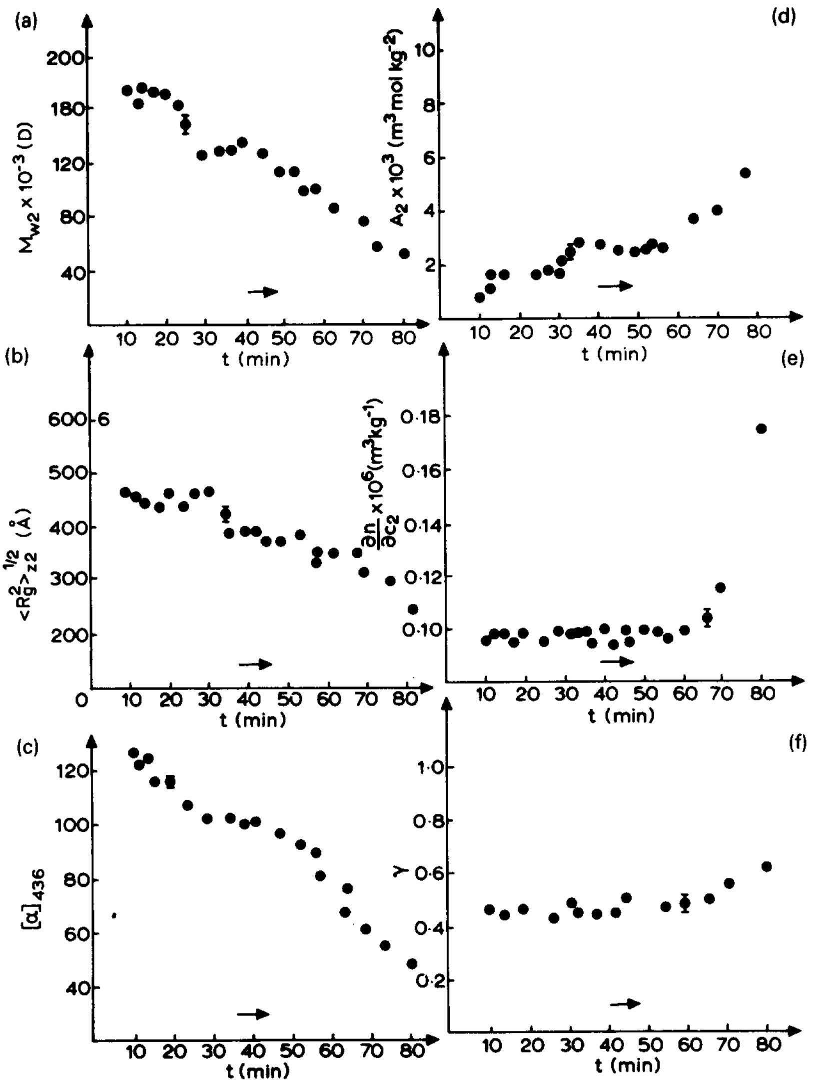

Fig. 4. Temperature dependence of the same characteristics as in Fig. 3, obtained on heating the sodium κ-carrageenan solutions in 0·1 M NaCl at a rate of 1·5°C $\min^{-1}$.

ing process begins to show a two-stage behaviour. The same phenomenon was reported elsewhere (Morris *et al.*, 1980*a*; Rinaudo & Rochas, 1981). The authors relate this phenomenon to the melting of the isolated and aggregated helices.

The wide temperature range over which the helix-coil transition occurs makes it impossible to discriminate between the principal steps of the κ-carrageenan solution structure disintegration.

Thus comparison of the processes associated with the formation and disintegration of the aggregates of macromolecules during the cooling and heating of the κ-carrageenan solutions, respectively, shows the presence of a significant temperature hysteresis due to the increase of structure formation and stabilisation of the helical conformation with solution ageing. These findings are consistent with the ideas of the origin of the temperature hysteresis discussed by other authors (Rees, 1969; Morris & Norton, 1983).

DISCUSSION

The present studies indicate that aggregation of the macromolecules of the sodium salt of κ-carrageenan during the cooling of its solutions occurs in two stages. The first stage consists of the aggregation of macromolecules having an unordered conformation. This takes place at 80–60°C, i.e. above the temperature range of the helix–coil conformational transition. The process involves a sharp decrease in the refractive index increment, which suggests the formation of multiple bonds in the system.

The data obtained point to the fact that at a sufficiently high concentration of ions promoting the gelation process helix-formation is not a prerequisite for macromolecules in solution to aggregate, contrary to what is postulated by the domain model advocated by other authors (Morris *et al.*, 1980*b*).

At low temperatures, when the κ-carrageenan macromolecules are mainly in the helical conformation, one can observe the occurrence of a second stage of aggregation. The interaction of the helical segments is likely to be its motive force. The process is not accompanied by a change in the refractive index increment, which may be due to the absence of any selective bonding of the sodium ions by the helical segments of the κ-carrageenan macromolecules (Grasdalen & Smidsrød, 1982; Smidsrød & Grasdalen, 1982).

Aggregation of macromolecules is, as a rule, a prerequisite for network formation in thermoreversible gels (Rogovina & Slonimsky, 1974). Both formation of aggregates and their association into a network stem from a deterioration of the thermodynamic quality of the solvent down to a certain optimal level (Tolstoguzov & Braudo, 1983). The data in Fig. 3(d) indicate that an increase in the molecular weight of the dissolved particles both at the low and high temperatures involves a drop in their second virial coefficient, i.e. with a lowering of their affinity for the solvent.

The literature contains two concepts for the mechanism of helix-formation in κ-carrageenan macromolecules in solution. On the one hand, the systematic studies of Rees, Morris and other researchers (Morris & Norton, 1983) showed the presence of a double helix in liquid solutions. At the same time, according to other authors (Smidsrød *et al.*, 1980), the change in optical rotation of κ-carrageenan between 60°C and 30°C, when they observed the helix–coil conformational transition, does not depend on concentration. Formation of macromolecule helices of κ-carrageenan does not involve a change in its molecular weight (Smidsrød *et al.*, 1980; Smidsrød & Grasdalen, 1982). These results were interpreted as favouring the idea that the process of helix-formation occurs within the single chains to give rise accordingly to single helices.

Our results suggest that in the temperature range studied by Smidsrød *et al.* (1980), i.e. 30–60°C, the aggregates of κ-carrageenan macromolecules are stable, their formation and disintegration occurring at higher temperatures. Therefore the data reported elsewhere (Smidsrød *et al.*, 1980; Smidsrød & Grasdalen, 1982) cannot be regarded as advocating the formation of a single helix.

Even at the stage preceding helix formation the κ-carrageenan solutions contain regions with a higher local concentration of the polymer (aggregates). The cooperative conformational transition takes place inside these aggregates (see also Grinberg *et al.*, 1980). Accordingly, the effective concentration of the unordered and helical forms is controlled by a conversion within the aggregate but is independent of that in the system as a whole. In other words, macroscopically the coil–double helix transition is zero order with respect to the polymer concentration.

The concentration independence of the specific optical rotation during the conformational transition of κ-carrageenan is another example of the independence of the properties of the microheterogeneous system from concentration. Among such systems, not only semi-diluted solutions and gels of some of the anionic polysaccharides (Yuryev *et al.*, 1981; Braudo *et al.*, 1984) and gels of some of the denaturated proteins (Bikbov *et al.*, 1981) but sometimes, as shown by this work, also diluted solutions may be cited.

CONCLUSIONS

The aggregation of macromolecules on cooling dilute solutions of a κ-carrageenan sodium salt in 0·1 M NaCl occurs in two stages. The first stage consists of the association of the disordered macromolecules. It is accompanied by a two-fold decrease in the refractive index increment.

The second, low-temperature stage involves the association of the helical macromolecules.

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