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Thermoreversible gelation of κ-Carrageenan: relation between conformational transition and aggregation

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Abstract

We have studied, by optical rotation dispersion, light scattering and rheology, the κ-Carrageenan system to elucidate the processes involved in gel formation (on decreasing the temperature) and gel melting (on increasing the temperature). Our results show that, on decreasing the temperature, a conformational transition from coils to double helices first occurs, followed by aggregation of the double helices into domains and gel formation at appropriate polymer concentration. Structural details of this sequence are better revealed by re-heating the system. Melting appears as a two-step process characterized by first a conformational change of helices involved in junction zones between aggregates, followed by the conformational transition of the helices inside the aggregates. These helices can regain the coil conformation only when the aggregates melt at higher temperature, in full agreement with the old 'domain' model. The full description of the sol–gel mechanism of this system can be useful in the search for new methods to control the gel texture, a relevant property for many industrial applications.

Keywords: Self-assembly; Gelation; Carrageenan

1. Introduction

The carrageenans are a family of linear water-soluble polysaccharides extracted from different species of marine red algae. Different types of carrageenans can be distinguished by their primary structure [1]: κ-Carrageenan is composed of alternating α-(1-3)-D-galactose-4-sulphate and β-(1-4)-3,6-anhydro-D-galactose; ι-Carrageenan differs only by the presence of an additional sulphate group at carbon 2 of 1,4 linked galactose unit [2,3]; λ-Carrageenan presents a third sulphate group at carbon 6 of 1,4 linked galactose unit. They are used extensively as thickening, gelling agents or texture enhancers, stabilizers, etc., in the food and pharmaceutical/cosmetic industries [4–6].

In aqueous solutions and in the presence of cations, both κ- and ι-Carrageenans easily form, on cooling, thermoreversible gels, whereas gelation of λ-Carrageenan has never been observed. All

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types of carrageenans act as stabilizer by modifying the rheological properties of the continuous aqueous phase [7].

It is known that thermoreversible gelation of κ-Carrageenan involves a coil–helix conformational transition, which may be followed by aggregation of the ordered molecules to form an infinite network [8,9]. The gel formation in aqueous solution is a complex process that depends on polysaccharide chemical structure, nature of co- and counterions, polymer concentration and temperature [10–12].

Due to the broad range of structural factors and the variety of experimental procedures used by different investigators, it is not surprising that several controversies on the kinetics of gel formation and nature of the ordered conformations of κ-Carrageenans persist. The type of conformational transition (coil–helix or coil–double helix), the relation between conformational transition and gelation, the sequence of steps leading to the gelation, have been, and still are, matter of debate [11,13,14].

In previous work on several polymeric or proteic systems [15–17], we have found that the macromolecular self-assembly is the result of mutual interaction between different processes such as conformational transition, phase demixing and cross-linking. Results have shown that the final gel structure is highly dependent on kinetic competition between these processes [17]. Here we present results obtained on water solution of κ-Carrageenan in presence of different amounts of KCl by combined measurements of static and dynamic light scattering, optical rotation dispersion (ORD) and rheology. Results reported here elucidate all processes involved in the formation of a gel (on decreasing temperature) and its melting (on increasing temperature). The causal relationship between conformational transition, aggregation and gelation is also elucidated. In fact, data show that on decreasing temperature: (i) a conformational change from coil to double helices is the leading step that allows the formation of domains of helices that aggregate, giving rise to network formation and consequent gelation; (ii) the aggregation process depends on κ-Carrageenan concentration and temperature, the latter affecting only the rate of aggregation kinetic. On increasing temperature we have observed in the ORD signal the presence of hump, which amplitude and position depend on the time allowed the system to stay at low temperature (below the transition temperature) where the aggregation develops. In this case ORD data can be fitted to a double sigmoid transition confirming that the melting can be described in terms of two processes occurring at two different temperatures: (i) change of conformation of helices involved in the junction zones and consequent breaking of the network (at lower temperature); (ii) melting of aggregates and consequent conformational change of helices involved in aggregates (at higher temperature).

The deeper understanding of the mechanism involved in the sol–gel–sol transition in this system can be useful to find new methods apt to control the texture of κ-Carrageenan gels. This possibility can be relevant for many industrial applications where κ-Carrageenan is used for its stabilizing and gelling properties [18].

2. Materials and methods

The κ-Carrageenan (type X-6913, Lot 63-80270) was a gift from Copenhagen Pectin A/S, Denmark. The sample was prepared in accordance with the procedure of other authors [12]. It consists in dissolving the κ-Carrageenan powder in hot Millipore deionised water (70 °C) including 200 ppm sodium azide as a batteriostatic agent, and stirring at the same temperature for 2 h. The pH of the solution was 8.7 to avoid the hydrolysis during preparation [19,20]. Then, the solution was dialyzed against Millipore water to eliminate excess salt. Hot Millipore water containing the appropriate amount of KCl was added to set the final ionic conditions. The solution was finally filtered at high temperature through a 0.22 μm membrane directly in the couvette or rheometer holder.

Static and dynamic light scattering measurements were done using a Brookhaven Instrument BI-9000 digital correlator and an ILT 550 Argon laser tuned at 514.5 nm. Samples prepared as described above were put into a thermostated cell compartment of a Brookhaven Instrument BI200-

SM goniometer system. Temperature was controlled to within ±0.1 °C using a thermostated recirculating bath. The shape factor $P(\theta)$ was obtained by recording light scattering intensity at a different scattering vector $q=4\pi n\lambda_0^{-1}\sin(\theta/2)$, where n is the refraction index of solution, λ_0 is the wavelength of the incident light and θ is the scattering angle. Time correlation function was analyzed by standard cumulant method [21]. A second- and third-order cumulant fit were performed on data. Both analyses give very close values of average diffusion coefficient and polydispersity index. An estimation of the average hydrodynamic radius was obtained by using the Stokes–Einstein relationship [22].

Optical rotation measurements were performed using a Jasco P-1020 polarimeter. All measurements were made at $\lambda=589$ nm (Na lamp) using a thermostated cell (optical path 10 cm). Also, in this case, the temperature was controlled to within ±0.1 °C using a thermostated recirculating bath.

Rheological measurements under low amplitude oscillatory shear were performed on a controlled stress AR-1000 rheometer (TA Instruments, UK) using a standard-size double concentric aluminium cylinder (rotor outer radius 21.96 mm, rotor inner radius 20.38 mm, stator outer radius 20.00 mm, cylinder immersed height 59.50 mm, gap 500 μm). All measurements were performed at a frequency of 0.5 Hz and a strain of 0.2. The hot solution was loaded into the cylinder set at 50 °C. The cylinder–cylinder upper gap was coated with silicone oil to minimize loss of solvent at high temperature. Storage modulus (G′) and loss modulus (G″) were monitored as functions of temperature and time.

Temperature scan experiments were performed using a thermostated circulating bath with a home-built temperature scan controller. The temperature rate was set to 0.2 °C/min in all experiments. For temperature quench experiments, we used two circulating baths thermostated at initial and final temperature values. The sample was firstly equilibrated at the initial temperature and, then, brought to the new temperature by switching the flow of thermostating liquid. In this way the time required to thermally equilibrate the sample is approximately 90 s.

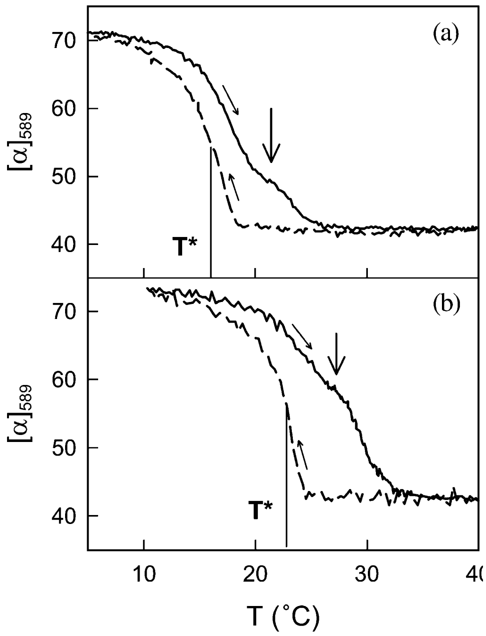

Fig. 1. Temperature dependence of the ORD signal of 0.1% (w/w) κ-Carrageenan solutions at two different salt concentrations (a: 10 mM KCl; b: 5 mM KCl) on cooling (broken line) and heating (continuous line). T^* indicates the midpoint transition temperature. A hump (indicated by an arrow) is visible on heating.

3. Results and discussion

The temperature induced conformational transition of κ-Carrageenan was monitored by optical rotation measurements. Results for samples at 0.1% w/w polymer concentration and two different amounts of KCl (5–10 mM) are shown in Fig. 1. Starting from high temperature (≈40 °C), the sample was first cooled and then re-heated at the same (0.2 °C/min) scanning rate. Data show that the transition midpoint temperature (T^*) in the cooling process shifts to higher values at increasing salt concentration, in agreement with literature results [8,10,14]. The process is thermoreversible with a hysteresis width that increases with KCl concentration. The presence of a hysteresis, taken as direct evidence of aggregation, has been widely discussed with relation to the gelation mechanism and nature of the ordered conformation [8,10,14]. Indeed, it is still not resolved whether conformational change involves a coil–helix or coil–double helix transition [8,13,14]. More recently, it has been proposed that, at increasing ionic concentration, the equilibrium between single helix and helical dimer changes in favor of dimeric form, which is the only one capable of undergoing aggregation [14]. Regardless of which conformational change actually occurs, the dependence of hysteresis width on ionic concentration evidences the role of K^+ ions in promoting aggregation between chains in ordered conformation. The existence of a hump (indicated by an arrow in the figure) in the heating process indicates that melting is a two-step process. This has been previously interpreted in terms of two populations of helices that undergo the conformational transition at different temperature: free helices in solution and helices involved in aggregates that can regain the coil conformation only when the aggregates melt at higher temperature [8]. Data also show that at increasing the K^+ concentration, the hump amplitude increases, further supporting the hypothesis that the hump is due to the presence of aggregates. To better verify this hypothesis, we performed a set of similar experiments at increasing polymer concentration and fixed salt content. Indeed, under these conditions, the conformational transition, the leading event in the aggregation, occurs at the same temperature [20,23]; this implies that samples at different polymer concentration became capable of undergoing aggregation at the same temperature value. Therefore, scanning experiments can be done in the same temperature range and effects of polymer concentration on aggregation can be consistently compared, temperature being equal.

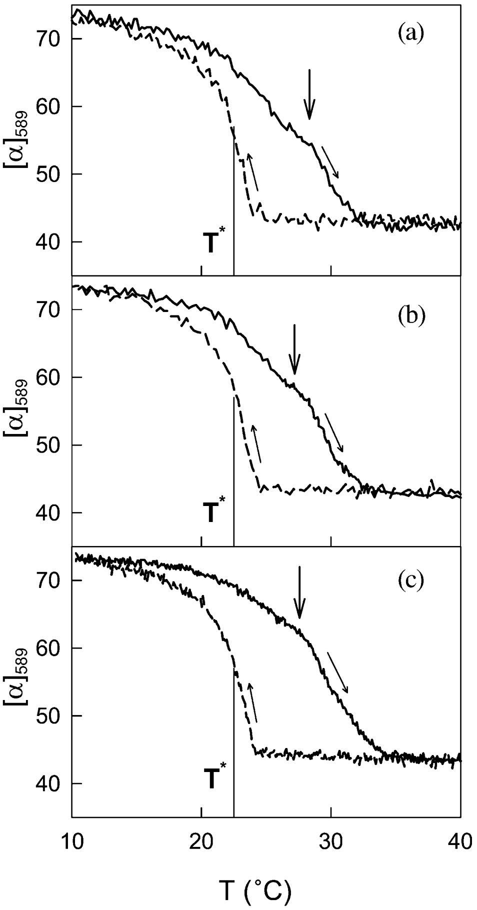

Fig. 2. Temperature dependence of the ORD signal at different κ-Carrageenan concentration (a: 0.05% w/w; b: 0.1% w/w and c: 0.2% w/w) and fixed salt amount (10 mM KCl). T^* indicates the midpoint transition temperature. Lines are as in Fig. 1. The arrow indicates the hump on heating.

ORD results on cooling and heating cycles are shown in Fig. 2. Data refer to samples with the same KCl concentration (10 mM) and different amounts of κ-Carrageenan (0.05, 0.1 and 0.2% w/w). The temperature of conformational transition on cooling is found independent on κ-Carrageenan concentration, in agreement with literature results [20,23]. Hysteresis width and hump amplitude observed in the heating run increase with polymer concentration, consistently with the hypothesis that both quantities are hallmarks of aggregation; indeed, it can be expected that at increasing polymer concentration, the population of helices taking part into aggregates increases so enhancing the hump contribution to ORD signal. The latter can be described by the sum of two sigmoids representing two inverse conformational transitions:

$$y=y_0+\frac{A_\mathrm{L}}{1+\exp\left[-\dfrac{T_\mathrm{L}-T}{W_\mathrm{L}}\right]}+\frac{A_\mathrm{H}}{1+\exp\left[-\dfrac{T_\mathrm{H}-T}{W_\mathrm{H}}\right]} \qquad (1)$$

where $A_{i=\mathrm{L,H}}$, $W_{i=\mathrm{L,H}}$ and $T_{i=\mathrm{L,H}}$ are amplitude, width and midpoint temperature; the subscripts L

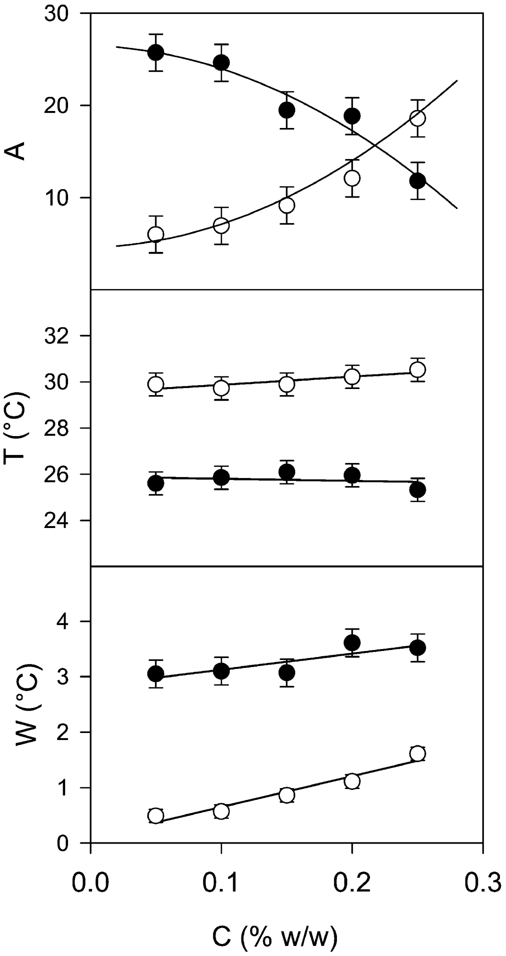

Fig. 3. Fit parameters of melting ORD signal to Eq. (1) in the text, vs. κ-Carrageenan concentration. Salt concentration is 10 mM for all samples. From top to bottom: amplitude, midpoint temperature and width of the sigmoid transitions at low (full symbols) and high temperature (open symbols).

and H refer, respectively, to low temperature transition of free helices and to high temperature transition of helices taking part into aggregates.

Data measured on samples at different polymer concentration were fitted to Eq. (1). Fit parameters A, T and W are reported in Fig. 3 vs. polymer concentration. The figure shows: (i) a progressive A_L decrease and complementary A_H increase (top panel); (ii) no appreciable change of T_L and T_H (middle panel); (iii) a small W_H increase, indicating a progressive decrease of cooperativity, and a nearly constant W_L value (bottom panel). Altogether the dependence of fit parameters on polymer concentration indicates that the increase of the hysteresis width is mainly due to the change of balance between the two populations. It is also useful noting that W_H is always smaller than W_L, that is the high temperature conformational transition is, in any case, more cooperative. This further supports the hypothesis that the second transition concerns chains involved in aggregates that hinder the restoring of disordered conformation; only when the aggregates melt, chains can suddenly regain the coil conformation.

As just discussed, hysteresis width and hump amplitude of ORD signal reflect the aggregation extent. This is, in turn, determined by the aggregation rate and waiting time for the process. The experiments discussed above had shown the effect of different aggregation rate as controlled by polymer concentration. To check the effect of different waiting time, a sample at 0.2% κ-Carrageenan and 10 mM KCl was cooled to 10 °C at 0.2 °C/min and kept at this temperature for 15 h before starting the heating run. Panel A of Fig. 4 shows ORD signal obtained in this case (curve no. 2) as compared with that obtained when the heating run starts just after the end of the cooling (curve no. 1). The hysteresis is larger and the hump is hardly visible. In the frame of a two-step inverse transition, as described by Eq. (1), this change could be ascribed to amplitude increase of the second transition that becomes predominant with respect to the first one. This suggests to fit curve no. 2 to Eq. (1), with T_L and W_L kept equals to values found for the curve no. 1, and A_L, A_H, T_H and W_H left as free parameters. Table 1 reports fit parameters for both curves. Best fit of curve no. 2 gives a sensibly smaller A_L and larger A_H, indicating that, waiting enough time for a larger aggregation extent, melting process is dominated by melting of chains involved in strong structures (aggregates). The effect of different waiting time is also studied by rheological experiments. Behaviors of the elastic modulus (G′) and viscous modulus (G″) are, respectively, shown in panels B and C of Fig. 4. The two vertical lines drawn on the figure in correspondence of T_L and T_H values of ORD curve no. 2 are of help for comparative

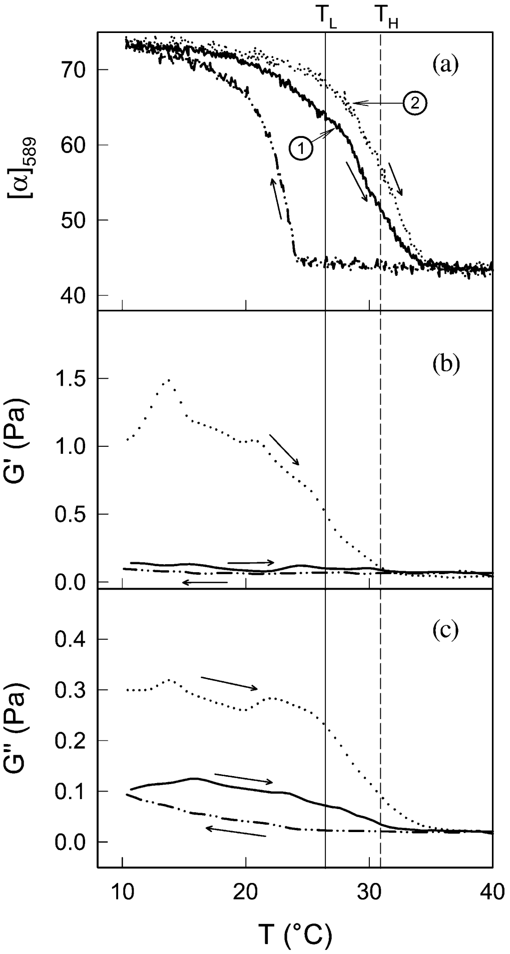

Fig. 4. Temperature scan experiments on 0.2% κ-Carrageenan in 10 mM KCl: (a) ORD signal; (b) elastic modulus (G′); (c) viscous modulus (G″). Dot-dashed line: cooling run; continuous line: heating run started just after the cooling; dotted line: heating run started after 15 h at low temperature. The two vertical lines drawn on the figure indicate the values of inverse transition temperatures T_L and T_H, as obtained by fitting the curve no. 2 to Eq. (1) in the text.

purpose with ORD data. When the heating run starts immediately, G′ does not change, while G″ displays a small hysteresis loop corresponding to that of the ORD signal, accordingly with the presence of small disconnected aggregates that do not affect the elastic component and slightly increase solution viscosity. When the heating run starts after 15 h at low temperature, both G′ and G″ are found sizably increased, and the sample has a gel-like character as indicated by the higher value of G′ with respect to G″. On increasing temperature, G′ decreases and reaches its initial value when the temperature is equal to T_H. At this point G″ is still larger than its initial value that is reached only when even the second conformational transition is complete, as indicated by ORD signal. This suggests that the elastic modulus is mainly due to the chains, which melt at lower temperature, while the viscous modulus includes even chains involved into aggregates, which melt at higher temperature. Altogether, these results support the main point of the ‘domain’ model [10] proposed for the gelation mechanism of κ-Carrageenan, that is the formation of domains of double helices that aggregates, giving rise to network formation. The inverse melting mechanism is schematically shown in Fig. 5. On increasing temperature, helices involved in junction zones change conformation and the network is broken, as indicated by the decrease of G′; when the aggregates, remained disconnected, melt at higher temperature (as indicated by the decrease of G″), helices involved in them can quickly change their conformation coming back to coil state.

To disentangle time- and temperature-dependence of conformational transition and aggregation, quench experiments from high temperature (higher than T^*) to low temperature (lower than T^*) were done. Combined measurements of static light scattering and ORD were performed on different aliquots of the same sample. Fig. 6 shows the time course of both signals for a sample at 0.1% κ-Carrageenan in 10 mM KCl, quenched from 48 to 15 °C. We recall that T^* is 22.6 °C at the chosen salt concentration, and the time required for the sample to thermally equilibrate is approximately 90 s, as already described in the experimental

Table 1
Fit parameters of ORD signal to Eq. (1) in the text (curves 1 and 2 of Fig. 4)

Waiting time at low temperature (h)	A_L	T_L	W_L	A_H	T_H	W_H
0	18.9	26.7	3.6	12.0	30.2	1.1
15	10.5	26.7	3.6	20.7	31.3	1.3

section. The figure shows that, following the temperature quench, the ORD signal reaches the steady state value in approximately 3 min; in the same time, the light intensity scattered at 90° becomes twice its initial value and then keeps on with increasing. The doubling of light scattered intensity, I, suggests that the conformational change of κ-Carrageenan is related to the formation of double helices. Indeed, it is well known that, for non-interacting scattering particles with dimension small with respect to the wavelength of the incident light, $I \propto cM^2$, where c is the concentration in number and M the molecular mass of the scattering particle. The formation of double helices should just imply halving of c and doubling of M, while the further increase of I, observed when the conformational change is already complete, could be ascribed to the ongoing aggregation process. The validity of this interpretation requires a closer inspection of the scattering particles. Indeed, aqueous solutions of κ-Carrageenan are usually reported as quite polydisperse systems and a fast growth of a population at higher molecular weight could be responsible for the intensity increase, being the initial doubling just a coincidence. Further, if the scattering particles are not so small compared to the wavelength, the intensity increase could reflect a change in particle's shape. Possible changes in size distribution and particle shape were therefore investigated by Dynamic and Static Light Scattering experiments on the same sample in Fig. 6 at both temperature values. Measurements at 15 °C were done 3 min after the temperature quench, just after having observed the doubling of static light scattered intensity. The experimental duration was of few minutes. Panel a of Fig. 7 shows low and high temperature field time correlation functions, $g_1(\tau)$, at 90° scattering angle. For comparative purpose, time values of $g_1(\tau)$ at low temperature have been normalized for the decay

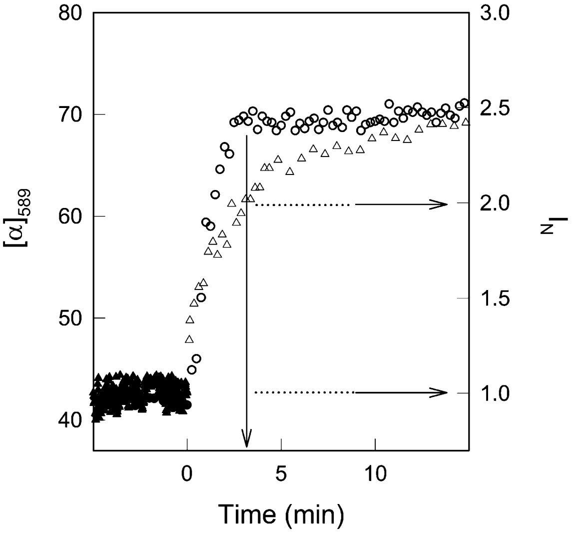

Fig. 6. Temperature quench experiment on 0.1% κ-Carrageenan in 10 mM KCl. Full symbols: $T=48$ °C; open symbols: $T=15$ °C; circles: optical rotation signal; triangles: scattered light intensity. The intensity data are normalized to the initial value at $T=48$ °C.

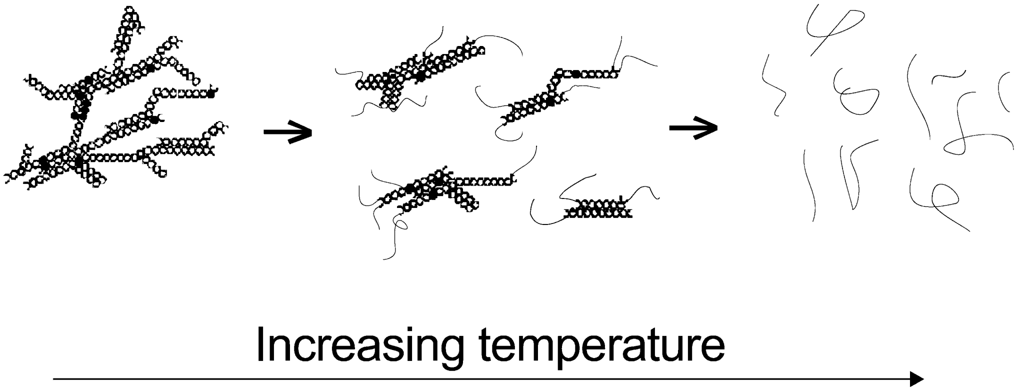

Fig. 5. Schematic model for the melting process of κ-Carrageenan.

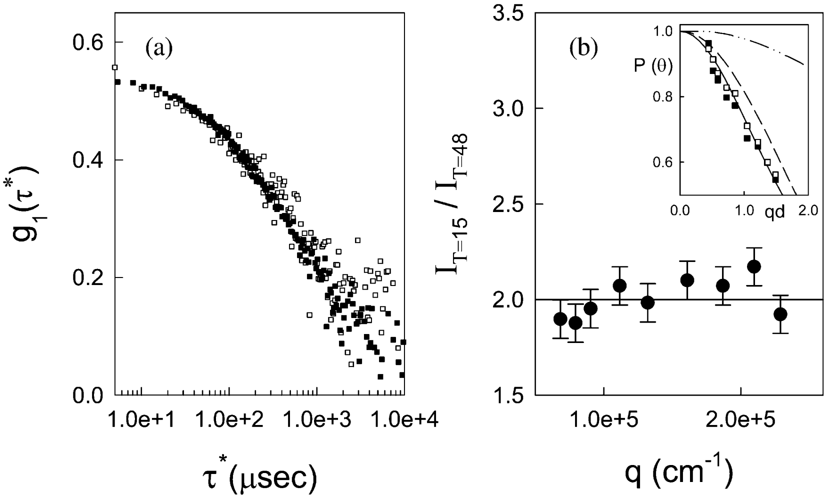

Fig. 7. (a) Field correlation function, $g_1(\tau)$, at 90° scattering angle for 0.1% κ-Carrageenan in 10 mM KCl. Full symbols: $T=15$ °C; open symbols: $T=48$ °C; time values of $g_1(\tau)$ at 15 °C have been normalized for the decay time dependence on temperature and solvent viscosity by using the relation: $\tau_2=\tau_1(T_2/T_1)(\eta_1/\eta_2)$ with $T_1>T_2$. (b) Ratio between scattered intensity at 15 and 48 °C vs. q-vector. The inset shows the factor form at both temperature values (symbols as in a) as compared with basic particle shape (continuous line: coil; dashed line: sphere; dotted line: rod).

time dependence on temperature and solvent viscosity by using the relation: $\tau_2=\tau_1(T_2/T_1)(\eta_1/\eta_2)$ with $T_1>T_2$. The resulting function appears identical to that measured at high temperature. Cumulant fit of raw data gives, in fact, the same value for both polydispersity (0.7) and hydrodynamic radius (30 ± 3 nm); the latter is in good agreement with literature results [11]. Panel b of Fig. 7 shows the ratio between excess scattering intensity at 15 and 48 °C vs. q-vector. A factor of approximately 2 is obtained at all q-values, implying that the q-dependence of I, that is the factor shape $P(\theta)$, does not change. $P(\theta)$ can be compared with idealized polymer shape by reporting intensity data, normalized by $I(q=0)$, vs. qR_G, where R_G is the gyration radius. The latter was found to be 65 ± 8 nm, as estimated from the initial slope of $P(\theta)^{-1}$ vs. q^2 [24]. The inset in panel b shows $P(\theta)$ as compared with functions for basic particle shape. Data match very well with the curve of a random coil. Since both size and shape of scattering particle do not change following the conformational transition, the initial doubling of intensity can be only ascribed to a doubling of local density. This suggests the occurrence of a dimerization process (on a length scale smaller than particle size) that can be reasonably assigned to double-helix formation between chain fragments, as already proposed by other authors [25]. The presence of ordered fragments promote, in turn, aggregation and/or macroscopic gelation at appropriate polymer concentration [25].

To study aggregation dependence on both polymer concentration and temperature, three samples at different κ-Carrageenan concentration (0.05, 0.1 and 0.2%) and equal amount of KCl (10 mM) were quenched each at three different temperature values (15, 11 and 7 °C), below the temperature of the conformational transition (22.6 °C). Fig. 8 shows the time course of ORD signal and I (left and right, respectively). Light scattering data were first normalized for polymer concentration and then scaled to the initial value at high temperature (48 °C). Following the temperature quench, the same fast change of ORD signal is observed, independently on polymer concentration and quenching temperature. Scattered intensity displays in all cases two visibly different kinetics of growth. The first one, corresponding to the intensity doubling and occurring on the same time scale of the

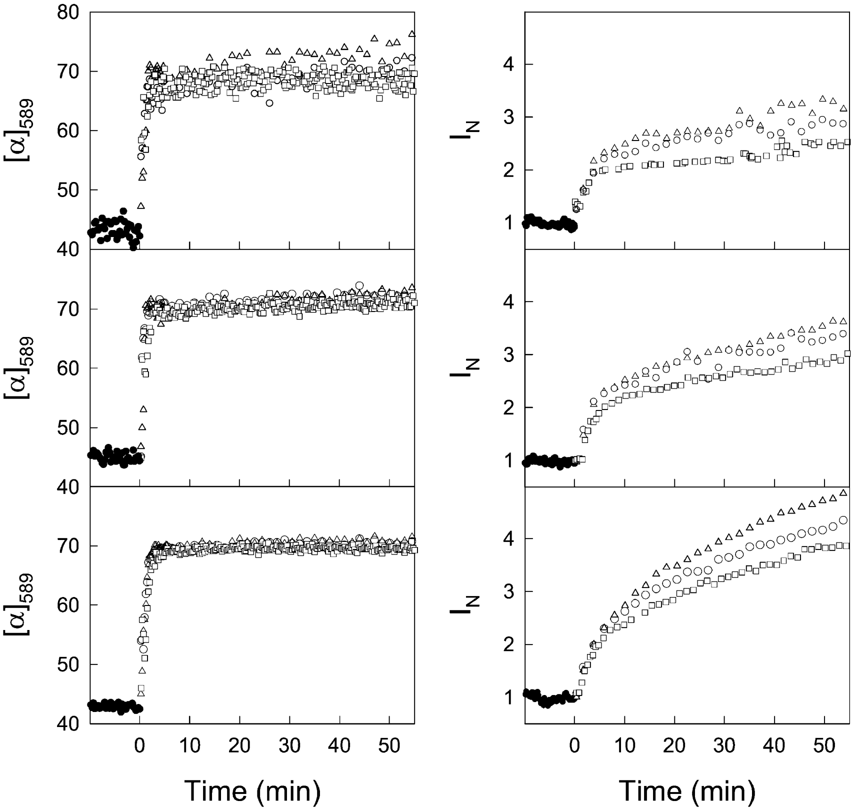

Fig. 8. Temperature quench experiments from $T=48$ °C to different temperatures (square: 15 °C; circle: 11 °C; triangle: 7 °C) on samples at different κ-Carrageenan concentration (top panels: 0.05% w/w, centre panels: 0.1% w/w, bottom panels: 0.2% w/w in 10 mM KCl). Left column: ORD signal. Right column: normalized light scattered intensity.

ORD change, is independent on temperature or polymer concentration, as expected if it is related to double helices formation. The second kinetics appears instead dependent on both concentration and temperature, as expected for aggregation. Temperature- and concentration-dependence of aggregation can be more closely analyzed by trying to distinguish the effects of both parameters on the kinetic rate and aggregate structures, as suggested by other authors [12]. The rationale is that, if only the kinetic rate has changed, data obtained at different parameter value should be superimposable on a single curve by time scaling. In our conditions, we found that this is true for the temperature-dependence as shown in Fig. 9, where data relative to samples at the same concentration and different quenching temperature, have been retraced on the same curve by time scaling (time factors are indicated in the figure). The initial points of each set have been omitted because, as seen above, they refer to the kinetics of conformational transition, which is temperature (and concentration) independent. Attempts to scale data relative to samples at different concentration and equal quenching temperature failed in any case, as already evident from the different time dependence of the three data sets shown in Fig. 9. This indicates that aggregate structure is strongly dependent on polymer concentration. Indeed, samples at 0.05 and 0.1% remain macroscopically liquid even after some days, while sample at 0.2% forms a macroscopic gel in short time. In Fig. 10 the time course of intensity scattered by the sample at 0.2% (10 mM KCl) quenched at 11 °C is

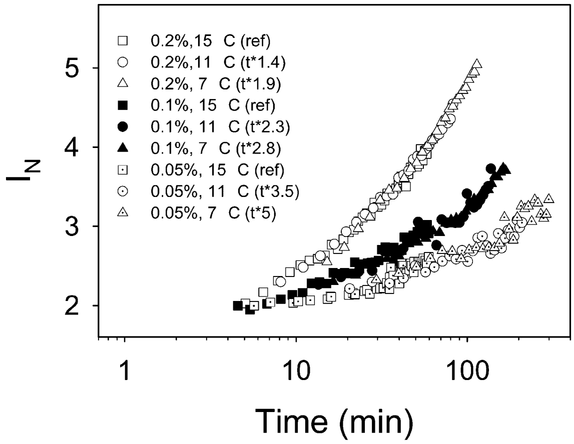

Fig. 9. Time scaling of light scattering intensity data of Fig. 8. Master curves are obtained for samples at the same concentration and different quenching temperature. Shift factors are indicated in the figure.

compared with that of G′ and G″. Onset of gelation is seen to occur after approximately 50 min as indicated by the crossing of G′ and G″ and by the appearance of large fluctuations in the scattered intensity [26].

4. Conclusions

Results on samples at different κ-Carrageenan concentration and temperature show the occurrence of a coil to double helix conformational transition, determined by the presence of K^+ ions and independent on polymer concentration. The conformational transition precedes and promotes the formation of large supramolecular aggregates that, in turn, at appropriate polymer concentration, can lead to gelation. The gel melting is characterized by the presence of a two-step process, which is the conformational change of helices involved in the network junction zones and, afterwards, the aggregates melting. Only when these aggregates, which hinder the restoring of the initial conformation, melt, all chains can suddenly undergo the transition from helix to coil. Results here presented give a more clear understanding of the dynamic of the sol–gel process which can be useful to control the structural properties of this system, with high interest for technological purposes.

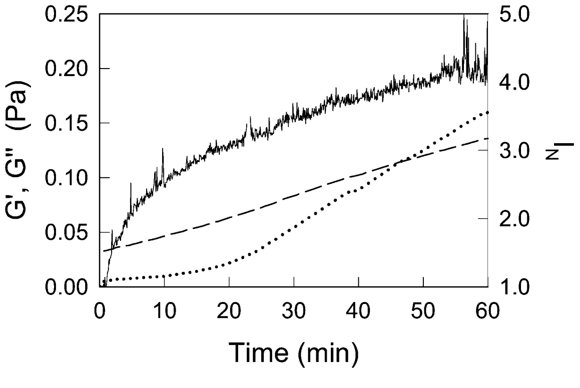

Fig. 10. Time course of normalized scattered intensity (continuous line), G′ (dotted line) and G″ (dashed line) for 0.2% κ-Carrageenan in 10 mM KCl quenched at $T = 11$ °C.

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