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K^+ and Na^+ effects on the gelation properties of κ-Carrageenan

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Abstract

The effects of K^+, Na^+ ions and their mixture on the conformational transition and macroscopic gel properties of κ-Carrageenan system have been studied using different experimental techniques. The macroscopic gelation properties of κ-Carrageenan were found to be dependent upon cosolute type. Indeed, a more ordered and strong gel was obtained in the presence of K^+ with respect to Na^+ ions. The gel properties obtained using mixtures of two cosolutes are shown to depend on the $[K^+]/[Na^+]$ ratio.

Keywords: κ-Carrageenan; Na^+ and K^+ ion effects; Self-assembly; Gelation; Fractal dimension

1. Introduction

The κ-Carrageenan comes from a family of linear water-soluble polysaccharides extracted from different species of marine red algae with a primary structure based on an alternating disaccharide repeating unit of α-(1-3)-D-galactose-4-sulphate and β-(1-4) 3,6-anydro-D-galactose. It is largely used as thickening, gelling agent or texture enhancer or stabilizer in food, pharmaceutical [1,2] and cosmetic [3] industries. Indeed, in aqueous solutions and in the presence of several cations, κ-Carrageenan forms, on cooling, thermoreversible gels.

It is known that, in the presence of K^+, thermoreversible gelation of κ-Carrageenan involves a coil-to-double helix conformational change, followed by aggregation of the ordered molecules in an infinite network [4].

One of the most interesting feature of this system is that macroscopic properties of κ-Carrageenan gels are affected by the concentration and species of cations in solution. Cations are known to affect the polymer conformational properties but the relation between conformational change and final gel structure in this system is still subject of debate. In addition to the practical interest, the understanding of mechanism leading from molecular conformational change to macroscopic aggregation is, at the present time, relevant in pathological protein coagulation [5,6] and polymeric aggregation [7,8].

In the present work, we study in detail, using different experimental techniques (static light scattering (SLS), optical rotatory dispersion (ORD), rheology and turbidity), the effects of K^+, Na^+ and their mixture on the conformation/gelation properties of κ-Carrageenan system.

Several studies are found in the literature, concerning the specific effect of some cations (K^+, Rb^+ and Cs^+) on gelation properties of κ-Carrageenan [9–11], while the effect of Na^+ on its conformational change and aggregation is still a matter of debate. In the presence of Na^+, κ-Carrageenan is found in the coil state at room temperature [12], whereas in the presence of K^+, it can be in the coil or helix state depending on salt concentration and temperature [13,14]. Potassium ions seem to have a specific effect and to bind to the polymer also in the disordered state [10]. More recently, it was suggested that the presence of Na^+ in solution can induce a conformational transition in κ-Carrageenan at high salt concentration and low temperature only [15–18]. Finally, only few literature data concern the effects of simultaneous presence of different cations on the gelation properties of κ-Carrageenan. Indeed, results obtained by

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Hermansson et al. [19] suggest that the addition of a mixture of K^+ and Na^+ ions may have a synergistic effect, although no detailed explanation is provided.

Results here presented suggest: (i) a mechanism responsible for gelation pathway in the presence of Na^+, and (ii) the possibility to modify the gelation pathway and the structural properties of the final gel by an appropriate choice of the $[K^+]/[Na^+]$ ratio.

2. Materials and methods

The κ-Carrageenan (type X-6913, Lot 63-80270) was a gift from Copenhagen Pectin, Denmark. The κ-Carrageenan powder was dissolved in hot Millipore deionised water at 70 °C (in the presence of 200 ppm sodium azide as batteriostatic agent) and stirred at the same temperature for 2 h [4]. The pH of the solution was 8.7 to prevent hydrolysis during preparation [18,20]. Then the solution was dialyzed against Millipore water to eliminate excess salt. Hot Millipore water, containing the appropriate amount of KCl and/or NaCl, was added to set the wanted ionic composition. The solution was finally filtered at high temperature through a 0.22-μm membrane directly in the cuvette or rheometer plate.

Temperature scan experiments were done using a thermostated circulating bath with a temperature scan controller. The scan rate was 0.2 °C/min. Temperature quench experiments were done using two circulating baths set at initial and final temperature values, respectively. The sample was first equilibrated at the initial temperature and then brought to the final temperature by switching the flow of thermostating liquid (thermal equilibration time is about 90 s). In all experiments, temperature was controlled within ±0.1 °C.

Static 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 Brookhaven Instrument BI200-SM goniometer system. The intensity of light scattered by gelled samples was measured probing different regions of the specimen by using a motor-driven cell holder. The structure function was obtained by recording light scattering intensity at different scattering vector $q=4\pi n\sin(\theta/2)/\lambda_0$, where n is the refraction index of solution, λ_0 is the wavelength of the incident light and θ is the scattering angle.

Viscoelastic spectra under low amplitude oscillatory shear were done on a controlled stress AR-1000 rheometer (TA Instruments, UK) using a titanium cone-plate geometry (angle <1°, radius 20 mm, gap 26 μm). All experiments were done in 0.02–30 Hz frequency range at 4×10^{-3} strain. The hot solution was loaded on the rheometer plate, set at 65 °C. Then, the temperature was quickly lowered to 20 °C. The sample was kept at this temperature for 1 h before the start of measurement. Temperature scan experiments were done in 65–10 °C temperature range at 0.5 Hz and 4×10^{-3} strain using the same geometry described above. The temperature was controlled by the built-in Peltier system. The thin sample–air interface was coated with silicone oil to avoid solvent evaporation.

ORD measurements were done using a Jasco P-1020 polarimeter. All measurements were taken at 589 nm wavelength (Na lamp) using a thermostated cell.

Turbidity was monitored using a Jasco V-530 UV–VIS spectrophotometer. Measurements were done at 450 nm wavelength using a thermostated cell holder.

3. Results and discussion

3.1. Effects of K^+ ion on gelation

Our previous studies, performed on κ-Carrageenan samples at low polymer concentration (0.1% (w/w)), had shown the occurrence, on cooling, of a coil-to-double helix conformational transition induced by the presence of K^+ ions [4]. Under those experimental conditions, the conformational transition was followed by the formation of large supramolecular aggregates (mesoscopic gelation) that, eventually, led to macroscopic gelation after an appropriate waiting time. The following results concern samples at 0.4% (w/w) κ-Carrageenan in 10 mM KCl that form quickly a macroscopic gel on cooling. Fig. 1 shows ORD results on a cooling and heating cycle as compared with similar data relative to a sample at 0.1% (w/w) polymer concentration with the same KCl amount (10 mM) [4].

The conformational transition temperature T_t, defined as the temperature of 50% ORD change on cooling scan, is found to be independent on κ-Carrageenan concentration, in agreement with literature data [4,21]; further, cooling profiles for both samples are very similar. The process is thermoreversible with a hysteresis area that is larger at

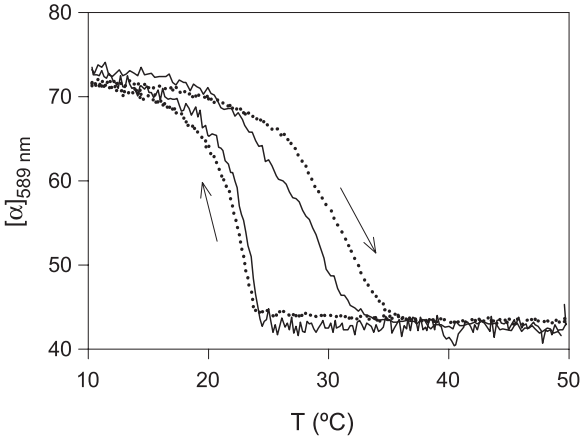

Fig. 1. Temperature dependence of the ORD signal of 0.1% (continuous line) and 0.4% (w/w) (dotted line) κ-Carrageenan solutions at 10 mM KCl.

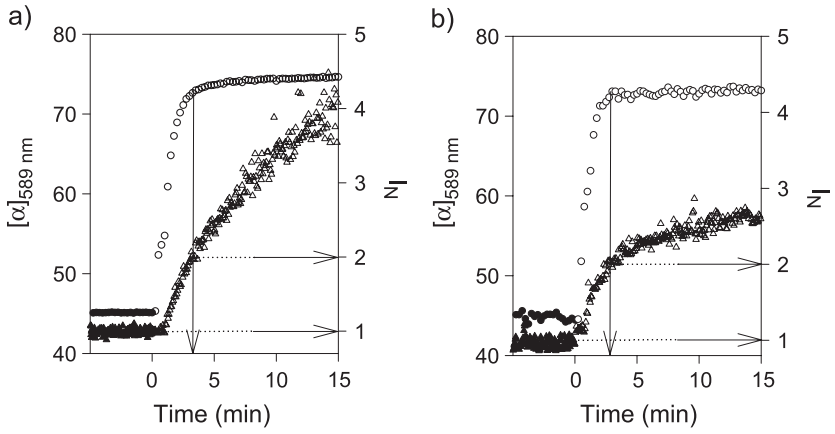

Fig. 2. Time course of ORD signal (circles) and scattered light intensity (triangles) upon a temperature quench from 48 °C (full symbols) to 7 °C (empty symbols) for 0.1% (panel a) and 0.4% (w/w) (panel b) κ-Carrageenan in 10 mM KCl. The intensity data are normalized to the initial value at T=48 °C. In both cases, reaching the ORD plateau corresponds to doubling of the I_N signals.

higher polymer concentration, consistently with the hypothesis of the hysteresis being related to aggregation [4,10,16].

In order to disentangle conformational transition and aggregation process, quench experiments from high temperature (higher than T_t) to low temperature (lower than T_t) were performed on the same sample. Time courses of ORD and SLS signals are shown in Fig. 2(a); similar results for 0.1% (w/w) sample at the same salt concentration are shown in Fig. 2(b). In both cases, the ORD signal reaches the steady-state value in few minutes after the temperature quench; at the same time, the normalized light intensity at 90° scattering angle (I_N) doubles its initial value and then increases with a rate dependent on polymer concentration. The intensity doubling can be confidently taken as evidence that the conformational change is related to double helices formation [4].

The internal structure of the gelled sample was studied by SLS measurements. Fig. 3 shows the structure function, $S(q)$, in the 50–500 nm interval. $S(q)$ is seen to be featureless, and its log–log plot is accurately represented by a straight line, as shown in the figure. The system is thus self-similar in this scale interval. From slope in Fig. 3, which is from power law dependence:

$$S(q) \propto q^{-d_f}$$

of the structure function upon q, the fractal dimension d_f is derived. The latter is defined by the relation $M \propto R^{-d_f}$, where M is the mass of the objects within a radius R [22]. We found $d_f \approx 1.1$ that is consistent with an overall loose packing of ramified structures [23]. Fractal dimension was measured 1 and 24 h after sample preparation and no difference was observed. Measurements at different KCl concentration ranging from 10 to 300 mM showed that fractal dimension values were almost independent on salt amount ($1.1 \leq d_f \leq 1.3$), suggesting that, on this q range, the internal structure of gel does not change in agreement with literature data [18].

3.2. Effects of Na^+ ion on gelation

A sequence of experiments similar to those discussed above was done in the presence of Na^+ ions. Fig. 4 shows

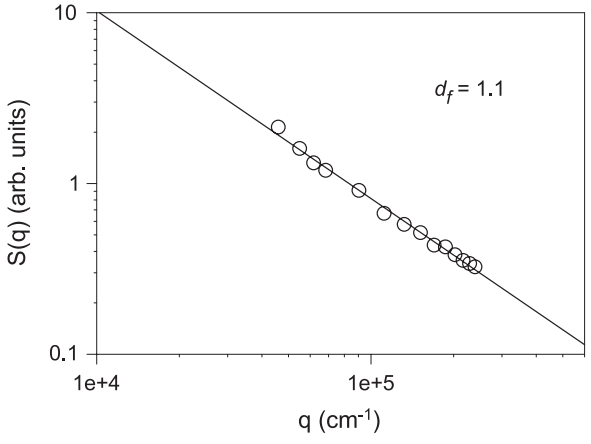

Fig. 3. Log–log plot of structure function, $S(q)$, vs. q of 0.4% (w/w) κ-Carrageenan in 10 mM KCl after a quench at T=20 °C.

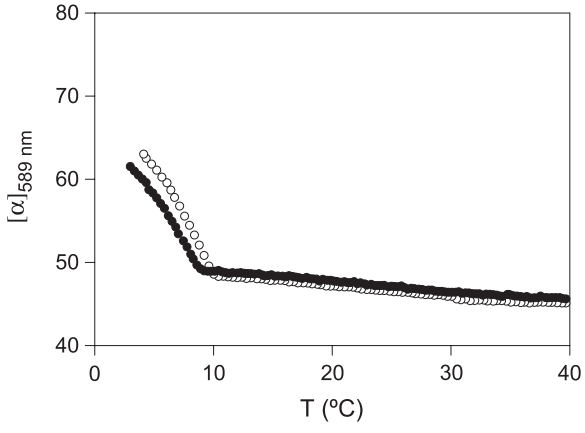

Fig. 4. Comparison between the temperature dependence of ORD signal during cooling of 0.4% (w/w) κ-Carrageenan sample in the presence (empty symbols) and absence (full symbols) of 10 mM NaCl.

ORD signal measured in a downward temperature scan on a sample at 0.4% (w/w) κ-Carrageenan and 10 mM NaCl. Differently K^+ case, only a small increase of the specific rotation power is observed at lower temperature (about 10 °C). Since Na^+ is reported as a non-specific ion for the Carrageenan conformational transition, a further experiment on a sample without salt was done to check if the signal growth could be due to the presence of other residual counterions, present in the κ-Carrageenan powder. In fact, cooling results for both samples are almost coincident, indicating that [α] increment is not determined by the presence of Na^+.

Quench experiments from high temperature (48 °C) to low temperature (7 °C) have been performed, as done in K^+ case. Fig. 5 shows the time course of SLS and ORD signals. Upon temperature quench, the ORD signal reaches in few minutes a steady-state value whereas the light scattered intensity at 90° does not change, indicating that the [α] increment cannot be related to double helix formation. Moreover, as a major difference with the K^+ case, molecular aggregation does not occur at this very low NaCl concentration (10 mM). In fact, this sample does not form a gel even if kept at low temperature for several days. Much larger Na^+ concentration (≈300 mM) is required for observing gelation of this system in convenient experimental time.

Absorbance, viscoelasticity and ORD data measured during a downward temperature scan on 0.4% (w/w) κ-Carrageenan with 300 mM NaCl sample are shown in Fig. 6. The elastic modulus (G') shows a rapid increase at about 30 °C where it becomes larger than the viscous modulus (G''); at the same temperature, which we define as aggregation temperature for this system, the absorbance rapidly increases as well. Instead, the ORD signal shows a slight monotonic increase down to 29 °C, below which it

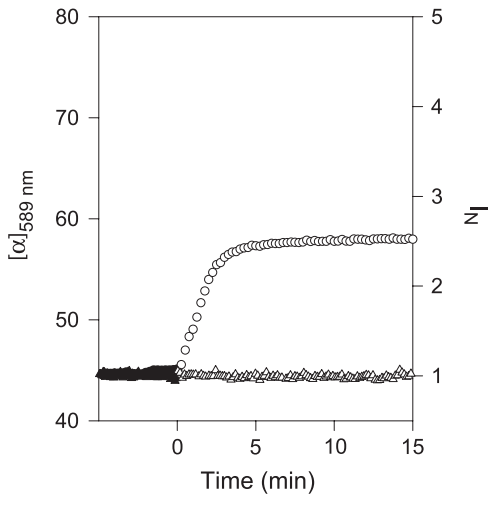

Fig. 5. Time course of ORD signal and scattered light intensity upon a temperature quench from 48 to 7 °C on 0.4% (w/w) κ-Carrageenan in 10 mM NaCl. Symbols as in Fig. 2.

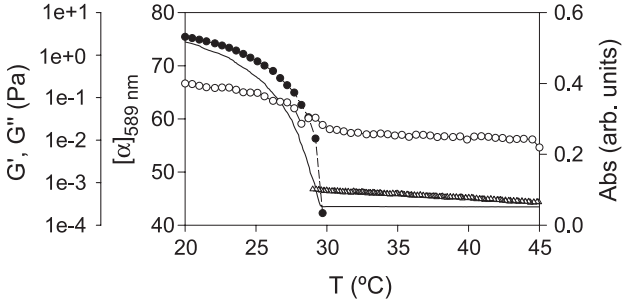

Fig. 6. Temperature dependence of turbidity (continuous line), elastic modulus, G' (full circles), viscous modulus, G'' (empty circles) and ORD (empty triangles) during a downward scan on 0.4% (w/w) κ-Carrageenan sample in 300 mM NaCl. The ORD signal is recorded only down to 29 °C because of the large turbidity increase. The G' signal is detectable starting from $T \approx 30$ °C.

cannot be further measured, due to the large turbidity increase. Under these experimental conditions, the macroscopic gelation occurs without evident sign of polymeric conformational transition. The structural properties of the same sample, upon a temperature quench at 20 °C, were probed by SLS and viscoelastic measurements. Fig. 7a shows the mechanical spectra taken 1 h after the quench; they are almost flat and G' is larger than G'', as typically found with gelled sample; the gel is very weak as indicated by the small G' value and has a fractal dimension, as shown in Fig. 7b, close to 3, which is much larger than that obtained for the sample with same amount of K^+. Dependence of the aggregation temperature on NaCl concentration in 0.4% (w/w) κ-Carrageenan is shown in Fig. 8 (continuous line is a guide to the eye). Each point was determined by measuring the turbidity during a scan experiment (see the inset in the figure) as described above. In all these experiments, no sign of conformational transition, as measured by ORD (data not shown), was detected prior to the aggregation process. The rapid increase of turbidity not preceded by a conformational change, as observed instead in K^+ case, suggests that a different mechanism could be responsible for the polymer aggregation in the presence of NaCl. In similar biopolymeric systems [24,25], a liquid–liquid phase transition was found to promote gelation even at very low polymer concentration. The same mechanism can be invoked in the NaCl case and the line in Fig. 8 can be thought as a phase separation line. When the system approaches the phase separation line during a temperature scan experiment, we observe an increase of scattered light and, consequently, of turbidity, due to the demixing of the solution in regions of higher- and lower-than-average concentrations. In the high concentration region, the cross-link of κ-Carrageenan is favoured and gelation starts leading to structures locally more compact (high d_f value), less interconnected (low G' value) and macroscopically heterogeneous (high turbidity).

Overall, these results suggest that a different pathway of polymeric self-assembly could be responsible for different gel structural properties; indeed, molecular aggregation of

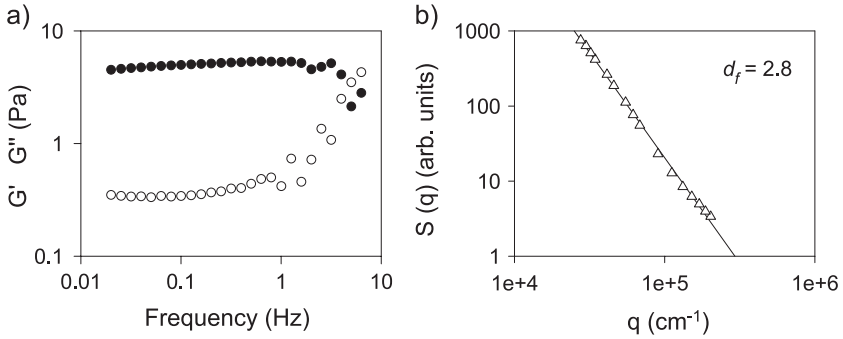

Fig. 7. Mechanical spectra (panel a) and structure function (panel b) of a gelled sample at 0.4% (w/w) κ-Carrageenan and 300 mM NaCl.

κ-Carrageenan chains without the ordering imposed by the conformational transition appears to be consistent with the more disordered and compact structure observed in gel formed in the presence of Na^+.

3.3. Combined Na^+ and K^+ effect

Combined effects of ions, K^+ and Na^+, were studied to investigate the feasibility of modulating the gelation pathways and the final gel structural properties with an appropriate choice of the $[K^+]/[Na^+]$ ratio.

We first studied the effect of Na^+ addition on the polymeric conformational transition promoted by K^+ presence. Downward temperature scans from high temperature to 20 °C were done on two samples at 0.4% (w/w) κ-Carrageenan and 20 mM KCl in the presence or absence of 100 mM NaCl. For comparison, a sample at the same polymer concentration and 120 mM KCl (that is, at the same ionic strength of the salt mixture) was also studied. ORD data in Fig. 9 show that the Na^+ addition does not change the T_t value (about 30 °C), in agreement with literature data [18], i.e., it does not affect the coil-to-double helix transition promoted by K^+. Only a slight change of the transition profile is observed. For the sample at 120 mM KCl, the conformational transition occurs at about 52 °C (data not shown). Thereafter, we studied the effects of Na^+ addition on gel structural properties. Preliminary turbidity measurements upon a temperature quench from 80 to 20 °C allowed to estimate gelation time of the three samples discussed above. They all reached a plateau in about 15 min, with an absorbance value, due to the gel cloudiness, dependent on salt composition. The absorbance plateau and fractal dimension values of these three samples (measured 1 h after the temperature quench) are reported in Table 1. We observe that gel formed in the presence of both salts displays the highest values of fractal dimension and absorbance indicating a more heterogeneous structure. Rheological properties of the same gels were also compared. Results in Fig. 10 show that: (i) the lower G' value is observed for the sample with 20 mM of KCl; this value is, nevertheless, about 30 times larger than that one obtained in the presence of 300 mM NaCl alone (see Fig. 7a); (ii) G' value increases with K^+ concentration; (iii) the higher value of G' is that one obtained in the mixture of 20 mM KCl and 100 mM NaCl. All these findings agree with the known difference between effects of K^+ and Na^+ on gel structure. In fact, when K^+ is the only cation, gelation is promoted by the formation of rigid double helices, which aggregate into coarse super-strands. In this case, the

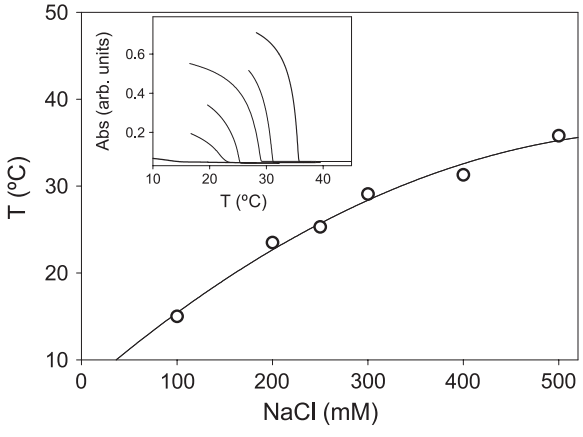

Fig. 8. Aggregation temperature of 0.4% (w/w) κ-Carrageenan samples in the presence of different amounts of NaCl. Each point was determined as the T at which the measured turbidity during a scan experiment starts to increase. The inset shows the turbidity raw data at increasing salt concentration (from left to right).

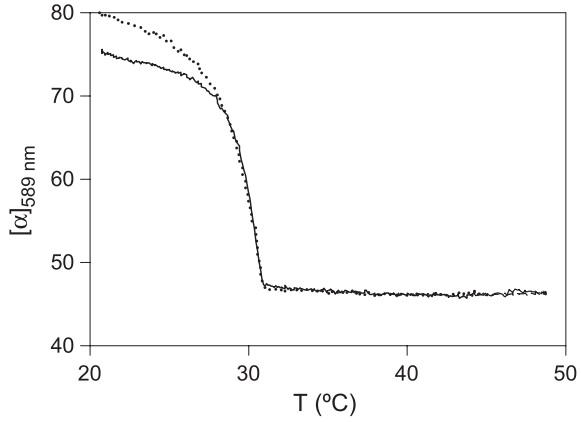

Fig. 9. Temperature dependence of ORD signal measured on 0.4% (w/w) κ-Carrageenan at different salt compositions: 20 mM KCl (continuous line) and 20 mM KCl+100 mM NaCl (dotted line).

Table 1
Fractal dimension (d_f) and absorbance (Abs.) of gelled samples of 0.4% (w/w) κ-Carrageenan at 20 °C

Salt concentration	d_f	Abs.
20 mM KCl	1.1±0.1	0.04
120 mM KCl	1.1±0.1	0.055
20 mM KCl+100 mM NaCl	1.8±0.1	0.075

network is formed by a moderate number of very good bonds. On the opposite, the gel formed in the presence of 300 mM of NaCl alone will contain a great number of very weak bonds, since the coils have the flexibility to arrange on a huge number of non-specific mutual contacts. Within this frame, it is easy to imagine how a mixture of K^+ and Na^+ can lead to a more elastic gel (synergic effect). The K^+-containing solution is still able to form strands and helices, but the presence of Na^+ will introduce a number of defects in these structures enhancing flexibility and number of bonds. In this case, in fact, we can obtain interconnected regions of partially formed double helices or more flexible and shorter super-strands, as suggested in the literature [19].

We also investigate the possible hypothyzed competition between the two types of mechanism responsible for polymeric aggregation by another set of experiments for different $[K^+]/[Na^+]$ ratios. In Fig. 11 (top panel), we report the d_f measured on samples containing 300 mM NaCl and different KCl concentrations, quenched at 20 °C. We recall that the gelation temperature for gels with 300 mM NaCl only is about 30 °C (continuous line in the bottom panel in the same figure). On increasing K^+ concentration, a transition from high to low d_f value is observed (as evidenced by the gray region in the figure). A possible explanation of this behavior is illustrated by the bottom panel in the same figure. In fact, in the region (gray area in the figure) where the temperature quench is higher than the correspondent conformational transition temperature (dashed line), we observe a higher d_f value, typical of gel formed using NaCl only; at higher K^+ concentrations, where the temperature quench is lower than both temperatures, the d_f value decreases approaching that of K^+-only system.

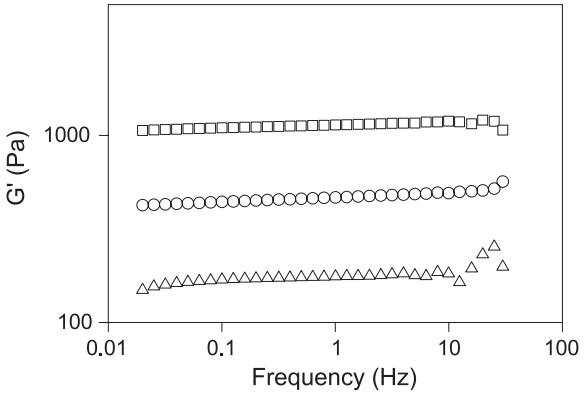

Fig. 10. Mechanical spectra at 20 °C of 0.4% (w/w) κ-Carrageenan samples with different salt compositions: 20 mM KCl (triangles), 120 mM KCl (circles) and 20 mM KCl+100 mM NaCl (squares).

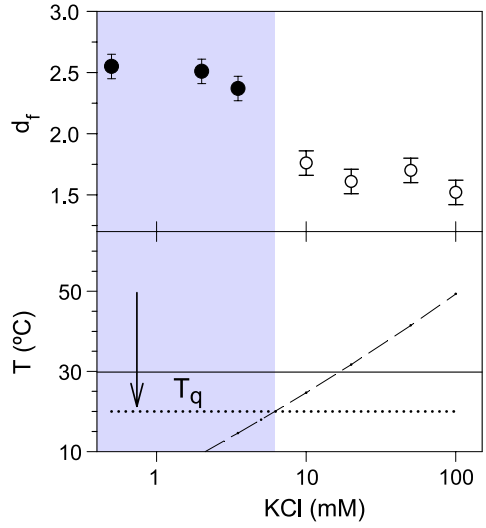

Fig. 11. Top panel: Fractal dimension of gels at 0.4% (w/w) κ-Carrageenan vs. KCl concentration in presence of a fixed amount of NaCl (300 mM). Gels were formed upon a quench at 20 °C as indicated in the bottom panel (dotted line). In the latter, the continuous and dashed lines indicate the aggregation temperature in absence of KCl and the conformational transition temperature in absence of NaCl, respectively. The grey area delimitates the region where the conformational transition temperature is below the chosen temperature quench (20 °C).

4. Conclusions

Our study highlights the different role played by K^+ and Na^+ ions in promoting κ-Carrageenan macroscopic gelation. In fact, K^+ induces a coil→double helix transition preceding and promoting polymer aggregation [4]. The final gel has a fractal dimension value close to 1, almost independent on the K^+ concentration, because of the ordered formation of linear structures (double helix).

Na^+ ion, although it does not promote the coil→double helix transition, takes part in the aggregation process probably through a phase separation mechanism, as found in many different biopolymeric systems [24–27], leading to the formation of a weak gel having a fractal dimension close to 3. The resulting structure is more disordered with respect to that one obtained in the presence of KCl, due to the different assembly.

All data here presented suggest that the two mechanisms that induce κ-Carrageenan gelation can compete when both ions are present in solution. The cross-linking and gel network structure are dominated by which process (formation and growth of the length of the helices induced by the K^+ ions or coil aggregation induced by phase separation) firstly occurs. Since the two mechanisms are controlled by the temperature and the latter, in turn, can be modulated by changing salt concentration, an appropriate choice of $[K^+]/[Na^+]$ ratio makes possible to obtain a gel with a desired

texture for industrial, food and pharmaceutical applications [28–30].

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