Synergistic effects of mixed salt on the gelation of κ-carrageenan

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ABSTRACT

The effect of the addition of calcium or sodium ions on the potassium induced gelation of κ-carrageenan (κ-car) is investigated using oscillatory shear rheology and turbidimetry. Both the gelation kinetics and the steady state shear moduli are investigated. Gelation in mixed salt solutions is compared with that in pure potassium and calcium solutions. It is shown that the elastic shear modulus increases with increasing pure KCl concentration, but decreases with increasing pure $CaCl_2$ concentration. In mixed salts, gelation of κ-car is induced by potassium and addition of $CaCl_2$ leads to an increase of the elastic modulus with increasing $CaCl_2$ concentration. κ-Car gelled at low mixed salt concentrations for which it remained liquid in pure salt. At equivalent ionic strengths, the effect of adding NaCl on potassium induced gelation is much weaker. In pure KCl solutions, κ-car gels are transparent, but in pure $CaCl_2$ they become increasingly turbid with increasing $CaCl_2$ concentration. The turbidity of gels formed in mixed salts is intermediate.

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1. Introduction

κ-Carrageenan (κ-car) is an anionic polysaccharide extracted from red algae and is used extensively for its capacity to form a gel in aqueous solutions (Piculell, 2006). Gelation of κ-car occurs upon cooling below a critical temperature (T_c) that depends on the concentration and the type of cations that are present (Rochas & Rinaudo, 1980). Gelation is induced by a conformational transition of the κ-car chains from a random coil to a helix. The transition occurs over a relatively narrow range of temperatures and the fraction of helices increases with decreasing temperature in this range. Chains with the helical conformation aggregate and if their concentration is sufficiently high they form a percolating network. T_c corresponds to the temperature where the concentration of helices is sufficient to form a percolating network. A particularly effective cation to induce the coil-helix transition of κ-car is potassium. The presence of only 10 mM potassium can induce gelation at room temperature, while a more than ten times larger amount is needed for sodium (Hermansson, Eriksson, & Jordansson, 1991; Mangione et al., 2005; Michel, Mestdagh, & Axelos, 1997; Nono, Durand, & Nicolai, 2012; Nono, Nicolai, & Durand, 2011). Gelation can be reversed by heating, but the melting temperature is often higher than the gelling temperature.

The effect of both the concentration and type of cation on the elastic modulus of κ-car gels has been studied in some detail for potassium and calcium, which are most commonly used to induce

gelation. It is found that when considering the effect of the ion concentration one also needs to take into account the activity of the counterions. Generally, it is found that the elastic modulus increases with increasing potassium concentration (Doyle, Giannouli, Philp, & Morris, 2002; Nguyen, Phan-Xuan, Benyahia, & Nicolai, 2012; Nono et al., 2011; Núñez-Santiago & Tecante, 2007; Thrimawithana, Young, Dunstan, & Alany, 2010), while it reaches a maximum when the calcium concentration is increased (Doyle et al., 2002; MacArtain, Jacquier, & Dawson, 2003; Thrimawithana et al., 2010). Another difference between gels induced by potassium and those induced by calcium is that the latter are increasingly turbid with increasing ion concentration (Doyle et al., 2002; MacArtain et al., 2003), while the former remain transparent.

The effect of mixed salts on the gelation of κ-car has been studied relatively little even though in applications often more than one type of salt is present. The most extensive study was reported by Hermansson et al. (1991) who found that adding NaCl to a κ-car solution containing 20 mM potassium led to an increase of the elastic modulus, whereas in the absence of potassium these solutions did not gel. An even stronger synergistic effect was found when $CaCl_2$ was added. Addition of as little as 2 mM $CaCl_2$ was found to increase the elastic modulus significantly. Mangione et al. (2005) reported that addition of 100 mM NaCl to a κ-car solution containing 20 mM KCl did not influence T_c, but led to a significant increase of the elastic shear modulus. These results clearly show that gelation of κ-car in mixed salt solutions cannot be deduced from that of the pure salt solutions.

Here we present a systematic investigation of the influence of adding $CaCl_2$ or NaCl on κ-car gelation induced by potassium and compare it with gelation induced by pure $CaCl_2$ and pure KCl. We

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have studied not only the effect on the elastic modulus at steady state, but also on the gelation kinetics and the turbidity.

2. Materials and methods

2.1. Materials

The sodium κ-carrageenan used for this study is an alkali treated extract from *Eucheuma cottonii* and was a gift from Cargill (Baupte, France). Using NMR it was found that the sample contained less than 5% ι-carrageenan. A freeze-dried sample of κ-car was dissolved by stirring for a few hours in Milli-Q water (70 °C) with 200 ppm sodium azide added as a bacteriostatic agent. The solution was extensively dialysed against Milli-Q water at pH 7 and subsequently filtered through 0.45 μm pore size Anatop filters. The pH of the solution was adjusted to 7 by adding small amounts of HCl 0.1 M. The κ-car concentration (C_K) was determined by measuring the refractive index using refractive index increment 0.145 mL/g. The molar mass (M_w) and radius of gyration (R_g) were determined by light scattering as described elsewhere (Meunier, Nicolai, Durand, & Parker, 1999): $M_w = 2.1 \times 10^5$ g/mol, $R_g = 52$ nm. The sample contained 5500 mg/100 g Na, 56 mg/100 g Ca and 300 mg/100 g K.

2.2. Methods

The shear moduli were determined as a function of the frequency and the temperature using a stress imposed rheometer (AR2000, TA Instruments) with plate – plate geometry (diameter 40 mm, gap 700 μm). The temperature was controlled by a Peltier system and the geometry was covered with paraffin oil to prevent water evaporation. In all cases the measurements were done in the linear response regime. For some systems we observed a decrease of the shear modulus during gelation, which is caused by partial detaching of the gels from the geometry. Here we only show results of measurements where this did not occur and the results were quantitatively reproducible.

Turbidity measurements were done as a function of the wavelength in rectangular air tight cells using a UV–visible spectrometer Varian Cary-50 Bio. Different path lengths were used depending on the turbidity of the samples in order to avoid saturation. Measurements were done at different temperatures that were controlled within 0.2 °C using a thermostated bath.

3. Results

3.1. Pure potassium induced gelation

Fig. 1 shows the evolution of the storage shear modulus (G') at 0.1 Hz during a cooling and subsequent heating ramp (2 °C/min) for aqueous solutions of κ-car in 10 mM KCl at various κ-car concentrations between 2 and 13.5 g/L. For $C > 4$ g/L, G' increased steeply at a critical temperature $T_c \approx 18$ °C. At lower concentrations, weaker gels are formed and the initial steep increase of G' is below the sensitivity of the apparatus. Therefore we observe for these solutions only the slower increase at lower temperatures. At lower concentrations no significant increase of G' was observed during the cooling ramp.

During subsequent heating, G' first increased weakly followed by a decrease at significantly higher temperatures than T_c caused by melting of the gel. The initial increase can be explained by the slow gelation kinetics that initially dominates the effect of decreasing gel strength with increasing temperature. As was reported elsewhere (Meunier et al., 1999), the kinetics of gelation can be very slow,
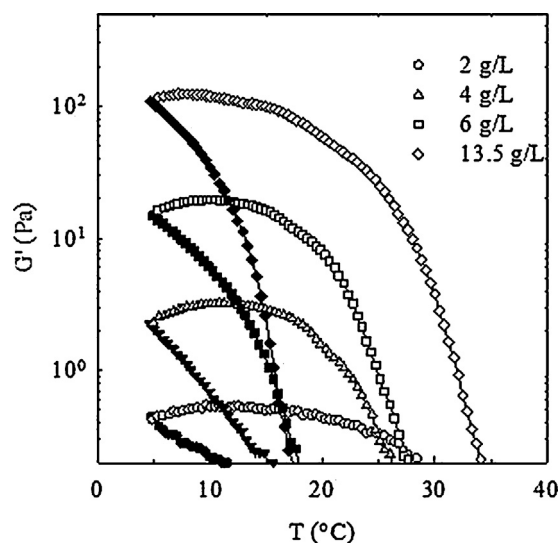

Fig. 1. Storage shear modulus at 0.1 Hz of different concentrations of κ-car at 10 mM KCl during a cooling (filled symbols) and subsequent heating (open symbols) ramp at a rate of 2 °C/min.
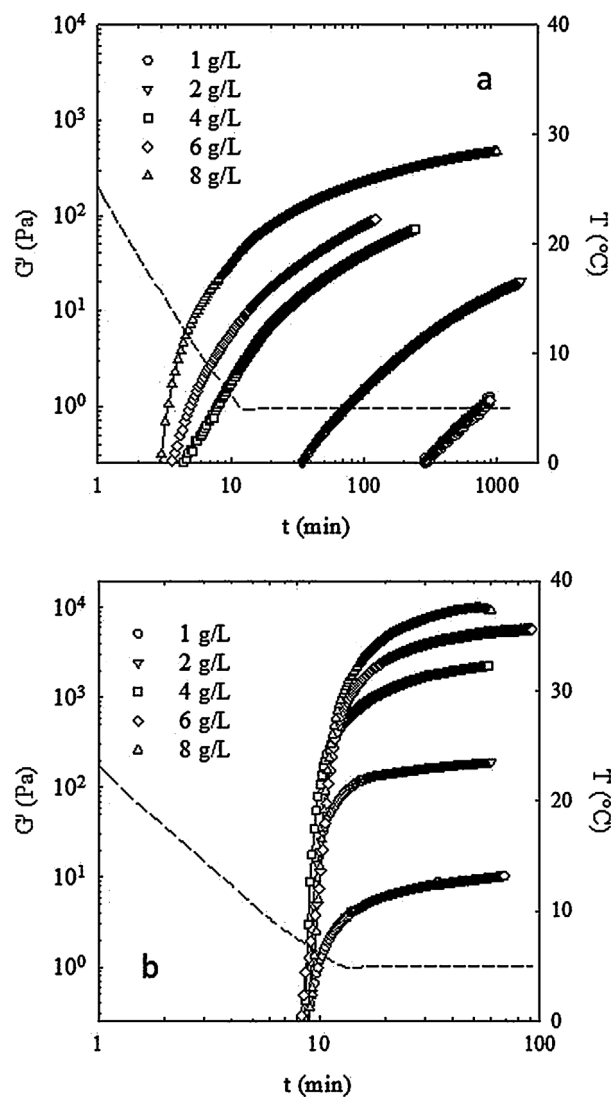

Fig. 2. Evolution of the storage shear modulus at 0.1 Hz for different concentrations of κ-car at 10 mM KCl (a) or 10 mM CaCl₂ (b) during and after rapid cooling to 5 °C. The dashed lines indicate the temperature of the sample.
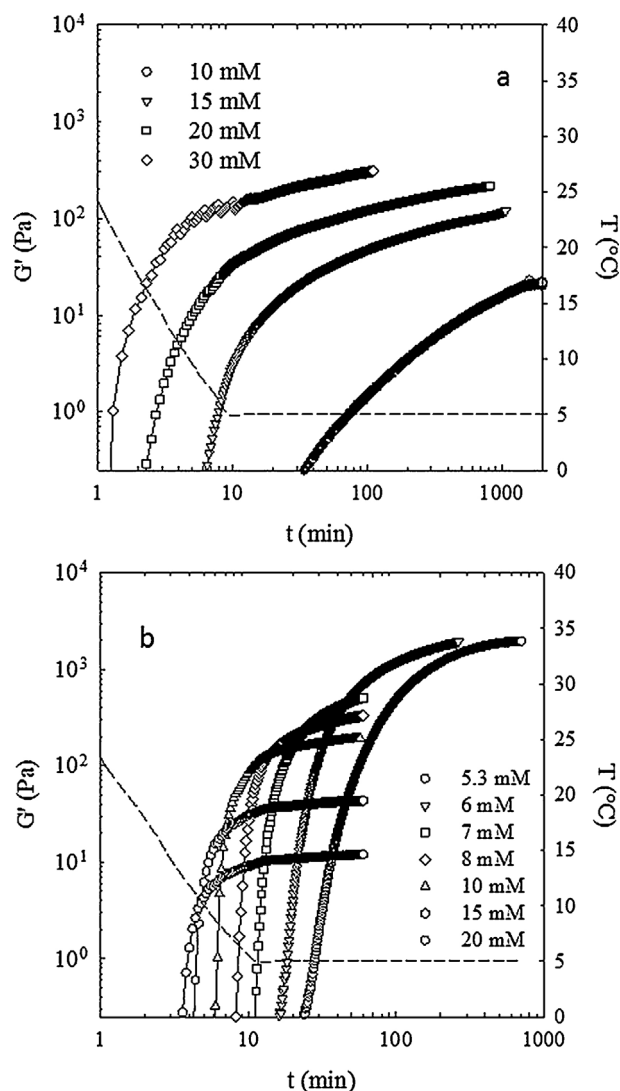
Fig. 3. Evolution of the storage shear modulus at 0.1 Hz of 2 g/L κ-car at different KCl concentrations (a) or different CaCl$_2$ concentrations (b) during and after rapid cooling to 5 °C. The dashed lines indicate the temperature of the sample.

which is illustrated in Fig. 2a where the evolution of G' is shown after rapid cooling to 5 °C from the liquid state at 50 °C.

The effect of the KCl concentration on the evolution of the shear modulus after rapid cooling to 5 °C at $C = 2$ g/L κ-car is shown in Fig. 3a. At low KCl concentrations gelation is extremely slow, but with increasing KCl concentration gelation becomes faster and the shear modulus becomes larger.

3.2. Pure calcium induced gelation

Fig. 2b shows that in the presence of 10 mM CaCl$_2$ gelation was fast even at low κ-car concentrations and the gel moduli were much higher than at 10 mM KCl even though the critical gelation temperature was lower ($T_c \approx 10$ °C). The evolution after rapidly cooling to 5 °C is shown for $C = 2$ g/L κ-car at different CaCl$_2$ concentrations in Fig. 3b. Clearly gelation induced by calcium ions is much faster than gelation induced by potassium ions except at 5.3 mM CaCl$_2$ where it took several hours to approach steady state. We did not observe gelation at [CaCl$_2$] = 4 mM in the fridge at 4 °C even after several weeks. Samples with higher κ-car concentrations up to 8 g/L did not show gelation at 4 mM CaCl$_2$ either. Trials with intermediate
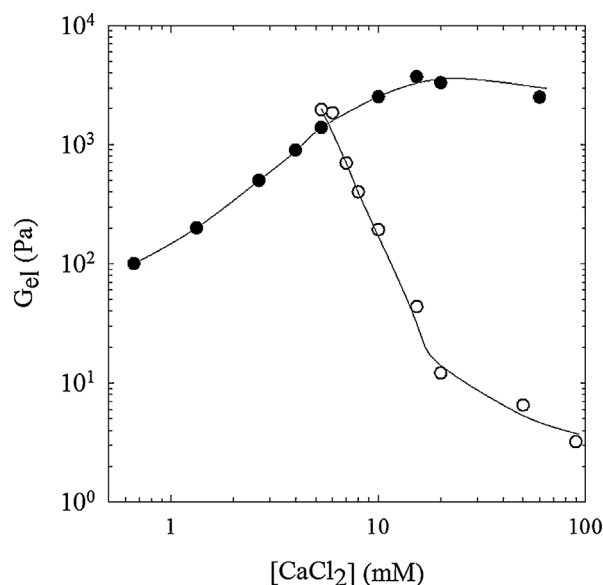

Fig. 4. Elastic modulus of κ-car gels at $C = 2$ g/L as a function of the CaCl$_2$ concentration at 5 °C in the absence (open symbols) and presence of 10 mM KCl (closed symbols).

CaCl$_2$ concentration showed that a gel was formed at 5.0 mM after 2 days, but no gels were formed at 4.7 mM.

The frequency dependence of the shear moduli was measured after the system was allowed to evolve at 5 °C. For all systems discussed here G' was larger than G'' and was almost independent of the frequency at lower frequencies where G' is equal to the elastic modulus (G_{el}) of the gels. The dependence of G_{el} near steady state on the CaCl$_2$ concentration is shown in Fig. 4. Remarkably, the gel modulus decreased with increasing CaCl$_2$ concentration, whereas it increased with increasing KCl concentration, see Fig. 3a. Another notable difference between gels induced by KCl and CaCl$_2$ was that the latter became increasingly turbid with increasing [CaCl$_2$], whereas the former remained transparent, see Fig. 5.
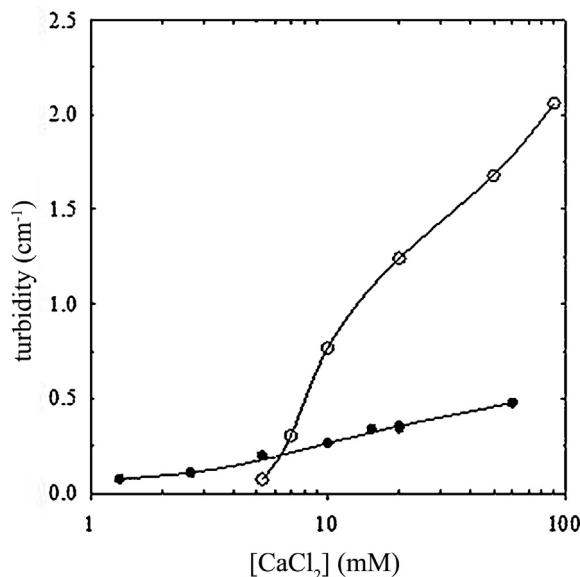

Fig. 5. Turbidity of κ-car gels at $C = 2$ g/L as a function of the CaCl$_2$ concentration at 5 °C in the absence (open symbols) and presence of 10 mM KCl (closed symbols).
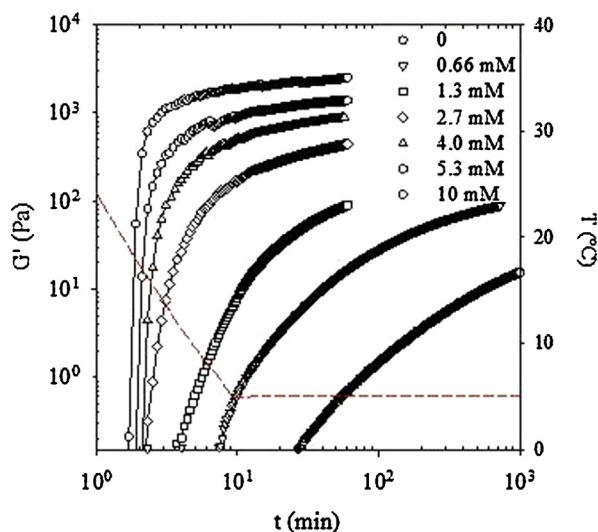
Fig. 6. Evolution of the storage shear modulus at 0.1 Hz of 2 g/L κ-car with 10 mM KCl at different CaCl$_2$ concentrations during and after rapid cooling to 5 °C. The dashed line indicates the temperature of the sample.

Fig. 7. Elastic modulus of κ-car gels at C = 2 g/L as a function of the KCl concentration at 5 °C in the presence of different CaCl$_2$ concentrations.

3.3. Influence of CaCl$_2$ on potassium induced gelation

Fig. 6 shows the evolution of G' after rapid cooling to 5 °C for κ-car solutions at C = 2 g/L in the presence of 10 mM KCl and various concentrations of CaCl$_2$. The critical gelation temperature of κ-car in 10 mM KCl is higher than that in pure CaCl$_2$ in the range used here. In fact, temperature ramps showed that addition of CaCl$_2$ did not increases T_c significantly. Therefore we may assume that for all systems gelation is induced by KCl. However, gelation was found to be much faster in the presence of CaCl$_2$ even if it was in very small amounts. In addition, the gel modulus at steady state increased with increasing CaCl$_2$ concentration in contrast to pure CaCl$_2$ solutions for which it decreased, see Fig. 3b.

In Fig. 4 the elastic modulus near steady state is shown as a function of the CaCl$_2$ concentration and compared to that in the absence of KCl. In the latter case, G_{el} decreased rapidly with increasing CaCl$_2$ concentration starting from about 2 kPa at 5.3 mM CaCl$_2$. In the presence of 10 mM KCl the elastic modulus increased with increasing CaCl$_2$ concentration until about 15 mM where it reached a value of 4 kPa. The presence of 10 mM KCl also led to a decrease of the turbidity of the gels, implying that they were more homogeneous, see Fig. 5.

The effect of the KCl concentration on the elastic modulus in the presence of different amounts of CaCl$_2$ is shown in Fig. 7. It appears that addition of as little as 0.5 mM KCl has a strong effect on κ-car gelation in the presence of CaCl$_2$. The presence of a small amount of KCl induced gelation at [CaCl$_2$] = 4 mM when in pure salts no gelation was observed. Adding more than 2 mM KCl does not further strengthen the gel significantly even though it led to an increase of T_c. A peculiar phenomenon was observed at 5.3 mM CaCl$_2$. In this case the gel in pure CaCl$_2$ was slightly stronger than when KCl was added. However, addition of KCl strongly increased the gelation rate, which was very slow in the absence of KCl, see Fig. 3b.

Fig. 8 shows the dependence of the gel modulus at 5 °C on the κ-car concentration in the presence of 10 mM CaCl$_2$ and 5.3 mM KCl. The results in the mixed salt are compared to that in pure 10 mM CaCl$_2$. We note that in pure 5.3 mM KCl gelation was very slow and the elastic modulus was very small. As might be expected, in both cases G_{el} increased with increasing κ-car concentration. However, the increase is weaker in the mixed salt and the effect of adding KCl decreased with increasing κ-car concentration.
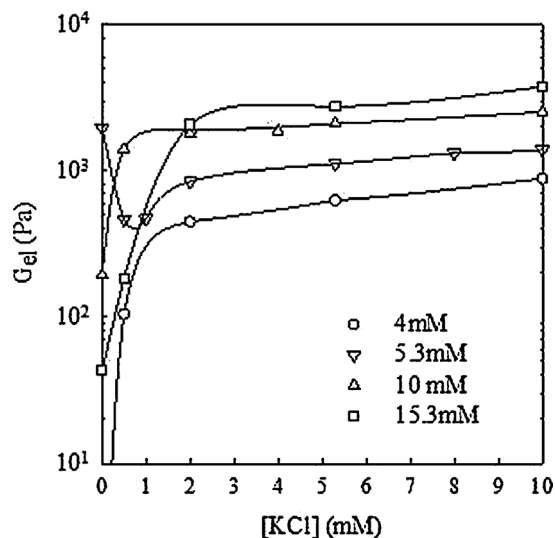
Clearly, the synergistic effect of mixing the two types of salt is less important when the gels are strong.

3.4. Influence of NaCl on potassium induced gelation

A possible origin of the strong influence of CaCl$_2$ on the potassium induced gelation of κ-car is screening of the electrostatic interactions between the chains. In this case addition of NaCl would have the same effect at the same ionic strength (I). Fig. 9 shows the effect of adding NaCl on the gelation of 2 g/L κ-car at 5 °C induced by the presence of 10 mM KCl. Qualitatively the effect of adding NaCl was the same as that of adding CaCl$_2$, i.e. acceleration of the gelation process and increase of the elastic modulus. However, quantitative comparison of the elastic modulus at the same ionic strength (I = [NaCl] and I = 3[CaCl$_2$]) shows that the influence of CaCl$_2$ was much stronger, see Fig. 10. Nevertheless, the presence of Na$^+$ was significant even at concentrations as low as 5 mM. This means that the contribution of the sodium counterions of the κ-car cannot be completely neglected, because we add roughly 2 mM Na$^+$ counterions per gram of κ-car.
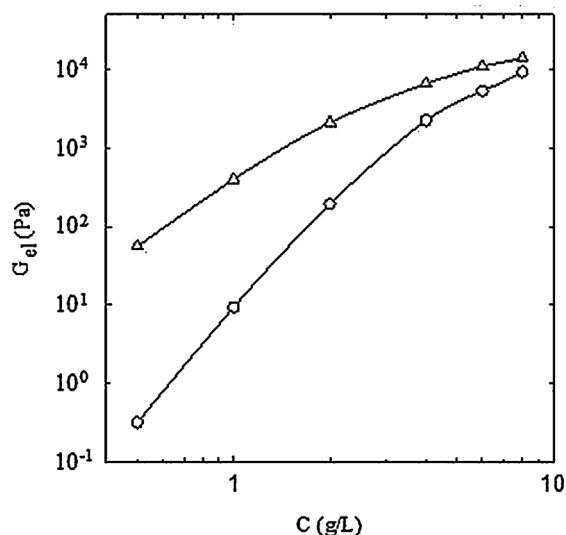

Fig. 8. Dependence of the elastic modulus at 5 °C on the κ-car concentration at [CaCl$_2$] = 10 mM with (△) and without 5.3 mM KCl (○).
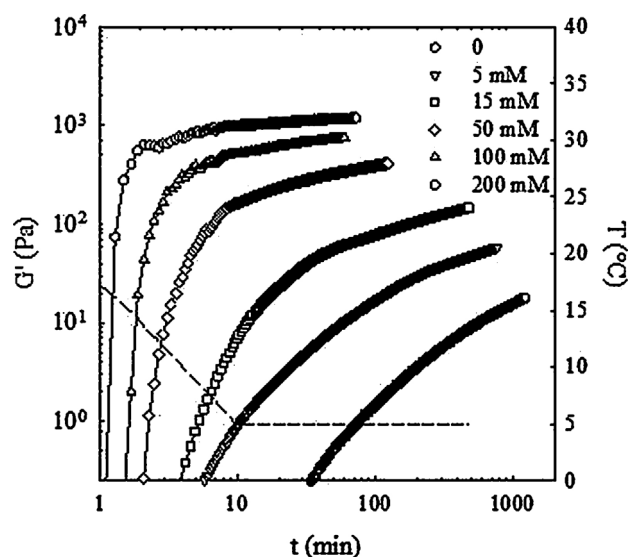
Fig. 9. Evolution of the storage shear modulus at 0.1 Hz of 2 g/L κ-car with 10 mM KCl at different NaCl concentrations during and after rapid cooling to 5 °C. The dashed line indicates the temperature of the sample.
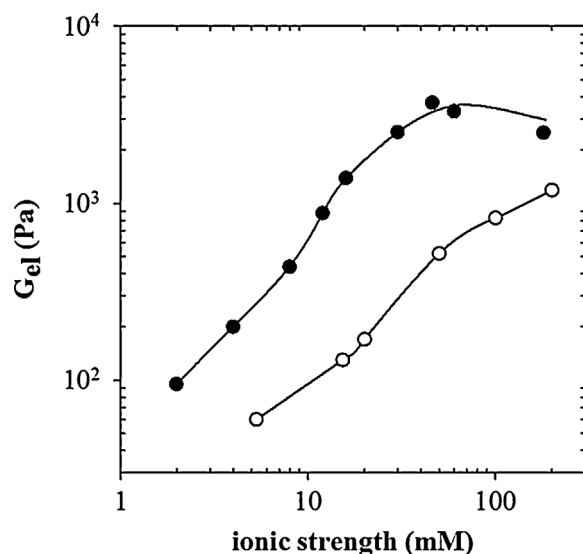

Fig. 10. Elastic modulus of 2 g/L κ-car with 10 mM KCl at 5 °C as a function of the ionic strength of added CaCl₂ (closed symbols) or added NaCl (open symbols). Solid lines are guides to the eye.

4. Discussion

κ-Carrageenan gels because helices from different chains associate laterally. Therefore a necessary condition to form a gel is to induce the coil–helix transition. This transition occurs below a critical temperature that is strongly dependent on the type and concentration of ions that are present including the counterions. However, the critical temperature is independent of the polysaccharide concentration as long as the contribution of the counterions can be neglected. The association of helices accelerates with increasing concentration and for a given concentration with decreasing temperature, as was reported in more detail elsewhere (Meunier et al., 1999).

It is remarkable that the gel stiffness increased with increasing the cation concentration in the case of pure K⁺, at least up to 100 mM, but the elastic modulus decreased in the case of pure Ca²⁺. As was mentioned in the introduction, it has been reported that G_{el} increases at low Ca²⁺ and decreases only at higher Ca²⁺

concentrations, while here we only observe a decrease. However, the initial increase may have been observed for samples for which G' had not yet reached its steady state value, because the gelation kinetics can be very slow at lower salt concentrations. Another remarkable difference between gels induced by pure K⁺ and by pure Ca²⁺ is that the latter become increasingly turbid when more ions are present, while the former remain transparent. The most likely cause for the difference between gelation induced by pure K⁺ and pure Ca²⁺ is that in the presence of higher Ca²⁺ concentrations the helices stack into thicker strands that form more heterogeneous gels.

In the presence of pure K⁺ the association of the helices was very slow at low polymer concentrations even 15 degrees below T_c. Most likely the association was inhibited by electrostatic repulsion between the helices, which explains why adding NaCl strongly sped up this process. NaCl did not modify the coil–helix transition induced by 10 mM KCl, at least below 0.2 M, but it screened the electrostatic repulsion. By itself NaCl induced the formation of weak turbid gels only above 0.1 M.

The synergistic effect on gelation of adding K⁺ and Ca²⁺ together was very strong and cannot be explained only in terms of the ionic strength. Especially at low ionic strengths the effect of adding Ca²⁺ on K⁺ induced gelation was much stronger than that of Na⁺ even though neither of the ions influenced the coil–helix transition at these lower ionic strengths. This observation indicates that specific binding of Ca²⁺ to κ-car occurred as was already suggested by Doyle et al. (2002), which reduced the charge density of the chains much more effectively than merely screening.

Inversely, addition of as little as 2 mM KCl completely modified Ca²⁺ induced gelation. Instead of decreasing with increasing Ca²⁺ concentration in pure CaCl₂, the elastic modulus increased with increasing Ca²⁺ concentration when KCl was present. We note that by itself 2 mM KCl did not give rise to a significant increase of the shear modulus. Clearly, such small amounts of K⁺ do not influence the electrostatic interactions significantly, so the effect must be attributed to the influence on the helix formation. The much lower turbidity in the presence of K⁺ even at high Ca²⁺ concentrations also indicates that different types of gels are formed that are much more homogeneous. Remarkably, gels were formed rapidly in 2 mM KCl even for [CaCl₂] < 5 mM, i.e. under conditions were pure salts did not cause a significant increase of the shear modulus.

The case of 5.3 mM CaCl₂ is peculiar in that addition of KCl led to a decrease of G_{el} up to 1 mM. At higher KCl concentrations G_{el} increased again, but remained slightly lower than without KCl However, the gels with added KCl were formed much more rapidly, compare Figs. 3b and 6. The minimum of G_{el} as a function of the KCl concentration at 5.3 mM CaCl₂ can be understood from the fact that when KCl is added the gel is no longer induced by Ca²⁺, but by K⁺. At higher CaCl₂ concentrations the K⁺ induced gel is stronger than the Ca²⁺ induced gel, but in the presence of 5.3 mM CaCl₂ it is slightly weaker.

The increase of the stiffness of K⁺ induced gels by the reduction of electrostatic repulsion should also apply to screening by KCl at temperatures significantly below T_c. The origin for the increase of the elastic modulus with increasing KCl concentration could therefore be, at least in part, the effect of screening rather than the effect of increasing T_c. In fact, if we consider the further addition of KCl in excess of 10 mM in the same way as that of NaCl and CaCl₂, we find that the increase of the G_{el} with increasing ionic strength by adding more KCl shown in Fig. 2a is intermediate between that by adding NaCl and by adding CaCl₂.

5. Conclusion

κ-Car gels are formed below the coil–helix transition temperature in the presence of KCl or CaCl₂, but with different structures.

In KCl the gel stiffness increases with increasing salt concentration and the gels remain transparent, while in CaCl$_2$ the gel stiffness decreases with increasing salt concentration and they become increasingly turbid.

Addition of NaCl reduces the electrostatic repulsion between the helices which facilitates their association for K$^+$ induced gelation. As a consequence the gel stiffness and the gelation rate increase with increasing NaCl concentration. A similar effect on K$^+$ induced κ-car gelation is observed when CaCl$_2$ is added. However, the effect is much stronger than for NaCl at the same ionic strength indicating specific binding of Ca^{2+} to κ-car.

The presence of a small amount of KCl strongly modifies the Ca^{2+} induced gelation of κ-car. Especially at higher CaCl$_2$ concentrations it leads to a strong increase of the elastic modulus and a reduction of the turbidity. The synergistic between Ca^{2+} and K$^+$ is most striking when the gels are weak in the pure salts.

Acknowledgement

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