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Viscoelastic properties of *κ*-carrageenan in saline solution

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Abstract Viscoelastic properties of *κ*-carrageenan in saline solution at various concentrations and pH were investigated by dynamic rheological techniques, viscosity, elasticity measurements, and IR spectrometry. The viscosity and elasticity at low concentrations of *κ*-carrageenan do not depend on pH, confirming that *κ*-carrageenan is in a disordered conformation. At 0.7% *κ*-carrageenan, the disordered confirmation transforms into an ordered helical confirmation with the possibility of weak-type gel formation. The transformation is also confirmed by dynamic measurements of loss and storage moduli. Furthermore, at this concentration, the viscosity and elasticity are highly dependent on pH. At higher concentrations of NaCl (0.5 M) at some pHs, we observed that storage moduli is greater than loss moduli for the entire frequency region. Hence, there is a possibility of structure transformation from weak-type gel to a somewhat intermediate gel. The lowest viscosity and elasticity were obtained at extreme pH, confirming that there are structural changes occurring at these pHs due to hydrolysis. This is confirmed by IR data.

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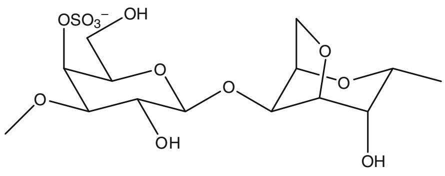
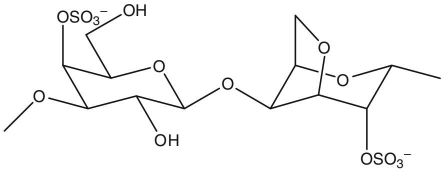
Introduction

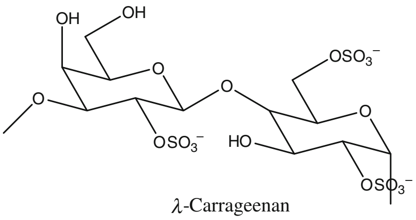
Carrageenan is a high molecular weight ($M_w = 400$–600 kDa) polysaccharide with a high degree of polydispersity. Information on viscosity has many advantages such as texture identification, manufacturing process control, which in turn helps to dictate the choice of pumps, heat exchangers, and mixing systems [1]. Alizadehfard et al. and Ipsen, have studied the viscosity of membrane produced by whey protein concentrates with and without carrageenan [2, 3]. Carrageenans and proteins containing small amount of carrageenans are used in a variety of commercial applications such as gelling, thickening, and stabilizing agents, especially in the food industry. They are also used in pharmaceutical formulations, especially in microencapsulation and controlled drug release, cosmetics, and industrial applications [4]. Carrageenans are anionic linear hetero-polysaccharides obtained commercially by extraction from red seaweeds. They are generally composed of a linear galactose backbone with varying degrees of sulfonation (between 15 and 40%). There are three types of commercially available carrageenans: *ι*-, *κ*-, and *λ*-carrageenan. They are composed of (see Fig. 1) alternating 3-linked *β*-D galactopyranose (G-units), 4-linked-D galactopyranose (D-units), or 4-linked 3,6-anhydrogalactose (DA-units). *ι*- and *κ*-carrageenan are gel-forming carrageenans, whereas *λ*-carrageenan is a thickener/viscosity builder [5]. *κ*-carrageenan and *ι*-carrageenan can occur in two forms: unstructured random coil conformation, typically at elevated temperatures or without counter



κ-Carrageenan



ι-Carrageenan



λ-Carrageenan

Fig. 1 Structure of carrageenan

ions, and structured double helices, which are usually formed upon cooling or upon addition of counter ions.

During gelation of κ-carrageenan, it undergoes a disorder-order (coil ↔ helix) conformational transition that is highly depended on the presence of counter ions ($Rb^+ > Cs^+ \geq K^+ >> Na^+ \geq Li^+$) [6–8]. Furthermore, these cations stabilize the double helix structure by shielding the charge of the sulfate group and promote helix-helix aggregation [5]. The mechanism of gel formation of κ-carrageenan (in KCl) starts with the formation of helical dimers (coil ↔ helical dimers), followed by aggregation of these dimers into a gel phase [9]. The exact gelation mechanism depends on the type of cations present. For example, in mixtures containing NaI and CsI, the gelation mechanism will undergo coil → helix → super helical rod → gel transitions for many polysaccharide families [10–12]. Moreover, anions play a vital role in promoting gelation due to increasing aggregation [13–15]. Their results show that the Cl^- has the lowest binding strength to κ-carrageenan, hence the lowest effect on gelling in κ-carrageenan. The pH is less important for κ-carrageenan gelation. It has no influence in gelation temperature [15]. Furthermore, in Eleya and Turgeon [15] results show that 1% κ-carrageenan in deionized water, the pH between 4.0 and 7.0 has little effect on gelation temperature. Recent studies have shown that the κ-carrageenan in NaCl solution is in the disordered conformation, and it has terminal relaxation with G'' (loss modulus) $>G'$ (storage modulus) [15–17].

As mentioned above, NaCl (saline solution) has little effect on κ-carrageenan gelation when compared with other salt solutions [17]. However, the gelation temperature can be increased with increasing the NaCl concentration [18]. There is less attention paid to how pH affects for the gelation in NaCl salt solution. In this study, we investigated the gelation of κ-carrageenan in NaCl salt solution at different pHs, in order to mimic gelation concepts in physiological conditions.

Materials and sample preparation

Materials

κ-carrageenan (Colloid 710H) was obtained from TIC Company. According to the manufacture, it contained Na^+ (7.09×10^{-9} mol), K^+ (1.30×10^{-3} mol), and Ca^{2+} (3.04×10^{-5} mol). It was used without further purification. These counter ions are below the required counter ion concentrations for κ-carrageenan gelation. For example, the minimum potassium ions required for gelation at 288 K is 4.5 eq m^{-3} [19]. Water used in the experiments was purified through Barnstead E-pure filter system and collected at 18 MOhm.

Sample preparation

κ-carrageenan concentration was ranged from 0.1% (mass/mass) to 0.7% (mass/mass) with saline solution (0.1, 0.2, and 0.5 M NaCl). The pH was adjusted to 3.0, 5.0, 7.4, 9.0, 11.0, and 13.0 with diluted HCl or NaOH. pH were measured using the Thermo Orion (Model 410) pH meter. Samples were heated to 323 K for 10–15 min to dissolve the carrageenan and then stored at room temperature for 10 min before loading to the rheometer. The same procedure was followed for each sample in order to avoid the aggregation and aging effects.

Instrumentation

Rheometer

The Vilastic-3 Rheometer was used to determine viscosity and elasticity at constant frequency and temperature. Low concentration samples were measured using a 1-mm diameter tube, while higher concentration samples a 1.8-mm diameter tube. Both tubes were 6.27 cm long. All the data were taken within the linear viscoelastic region. The data reported are at least three replicates and the data reproducibility is within 5–10%.

IR spectrometer

1 mg of 0.7% κ-Carrageenan in different pHs were mixed with KBr, made into 0.5 cm pellets, and measured in the Magna 560 IR spectrometer.

Results and discussion

κ-carrageenan can be used in medical application due to its biocompatibility. For these applications, it is important to understand under which conditions gels form. Therefore, the gel point was determined under biological ionic strength. In order to find the gel concentration and gel point, viscosity and elasticity for 0.1 to 0.7% (mass/mass) were measured at different pHs.

The intrinsic viscosity for different pH values and hence viscosity average molecular mass (MM) were calculated using the Mark–Houwink relationship $\eta = K\,M^a$ with $a = 0.95$ and $K = 3.1 \times 10^{-3}$ (ml g $^{-1}$) (Table 1) [20]. The lowest MM values were obtained for pH 3.0 and pH 13.0, whereas the highest MM were obtained for pH 5.0 and pH 7.4 where the viscosity was found to be the highest.

Viscosity was determined at shear rates between 1 and 1000 (1/s). At 0.1%, viscosity is greater than elasticity (Fig. 2). Further, at this concentration viscosity is very close to the viscosity of water (1×10^{-3} Pa s). Figure 2 confirms that viscosity and elasticity do not change significantly with pH except for the sample at pH 3.0, which has the lowest viscosity. Viscosity is increasing with concentration (Fig. 3). Elasticity is still lower than viscosity, but at this concentration elasticity is decreasing with applied shear rate. The lowest viscosity was found at pH 3.0 and pH 13.0.

Figure 4 shows viscosity and elasticity versus shear rate for different pH values at 0.5% (mass/mass). We can clearly see a maximum viscosity and elasticity between pH 5.0 and pH 11.0. At this concentration, elasticity is also highly dependent on the shear rate.

As mentioned earlier, κ-carrageenan in NaCl solution is in disordered form [16]. Changing the pHs in low

Table 1 The calculated intrinsic viscosity and MM for different pH values

pH	Intrinsic viscosity	Molecular mass/$\times 10^5$ g mol^{-1}
3.0	11.04	0.1
5.0	528.19	3.2
7.4	412.72	2.5
9.0	204.39	1.2
11.0	373.37	2.2
13.0	158.82	0.9

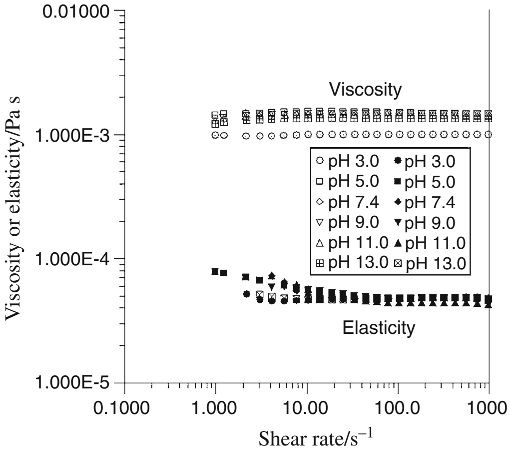


Fig. 2 κ-carrageenan 0.1% (mass/mass) for different pH values at temperature 298 K, viscosity and elasticity vs. shear rate

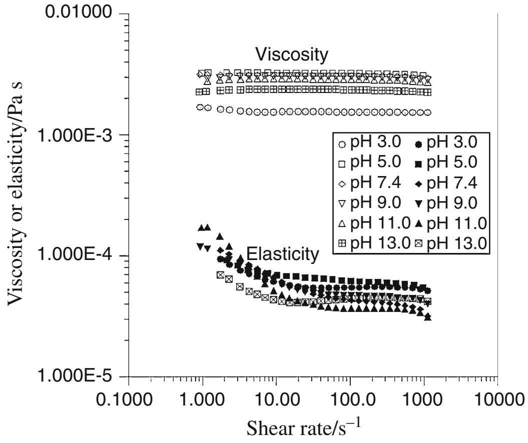


Fig. 3 κ-carrageenan 0.3% (mass/mass) for different pH values at temperature 298 K, viscosity and elasticity vs. shear rate

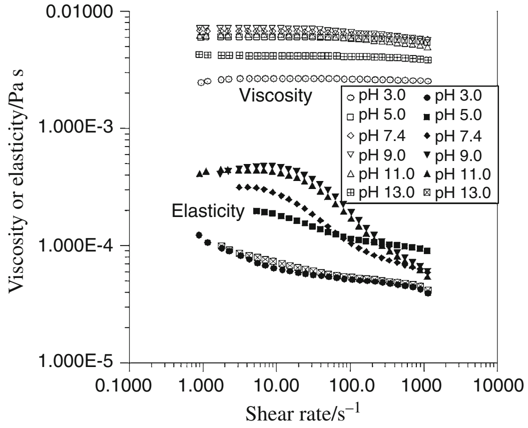


Fig. 4 κ-carrageenan 0.5% (mass/mass) for different pH values at temperature 298 K, viscosity and elasticity vs. shear rate

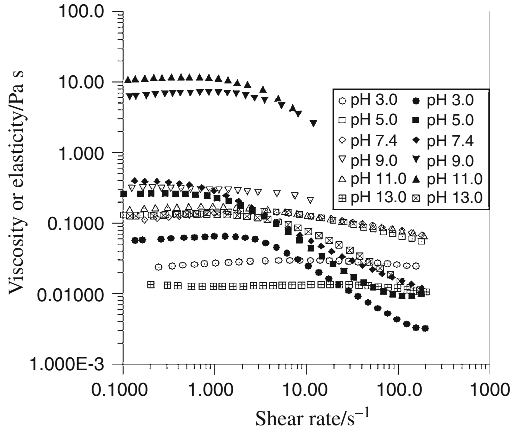


Fig. 5 κ-carrageenan 0.7% (mass/mass) for different pH values at temperature 298 K, viscosity (*open symbols*) and elasticity (*solid symbols*) vs. shear rate

concentration (0.1, 0.3, and 0.5%), it is still in disordered conformation and does not affect to change into the ordered double helical conformation. Furthermore, viscosity is almost constant for low concentrations (Fig. 4) and is independent of applied shear rate, further confirming the disordered conformation. However, elasticity is highly dependent on shear rate (0.5% κ-carrageenan—Fig. 4), confirming that there is a possibility to form temporary networks within this disordered conformation. At pH 3.0, due to acid hydrolysis, the structure is changed and the viscosity reduced [21–23]. This is also confirmed from our molecular mass data (Table 1) where we obtained the lowest MM at pH 3.0, and IR data (Fig. 7) that shows the loss of sulfate group and possible the lactone.

At 0.7%, κ-carrageenan gel formation (weak gels) was observed (Fig. 5). Various factors can influence the conditions of gel formation and the gel strength achieved. From our rheological measurement of viscosity data shows that at least 0.01 Pa s needed in order to form gels. Furthermore, viscosity and elasticity are very close to each other except at pH 9.0 and pH 11.0. Highest elasticity was found at these pHs as well. Additionally, viscosity is highly dependent on shear rate with the exception of the extreme pHs. This is confirmed that at higher concentration (0.7%) κ-carrageenan in NaCl can form ordered confirmation. It is possible that pH change can form additional hydrogen bonds along with ionic interactions and thus become strong.

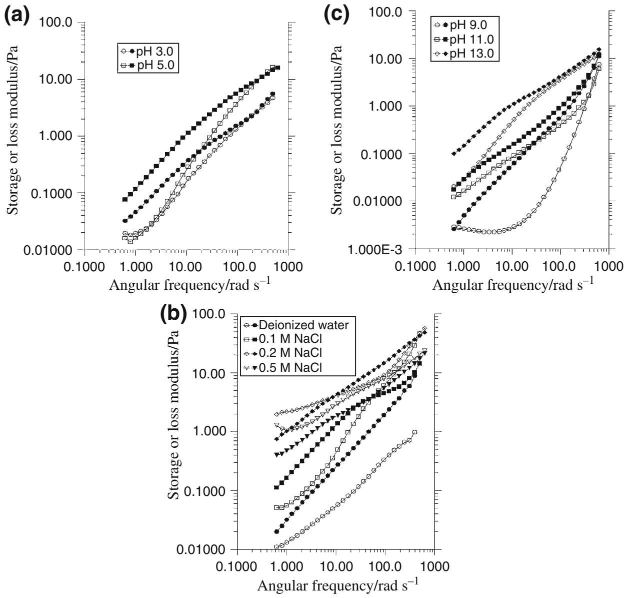


Fig. 6 κ-carrageenan 0.7% (mass/mass) for different pHs values, storage module (G' *open symbol*) and loss module (G'' *solid symbol*) vs. frequency at shear rate 6 (s^{-1}) and temperature 298 K. **a** pH 3.0 and pH 5.0, **b** pH 7.4 with different NaCl concentration, **c** pH 9.0, pH 11.0, and pH 13.0

The frequency dependence of G' (storage modulus) and G'' (loss modulus) for 0.7% κ-carrageenan is shown in Fig. 6, which is measured with fixed shear rate and well within the linear regime. With low pHs (pH 3.0 and pH 5.0) (Fig. 6a), the crossover point has shifted to high frequency with $G'' > G'$ for entire frequency range with $G'' \sim \omega^{0.8}$, $G' \sim \omega^{1.0}$ and $G'' \sim \omega^{0.8}$, $G' \sim \omega^{1.3}$ for pH 3.0 and pH 5.0, respectively. In dilute or semi-dilute polymer solution at low frequency, G' and G'' are proportioned to ω^2 and ω^1, respectively. Figure 6b shows G' and G'' at pH 7.4 for different NaCl concentration. Lowest G' and G'' was obtained with $G' > G'$ ($G'' \sim \omega^{1.6}$ and $G' \sim \omega^{2.7}$) for entire frequency range in deionized water, confirming that NaCl has a significant effect in κ-carrageenan gelation. Furthermore, it is also confirmed that other counter ions in our sample have less effect on gelation because we have not observed any gelation in deionized water. The crossover frequency, at which $G' = G''$, indicates gel formation and can be seen clearly for 0.1 M NaCl solution (which is around 35 rad s^{-1}). Hence, the calculated relaxation time of the gel is about 0.1 s. Furthermore, for the same NaCl concentration, we observe $G'' > G'$ at low frequencies ($G'' \sim \omega^{0.8}$ and $G' \sim \omega^{1.1}$) and $G' > G''$ ($G' \sim \omega^{1.0}$ and $G'' \sim \omega^{0.4}$) for higher frequencies. A typical gel should exhibit solid-like mechanical properties, i.e. $G' > G''$, with low frequency dependence of the modulus [16]. But in our case, $G'' > G'$, which confirms that the gel we observed is a weak gel. This is also confirmed by the large value of tan δ ($=G''/G'$), which is >0.1 (data not shown here) for the entire frequency range. These mechanisms may be explained in the following way. The macromolecular chains of the κ-carrageenan can disentangle during a long period of oscillation at a lower frequencies. Thus the system behaves like a solution, which explains why G'' is higher at lower frequencies. But at the same time the entangled macromolecular chains do not disentangle during a short period at a higher frequency. Therefore, the entanglement point plays a role of a kink or knot of 3-D network formation, and the system may well show solid-like behavior.

As the NaCl concentration increases (0.2 M) the cross-over frequency was shifted to lower values (8 rad s^{-1}) with $G' > G''$ ($G'' \sim \omega^{0.6}$ and $G' \sim \omega^{0.3}$) at low frequency and $G'' > G'$ at higher frequency. Furthermore, at 0.5 M NaCl solution, we observed $G' > G''$ ($G'' \sim \omega^{0.6}$ and $G' \sim \omega^{0.5}$) for the entire frequency range. At this NaCl concentration we observed a weak gel, but still much stronger than the gel at lower NaCl concentration (0.1 and 0.2 M). The dependence of storage modulus (G') on frequency for all the samples of 0.7% κ-carrageenan with 0.1, 0.2 and 0.5 M NaCl were fitted by a power-law relation, but with very small exponents less than 1. Thus, G' can be approximately viewed as less dependent of frequency.

An apparent feature was evidenced in Fig. 6b and is the existence of a minimum in the G'' (loss modulus) in all the gels, irrespective of weak or intermediate, at higher frequencies. This is also confirmed by the low values of ω dependency of G' and G''. We may call it as "intermediate" gel. Similar results (weak-type gel) were obtained for 1.5% κ-carrageenan in NaI solution with $G' > G''$ for entire frequency range [24]. Figure 6c, pH 9.0 (except low frequency), pH 11.0 (G', $G'' \sim \omega^{1.0}$), and pH 13.0 ($G'' \sim \omega^{0.8}$ and $G' \sim \omega^{1.2}$) show no crossover frequency and hence there is a terminal relaxation at or around $G'' > G'$ for the entire frequency. The same results were obtained for 1.2% (mass/mass) κ-carrageenan in 0.1 mol dm^{-3} NaCl solution with terminal relaxation time with a $G'' > G'$ [16]. However, Pelletier and Viebke G'' and G' values are less than 1 Pa compared with our values. We obtained the higher values for G'' and G' even with low κ-carrageenan concentration (0.7% mass/mass). This is due to changes in pH, which changes the disordered system into somewhat well-ordered system, thus increasing the moduli.

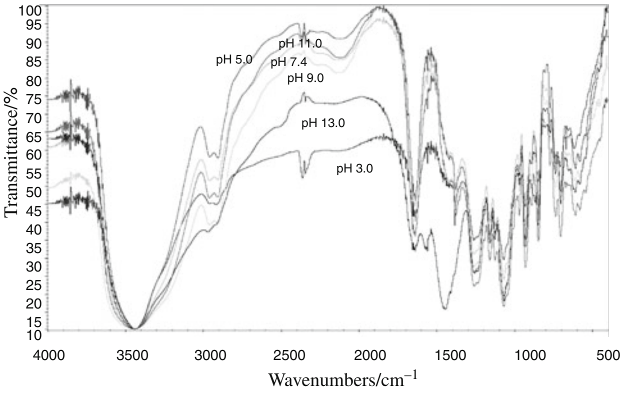


Fig. 7 IR spectroscopy data for κ-carrageenan 0.7% (mass/mass) for different pH values at 0.1 M NaCl

IR spectroscopy to determine structural changes in the gel

IR spectroscopy data for 0.7% κ-carrageenan are shown in Fig. 7. This data show that at pH 3.0 and pH 13.0 there are structural changes. At both pHs the peak at 2000–2300, due to the sulfate group, disappears, i.e. hydrolyzed off. Additionally, at pH 3.0 a strong peak at 1450 grows in which might be due to the loss of the lactone.

Conclusions

Viscoelastic properties of κ-carrageenan at different concentrations in saline solution were investigated at different pH values. Regardless of pH, at low concentration κ-carrageenan is in its disordered confirmation. Furthermore, viscosity and elasticity is not dependent on pH. At 0.7% κ-carrageenan concentration, the elasticity is very close to viscosity confirming that the formation of gel not strong gels. But at this concentration, κ-carrageenan is in its ordered conformation depending on the pH. This is also confirmed by the dynamic measurement of G' and G''. Furthermore, at this concentration, viscosity and elasticity are highly dependent on pH. Increasing NaCl concentration (0.5 M), weak type of gels can be transformed into intermediate gels with $G' > G''$ for the entire frequency range. Lowest viscosity and elasticity values were obtained at pH 3.0 and pH 13.0 which correlate with the lowest molecular mass values. These data are further confirmed by IR data, showing the possibility of structural changes of κ-carrageenan, such as the hydrolysis of the sulfate group at pH 3.0 and pH 13.0.

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