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# Rheological and calorimetric study of the sol–gel transition of κ-carrageenan

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## Abstract

Rheological and DSC techniques were used to study the effect of κ-carrageenan and KCl concentrations, 0–300 mM, on the sol–gel transition as well as on the linear viscoelasticity, at 25 °C, of the resulting gels. In heating and cooling DSC tests, the peak temperature was taken as the sol–gel transition point. In rheological tests, sol–gel transitions were determined from the variation of dynamic moduli with frequency and temperature, the independence of the phase angle on frequency and the evolution with temperature of dynamic moduli on cooling and heating at constant frequency and strain. Transition temperatures from DSC and rheology were in good agreement among them and with those previously reported. The three procedures yielded similar results, but the transition temperatures were more easily determined through the independence of the phase angle on frequency. Frequency sweeps showed gel behavior with stiffness increasing with polysaccharide and salt concentration. Below 100 mM KCl, $G'$ increased notably, whereas higher concentrations produced only marginal increases.

*Keywords:* κ-Carrageenan; Gels; Sol–gel transition; Viscoelasticity; DSC

## 1. Introduction

Carrageenans are sulfated polymers extracted from red algae that consist of alternating (1 → 3) α-D-galactose and (1 → 4)-β anhydro-D-galactose forming a linear chain. The κ, ι, and λ types exist depending on the number of sulfate groups and their position in the repetitive unit. κ-carrageenan forms gels with $Ca^{+2}$, $Na^+$ and $K^+$ ions, but the latter produce stronger gels (Mangione et al., 2004). This polysaccharide is used as a gelling agent in many prepared foods such as pies, paddies, flans, puddings, canned meats, pet foods and frankfurters (Reginald, 1998). In κ-carrageenan gels, the ionic interaction between $SO^{-3}$ groups and $K^+$ ions together with intra and interchain hydrogen bonds give rise to helical structures. When the total ionic concentration exceeds a critical value, $C^*$, of about 0.007 equiv/L, thermal hysteresis appears as a consequence of aggregation of helical structures into three-dimensional networks, while below such value only a coil–helix transition takes place without gelation (Rochas, 1982). It has been claimed that κ-carrageenan gelation can be described by the domain model (Mangione, Giacomazza, Bulone, Martorana, & San Biagio, 2003) and that complete gel setting is time-dependent (Tecante & Doublier, 1999). The sol–gel transition diagram, which is the relationship between log $C_T$ vs $T^{-1}$, with $C_T$ being total ionic concentration (salt + polymer) and $T$ absolute temperature, for the potassium salt of purified κ-carrageenan is well known (Rochas, 1982). κ-Carrageenan assumes a random coil conformation in the sol state, and low temperatures induce twisting of anhydro-galactose sequences into double helices. A portion of the hydrated sequences functions like a helix cross-linking domain that leads the infinite network structure to complete gelation (Yuguchi, Thuy, Urakawa, & Kajiwara, 2002). Gelation of κ-carrageenan has been

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investigated by a variety of techniques such as small angle neutron scattering (Sugiyama et al., 1998), rheology (Chen, Liao, & Dunstan, 2002; Mangione et al., 2003; Nishinari & Takahashi, 2003; Takemasa & Chiba, 2001), optical rotation and dispersion (Mangione et al., 2003), light scattering (Mangione et al., 2003), photon transmission (Kara, Tamerler, Bermek, & Pekcan, 2003), small angle X-ray scattering (Yuguchi et al., 2002; Yuguchi, Urakawa, & Kajiwara, 2003), and micro differential scanning calorimetry (Nayouf, 2003). However, assessment of the applicability of different rheological procedures to determine the sol–gel transition as well as the viscoelastic properties of the resulting gels in the presence of a wide range of counter-ion concentration are not documented in the literature.

The gelling temperature, $T_{\text{gel}}$, can be determined from rheological data by several procedures. Two of them arise from the works of Winter and Chambon (1986) and Chambon and Winter (1987) with chemical gels. They found that at the gel point, $G''$ and $G'$ were proportional to $\omega^n$ over a wide range of temperature and frequency. They also showed that $G(t) = St^{-n}$, where $G(t)$ is the relaxation modulus, $t$ is time, $S$ the gel strength and $n$ the relaxation exponent. This implies that at the gel point the phase angle between stress and strain, $\delta$, is independent of frequency and proportional to $n$, i.e. $\delta = n\pi/2$. Initially, they found $n$ to be 0.5 for cross-linking gels of polydimethylsiloxane under stoichiometric conditions. However, $n$ varies from 0.19 to 0.92 for end-linked polydimethylsiloxane polymers prepared with various stoichiometry, chain length and concentration (Scanlan & Winter, 1991). The exponent $n$, has shown to be 0.62 to 0.69 for whey protein gels (Labropoulos & Hsu, 1996), 0.13 for soy protein gels (Hsu, 1999) and 0.56 for egg yolk gels (Cordobés, Partal, & Guerrero, 2004). Winter's analysis has been used also to investigate physical gels of polysaccharides near the gel point (Nishinari, 1997). In such systems the exponent ranges from 0.18 to 0.21 and 0.17 to 0.24 for the sol–gel transition of ι-carrageenan without and with addition of potassium ions (Hossain, Miyanaga, Maeda, & Nemoto, 2001) and 0.5 for starch gels (Hsu, Lu, & Huang, 2000). Thus, the value of $n$ depends largely on the particular biopolymer as well as on the conditions of the aqueous environment. The gel point, $T_{\text{gel}}$ in our case, can be determined when the phase angle becomes independent of frequency (Chambon & Winter, 1987). This treatment, hereinafter called the critical phase angle procedure, has been used to determine the critical conditions, i.e. time, temperature and polymer concentration, during gelation or melting of ι-carrageenan, xanthan-locust bean mixtures, gelatin and pectin–calcium systems (Doublier & Cuvelier, 1996). The power law dependence of $G'$ and $G''$ with frequency and the critical phase angle arise from the same principle, however, handling of rheological data is different. It is this difference in data treatment that has allowed different authors to determine the value of $n$ for a given system. For example in gelatin gels, Michon, Cuvelier, and Launay (1993), determined first the gelation time and the exponent $n$ through the independence of $\tan\delta$ with frequency and compared this value with that determined from frequency sweeps at different aging times near the gel point as originally reported by Winter and Chambon (1986). On the other hand, Ross-Murphy (1991) described four ways from which the gelling time can be determined: (1) when viscosity becomes infinite in a steady shear test, (2) when $G'$ diverges from a characteristic "signal noise", (3) when $G''$ shows a maximum, and (4) when $G' = G''$ ($\tan\delta = 1.0$) during time evolution of both moduli in gel cure experiments. The first procedure is excluded because it is unrealizable in practice and only the other three seem feasible.

The question is whether the aforementioned procedures can be applied to κ-carrageenan in order to assess their applicability to study the effect of polymer and counter-ion concentration on the sol–gel transition temperature. Therefore, the purpose of our work is two-fold: (1) to analyze the feasibility and applicability of different procedures to determine $T_{\text{gel}}$ for κ-carrageenan, and (ii) to study the effect of κ-carrageenan and total $K^+$ ion concentration on the sol–gel transition and on the viscoelasticity of the resulting gels. We have used micro-DSC in order to monitor the transition and compare it with that determined from the evolution with temperature of $G'$ and $G''$ under low amplitude oscillatory shear. Our goal is to understand the way in which the variation of the previously mentioned factors determines the transition as well as the viscoelastic properties of the resulting gels of this polysaccharide.

## 2. Materials and methods

Food grade κ-carrageenan was kindly provided by Degussa Texturant Systems France SAS (Baupte, France). KCl (Sigma–Aldrich) was reagent grade and deionized water was used throughout. Polymer solutions were prepared on a dry weight basis. The molecular weight was $5.63 \times 10^5$ as determined from intrinsic viscosity in 0.1 M NaCl + 0.005 M EDTA at 25 °C, using the relation $[\eta_0] = 3.1 \times 10^{-5}\ \text{M}^{0.95}$ (Rochas & Landry, 1987).

### 2.1. Micro-DSC

The sol–gel transition was monitored in aqueous solutions of 0.5% κ-carrageenan in a microcalorimeter (Micro DSC III, Setaram, France) in the presence of 0–80 mM KCl. A mass of 500 mg of sample was weighted into a stainless steel cell and scanned at the maximum rate of 1.0 °C/min from 5 to 80 °C in two heating–cooling cycles. The reference line was obtained by scanning KCl solutions with the same salt concentration as in the samples. The transition enthalpy was calculated per gram dry weight of κ-carrageenan.

### 2.2. Rheology

In rheological tests, polymer concentrations were 0.1, 0.5, and 1.0% with KCl from 0 to 300 mM. In micro-

DSC and rheological measurements, KCl was dissolved first in water and then κ-carrageenan was dispersed at 25 °C. The dispersion was heated to 80 °C and held at this temperature for 15 min. The sol–gel transition was determined under low amplitude oscillatory shear in a strain rheometer (ARES-RFS III, TA Instruments, USA). The hot solutions were examined in serrated parallel plates (25 mm diameter, 1 mm gap) previously equilibrated to 80 °C with their rim covered with paraffin oil to prevent water evaporation. The change of $G'$ and $G''$ with temperature was determined during cooling (2.5 °C/min) to 25 °C at 1 Hz in the zone of linear viscoelasticity. The extension of this zone depended on polymer and salt concentration. Once at 25 °C, the viscoelastic properties were examined under low amplitude oscillatory shear from 0.1 to 100 rad/s. Finally, the sample was heated (3.0 °C/min) to 80 °C. Temperature was controlled by a circulator bath (FS18-MV, Julabo Ltd., Germany). The linear viscoelasticity zone was determined from strain sweeps at constant temperature. The sol–gel transition was examined by measuring the temperature and frequency dependence of $G'$ and $G''$ in the range of 80 to 10 °C.

## 3. Results

### 3.1. Sol–gel transition from DSC measurements

The heating and cooling signals were fully reproducible during the first and second scans and for this reason only the latter are reported. Fig. 1 shows the DSC heating and cooling thermographs for 0.5% κ-carrageenan containing 0–80 mM KCl. Only one "endo" and one "exo" peak was observed on heating and cooling, respectively. During heating, hydrogen bonds are broken and double helices change their conformation giving rise to fusion of aggregates and network break up. The transition occurs over a temperature range but the peak temperature was taken as the melting temperature, $T_m$ (Fig. 1(a)). On cooling (Fig. 1(b)) an exothermic random coil to helix transition occurs, leading to gel formation at a particular gelation temperature, $T_{gel}$, represented by the peak in the DSC signal. The difference between $T_m$ and $T_{gel}$ is known as "thermal hysteresis" which is associated with aggregation of doubles helices during gel formation (Mangione et al., 2003; Nayouf, 2003). Our results show that such aggregation is enhanced when $K^+$ ions are added and this resulted in $T_m$ greater than $T_{gel}$ for the same KCl concentration, for all salt concentrations. Thermal hysteresis has also been reported for κ-carrageenan with and without added KCl but for polymer and salt concentrations of 1–4% and 0, 27, 54 and 80 mM KCl, respectively (Kara et al., 2003). Hysteresis can be due to the different energy levels between the packed helices as it has been pointed out for gellan (Milas & Rinaudo, 1996). Fig. 2 shows the variation of $T_m$, $T_{gel}$, as well as the melting, $\Delta H_m$, and gelling, $\Delta H_{gel}$, enthalpies with KCl concentration during cooling and heating for 0.5% κ-carrageenan. The melting, $T_m$, and gelling, $T_{gel}$, temperatures were clearly different and increased with the increase in KCl concentration (Fig. 2a). Aggregation of helical segments of κ-carrageenan can lead to an increase of up to 10 °C in the melting temperature over that of isolated segments of the helix (Rochas & Rinaudo, 1982). The increase in KCl concentration also leads to larger thermal hysteresis. This fact suggests that the number of junction zones increases with KCl concentration. The variation of $\Delta H_{gel}$ and $\Delta H_m$ with salt concentration was similar (Fig. 2(b)).

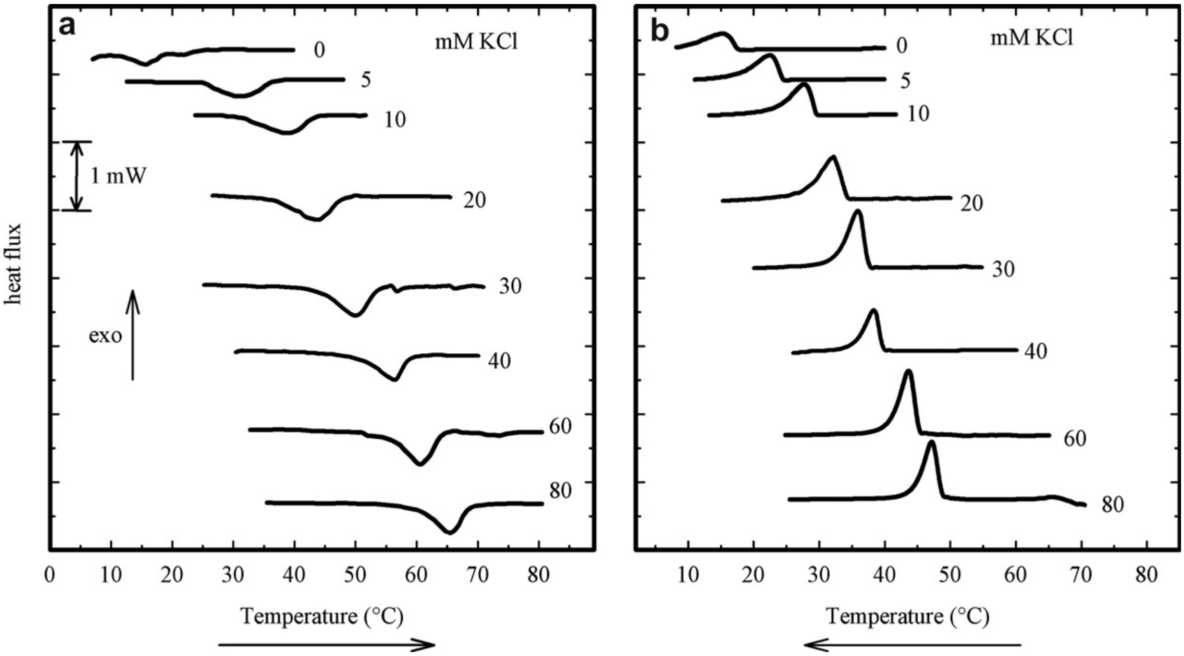

Fig. 1. Heating (a) and cooling (b) thermographs for 0.5% κ-carrageenan with different KCl concentrations. Rate = 1.0 °C/min.

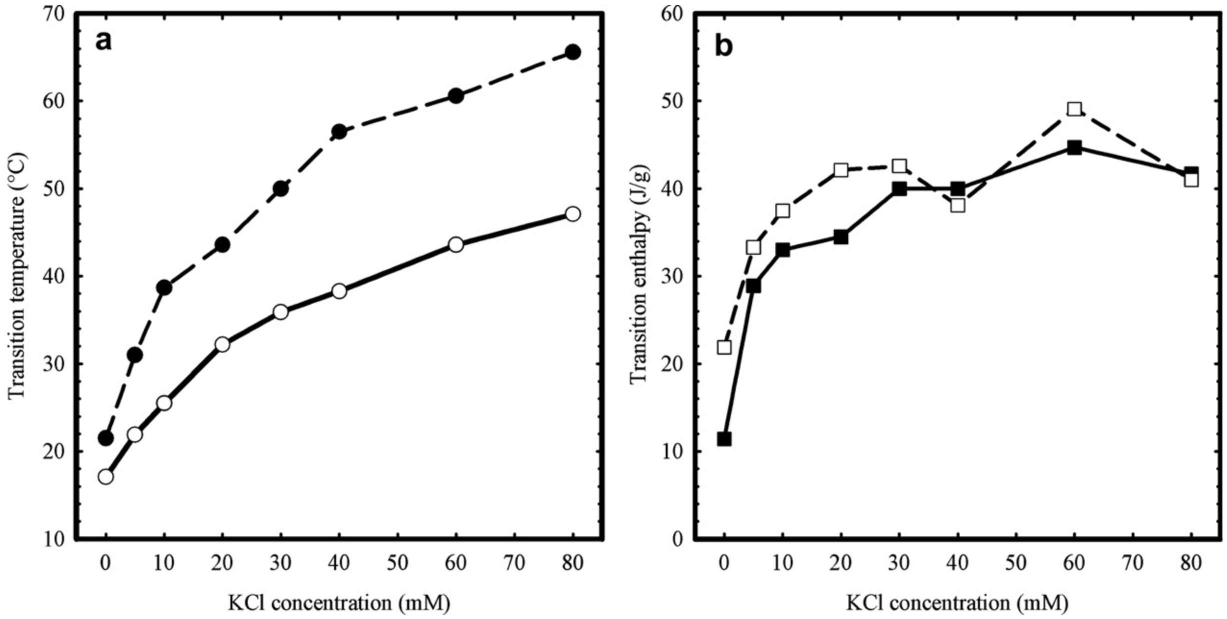

Fig. 2. Variation with KCl concentration of the melting (black symbols) and gelling (white symbols) temperatures (a) and transition enthalpies (b) for 0.5% κ-carrageenan.

$\Delta H_{\mathrm{gel}}$ was slightly superior to $\Delta H_{\mathrm{m}}$, because double helix aggregation releases more energy than that required to melt the aggregates as observed by photon transmission for κ-carrageenan with potassium ions (Kara et al., 2003).

### 3.2. Analysis of the variation of $G'$ and $G''$ with frequency and temperature

Fig. 3(a) shows the variation with frequency of the dynamic moduli from 38.6 to 42.7 °C for 0.5% κ-carrageenan with 40 mM KCl. These data were treated according to the procedure of Winter and Chambon (1986). At 42.7 and 41.2 °C the rheological behavior was typical of macromolecular solutions with $G''$ superior to $G'$ and both moduli dependent on frequency (Ferry, 1980). At 40.8 °C, both moduli were practically equal ($\tan\delta \approx 1.0$). At 39.6 and 38.6 °C, a characteristic gel behavior appeared with $G'$ greater than $G''$, $\tan\delta = 0.2$ to 0.4 and 0.05, respectively, and both moduli virtually independent of frequency (e.g. $G' \sim \omega^{0.02}$). Solutions with 1% κ-carrageenan without

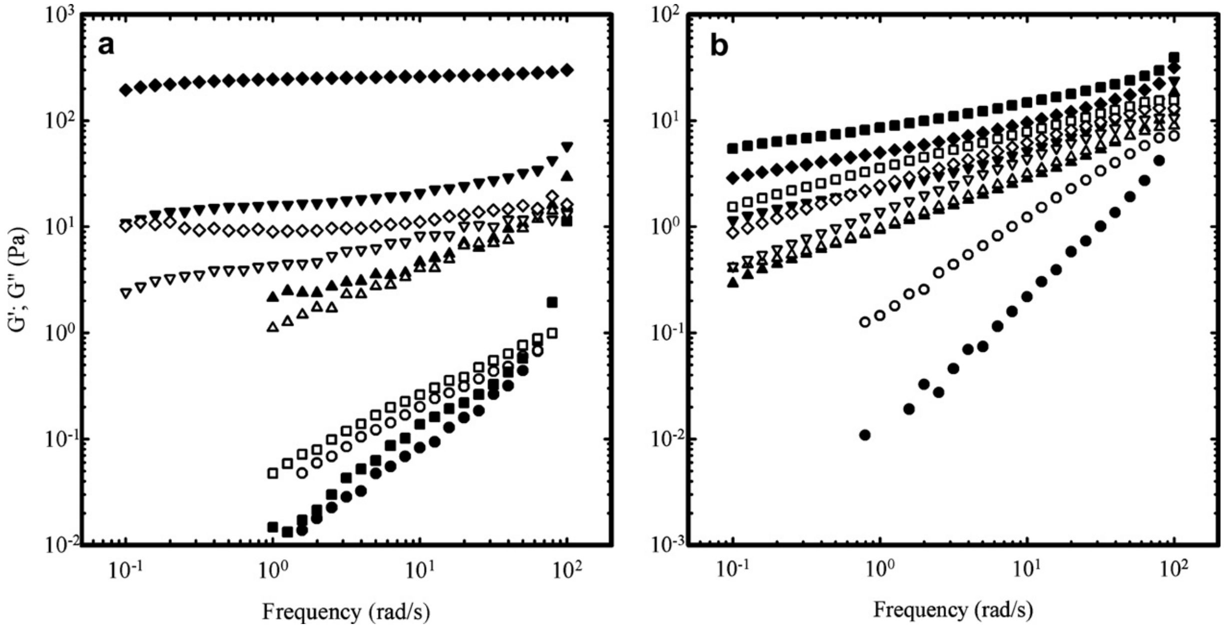

Fig. 3. Variation with frequency of the dynamic moduli at different temperatures. (a) 0.5% κ-carrageenan + 40 mM KCl, at 42.7 °C (circles), 41.2 °C (squares), 40.8 °C (triangles), 39.6 °C (inverted triangles) and 38.6 °C (diamonds). (b) 1.0% κ-carrageenan without added salt at 24.2 °C (circles) 22.3 °C (triangles), 21.4 °C (inverted triangles), 20.5 °C (diamonds) and 19.5 °C (squares). $G'$ (black symbols) and $G''$ (white symbols).

added ions exhibited a similar behavior during the sol–gel transition (Fig. 3(b)). However, at the transition temperature of 22.3 °C, both moduli were lower than those for 0.5% κ-carrageenan with added ions. This shows that for 0.5% κ-carrageenan + 40 mM, KCl aggregation was larger than for 1% κ-carrageenan without added ions. At 24.2 °C, 1% κ-carrageenan behaved as a macromolecular solution, while at 22.3 °C both moduli were nearly equal ($\tan\delta = 1.0$). Below 22.3 °C, $G'$ was superior to $G''$ and both moduli were slightly dependent on frequency (e.g. $G'$ and $G''$ α $\omega^{0.25}$). This is in agreement with the behavior reported for 1% κ-carrageenan between 20 and 5 °C (Chen et al., 2002). Near the gel point the variation of $G'$ and $G''$ with frequency obeyed a power law.

The power law exponent was calculated from the mechanical spectra for both carrageenan concentrations. Fig. 4(a) shows the variation of the power law exponents with temperature during cooling of 0.5% κ-carrageenan + 40 mM KCl. At 38.6 °C, the value of the power law exponent was low because at this temperature a strong gel was formed in which, as expected, the dynamic moduli were practically independent of frequency (Ferry, 1980). The power law exponent increased significantly with temperature; at about 40.8 °C, it was 0.62 and the dynamic moduli overcrossed setting the value of $n$ for this system. Our data show that the power law exponent is not constant, but near the sol–gel transition it increased rapidly with temperature. For example, it was about 0.1 at 39.6 °C and 0.8 at 42 °C.

In accordance with Winter and Chambon (1986), at the critical gel point, $G'$ and $G''$ show the same power law dependence over the entire frequency range and $n$ obtained from the particular mechanical spectra, as $G'$ and $G'' \sim \omega^n$, would agree with $n$ obtained from the condition $\tan\delta = G''/G' = \text{constant} = \tan(n\pi/2)$. However, as shown for the mechanical spectra at 40.8 °C (triangles in Fig. 3(a)) $G'$ and $G''$ had a small dependence on frequency and in consequence, $\tan\delta$ varied from 0.6 to 0.9. A similar analysis for 1% κ-carrageenan (Fig. 4(b)) yielded a power law exponent of 0.3 for temperatures below 20 °C. This value indicates a slight dependence of $G'$ and $G''$ on frequency for these gels, whereas at 24 °C the power law exponent increased rapidly for both moduli. At 22.1 °C, taken as the gelling temperature according to Winter and Chambon (1986), $G'$ and $G''$ had the same exponent with $n = 0.48$. However, the mechanical spectra for which $\tan\delta$ was independent of frequency (data not shown) with $\tan\delta = 1$ was that at 22.3 °C (Fig. 3(b)). The Winter and Chambon procedure was proposed for chemical gels in which the exponent $n$, which takes a fixed value of 0.5, is related to the stoichiometric coefficient. This criterion has been employed indistinctly in physical gels, in which $n$ depends on the nature of the system. For example, $n = 0.5$ for $\beta - 1.3/1.6$ glucan schizophyllan (Fuchs, Richtering, Buchard, Kajiwara, & Kitamura, 1997), and $n = 0.22$ for xanthan–Al (III) (Rood, Dunstan, Ross-Murphy, & Boger, 2001) and ι-carrageenan (Hossain et al., 2001). In the case of xanthan–Al (III), authors point out that the value of $n$ is not in agreement with previous work for systems at a limiting stoichiometric ratio. The inadequacy of constraining $n$ to 0.5 for determining the gelling temperature in physical gels is evident. However, the criterion has been successfully applied for whey proteins gels ($n = 0.62$ to 0.69) (Labropoulos & Hsu, 1996), for soy proteins ($n = 0.13$) (Hsu, 1999) and to gels obtained by heat-induced protein denaturation of egg yolk ($n = 0.56$) (Cordobés et al., 2004). In our opinion, this way of analyzing data to get $T_{\text{gel}}$ in physical gels is not straightforward because, as shown in Fig. 4, the power

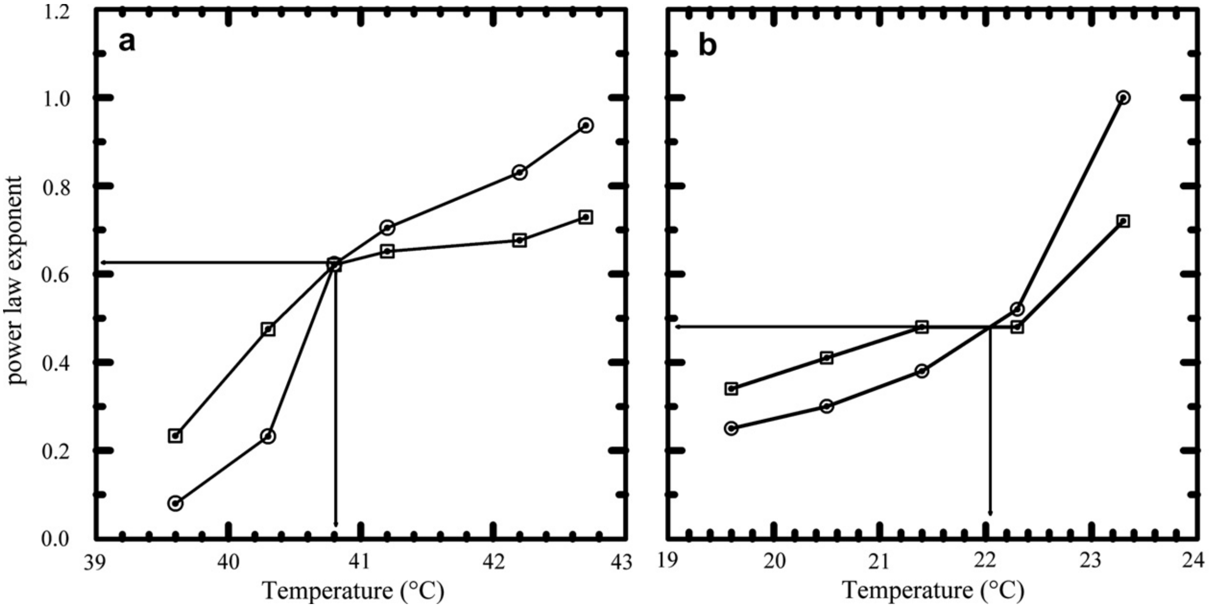

Fig. 4. Variation of the power law exponent with temperature for (a) 0.5% κ-carrageenan + 40 mM KCl and (b) 1.0% κ-carrageenan without added salt. $G'$ (circles) and $G''$ (squares). The lines joining experimental data points are included only as a guide for better localizing the transition temperature.

law exponent changes radically within a narrow temperature range requiring precise temperature control together with a system in which gelation can be stopped at a given temperature, as for egg yolk proteins (Cordobés et al., 2004). Besides, the transition of κ-carrageenan proceeds rapidly and cannot be stopped (Takemasa & Chiba, 2001).

### 3.3. Analysis of the critical phase angle

Fig. 5 shows the temperature and frequency dependence of tan $\delta$ for the mechanical spectra shown in Fig. 3. For 0.5% κ-carrageenan + 40 mM KCl, $T_{\text{gel}} = 40.75$ °C, while for 1% κ-carrageenan $T_{\text{gel}} = 22.1$ °C, which correspond to tan $\delta$ of 1.3 and 0.95, respectively. This method has been successfully used in whey protein isolates (Labropoulos & Hsu, 1996), mesquite seed galactomannan and deacylated xanthan mixtures in 5 NaCl (Goycoolea, Milas, & Rinaudo, 2001), tamarind seed xyloglucan + sodium gellan (Nitta, Kim, & Nishinari, 2003) and ι-carrageenan (Michon, Cuvelier, Launay, & Parker, 1995). The difference in temperature values between Winter's criterion and the critical phase angle procedure was less than 1%; actually it was 0.36 and 0.68% for 0.5% κ-carrageenan + 40 M KCl and 1% κ-carrageenan without added salt, respectively. In our opinion, the analysis of the critical phase angle is simpler than the analysis of the variation of $G'$ and $G''$ with frequency.

### 3.4. The temperature sweep procedure

Fig. 6 shows the typical evolution of $G'$ and $G''$ with cooling from 60 to 25 °C and heating from 25 to 60 °C for 0.5% κ-carrageenan + 40 mM KCl. The evolution of moduli during temperature-scanning rheometry showed three different regions. Region I, above 45 °C before the beginning of gelation, was characterized by an unstable signal of both moduli with "noise" at high temperatures owing to insufficient cross-linking necessary to form stable

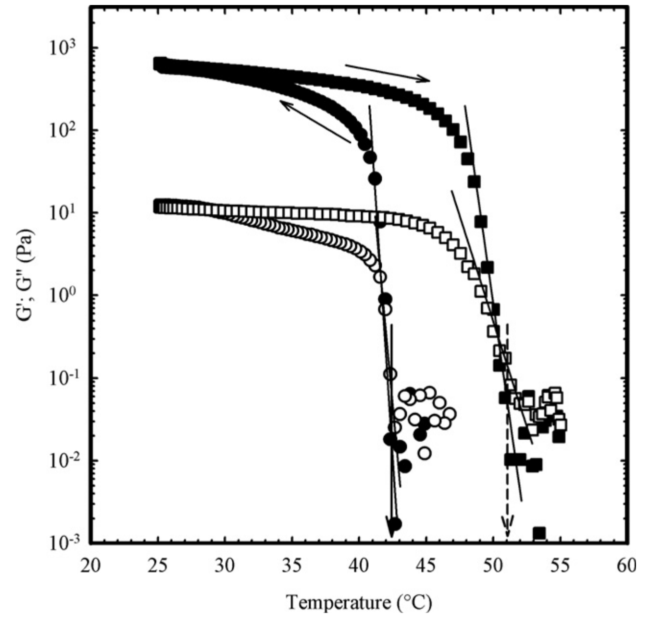

Fig. 6. Gelling–melting kinetics of 0.5% κ-carrageenan + 40 mM KCl at 1 Hz and 10% strain on cooling (circles) and heating (squares). $G'$ (black symbols) $G''$ (white symbols). Lines indicate the variation of moduli with temperature near the "noisy" region. Arrows point the temperature at which moduli departed from "noise" for gelling (continuous) and melting (dashed).

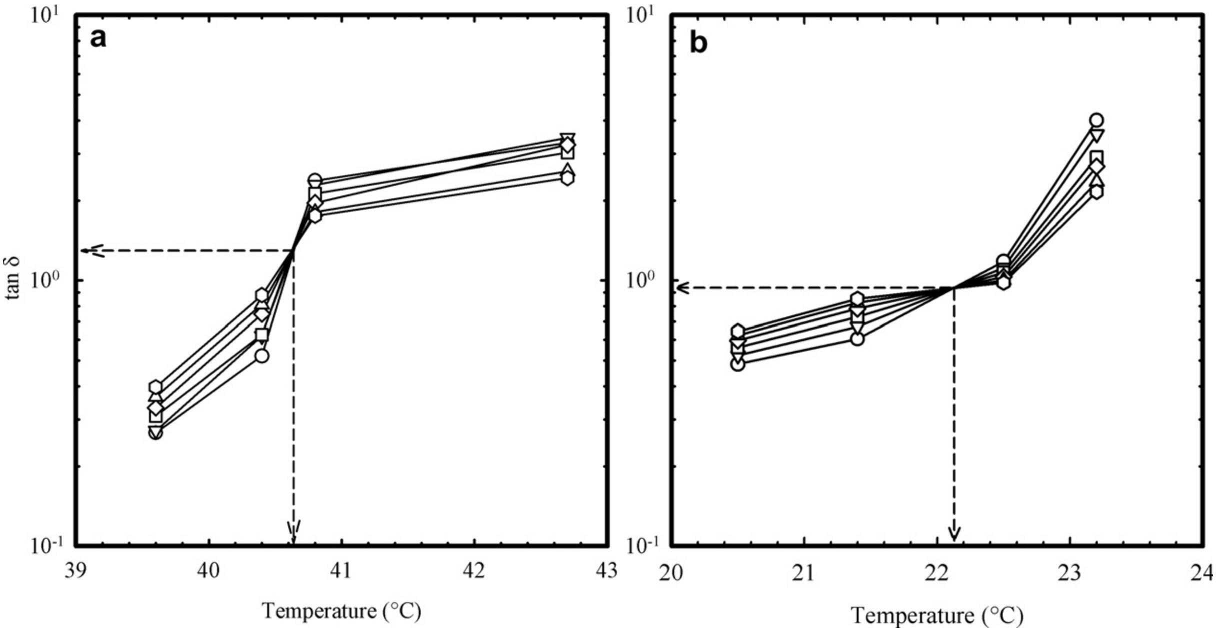

Fig. 5. Temperature and frequency dependence of tan $\delta$ for (a) 0.5% κ-carrageenan + 40 mM KCl and (b) 1.0% κ-carrageenan without added salt at different frequencies (rad/s); 1.0 (circles), 1.6 (squares), 2.5 (triangles), 3.9 (inverted triangles), 6.3 (diamonds) and 10 (hexagons). Data correspond to the mechanical spectra shown in Fig. 3.

junction zones. In this region, detection of clear signals of $G'$ and $G''$ was not possible. Region II, below 44 °C, was characterized by a remarkable increase in $G'$ and $G''$ as well as a noticeable decrease in $\tan\delta$ from 10 to 0.02. The crossover point between $G'$ and $G''$ ($\tan\delta = 1.0$) was typically located in this region. Moreover, the evolution of $G'$ and $G''$ with temperature indicates that a very rapid process took place essentially in this region. In agreement with the gelation mechanism of κ-carrageenan, near the transition there is a conformational change from random coils to double helices that allows aggregation, giving rise to network formation and consequently to gel formation. We can affirm that near the temperature at which moduli began to increase, incipient gelation occurred. At that point, the system has sufficient cross-links to cause an increase in $G'$ and $G''$ upon cooling. Region III, below 41 °C, was characterized by a monotonic increase in both moduli, with $G'$ superior to $G''$ and low $\tan\delta$. However, a plateau in $G'$ and $G''$ was not observed. Gelation was completed in this region, but for κ-carrageenan the time for moduli to reach their equilibrium values has been reported to be about 15 h (Tecante & Doublier, 1999).

It was difficult to observe $G'$ and $G''$ crossover from their "noisy" signals, so in agreement with the second criterion described by Ross-Murphy (1991), the transition temperature was estimated when "noise" disappeared and therefore, the temperature at which $G'$ and $G''$ suddenly increased, $T_{\text{onset}}$, was taken as the sol–gel transition temperature. The vertical continuous arrow in Fig. 6 indicates the temperature at which a stable signal was observed on cooling. Therefore, 42.5 °C was considered the sol–gel transition temperature. When temperature increases, helices involved in junction zones change their conformation and the network is broken up, as indicated by the decrease in $G'$. When disrupted aggregates melt at higher temperature, as indicated by the decrease in $G''$, the helices forming them quickly change their conformation returning to the coil state (MacArtain, Jacquier, & Dawson, 2003). The "noise" appears because the previously formed gel at 25 °C undergoes a gel–sol transition with a melting temperature, $T_{\text{m}}$, of about 51 °C, as indicated by the vertical dotted arrow. Thermal hysteresis was observed in temperature-scanning rheology as well as in micro-DSC. A possible source of error in determining $T_{\text{gel}}$ and $T_{\text{m}}$ by this method can be attributed to the sensitivity of the rheometer to measure $G'$ and $G''$ and their changes during cooling and heating. In Fig. 6, lines were drawn to show the change with temperature of the dynamic moduli and for an easier determination of the transition temperatures.

### 3.5. Conformational transition diagram

Fig. 7(a) shows our transition temperatures together with those reported by Rochas (1982) using optical rotation and conductivity on a purified preparation of K$^+$–κ-carrageenan. This type of polyelectrolytes exhibits a linear relationship between $\log C_{\text{T}}$ and $T^{-1}$ (Rinaudo, 2001), where $C_{\text{T}}$ is total ionic concentration (equiv/L) calculated as $C_{\text{s}} + \overline{\gamma} C_{\text{p}}$, with $C_{\text{s}}$ being the added ion content, $C_{\text{p}}$ the polymer concentration (equiv/L) and $\overline{\gamma}$ the mean activity coefficient; 0.55 for K$^+$–κ-carrageenan (Rochas, 1982). The slope of this line is particular to each polysaccharide and added salt. Our data, during cooling, yield slopes of −3456 and −3251 K for rheometry and DSC, respectively (Table 1), while for succinoglycan, Boutebba, Milas, and Rinaudo (1997) report a slope of −14,600 K, and Rinaudo (2001) reports a slope of −2000 K for xanthan with Na$^+$. For K$^+$–κ-carrageenan a slope of −8080 K, using a $\ln C_{\text{T}}$ vs $T^{-1}$ graph, has been reported (Rochas, 1982), which corresponds to −3508 K when $\log C_{\text{T}}$ is used. Table 1 also shows the regression parameters for previously reported values by Kara et al., 2003. The heating and cooling lines converge to a critical concentration, $C^*$, and temperature, $T^*$, below which there is no thermal hysteresis. Simultaneous solution of the equation in Table 1 using Rochas' data yields $C^* = 0.0066$ equiv/L (0.007 equiv/L as reported by Rochas, 1982) and $T = 18.1$ °C, while our rheometry data yield $C^* = 0.0086$ equiv/L and $T = 22.0$ °C. Our transition temperatures are in good agreement with those reported by Rochas (1982) even though we used rheology and calorimetery as well as a commercial preparation of κ-carrageenan without further purification. Fig. 7(b) shows the transition temperatures reported for cooling of purified 1% K$^+$–κ-carrageenan with no KCl added (Chen et al., 2002), 0.05%, 0.1% and 0.2% κ-carrageenan with 5 and 10 mM KCl (Mangione et al., 2003) and 2.9% κ-carrageenan without added salt (Yuguchi et al., 2003). The figure also includes data for cooling of 3% κ-carrageenan with 100 mM KCl (Sugiyama et al., 1998), heating and cooling of 4% κ-carrageenan with 27, 54 and 80 mM KCl (Kara et al., 2003) and those reported by Rochas (1982). Considering the systems examined by Kara and coworkers, it is apparent that their melting values deviate notably from Rochas' and our data, while those for gelling are in closer agreement (Table 1). However, their cooling and heating data do not converge to $C^*$ and $T^*$.

### 3.6. Viscoelastic behavior

At the end of temperature-scanning tests, frequency sweeps were carried out on the resulting gels at 25 °C in the linear region of viscoelasticity. Fig. 8 shows the frequency and strain sweep (Fig. 8, inset) for 0.5 % κ-carrageenan + 40 mM KCl. The linear viscoelasticity zone was particular to each gel. Frequency sweeps showed typical gel behavior (Ferry, 1980; Nishinari, 1997) with moduli independent on frequency (e.g. $G' \propto \omega^{0.03}$) from 0.1 to 100 rad/s, $G'$ greater than $G''$ and $\tan\delta$ well below 1.0. Chen et al. (2002) report a "weak gel" behavior under low amplitude examination for 1% κ-carrageenan without added salts. κ-Carrageenan exists as a gel when $C_{\text{T}}$ is higher than $C^*$ (0.007 equiv/L) and T is lower than $T_{\text{gel}}$. From the values of $G'$ at 1 Hz in the frequency sweep at 25 °C, the effect of κ-carrageenan and KCl concentration

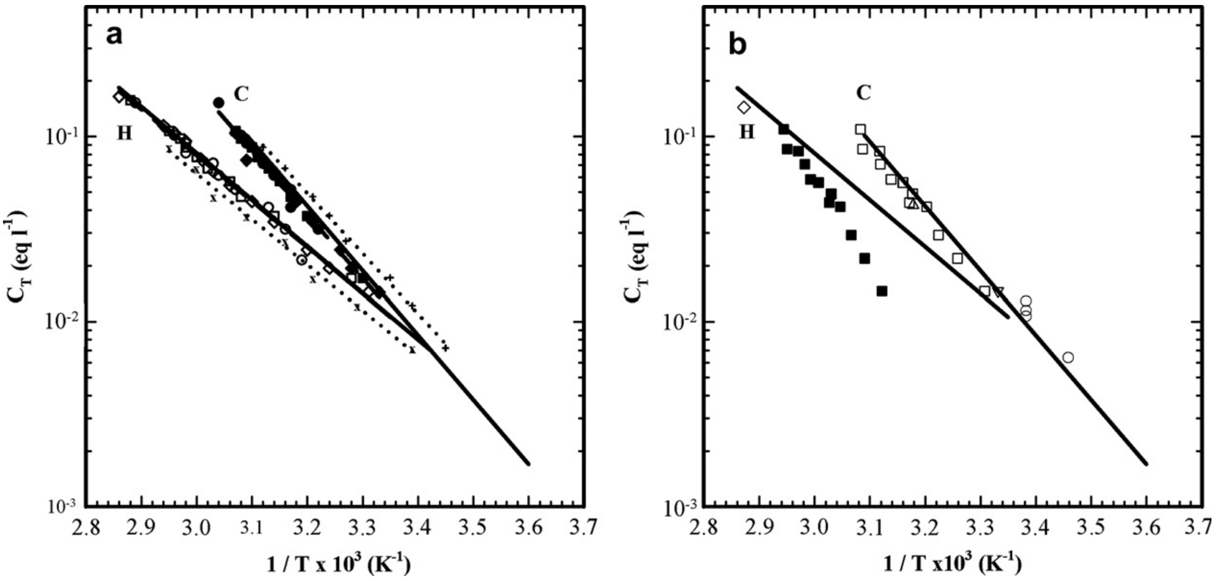

Fig. 7. Conformational transition diagram for κ-carrageenan with and without added KCl. (a) Rheology: cooling (black symbols), heating (white symbols) for polymer concentrations of 0.1% (circles), 0.5% (squares), 1% (diamonds) and regression (dashed line). Micro-DSC: cooling (plus), heating (cross) for 0.5% polymer and regression (dotted line). (b) Reported data: diamond (Sugiyama et al., 1998), inverted triangle (Chen et al., 2002), triangle (Yuguchi et al., 2002), circles (Mangione et al., 2003), black squares (heating), white squares (cooling) (Kara et al., 2003). The continuous lines are the regressions of transition temperatures determined by optical rotation and ion conductivity for cooling (C) and heating (H) (Rochas, 1982).

Table 1
Regression slope and heat of reaction of the equation $\log C_T = -\Delta H/2.303RT + \text{constant}$

| Reference | Technique | Cooling | | | Heating | | |
|---|---|---|---|---|---|---|---|
| | | $m$ (K) | $r$ | $\Delta H$ (kJ/mol) | $m$ (K) | $r$ | $\Delta H$ (kJ/mol) |
| Rochas (1982) | Optical rotation, conductivity | −3507 | 0.9988 | 67.1 | −2522 | 0.9989 | 48.3 |
| Kara et al. (2003) | Photon transmission | −3726 | 0.9889 | 71.3 | −4587 | 0.9896 | 87.8 |
| This work | Rheometry | −3456 | 0.9945 | 66.1 | −2486 | 0.9910 | 47.6 |
| This work | Micro-DSC | −3251 | 0.9985 | 62.2 | −2458 | 0.9915 | 47.0 |

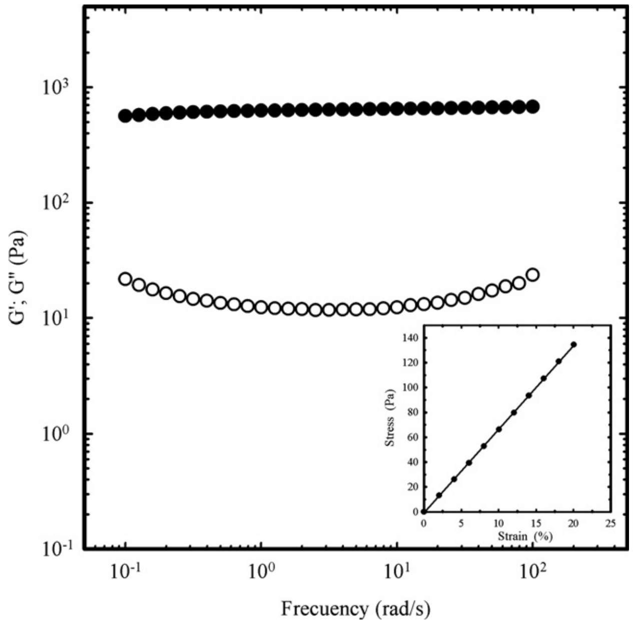

Fig. 8. Mechanical spectra for 0.5% κ-carrageenan + 40 mM KCl at 25 °C and 10% strain. $G'$ (black symbols), $G''$ (white symbols). (Inset) Strain sweep at 1 Hz and 25 °C.

was analyzed. Fig. 9 shows the effect of polysaccharide and KCl concentration on $G'$ for the corresponding gels. The stiffness of the gels increased with polysaccharide and salt concentration. For 0.1% κ-carrageenan, $G'$ increased noticeably for KCl concentrations lower than 60 mM, however, higher concentrations produced only a marginal increase. A similar behavior was observed for 0.5% and 1% κ-carrageenan for which the limits of KCl concentration were from about 100 to 150 mM KCl, respectively. Higher κ-carrageenan and KCl concentrations resulted in stronger gels because the proportion of aggregated helices increases with salt concentration. The marginal increase in $G'$ above a given KCl concentration, whose value depends on κ-carrageenan concentration, can be due to an oversaturation of potassium ions. In the sol–gel transition of κ-carrageenan with $Ca^{+2}$ ions, some authors (MacArtain et al., 2003) observed a maximum $G'$ for a 1.2 mol $Ca^{+2}$/mol κ-carrageenan ratio but not a flat zone as that shown in Fig. 9. This behavior was attributed to precipitation of κ-carrageenan by an excess of $Ca^{+2}$. Based on our observations and considering that even for a polymer concentration of 0.1%, $G'$ and $G''$ were practically independent of frequency ($G' \sim \omega^{0.02}$; $G'' \sim \omega^{0.06}$ from 60 to 100 mM

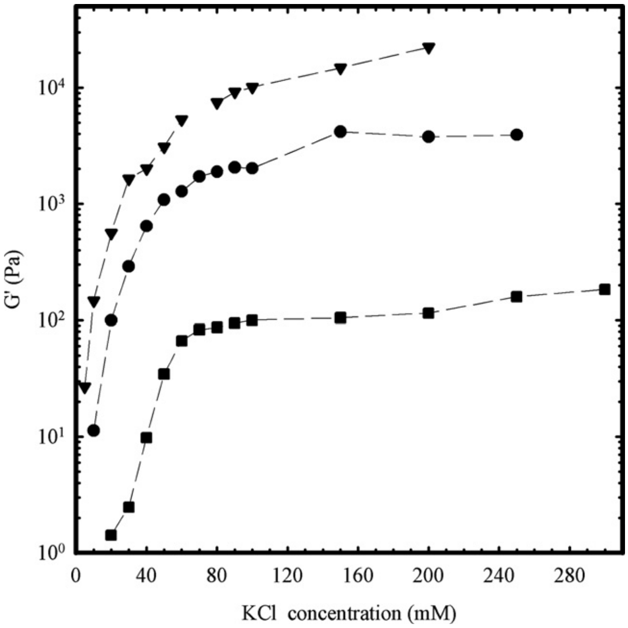

Fig. 9. Effect of κ-carrageenan and added KCl concentrations on storage moduli, $G'$, at 25 °C and 1 Hz: 0.1% (squares), 0.5% (circles) and 1% (inverted triangle).

KCl) with tan $\delta$ well below 1.0, i.e. $G'$ considerably superior to $G''$, network formation occurred leading to gelation with $G'$ values dependent on polysaccharide and ion concentrations. In such gels, molecular junction zones have a high energy content with large time stability. It is worth mentioning that the effect of KCl concentration on $G'$ of gels with 0.5% κ-carrageenan examined after 48 h was similar (data not shown). When salt concentrations were below 50 mM, $G'$ of 48-h-old gels was on the average 35% greater than for fresh gels. From 50 to 100 mM there was no difference, while above 100 mM, $G'$ of 48-h-old gels was on the average 20% lower than for fresh gels. However, both gels exhibited the trend shown in Fig 9.

## 4. Discussion

In spite of the fact that the analysis of $G'$ and $G''$ vs frequency and the critical phase angle stem from the same principle discussed by Winter and Chambon, the analysis of our rheological data to determine the sol–gel transition temperature shows that the power law dependence of the dynamic moduli with frequency and temperature is difficult to apply to κ-carrageenan, probably because gelation is time dependent and cannot be stopped once started. It is important to point out that the power law exponent changes drastically within a very narrow temperature range of about 2 °C. Hossain et al. (2001) applied Winter's criterion to ι-carrageenan and found a difference of 8 to 12% with respect to $T_{\text{gel}}$ measured by DSC. The critical phase angle procedure was easy to apply despite working with a system in which gelation is time dependent. Temperature-scanning rheology has been used to examine gelation of egg yolk (Cordobés et al., 2004); $Ca^{+2}$–κ-carrageenan (MacArtain et al., 2003); gellan (Miyoshi, Takaya, & Nishinari, 1996; Nishinari, 1997); xyloglucan–epigallocatechin gallate (Nitta, Fang, Takemesa, & Nishinari, 2004) and ι-carrageenan (Takemasa & Chiba, 2001). In our case, "noisy" signals in $G'$ and $G''$ from which it was difficult to observe their crossover, were the result of the low κ-carrageenan concentrations (0.1% and 0.5%), the random coil conformation adopted by polymer chains above $T_{\text{gel}}$ on cooling and $T_{\text{m}}$ on heating and the low strain applied during temperature scanning. The method allows thermal hysteresis to be determined.

The transition temperatures determined by DSC and rheology showed mutual agreement and were similar to those determined by others (Rochas, 1982). Our data show that the total critical concentration, $C^*$ in Fig. 7(a), which is the minimum value for gelation to take place, is about 0.0086 equiv/L. Concentrations below this value produced $G'$ and $G''$ vs $T$ curves in which moduli increased upon cooling but did not crossover (data not shown) because in this region there is only a coil–helix transition without helix aggregation or gelation. The techniques used here made possible to obtain the conformational transition diagram which is an important piece of information about the state of a given system as a function of temperature, polymer and added ion concentrations. Other transition diagrams have been obtained for succinoglycan (Boutebba et al., 1997), gellan (Milas & Rinaudo, 1996), κ-carrageenan–$Ca^{+2}$ (MacArtain et al., 2003) and tamarind seed xyloglucan with epigallocatechin gallate (Nitta et al., 2004). The number of investigations aimed at describing the effect of potassium and other ions on κ-carrageenan gels is significant (Chen et al., 2002; Kara et al., 2003; Mangione et al., 2003; Nishinari & Takahashi, 2003; Takemasa & Chiba, 2001; Yuguchi et al., 2003). However, authors do not usually refer their results to the conformational transition diagram. In our opinion, interpretation of their experimental data on the basis of this diagram would contribute to a better understanding of the state of the systems and the behaviors observed. As shown in Fig. 7(b) when data reported by others (Chen et al., 2002; Kara et al., 2003; Mangione et al., 2003; Sugiyama et al., 1998; Yuguchi et al., 2002) are included in the transition diagram, it is possible to situate the condition of the studied system. For example, data reported by Mangione et al. (2003) for 0.05%, 0.1% and 0.2 % κ-carrageenan with 10 mM added KCl, lie close to the transition line for cooling according to Rochas' data, although in the gel zone, whereas 0.1% κ-carrageenan with 5 mM added KCl lies near the critical concentration, $C^*$, and its corresponding temperature. On the other hand, Sugiyama's, Chen's and Yuguchi's data are consistent with those of Rochas (1982) and ours. However, Kara's data for cooling deviate slighlty at lower temperatures and concentrations, but their data for heating diverge significantly. The reason of such inconsistencies is not apparently due to the use of different techniques from which the transition temperature is determined because, as we show in Fig. 7, data from different techniques are in many cases consistent. It seems that the way in which the transition temperature is determined from a specific signal could explain the differences. For example, Yuguchi et al. (2002) took the crossover between the maximum scattering vector in SAXS, $q_{\max}$, and the cross-sectional radius of gyration, $R_{G,c}$, Kara et al. (2003) the peak of the first derivative of $dI_{tr}/dT$ in photon transmission measurements, Mangione et al. (2003) the transition midpoint temperature, $T^*$, in optical rotation dispersion, Sugiyama et al. (1998) the endothermic peak in micro-DSC signals, and Chen et al. (2002) the $T_{\text{onset}}$ of the temperature dependence of $G'$.

On the other hand, a linear relationship between $\log C_T$ and $T^{-1}$ was obtained. From the slope on cooling, the activation enthalpy for the sol–gel transition can be calculated according to the equation $\log C_T = -\Delta H/2.303RT +$ constant, where $C_T$ is the total concentration, T is the transition temperature, $R$ is the gas constant and $\Delta H$ is the heat of reaction of a mole of junction zone at the transition temperature (Eldridge & Ferry, 1954). Table 1 shows the enthalpy values for heating and cooling for our data together with those calculated from data reported by others. One cannot compare $\Delta H_{gel}$ with $\Delta H$ because the former was calculated over a wide range of temperatures of a broad endotherm during which association–dissociation of junction zones, accompanied by recombination of network strands, is likely to occur in thermo-reversible gels of κ-carrageenan (Hossain et al., 2001).

Rheological studies on the interaction between carrageenan and ions deal mostly with the effect of ion concentration on carrageenan gelation (Chen et al., 2002). High κ-carrageenan and KCl concentrations resulted in stronger gels. However, higher KCl concentrations as those used here have not been studied previously. It was possible to obtain gels with 0.1% κ-carrageenan and KCl only for total concentration, $C_T$, superior to the critical concentration, $C^*$. However, there is a limit in gel strength, $G'$, for a constant concentration of κ-carrageenan. Once the limiting value of $G'$ is reached, further addition of $K^+$ did not increase the strength. Nevertheless, the increase depends on κ-carrageenan concentration. For example, for 0.1% carrageenan, the limiting value of $G'$ is about 100 Pa for 80 mM KCl, for 0.5% κ-carrageenan, it is 300 Pa for 100 mM KCl, and for 1% κ-carrageenan it is about 15,000 Pa for 150 mM KCl (Fig. 9). A possible way of explaining this behavior is that the polymer becomes saturated with $K^+$, so that maximum aggregation of double helices occurs. These observations are compatible with micro DSC for 0.5% κ-carrageenan (Fig. 2). The enthalpy values on heating and cooling did not increase above 60 mM KCl.

## 5. Conclusions

In the literature, there are several methods to evaluate the gelling temperature from rheological data. Winter and Chambon's method was developed for chemical gels but has been used for physical gels too. However, this method requires further analysis of rheological data near the gel point in the case of physical gels. The analysis based on the variation of the dynamic moduli with frequency and temperature was less straightforward probably because of the rapid transition of κ-carrageenan. On the other hand, the critical phase angle method was easier to use and feasible to apply to physical gelling even in a system with a rapid transition and time-dependent gelation. The temperature sweep method at constant frequency and strain produced results comparable to micro-DSC both for gelling and melting and allowed thermal hysteresis to be observed. Results were in close agreement with the reported conformational transition diagram for κ-carrageenan obtained from optical rotation and conductivity measurements. Finally, at 25 °C, individual κ-carrageenan gels exhibited rheological behaviors typical of solid-like materials. The different combinations of KCl and κ-carrageenan concentrations lead to formation of three-dimensional networks of different degrees of rigidity as evidenced by the existence of a threshold salt concentration.

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