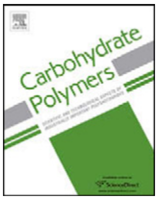
Carbohydrate Polymers 85 (2011) 429–438

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol



Effect of molecular weight and chemical structure on thermal and rheological properties of gelling κ/ι-hybrid carrageenan solutions

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ARTICLE INFO

Article history:
Received 1 December 2010
Received in revised form 25 February 2011
Accepted 2 March 2011
Available online 9 March 2011

Keywords:
κ/ι-Hybrid carrageenan
Ionic strength
DSC
Rheology

ABSTRACT

Three κ/ι-hybrid carrageenan polysaccharides with distinct molecular mass and content of ι-carrageenan disaccharide units were isolated from *Mastocarpus stellatus* seaweeds. The viscoelastic and thermal properties of 2 wt% κ/ι-hybrid carrageenan solutions with ionic strength tuned by the addition of NaCl salt were studied by means of rheological tests and differential scanning calorimetry (DSC). All solutions form a gel upon cooling. The gel elasticity decreases with the content in ι-carrageenan disaccharide units, and does not correspond to the additive elasticity of a ι- and a κ-carrageenan networks. The highest gel elasticity is obtained with the lowest molecular mass. In 0.1 M NaCl, two gelling processes are evidenced. The first increase in solution viscosity upon cooling coincides with a thermal transition process, which is assigned to a coil-to-helix conformational transition. The transition depends on both molecular mass distribution and chemical structure. The same dependencies are observed for the gel melting behaviour as all rheological and thermal processes determined are shifted to higher temperature with decreased molecular mass or increased content in ι-carrageenan disaccharide units.

1. Introduction

Carrageenans are sulfated galactans extracted from specific red seaweeds species, and are increasingly used in industrial applications as stabilizing or texturing agents (Bixler, 1996; McHugh, 2003; Piculell, 1995). Gelling carrageenans are usually classified into two main groups, namely κ-carrageenan and ι-carrageenan, as their chemical structures differ, resulting in distinctive gel properties. The latter essentially results from the aggregation of helical carrageenan conformers obtained by cooling hot carrageenan aqueous solutions. However, the mechanism for helix aggregation, the structure of gelling aggregates and the interplay between gel properties and gel structure are still a subject of vivid research (Funami et al., 2007; Guenet, 2000; Hermansson, 1989; Hilliou, Wilhelm, Yamanoi, & Gonçalves, 2009; Ikeda, Morris, & Nishinari, 2001; Meunier, Nicolai, & Durand, 2000; Piculell, 1995; Viebke, Piculell, & Nilsson, 1994).

A third group, namely κ/ι-hybrid carrageenans, has recently received increased interest (Guibet et al., 2008; Hilliou & Gonçalves, 2007; Hilliou, Larotonda, Abreu, et al., 2006; Hilliou, Larotonda, Sereno, & Gonçalves, 2006; van de Velde, 2008; van de Velde et al., 2005; van de Velde, Peppelman, Rollema, & Tromp, 2001; Villanueva, Hilliou, & Sousa-Pinto, 2009; Hilliou, Wilhelm, Yamanoi, & Gonçalves, 2009). The reason for this is that most of carrageenophyte seaweeds actually produce κ/ι-hybrid carrageenans (van de Velde, 2008), and alternative algal resources for carrageenan production are needed to cope with the steadily increasing demand in food texturing agents (Bixler, 1996; McHugh, 2003). In addition, κ/ι-hybrid carrageenans appear to be a good alternative to mixtures of κ- and ι-carrageenans conventionally used in dairy applications (Bixler, Johndro, & Falshaw, 2001; Villanueva, Mendoza, Rodrigueza, Romero, & Montano, 2004). κ/ι-Hybrid carrageenans are natural copolymers made of blocks of κ or ι-carrageenan disaccharide units statistically distributed in the chain (Guibet et al., 2008; van de Velde, 2008; van de Velde et al., 2005, 2001), together with minor amounts of non gelling disaccharide units (biological precursors). The relative amount of κ- and ι-carrageenan blocks depends on a complex interplay between the seaweeds biology (Chopin, Kerin, & Mazerolle, 1999; Lahaye, 2001; Piculell, 1995; van de Velde et al., 2005) and the biopolymer extraction procedure (Hilliou, Larotonda, Abreu, et al., 2006; Lahaye, 2001; McHugh, 2003; Villanueva et al., 2009). The chemi-

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doi:10.1016/j.carbpol.2011.03.006

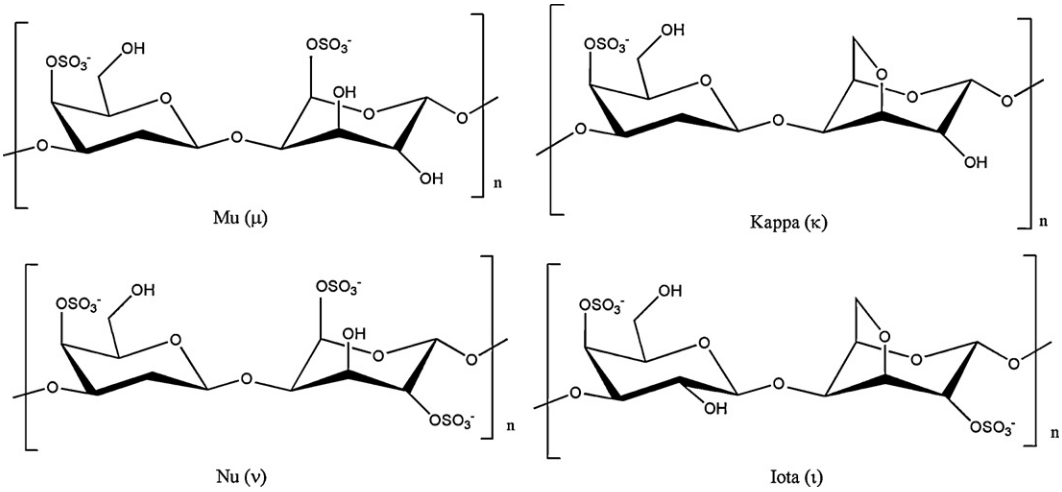

Fig. 1. Chemical structure of Mu (μ), Nu (ν), kappa (κ) and iota (ι) carrageenans.

cal structure of κ/ι-hybrid carrageenans has a direct impact on the gel properties of the polysaccharide. A recent study (van de Velde et al., 2005) performed with a set of seaweeds containing κ/ι-hybrid carrageenans with different chemical structures reported a continuous relation between the increasing content in κ-carrageenan disaccharides and the increasing shear elastic modulus of the gels, whereas the gelling temperature showed no dependence on the biopolymer chemistry, for polysaccharides containing more that 50 mol% κ-carrageenan disaccharide units. High-sensitivity differential scanning calorimetry (DSC) indicated that the helix-to-coil transition temperature for κ-carrageenans disaccharide blocks and ι-carrageenans disaccharide blocks were not affected by the chemical composition of the κ/ι-hybrid carrageenans, whereas the total enthalpy of the transition does not correspond to the simple weighted sum of the enthalpies of pure ι- and κ-carrageenans.

We recently explored the chemistry–gel properties relationships in a set of κ/ι-hybrid carrageenans extracted from a single seaweed, namely *Mastocarpus stellatus* (Hilliou, Larotonda, Abreu, et al., 2006; Hilliou, Larotonda, Sereno, et al., 2006). The chemical structure was tuned by varying parameters of both alkaline pre-treatment and extraction, resulting in κ/ι-hybrid carrageenans containing non-gelling carrageenan disaccharide units (namely ν- or μ-carrageenans) (see Fig. 1) and showing gel elasticity ranging from 250 to 2500 Pa. As expected, correlations between gel elasticity and polysaccharide chemical structure were evidenced, but results also indicated that gel properties depend on the molecular mass. Here we further explore the interplay between κ/ι-hybrid carrageenans chemical structure and gel properties, as this is still poorly documented in the literature (van de Velde, 2008). Three extracts of κ/ι-hybrid carrageenans were selected, which differ either by molecular mass or by the content in ι-carrageenans disaccharide units. The ionic strength in the gelling solutions was varied from 0.1 to 1 M in order to shift all transition temperatures into the experimental window. In contrast to earlier reports (Hilliou & Gonçalves, 2007; Hilliou, Larotonda, Abreu, et al., 2006; Hilliou, Larotonda, Sereno, et al., 2006; van de Velde, 2008; van de Velde et al., 2005) where a mixture of salts is used to obtain gelling samples exhibiting high enough elasticity without water syneresis, we used a single salt, namely NaCl. Such well defined salt conditions stem from the extraction process, and are critically needed to separate the contributions of respective κ- and ι-carrageenans disaccharide units to the rheological and thermal behaviour of the gelling solutions (Chanvrier et al., 2004). This is due to the salt specificity of κ-carrageenan which shows higher gel elasticity in the presence of potassium salt. Using such salt could mask the gel elasticity associated with ι-carrageenan disaccharide units (Chanvrier et al., 2004; Parker, Brigand, Miniou, Trespoey, & Vallée, 1993). In addition, we study the thermal transitions of gelling and melting solutions by means of DSC, thus extended previous calorimetric studies (van de Velde, 2008; van de Velde et al., 2005) to concentrations where κ/ι-hybrid carrageenans actually form aggregates and eventually gel.

2. Experimental

2.1. Materials

The recovery of κ/ι-hybrid carrageenans from *M. stellatus* seaweeds has been detailed elsewhere (Hilliou, Larotonda, Abreu, et al., 2006; Hilliou, Larotonda, Sereno, et al., 2006). Prior to carrageenan extraction, seaweeds are submitted to an alkali treatment where Na_2CO_3 is used to partially convert biological carrageenan precursors into gelling carrageenan disaccharide units. Inductively Coupled Plasma-Atomic Emission Spectroscopy showed that the κ/ι-hybrid carrageenans are essentially recovered in the sodium form (roughly 3 wt% Na^+, whereas all other cations remain below the sensitivity of the apparatus). However, the pure sodium form comes at the expense of the non gelling monomers. Indeed, proton NMR indicates that ν- or μ-carrageenan disaccharide units are still present on the κ/ι-hybrid carrageenans chains. The chemical structure of the 3 samples used in the present study is summarized in Table 1. Samples A and B show a significant difference in their molecular mass distributions, and as such are suited to study the effect of this parameter on the thermal and gel properties.

Table 1
Molecular mass distribution (weight averaged mass M_w in g/mol, polydispersity index I), and content (in mol%) in carrageenan disaccharide units for the three κ/ι-hybrid carrageenans samples extracted from *M. stellatus* seaweeds.

Sample	M_w ($\times 10^6$)[a]	I[a]	ι[b]	κ[b]	PREC[b]
A	0.431	2.4	32.7	48.0	19.3
B	2.199	4	31.2	50.9	17.9
C	2.590	3.2	37.6	46.1	16.3

[a] Determined by size exclusion chromatography performed with 0.1 wt% polysaccharide solutions in 0.1 M NaCl at 40 °C Hilliou, Larotonda, Abreu, et al. (2006).

[b] Determined by ^{1}H NMR (integrated area relative to the total integrated area). ι stands for iota carrageenan disaccharide units, κ stands for kappa carrageenan disaccharide units and PREC stands for biological carrageenan precursors such as μ- and ν-carrageenan disaccharide units.

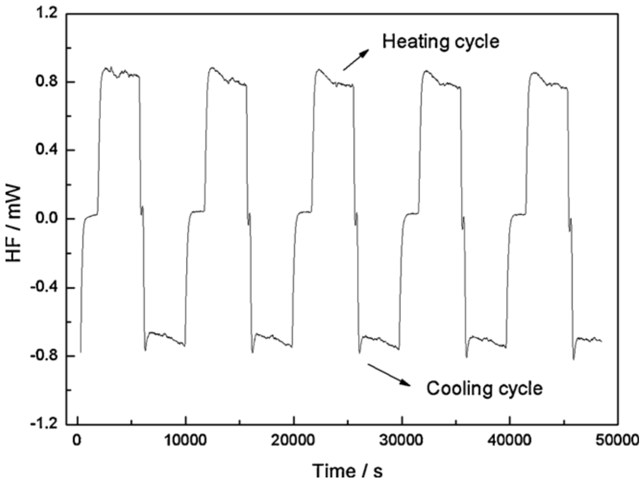

Fig. 2. Heat flow as a function of time for consecutive heating/cooling cycles as obtained with sample A, in deionised water.

Such difference was achieved by tuning the extraction temperature and duration while keeping the alkali treatment constant, as prolonged extraction at higher temperature results in depressed molecular mass (Hilliou, Larotonda, Abreu, et al., 2006). On the other hand, samples B and C essentially differ by their relative content in ι-carrageenans units, and thus allow the study of the effect of such chemical asset on the polysaccharide functional properties. This was achieved by a prolonged soaking of seaweeds in alkali, thereby increasing the conversion of ν-carrageenans biological precursors into ι-carrageenans disaccharide units (Hilliou, Larotonda, Abreu, et al., 2006). Sodium chloride (NaCl) (Merck; 99.5%) was analytical grade and used without further purifications.

2.2. Solution preparation

κ/ι-Hybrid carrageenan solutions were prepared by dissolving 2.0 wt% polysaccharide in deionised H_2O, NaCl 0.1 M solution or NaCl 1 M solution. The solutions were stirred at 80 °C during 60 min in order to ensure complete carrageenan dissolution. Solutions were studied by microDSC and rotational rheometry right after their preparation.

2.3. Differential scanning microcalorimetry (microDSC)

Differential scanning calorimetry experiments were made using a Micro DSC III Setaram (France) instrument, which has shown to be suitable for working with dilute solutions of biological macromolecules (Beezer, 1980). The time dependence of the heat flow was recorded during consecutive heating/cooling cycles, between 15 and 80 °C, using a heating/cooling rate of $\beta = \pm 1\,^{\circ}\mathrm{C\,min^{-1}}$. Between each consecutive cooling and heating scan, the sample was equilibrated at 15 °C during 30 min (see Fig. 2), and at 80 °C during 5 min. The measurements were performed in standard Hastelloy cells with nominal volume 1 $\mathrm{cm^3}$, using nitrogen as the carrying gas. The measuring cell was loaded with 0.8 mL of κ/ι-hybrid carrageenan solution and was rigorously weighted (to ±0.0005 g). The weight of the reference cell's content (filled with buffer (H_2O, NaCl 0.1 M or NaCl 1 M)) was adjusted to the weight of the measuring cell's content to within ±0.0005 g. The calorimetric profile for the κ/ι-hybrid carrageenan solutions was collected against buffer, and buffer–buffer runs were performed to be used as blank experiments. The instrument software (SETSOFT, Setaram) was used for data processing and calculation of the heat capacity associated with each thermal process. The transition temperature was determined as temperature of maximum heat capacity. The reported peak temperature and heat capacity values are averages (with corresponding standard error) over three consecutive heating/cooling cycles, recorded after the first cycle.

2.4. Rotational rheometry

The experimental protocol used to characterize the viscoelastic properties of cooling or heating κ/ι-hybrid carrageenan solutions and corresponding gels is illustrated in Fig. 3. Hot carrageenan solutions were directly loaded in the preheated (80 °C) plate–plate geometry of stress-controlled rheometer (TA Instruments Inc., New Castle, DE, USA). The geometry was covered with paraffin oil to prevent water loss. During the cooling of the solution ($\beta = -1\,^{\circ}\mathrm{C\,min^{-1}}$ as in DSC test) a small amplitude oscillatory shear was applied (with 5% strain at a frequency $\omega = 1$ Hz) in order to probe the temperature evolution of linear viscoelastic properties such as tan $\delta = G''/G'$ (Fig. 3a). The temperature where tan $\delta = 1$ was defined as the gel setting temperature T_{gel}. T_{ON} is defined as the inflexion point in G'' temperature dependence (change in slope, related to a change in the viscous component of the solution rheological properties). Following the cooling and gel setting, the time evolution at 15 °C of shear complex moduli G' and G'' was measured (with 0.5% strain at 1 Hz) during 30 min to probe the gel kinetics. Data recorded for all samples indicated that 30 min was sufficient to obtain gel equilibrium conditions as both G' and G'' reached steady values (see Fig. 3b). Frequency sweeps were then performed with a 5% strain, which ensured the measurement of equilibrated gels' mechanical spectra in the linear regime (see Fig. 3c) and the determination of the elastic storage modulus at 1 Hz, thereafter labelled as G_0. Fourier transform analyses of stress periodic responses performed as described elsewhere (Hilliou, Wilhelm, Yamanoi & Gonçalves, 2009) indicated that the second and third harmonics were quantitatively similar to the virtually sinusoidal strain excitation. This result confirms that rheological data are essentially free of slip or other non linear effects, which is in harmony with the absence of water syneresis in the present κ/ι-hybrid carrageen gels. Finally, the gels were heated ($1\,^{\circ}\mathrm{C\,min^{-1}}$) up to 80 °C and G' and G'' were simultaneously measured at a 5% strain and a frequency of $\omega = 1$ Hz, enabling the characterization of the gel melting behaviour and of the gel melting temperature T_{melt} defined as the temperature where tan $\delta = 1$. In addition, a temperature T_{end} corresponding to the last inflexion point (highest temperature) in the temperature dependence of G'' was defined as the temperature for which the gel melting process is completed (see examples of such process in Fig. 6a and b). For all mechanical tests, linearity between imposed strain and recorded stress was assessed by the on-line recording of sinusoidal waveforms for the torque response.

3. Results

3.1. Thermal reversibility and mechanical properties of equilibrated gels

As a preliminary test, we have studied the thermal behaviour of κ/ι-hybrid carrageenan samples with different ionic strength solutions by performing consecutive heating/cooling cycles in the microDSC. Fig. 2 shows the calorimetric curves for 5 successive heating/cooling cycles obtained with sample A in deionised water. Results indicate that heating/cooling cycles are reproducible, and suggest that the observed thermal transitions are fully reversible. The heating/cooling cycle thermograms for samples B and C also show reversible processes, for all studied ionic strengths. This is in agreement with the rheological tests, which also show thermal

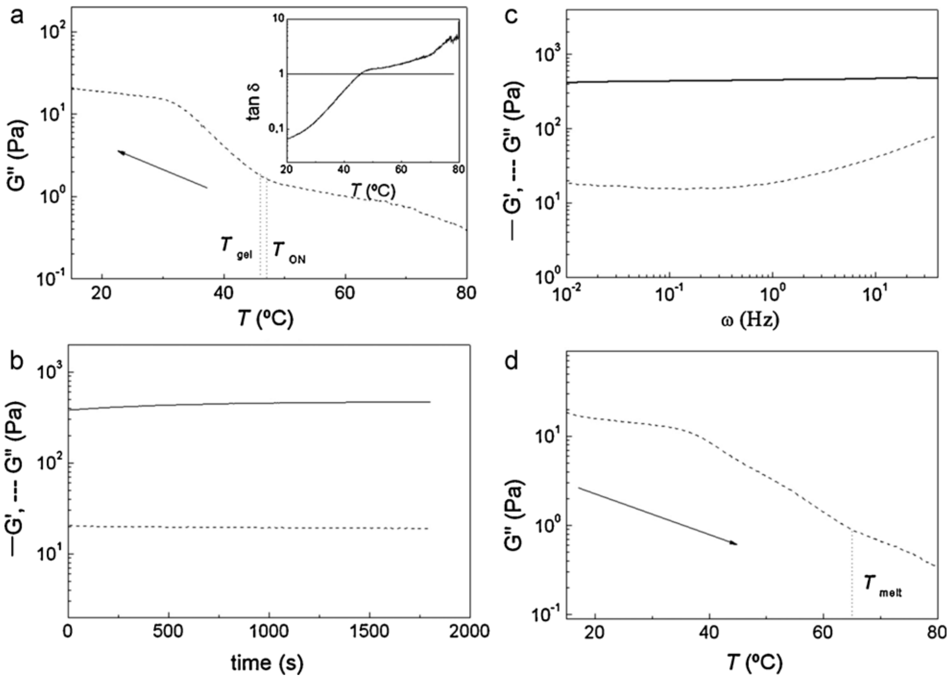

Fig. 3. Rheological protocol used for the viscoelastic characterization of sample B, with concentration 2 wt% in NaCl 1 M: (a) temperature dependence of loss modulus G'' recorded during first cooling performed with $\beta = 1\ \mathrm{K\,min^{-1}}$, $\omega = 1$ Hz and strain 5%; (b) time dependence of G' (solid line) and G'' (dotted line) at $T = 15\,^\circ\mathrm{C}$; (c) frequency sweep performed at $T = 15\,^\circ\mathrm{C}$ (strain 5%) 30 min after the cooling step pictured in (a): G' (solid line), G'' (dotted line); and (d) temperature dependence of G'' recorded during heating performed with $\beta = 1\ \mathrm{K\,min^{-1}}$, $\omega = 1$ Hz and strain 5%.

reversibility, as illustrated in Fig. 3 for sample B: G'' modulus measured at 80 °C during the cooling (see Fig. 3a) nicely matches the value measured at the same temperature during the heating (see Fig. 3c). Repeated cooling after 5 min curing at 80 °C also shows satisfactory reproducibility (results not shown), which also validates the thermal reversibility of rheological behaviour even with samples for which no T_{end} could be measured (see Table 3) within the experimental range of temperatures.

The mechanical spectra of equilibrated gels at 15 °C are displayed in Fig. 4 for all samples. In the low frequency regime, the shear storage modulus G' is nearly constant (weak frequency dependence) and larger than the shear loss modulus G''. This is the rheological signature of a viscoelastic solid behaviour and as such indicates that gels were formed for all experimental conditions. The mechanical spectrum of sample B in the salt free solution (Fig. 4a) shows a cross over between G' and G'' at nearly 1 Hz. This behaviour contrasts with the two other samples under similar ionic strength, as such cross over is shifted to higher frequencies for sample A, and is even not resolved for sample C. This result points to the fact that the gel obtained with sample B shows a distinct microstructure and a larger viscous component. As expected (Piculell, 1995), gels formed with larger amount of salt show increased elasticity. The addition of salt also changes the spectrum of relaxation times in the gel, as a relative minimum in G'' shows up in spectra measured with NaCl 1 M. Such minimum, which is not measured at lower ionic strength, was assigned to the existence of two relaxation times at frequencies larger and lower than the experimentally accessed ones (Meunier, Nicolai, & Durand, 1999), and is indicative of a salt-induced structural change in the gel. Note also that the minimum is shifted to higher frequencies with increasing gel elasticity.

3.2. Cooling cycles

Fig. 5 shows representative heat flow scans along with the loss modulus G'' measured during the cooling of solutions of sample C for different ionic strengths. Arrows in Fig. 5 indicate the transition temperatures as determined by DSC. The increase in G'' with cooling is not monotonic. Instead, two processes characterized by two temperatures T_{ON} corresponding to two step-like increases in the viscous component of the shear modulus are clearly resolved in NaCl 0.1 M (see Fig. 5b). These temperatures are reported in Table 2, along with temperatures T_{gel} for all samples and ionic strengths. Note that for NaCl 1 M and for the salt free solution, a single temperature T_{ON} is reported, as the second inflexion point is shifted to temperatures higher than 80 °C (see the inflexion point at roughly 79 °C in Fig. 5c) or lower than 15 °C (see Fig. 5a), respectively. As a result, two values are reported in Table 2 for T_{ON} at NaCl 1 M, but with the highest temperature denoted as >80. Sample B in H_2O did not show any gel point. Instead a gel was formed (see Fig. 4a) during the 30 min of the equilibrium step at 15 °C.

T_{ON} appears at similar temperatures as the ones found for the first exothermic thermal process in the calorimetric experiments, reported as T_{CH} in Fig. 5 for the experiments in water and NaCl 0.1 M. This temperature was assigned to a coil-to-helix conformational transition. However, at the highest ionic strength studied (1 M), T_{CH} is not related to any rheological transition (see Fig. 5c and Table 2). For all samples with ionic strengths below 1 M, T_{gel} shows up at temperatures lower than T_{CH}. This is in harmony with the gel mechanism devised for carrageenan polysaccharides which is based on the aggregation of helical conformers into a three dimensional network (Piculell, 1995). As a result, comparing the rheological data with the DSC data facilitates the assignment of the exothermal peaks obtained during the cooling cycles.

3.3. Heating cycles

Fig. 6 presents the microDSC scan and the loss modulus G'' measured during the heating of sample C. Qualitatively similar results were obtained for both rheological and thermal responses of samples A and B. Two rheological processes (T_{end}) are seen in Fig. 6b for NaCl 0.1 M, suggesting that two networks are melting within the temperature range accessed experimentally. This is in agreement

Table 2
Parameters obtained from the cooling cycle – temperature of coil-to-helix transition (T_{CH}) and corresponding enthalpy change (ΔH) as obtained from DSC experiments and rheological proprieties (T_{ON} and T_{gel}) of the A, B and C κ/ι-hybrid carrageenans, 2% (w/w), in water, NaCl 0.1 M and NaCl 1 M.

Sample	Deionised water				NaCl 0.1 M					NaCl 1 M				
	T_{ON}		T_{gel}	T_{CH}	ΔH (J g^{-1})	T_{ON}		T_{gel}	T_{CH}	ΔH (J g^{-1})	T_{ON}		T_{gel}	T_{CH}	ΔH (J g^{-1})
A	49.6 ± 0.6	<15	30.7 ± 0.1	48.5 ± 0.5	−10.13	54.2 ± 0.2	23.3 ± 1	44.3 ± 0.1	50.9 ± 0.5	41.96	>80	56.5 ± 0.3	56.6 ± 0.1	38.9 ± 0.5	85.83
B	27.6 ± 0.2	<15	<15	23.8 ± 0.5	−12.84	44.2 ± 0.2	18 ± 2	30.6 ± 0.1	41.9 ± 0.5	36.49	>80	46.8 ± 0.1	45.6 ± 0.1	34.6 ± 0.5	93.55
C	36.8 ± 0.2	<15	23.2 ± 0.1	34.8 ± 0.5	−7.13	48.3 ± 0.3	21.7 ± 1	34.7 ± 0.1	47.2 ± 0.5	12.71	>80	43.9 ± 0.3	62.7 ± 0.1	35.9 ± 0.5	53.83

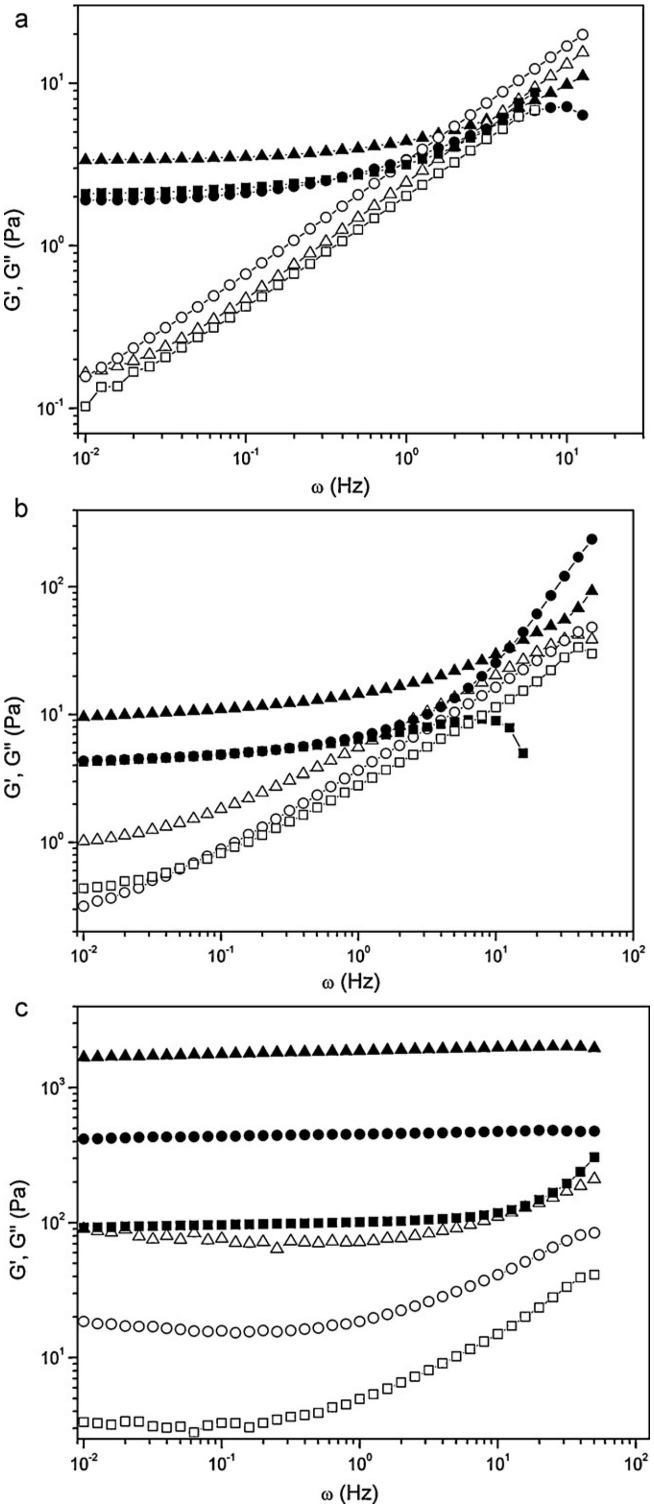

Fig. 4. Storage modulus G' (solid symbols) and loss modulus G'' (empty symbols) as a function of the oscillation frequencies for samples A (triangles), B (circles) and C (squares), 2% (w/w), in deionised water (a), NaCl 0.1 M (b), and NaCl 1 M (c), at 15 °C.

with the cooling run, where two onsets of gelling were observed and the overall thermal reversibility was assessed (Section 3.1). Similarly to the cooling cycle, the two processes cannot be resolved at higher or lower ionic strength as one of the melting is shifted to higher or lower temperature (see Fig. 6a and c). The second T_{end} at higher temperature corresponds to the end of the 2nd transition probed by DSC (change of slope following the first continuous

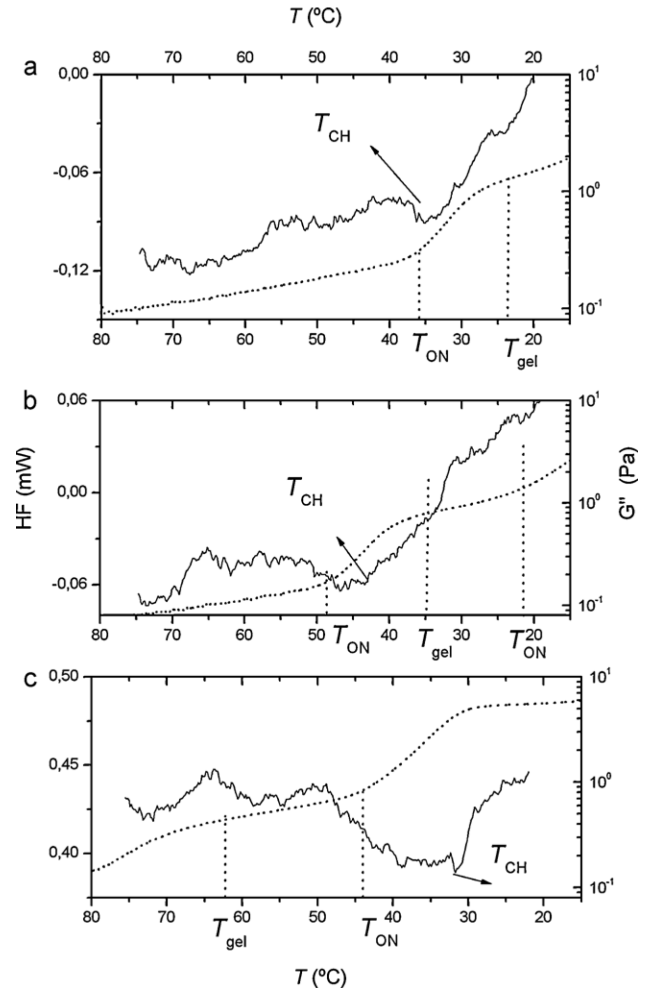

Fig. 5. Temperature dependence of the heat flow (solid lines) and the shear loss modulus G'' (dashed lines) recorded during the cooling of solutions of sample C in water (a), NaCl 0.1 M (b) and NaCl 1 M (c). Arrows indicate the temperatures at which corresponding labelled transitions occur (T_{CH}), whereas vertical dotted lines indicate temperatures at which rheological transitions occur.

increase in the DSC trace). The lower melting process T_{end} is a partial one as the heat flow is still continuously evolving with no change of slope in the DSC curve, and the gel melting point T_{melt} is not yet reached. The coincidence between T_{end} and DSC data is also resolved in H_2O (see Fig. 6a) and suggests that the gel melting is actually completed before any conformational transition shows up (as a peak) in the DSC tracing (Denef et al., 1998; van de Velde et al., 2005). Data displayed in Fig. 6 are qualitatively similar to DSC traces obtained for pure ι-carrageenan samples in NaCl but at much lower polymer concentration, with a similar experimental set-up (Denef et al., 1998). The similarity includes the noise which was assigned to the relatively short (30 min at 15 °C) thermal history of the samples. Temperatures T_{HC} related to peaks in DSC traces are reported for the three samples in Table 3, together with T_{melt} and T_{end} obtained with rheology. Note that in H_2O, a single T_{HC} is measured. This is in contrast to data obtained at higher ionic strengths where two peaks can be resolved.

In NaCl 1 M, the helix-to-coil transitions observed in the DSC tracings always precede the gel melting temperature T_{melt} (see Fig. 6c). This is in contrast to the gel melting processes observed in rheological thermograms for H_2O and 0.1 M NaCl, where T_{melt} is located below any T_{HC}.

Table 3
Parameters obtained from the heating cycle – temperature of helix-to-coil transition (T_{HC}) and corresponding enthalpy change (ΔH) as obtained from DSC experiments and rheological proprieties (T_{ON} and T_{melt}) of the A, B and C κ/ι-hybrid carrageenans, 2% (w/w), in water, NaCl 0.1 M and NaCl 1 M.

Samples	Deionised water				NaCl 0.1 M							NaCl 1 M						
	T_{end}	T_{melt}	T_{HC}	ΔH (J g^{-1})	T_{end}		T_{melt}	T_{HC}		ΔH (J g^{-1})		T_{end}		T_{melt}	T_{HC}		ΔH (J g^{-1})	
A	51.4 ± 0.5 23 ± 2	34.7 ± 0.1	50.9 ± 0.5	17.32	58 ± 1	26.3 ± 0.5	45.5 ± 0.3	63.8 ± 0.5	69.5 ± 0.5	5.04	9.88	<15	>80	73 ± 1	47.7 ± 0.5	54.8 ± 0.5	6.87	6.22
B	30.3 ± 0.1	17.9 ± 0.3	28.3 ± 0.5	14.10	46.0 ± 0.5	23 ± 1	33.4 ± 0.2	44.65 ± 0.5	58.8 ± 0.5	16.96	4.75	<15	>80	61.2 ± 0.2	45.3 ± 0.5	57.6 ± 0.5	20.48	3.17
C	38.3 ± 0.2	27.3 ± 0.1	61.7 ± 0.5	7.75	50.5 ± 0.5	24 ± 1	37.4 ± 0.3	52.4 ± 0.5	60.8 ± 0.5	22.45	12.79	<15	>80	65 ± 1	50.6+ ± 0.5	63.9 ± 0.5	25.27	4.48

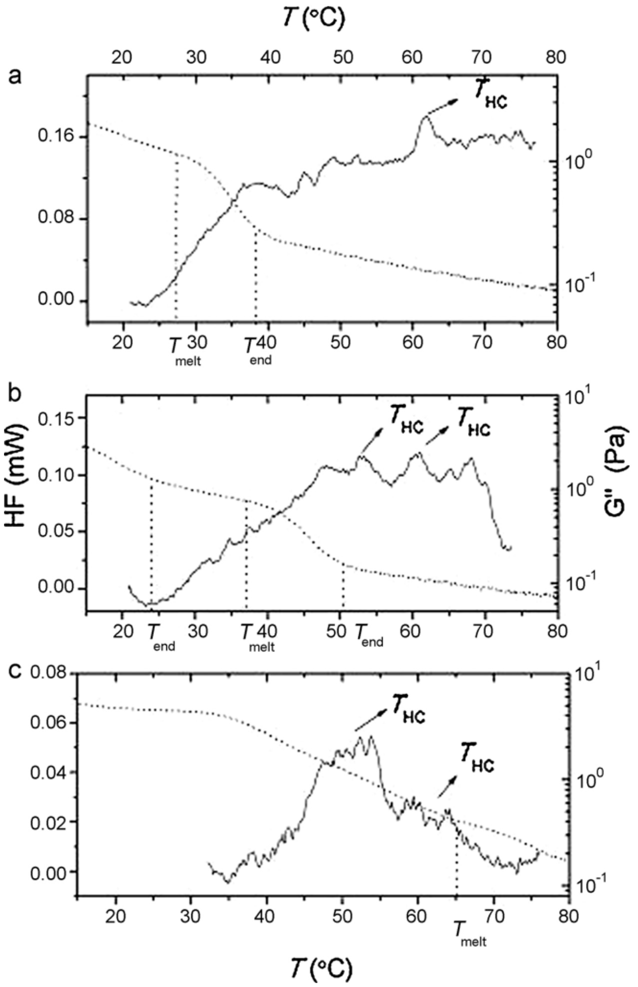

Fig. 6. Temperature dependence of the heat flow (solid lines) and the shear loss modulus G'' (dashed lines) recorded during the heating of gels of sample C obtained in deionised water (a), NaCl 0.1 M (b) and NaCl 1 M (c). Arrows indicate the temperatures at which corresponding labelled transitions occur (T_{HC}), whereas vertical dotted lines indicate temperatures at which rheological transitions occur.

4. Discussion

Results displayed in Figs. 5 and 6 indicate that at 0.1 M NaCl, the rheological curves are characterized by a two-step gelation and a two-step melting processes (see temperatures T_{ON} and T_{end}). The increase in T_m and T_{CH} with increasing ionic strength has long been demonstrated for pure κ-carrageenan (Rochas & Rinaudo, 1984) and ι-carrageenans (Nilsson & Piculell, 1989) and this actually motivated the present tuning of salt conditions for shifting both gelling and melting processes within our experimental window. Interestingly, mixtures of pure κ-carrageenans and pure ι-carrageenans also present a two-step gelation behaviour (Parker et al., 1993; Ridout, Garza, Brownsey, & Morris, 1996), which has been related to the formation of two phase separated networks which are actually interpenetrated (Ridout et al., 1996) Transition temperatures gathered in Tables 2 and 3 span the whole range of experimentally accessible temperatures, and show a significant variation between samples. This is in contrast with the transition temperatures recorded during the heating of a series of non gelling κ/ι-hybrid carrageenans solutions which showed little variation with the polysaccharide chemical composition (van de Velde et al., 2005). The total enthalpy of the two transitions which were assigned to helix to coil conformational changes of κ-carrageenan and ι-carrageenan blocks (heating scan), did not show the linear relationship with the chemical composition which is expected for ideal mixtures (Chanvrier et al., 2004; van de Velde et al., 2005). Moreover, an overlap of thermal processes resulting in the merging of peaks was pointed out as the hallmark of κ/ι-hybrid carrageenans (van de Velde et al., 2005) as it is in sharp contrast with the thermal behaviour of pure carrageenan mixtures, which show well separated thermal processes at constant temperatures (Chanvrier et al., 2004; Ridout et al., 1996; van de Velde et al., 2005). Accordingly, the melting temperatures characterized by DSC experiments and the temperatures for onset of network build up obtained by rheometry do not depend on the mixture composition (Parker et al., 1993; Ridout et al., 1996).

The enthalpy originating from the breaking-up of aggregated helices is known to significantly contribute to the total enthalpy of melting (Viebke, Borgstrom, Carlsson, Piculell, & Williams, 1998). We therefore conjecture that the size of blocks of κ- or ι-carrageenan disaccharide units, and inherently the molecular mass M_w of κ/ι-hybrid carrageenan macromolecules also contribute to the aggregation, and as such to the gel mechanical behaviour and gel melting properties. As a result, we now discuss separately the effects of M_w and chemical composition.

4.1. Effect of molecular mass M_w

Mechanical properties of pure κ-carrageenan gels are a function of the polysaccharide molecular mass M_w. Below a critical molecular mass $M_{c\kappa} = 3 \times 10^4$ g/mol, no κ-carrageenan gel is formed. However, gel elastic properties are independent of M_w, as soon as the latter is above 2×10^5 g/mol (Rochas, Rinaudo, & Landry, 1990; Smidsrod & Grasdalen, 1984). Highly sensitive DSC experiments carried in 0.2 M NaCl showed that a block of ι-carrageenan made of at least 5 disaccharide units was sufficient to allow a coil to helix conformational transition in κ/ι-hybrid carrageenan non gelling solutions (van de Velde et al., 2005). We therefore conjecture that a critical minimum block size with $M_{c\iota}$ of roughly 2000 g/mol is necessary to induce the formation of a ι-carrageenan-based network in the κ/ι-hybrid carrageenan.

Rheological data displayed in Fig. 4 indicate that all samples present κ-carrageenan or ι-carrageenan blocks with corresponding M_w larger than $M_{c\kappa}$ and $M_{c\iota}$, as gels could be obtained for all experimental conditions. In addition, the two-step gelation measured at 0.1 M NaCl is characterized by a T_{gel} which is sandwiched between the two T_{ON}. This result suggests that the first elastic network formed at higher temperature is completed before the onset of the second network formation, which takes place at lower temperature. G_0 values displayed in Fig. 4b are orders of magnitude lower than the elastic moduli G_0 measured with gels made with pure ι-carrageenan or κ-carrageenan under similar experimental gelling conditions (Hilliou, Gonçalves, et al., 2009) Such quantitative difference in gel elasticity is in favour of the block copolymer structure proposed earlier for κ/ι-hybrid carrageenans (van de Velde et al., 2005), as opposed to two phase separated or interpenetrated networks (Parker et al., 1993; Ridout et al., 1996) a 2 wt% mixture made of 30% pure ι-carrageenan, 50% pure κ-carrageenan and 20% non gelling carrageenan in 0.1 M NaCl should roughly show the mechanical properties of gel corresponding to 1 wt% κ-carrageenan gel (Ridout et al., 1996). This is essentially because the network made by 0.6 wt% ι-carrageenan in 0.1 M NaCl is much softer and thus does not significantly contribute to the overall gel elasticity. However, it has been recently reported that a 1 wt% κ-carrageenan gel shows a storage modulus G_0 as high as 4000 Pa in 0.1 M NaCl (Hilliou, Gonçalves, et al., 2009). This is still 2 orders of magnitude more elastic than gels displayed in Fig. 4b, and calls for a gel structure which differs from two interpenetrated networks which gel independently.

The molecular mass distribution critically affects both G_0 and the gel structure (see for instance curves for samples A and B in Fig. 4b). However, the relationship between M_w and G_0 is rather counterintuitive as the sample showing the larger M_w form less elastic gels. The negative correlation between these two parameters was indeed suggested in an earlier study where a larger set of κ/ι-hybrid carrageenan with multiple interdependent properties (both chemical and physical) was studied (Hilliou et al., 2006b). Such behaviour may relate to the polydispersity index I of each material and to the intrinsic distribution of block lengths for each carrageenan type. Sample B shows the widest distribution in M_w, and as such short non-gelling blocks of ι- and κ-carrageenan may coexist with longer gelling blocks. The viscous component seen in the mechanical spectrum of sample B (see the crossover in Fig. 4a) is in favour of non gelling blocks which lower the physical crosslink density and accordingly decrease the gel elasticity. Alternatively, marked differences in the distribution of biological precursors along the macromolecule due to different degrees of hydrolysis during extraction of samples A and B, may lead to the formation of long non-gelling sub-chains which act as dandling ends with plasticizing effects on G_0 (Hilliou et al., 2006b).

The thermal hysteresis, T_{melt}–T_{gel}, is not affected by M_w, as both samples A and B show a small hysteresis (nearly 3 °C in H_2O and 0.1 M NaCl) which is reminiscent from the slight hysteresis reported for a commercial κ/ι-hybrid carrageenan (Chanvrier et al., 2004).

On the other hand, a hysteresis as large as 16 °C is measured with 1 M NaCl. This is indicative of a larger helix aggregation leading to improved gel elastic properties with respect to pure ι-carrageenan gels (Piculell, 1995) It is worth mentioning here that no water syneresis could be detected throughout the study, which is in agreement with the rather low elasticity of the gels (see Fig. 4). However water release from gelling mixtures of pure ι- and κ-carrageenans showing elastic moduli ranging from 100 Pa to 200 Pa has been reported (Parker et al., 1993). These contrasting properties again point to the peculiar copolymer structure of κ/ι-hybrid carrageenans which impacts on the gel structure.

DSC cooling thermograms of samples A and B are qualitatively essentially identical as a single process is evidenced. However, quantitative differences in T_{CH} and corresponding transition enthalpies suggest that thermal transitions resolved in Fig. 5 are to some extent related to cooperative processes and not to independent helix-to-coil transitions. The higher transition temperature displayed by sample A suggests that smaller polysaccharides facilitate the conformational transition, whereas larger chains hinder the subsequent aggregation of formed helices, thus leading to smaller transition enthalpies. Comparing data in Fig. 5 with the literature is rather difficult as most reports use heating scans performed at different aggregating conditions (both salt and concentration). In this respect, heating scans presented in Fig. 6 are easier to discuss in light of data obtained under similar salt conditions as T_{HC} is known to depend on the ionic strength (Grinberg et al., 2001; Nilsson & Piculell, 1989; Piculell, 1995). Though the total enthalpy of the two helix-to-coil transitions measured at higher ionic strengths agree satisfactorily with enthalpies reported for a set of κ/ι-hybrid carrageenans solutions in 0.2 M NaCl (see Fig. 9 in van de Velde et al. (2005)), the scattering in T_{HC} values gathered in Table 3 for 0.1 M NaCl is at odds with the constant transition temperatures of 34 °C and 57 °C documented for the helix-to-coil transition of κ- and ι-carrageenan blocks in hybrid carrageenans (van de Velde et al., 2005), respectively. Helix aggregation is known to interfere with both T_{HC} and respective enthalpies in κ-carrageenan (Viebke et al., 1998). Moreover, it has long been recognised that the molecular mass distribution and rather large polydispersity bring additional complication to the helix-to-coil transition profile, and consequently a dispersion to the thermodynamic parameters derived thereof (Nilsson & Piculell, 1989). An additional transition with enthalpy of roughly 7 J g^{-1} and occurring at temperatures much lower than T_{HC}, has even been reported for ι-carrageenan solutions in 0.2 M NaCl. Helix aggregation was claimed to be at the origin of this transition as the corresponding temperature was shown to be concentration dependent (Grinberg et al., 2001) As such, the differences in both T_{HC} and enthalpies reported in Table 3 for samples A and B comes as no surprise, as soon as the formation of superhelical structures is considered. Both T_{HC} and enthalpies measured at low ionic strength actually depend on the polysaccharide concentration, as salt-induced screening of polysaccharide interactions is less effective (Nilsson & Piculell, 1989). Thus, data obtained in salt free conditions reveal the largest difference in T_{HC} between samples A and B: larger and more polydisperse polysaccharides form smaller and inherently less thermally stable aggregates of helices, which is in harmony with the looser network structure inferred from the rheology.

4.2. *Effect of chemical structure*

We now focus on the interplay between the relative content in ι-carrageenan disaccharide units and the thermo-mechanical properties of gels, comparing rheological and DSC data of samples B and C. By doing so, we discard any of the effects assigned to molecular mass distributions. If one assumes that the alkali treatment does not affect the statistical distribution of biological precursors along the polysaccharide, then we can consider that samples B and C only differ by their relative amount in ι- and κ-carrageenan disaccharide units.

The effect of chemical structure on gel mechanical properties is only seen at the highest ionic strength, as mechanical spectra for samples B and C are essentially overlapping in Fig. 4a and b. For 1 M NaCl, the gel made with sample B is 4 times more elastic, and this is in harmony with the corresponding larger amount of κ-carrageenan disaccharide units. In light of results presented in Fig. 4, we expect any difference in thermal behaviour to be seen only when enough NaCl is used to discriminate between the two chemical structures. Data in Tables 2 and 3 show that both gelling and melting occur at higher T for the iota-rich sample, and this conforms to the generally reported higher T_{gel} and T_{melt} for ι-carrageenan gels with no (or very small) thermal hysteresis (Piculell, 1995). Indeed, thermal hysteresis, namely T_{melt}–T_{gel}, lies in the range of 2–4 °C for all gels. This conforms to earlier rheological data obtained with a commercial sample containing 50 mol% ι-carrageenan (Chanvrier et al., 2004), and to the picture of less aggregated iota-carrageenan helices, thus imparting lower elasticity to the gel. However, sample B in 1 M NaCl shows a 15 °C thermal hysteresis, which is as large as the hysteresis found in κ-carrageenan gels. Such hysteresis is related to the lower gel-setting temperature T_{gel} which clearly shows dependence with the relative content in ι-carrageenan.

Thermal transitions derived from DSC show an overall similar trend, as an increase in the ι-carrageenan content is accompanied by an increase in all transition temperatures. This result is opposite to the documented invariance of T_{HC} with the chemical composition of the κ/ι-hybrid carrageenans (van de Velde et al., 2005) and again points towards the proposed helix association process as resolved in the present DSC experiments. Indeed, since blocks of κ-carrageenans are covalently linked to blocks of ι-carrageenans, any cooperative association of high temperature helical conformers will affect the coil-to-helix transition and subsequent transition of the low temperature conformers. The thermal behaviour of samples B and C is reminiscent from the crystallization of synthetic diblock copolymers in the ordered state. In these systems, the crystallization temperature of each block is significantly shifted with respect to its homopolymer counterpart. This is due to the competition between crystallization and microphase separation, as all structuring processes are hindered by the covalently linked and chemically different blocks which tends to phase separate (Müller, Balsamo, & Arnal, 2005).

5. Conclusion

We studied the separate effects of the molecular mass distribution and the relative content in ι-carrageenan disaccharide units on the elastic and thermal properties of a set of κ/ι-hybrid carrageenans gels. The tuning of the extraction parameters allowed the isolation of three κ/ι-hybrid carrageenans in the sodium form with controlled M_w and content in ι-carrageenan. However, the extraction of the biopolymers from *M. stellatus* comes at the expense of a rather high content (between 16 and 19 mol%) in non-gelling biological precursors. The main output of the rheological and DSC characterizations of gelling κ/ι-hybrid carrageenans solutions in the presence of a single type of salt, namely Na^+, is that the molecular mass distribution significantly affects the gelling properties, even when M_w is much larger than the critical molecular mass $M_{c\kappa}$ or $M_{c\iota}$. Therefore, it is not enough to solely consider the chemical composition of κ/ι-hybrid carrageenans to predict the gelling properties. A two-step gelation mechanism is evidenced in 0.1 M NaCl, where the two gel-setting temperatures are shifted into the present experimental window. The first increase in solution viscosity coincides with a coil-to-helix transition resolved by DSC, whereas a second viscosity upraise occurs at a temperature lower than the gel-setting temperature. The comparison of thermal properties of two κ/ι-hybrid carrageenans possessing nearly identical molecular mass distributions reveals that the gel-setting temperature actually depends on the chemical composition of the polysaccharide. Indeed, higher T_{gel} and T_{melt} are measured for the sample with higher content in ι-carrageenan disaccharide units, which in turn showed the weakest elastic modulus. All gels have an elasticity which is much weaker than the additive elasticity of two phase separated ι- and κ-carrageenan networks with polysaccharide concentrations corresponding to the κ/ι-hybrid carrageenans chemical composition. This result confirms the specific copolymer structure of κ/ι-hybrid carrageenans, which is at the origin of the formation of gels without water syneresis. The melting behaviour of κ/ι-hybrid carrageenans gels is in qualitative agreement with earlier reports, as two processes are observed in the DSC curves recorded in 0.1 M NaCl. However, the corresponding transition temperatures depend on both chemical composition and molecular mass distribution, thus suggesting that such transitions involve helix aggregation in addition to the helix-to-coil transitions of separate κ-carrageenan and ι-carrageenan blocks. Additional experimental work is however needed to better understand to what extent biological precursors are involved in the peculiar thermal and gelling properties of κ/ι-hybrid carrageenans showing κ-carrageenan contents close to 50 mol%.

Acknowledgments

Thanks are due to FCT for financial support to REQUIMTE and CIQ(UP) and project PTDC/CTM/100076/2008, and for Post-Doctoral grant to H.K.S.S. SFRH/BPD/37514/2007.

References

Beezer, A. E. (1980). *Biological microcalorimetry*. London: Academic Press.

Bixler, H. J. (1996). Recent developments in manufacturing and marketing carrageenan. *Hydrobiologia*, *326/327*, 35–57.

Bixler, H. J., Johndro, K., & Falshaw, R. (2001). Kappa-2 carrageenan: Structure and performance of commercial extracts. II. Performance in two simulated dairy applications. *Food Hydrocolloid*, *15*, 619–630.

Chanvrier, H., Durand, S., Garnier, C., Sworn, G., Bourriot, S., & Doublier, J. L. (2004). Gelation behaviour and rheological properties of κ/ι hybrid carrageenans. In P. A. Williams, & G. O. Phillips (Eds.), *The royal society of chemistry* (pp. 139–145). Cambridge: Gums and Stabilisers for the Food Industry.

Chopin, T., Kerin, B. F., & Mazerolle, R. (1999). Phycocolloid chemistry as a taxonomic indicator of phylogeny in the Gigartinales, Rhodophyceae: A review and current developments using Fourier transform infrared diffuse reflectance spectroscopy. *Phycological Research*, *47*, 167–188.

Denef, B., Gamini, A., Delben, F., Paoletti, S., Reynaers, H., & Vanneste, K. (1998). Thermodynamics of conformational transition and chain association of ι-carrageenan in aqueous solution: Calorimetric and chirooptical data. *Biopolymers*, *45*, 105–117.

Funami, T., Hiroe, M., Noda, S., Asai, I., Ikeda, S., & Nishinari, K. (2007). Influence of molecular structure imaged with atomic force microscopy on the rheological behavior of carrageenan aqueous systems in the presence or absence of cations. *Food Hydrocolloids*, *21*, 617–629.

Grinberg, V. Y., Grinberg, N. V., Usov, A. I., Shusharina, N. P., Khokhlov, A. R., & Kruif, K. G. (2001). Thermodynamics of conformational ordering of ι-carrageenan in KCl solutions using high-sensitivity differential scanning calorimetry. *Biomacromolecules*, *2*, 864–873.

Guenet, J. M. (2000). Structure versus rheological properties in fibrillar thermoreversible gels from polymers and biopolymers. *Journal of Rheology*, *44*, 947–960.

Guibet, M., Boulenguer, P., Mazoyer, J., Kervarec, N., Antonopoulos, A., & Lafosse, M. (2008). Composition and distribution of carrabiose moieties in hybrid κ/ι-carrageenans using carrageenases. *Biomacromolecules*, *9*, 408–415.

Hermansson, A.-M. (1989). Rheological and microstructural evidence for transient states during gelation of kappa-carrageenan in the presence of potassium. *Carbohydrate Polymers*, *10*, 163–181.

Hilliou, L., & Gonçalves, M. P. (2007). Gelling properties of a κ/ι hybrid carrageenan: Effect of concentration and steady shear. *International Journal of Food Science and Technology*, *42*, 678–685.

Hilliou, L., Gonçalves, M. P., & Wilhelm, M. (2009). Interplay between microstructure and mechanical properties in carrageenan gels in NaCl salt and in gelatin gels. In P. Fischer, M. Pollard, & E. J. Windhab (Eds.), *Proceedings of the 5th international symposium on food rheology and structure* , ISBN 978-3-905609-43-1 (pp. 306–309). Laboratory of Food Process Engineering.

Hilliou, L., Larotonda, F. D. S., Abreu, P., Ramos, A. M., Sereno, A. M., & Gonçalves, M. P. (2006). Effect of extraction parameters on the chemical structure and gel properties of κ/ι-hybrid carrageenans obtained from *Mastocarpus stellatus*. *Biomolecular Engineering*, *23*, 201–208.

Hilliou, L., Larotonda, F. D. S., Sereno, A. M., & Gonçalves, M. P. (2006). Thermal and viscoelastic properties of κ/ι-hybrid carrageenan gels obtained from the Portuguese seaweed *Mastocarpus stellatus*. *Journal of Agricultural and Food Chemistry*, *54*, 7870–7878.

Hilliou, L., Wilhelm, M., Yamanoi, M., & Gonçalves, M. P. (2009). Structural and mechanical characterization of κ/ι-hybrid carrageenan gels in potassium salt using Fourier transform rheology. *Food Hydrocolloids*, *23*, 2322–2330.

Ikeda, S., Morris, V. J., & Nishinari, K. (2001). Microstructure of aggregated and nonaggregated kappa-carrageenan helices visualized by atomic force microscopy. *Biomacromolecules*, *2*, 1331–1337.

Lahaye, M. (2001). Developments on gelling algal galactans, their structure and physicochemistry. *Journal of Applied Phycology*, *13*, 173–184.

McHugh, D. J. (2003). *A guide to the seaweed industry*. Italy: FAO Fisheries Technical Paper, 441.

Meunier, V., Nicolai, T., & Durand, D. (1999). Light scattering and viscoelasticity of aggregating and gelling κ-carrageenan. *Macromolecules*, *32*, 2610–2616.

Meunier, V., Nicolai, T., & Durand, D. (2000). Structure and kinetics of aggregating kappa-carrageenan studied by light scattering. *Macromolecules*, *33*, 2497–2504.

Müller, A. J., Balsamo, V., & Arnal, M. L. (2005). Nucleation and crystallization in diblock and triblock copolymers. *Advances in Polymer Science*, *190*, 1–63.

Nilsson, S., & Piculell, L. (1989). Helix–coil transitions of ionic polysaccharides analysed within the Poisson–Boltzmann cell model. 2. Effects of salt concentration on the thermal transition. *Macromolecules*, *22*, 3011–3017.

Parker, A., Brigand, G., Miniou, C., Trespoey, A., & Vallée, P. (1993). Rheology and fracture of mixed ι- and κ-carageenan gels: Two step gelation. *Carbohydrate Polymers*, *20*, 253–262.

Piculell, L. (1995). Gelling carrageenans. In A. M. Stephen (Ed.), *Food polysaccharides and their applications* (pp. 205–244). New York: Marcel Dekker Inc.

Ridout, M. J., Garza, S., Brownsey, G. J., & Morris, V. J. (1996). Mixed iota-kappa carrageenan gels. *International Journal of Biological Macromolecules*, *18*, 5–8.

Rochas, C., & Rinaudo, M. (1984). Mechanism of gel formation in kappa-carrageenan. *Biopolymers*, *23*, 735–745.

Rochas, C., Rinaudo, M., & Landry, S. (1990). Role of molecular weight on the mechanical properties of kappa carrageenan gels. *Carbohydrate Polymers*, *12*, 255–266.

Smidsrod, O., & Grasdalen, H. (1984). Polyelectrolytes from seaweeds. *Hydrobiologia*, *116/117*, 19–28.

van de Velde, F. (2008). Structure and function of hybrid carrageenans. *Food Hydrocolloids*, *22*, 727–734.

van de Velde, F., Antipova, A. S., Rollema, H. S., Burova, T. V., Grinberg, N. V., Pereira, L., et al. (2005). The structure of κ/ι-hybrid carrageenans. II. Coil–helix transition as a function of chain composition. *Carbohydrate Research*, *340*, 1113–1129.

van de Velde, F., Peppelman, H. A., Rollema, H. S., & Tromp, R. H. (2001). On the structure of κ/ι-hybrid carrageenans. *Carbohydrate Research*, *331*, 271–283.

Viebke, C., Borgstrom, J., Carlsson, I., Piculell, L., & Williams, P. (1998). A differential scanning calorimetry study of κ-carrageenan in the NACl/NaI/CsI/CsCl systems and analysis by Poisson–Boltzmann calculations. *Macromolecules*, *31*, 1833–1841.

Viebke, C., Piculell, L., & Nilsson, S. (1994). On the mechanism of gelation of helix-forming biopolymers. *Macromolecules*, *27*, 4160–4166.

Villanueva, R. D., Hilliou, L., & Sousa-Pinto, I. (2009). Postharvest culture in the dark: An ecofriendly alternative to alkali treatment for enhancing the gel quality of κ/ι-hybrid carrageenan from Chondrus crispus (Gigartinales, Rhodophyta). *Bioresource Technology*, *100*, 2633–2638.

Villanueva, R. D., Mendoza, W. G., Rodrigueza, M. R. C., Romero, J. B., & Montano, M. N. E. (2004). Structure and functional performance of gigartinacean kappa-iota hybrid carrageenan and solieriacean kappa-iota carrageenan blends. *Food Hydrocolloids*, *18*, 283–292.