Helicity degree of carrageenan conformation determines the 2 polysaccharide and water interactions

by Elmarhoum, S., Ako, K., Munialo, C.D. and Rharbi, Y.

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Helicity degree of carrageenan conformation determines the polysaccharide and water interactions

Authors and affiliations:

Said ELMARHOUM[a], Komla AKO *[a], Claire D. MUNIALO[b] & Yahya RHARBI[a]

a) Univ. Grenoble Alpes, CNRS, Grenoble INP, LRP, 38000 Grenoble, France

b) Food Land and Agribusiness Management Department Harper Adams UniversityNewport, Shropshire, TF10 8NB, UK

*__Corresponding author__: *komla.ako@univ-grenoble-alpes.fr*; *akokomla@hotmail.com*

Abstract:

The polysaccharide in solution at critical concentration, Cc (g/L), is assimilated to a nano hydrogel (nHG) made of a single polysaccharide chain. Taking as reference the characteristic temperature of 20 ± 2 °C at which kappa-carrageenan (κ-Car) nHG swelling is greater with a Cc = 0.55 ± 0.05 g/L, the temperature of the minimum deswelling in presence of KCl was found at 30 ± 2 °C for 5 mM with a Cc = 1.15 ± 0.05 g/L but not measurable above 100 °C for 10 mM of which Cc = 1.3 ± 0.05 g/L. Lowering the temperature to 5 °C, contraction of the nHG and further coil-helix transition with self-assembly increases the sample's viscosity, which steadily evolves with time in a logarithmic scale. Accordingly, the relative increment of the viscosity per unit of concentration, R_v (L/g), should increase in agreement with increasing polysaccharide concentration. But the R_v decreases for κ-Car samples above 3.5 ± 0.5 g/L in the presence of 10 mM KCl under steady shear 15 s^{-1}. This reflects a decrease of κ-Car helicity degree knowing that the polysaccharide is rather hydrophilic when its helicity degree is the lowest.

Keywords: Polysaccharide; Nanohydrogel; Kinetic; Coil-helix; Reduced viscosity

1 Introduction

As dissolution of the polysaccharides in aqueous phase precede most of the polysaccharide applications in food and nonfood areas, understanding their solubility, therefore, becomes critically important (Elfaruk, Wen, Chi & Li, 2021; Gerentes, Vachoud, Doury & Domard, 2002). For example, pectin, starch, agarose and chitosan powders have to be dissolved in aqueous media prior to any application (Einhorn-Stoll, 2018; Felfel, Gideon-Adeniyi, Hossain, Roberts & Grant, 2019; Saeedi Garakani et al., 2020; Yousefi & Ako, 2020). Solubilization of polysaccharide in water is currently performed at high temperature (> 60 °C) then cooled for applications such as stabilizer in food, scaffold in tissue engineering and matrix in personal care (Fabra, Hambleton, Talens, Debeaufort, Chiralt & Voilley, 2009; Jafari, Bernaerts, Dodi & Shavandi, 2020; Weiss, Salminen, Moll & Schmitt, 2019).

For ionic polysaccharide like kappa-carrageenan (κ-Car), the upper critical solution temperature (UCST) or the temperature above which a clear solution is observed strongly depends on the ionic strength and the type of counter ions (Heyda, Soll, Yuan & Dzubiella, 2014). The charges on the polysaccharides interact with the charges on the other components of mixed systems, for example, polysaccharides and proteins by repulsion or attraction and this determines the texture of the system's steady state (Ako, Durand & Nicolai, 2011; Huang, Mao, Li & Yang, 2021). In addition to the electrostatic interactions, polar and non-polar interactions are also determinant in the equilibrium of the systems (Derkach, Kuchina, Kolotova & Voron'ko, 2020). It has been shown that polar and non-polar interactions between the polysaccharides and proteins are significantly affected by for example the conformational transition (such as the coil-helix transition) of both components including unfolding of proteins (Derkach, Kuchina, Kolotova & Voron'ko, 2020; Voron'ko, Derkach, Vovk & Tolstoy, 2017; Weiss, Salminen, Moll & Schmitt, 2019). Since proteins may unfold and

expose polar and non-polar functional groups, attraction or repulsion interactions can emerge following or opposing the electrostatic forces. Phase separation (segregation or association) takes place if the mixed state correspond to a greater free energy than two separates phases (Ako, Durand & Nicolai, 2011; Mezzenga & Fischer, 2013).

Below the UCST, the polysaccharide solutions can turn to aggregate suspensions, fluid gels and gels, likewise, the viscosity of the solution increases to infinity at the gel point (Garrec, Guthrie & Norton, 2013; Rochas & Rinaudo, 1984). The rheological properties of the polysaccharide phase are crucial for the blending process, given that they contribute to the stability of the suspensions (emulsion) and the microstructure of the systems (Derkach, Kuchina, Kolotova & Voron'ko, 2020; Weiss, Salminen, Moll & Schmitt, 2019). However, aggregation and gelation processes may not be achieved on a time-scale of processing and observation, if the interactions between polysaccharide and water are still favorable even below the UCST (Elfaruk, Wen, Chi & Li, 2021; Meunier, Nicolai & Durand, 2000). Therefore, understanding the viscosity kinetic of the polysaccharide solutions and the underlying mechanisms are necessary to predict the long-term stability of many polysaccharide-based aqueous systems. The numerous works on polymer suspensions have shown that the interactions between the polymers and solvent, thereby, intra/interchain interactions, are determinant in the suspension viscosity (Bongaerts, Reynaers, Zanetti & Paoletti, 1999; Colby, 2010; Croguennoc, Meunier, Durand & Nicolai, 2000; Elmarhoum & Ako, 2022a).

The coil and helix states of carrageenan namely ι-Car, κ-Car and similar polysaccharide like agarose has been extensively investigated by many authors in various aqueous and thermal conditions using the optical rotation measurements or circular dichroism (Fujii, Yano, Kumagai & Miyawaki, 2000; Gilsenan, Richardson & Morris, 2000; Mangione, Giacomazza, Bulone, Martorana & San Biagio, 2003). At temperature below and equal the UCST, the

helices start to aggregate as soon as they are formed (Meunier, Nicolai & Durand, 2000; Norton & Goodall, 1983). The correlation of the coil-helix transition with the abrupt change of the suspension viscosity and visco-elasticity is well known from the numerous work reported in the literature (Elmarhoum, Mathieu, Ako & Helbert, 2023; Meunier, Nicolai & Durand, 2000; Rochas & Rinaudo, 1984). We took an advantage of existing research findings to investigate the conformational change and aggregation kinetic of polysaccharides using rheological methods. In a mixture of κ-Car and denatured β-lactoglobulin, the formation of helices and aggregation of κ-Car were found to cause spherical domains of denatured β-lactoglobulin similar to an emulsion (Ako, Durand & Nicolai, 2011). We hypothesize that the control of the degree of helicity through the polysaccharide and water interactions could lead to a better control of the structure of polysaccharide and protein mixtures. On one hand, a lower helicity degree may cause lower aggregation/gelation rate (Meunier, Nicolai & Durand, 2000). On the other hand, a decrease of the polysaccharide and water attractive interactions is expected to take place with an increase of the helicity degree (Tanaka & Wada, 1973).

2 Materials and methods

2.1 The Polysaccharides

The carrageenan polysaccharide is made of a repetition of disaccharide unit called carrabiose. The carrabiose units, that form the neutral backbone of α-Car and κ-Car are composed of 3-linked β-D-galactose (G) and 4-linked 3,6-anhydro-D-galactose (DA). For the two selected carrageenans, only the position of the unique sulfate (S) group on the carrabiose is different between them (Elmarhoum, Mathieu, Ako & Helbert, 2023). It is worth noting that the α-Car as native polysaccharide does not exist naturally, but is made by a desulfation enzymatic reaction from iota-carrageenan (ι-Car) coded as G4S-DA2S (Prechoux, Genicot, Rogniaux &

Helbert, 2013). Therefore, the κ-Car and α-Car are represented as G4S-DA and G-DA2S respectively. The production of α-Car is a breakthrough in the study of carrageenan functional properties, because they provide chemical or molecular origin information on the physical properties of the widely studied κ-Car and ι-Car systems, namely the interaction between these polysaccharides and water.

The κ-Car was a gift from RhodiaFood Switzerland (product name and reference: MEYPRO-GEL 01/2001 WG95-37 K-Car) of an average molecular weight of 3.3×10^5 g/mol and polydispersity index of $M_w/M_n = 2.0$, where M_w and M_n are the weight and number average molar mass respectively.

The α-Car was a gift from Cermav (Centre de recherche sur les macromolecules végétales) of average molecular weight of 3.8×10^5 g/mol and polydispersity index of $M_w/M_n = 1.8$.

2.2 Preparation of the Samples

The solutions of the two polysaccharides were prepared following the same preparation as reported in our previous work (Elmarhoum, Mathieu, Ako & Helbert, 2023). The stock solutions of the polysaccharide are dialyzed and free of added salts. The samples were prepared by dilution to different concentrations of polysaccharide and salt (KCl) where applicable. The samples with 0 mM salt indicate dilution of the stock solution with demineralized water or as the polysaccharide solution in dialyzed form. The solutions with salt were heated between 70 and 90 °C until they became completely transparent, ≈ 15 min, then they were cooled prior to any use. These samples are considered as fresh samples only during cooling and are no longer fresh if the cooling process was stopped. The cooling ramp was 2 °C/min. The conservation of the sample before the measurements could last from 10 s to 10 weeks. The aged samples were reset by heating before new measurements were performed.

2.3 Rheological measurements

The viscosity of hydrocolloidal suspensions changes with the hydrocolloid's structural properties. Therefore, the viscosity quantity is consistent for characterizing the aggregation of hydrogel-forming polysaccharide in aqueous phase. Kinetics of the viscosity at different shear rate and shear rate sweep of fresh and aged samples were performed in Couette geometry using a DHR3 Rheometer (TA Instrument) with a thermo regulator (Microcool LAUDA MC600) to control the temperature of the samples. The Couette geometry consisted of a concentric cylinder geometry of an inner rotor cylinder (bob) and an outer stator cylinder (cup) with radii R_1 of 14 mm and R_2 of 15 mm, respectively, defining a horizontal gap (R_2 - R_1) of 1 mm and an average radius R of 14.5 mm as (R_1 + R_2)/2. The height of the bob was 42 mm, and the vertical gap between the bob and cup was set at 2 mm. The measurements were done using similar experimental conditions as previously reported (Elmarhoum & Ako, 2021).

2.4 Determination of critical concentration and statistical conditions

The amount of solution of each concentration was prepared sufficiently to make at least three samples. The measurements of the viscosity were performed on loaded sample with the same run program more than twice after several cooling and heating, because the system is thermoreversible. At least three samples of same polysaccharide / salt concentration were analyzed at different days following the same protocol of mapping of the rheometer before the samples were loaded onto the rheometer. In this experimental condition, the deviation of the viscosity measurements is restricted to the size of the plot symbols except if indicated. The viscosity (η_C) was plotted as function of polysaccharide concentrations for a given temperature (T). The Kraemer specific viscosity was computed as $Ln(\eta_C / \eta_0)$, where η_0 is the solvent viscosity, then this was divided by C and plotted as function of $Ln(C)$. The details on

this method have previously been reported (Ako, Elmarhoum & Munialo, 2022). We called this type of representation Kraemer-LogC (K-LogC) in relation with the Kraemer equation (Eq.1) commonly used to determine the Kraemer intrinsic viscosity $[\eta]_K$.

$$\frac{Ln(\eta_C/\eta_0)}{C} = [\eta]_K + k_K[\eta]_K^2 C \quad (1)$$

Since the concentration dependence of the viscosity shows the dilute and semidilute regimes, the linear regression of the data in K-LogC representation of the two regimes cross at a concentration termed critical. The error bar or deviation applied to this critical concentration (Cc) is determined by an acceptable coefficient of determination which was here R^2 between 0.85 and 1. Therefore, all the Cc values displayed in this work were obtained with $R^2 \geq 0.85$.

2.5 Temperature dependence of the viscosity model

The model is based on lower-viscosity-temperature (LVT) concept used to characterize the thermal property of the polysaccharide solutions.

$$\eta^T = \eta^{Tref} \cdot \exp\left[B_2 \cdot (T - T_{ref})(T - T_2)\right] \quad (2)$$

where

$$T_2 = 2T_c - T_{ref} \quad (3)$$

and B_2 with T_c are adjusting parameters in respect of Ako et al., (2022) definition of these parameters (Ako, Elmarhoum & Munialo, 2022; Elmarhoum & Ako, 2022b). The quantity $B_2 \times T_c$ ($°C^{-1}$) is less dependent on the adjustment, it was defined as the thermal expansion coefficient of the polymer chains. The T_c gives information on the free energy cost to break the interactions between polymer chains, therefore T_c is a bulk dependent phenomenon. However, to some extent we can hypothesize that monomer-monomer interchain interactions

could work for monomer-monomer intrachain interactions. For pure liquid, T_c characterizes the temperature where molecular interactions become ineffective. But for small molecular weight liquid like water, T_c could characterize the boiling point.

3 Results and discussion

3.1 The κ-Car and α-Car polysaccharide solutions

Viscous flow is a kinetic phenomenon, whereas conformational characteristic is a result of thermodynamic properties of the polymers in solution (Colby, 2010). Therefore, the correlation between viscosity and conformational characteristic of the polysaccharide herein is derived from Newtonian's flow of the polysaccharide solutions. The relationship between the temperature and viscosity of κ-Car and α-Car solution characterizes the inter/intra molecular interactions at work in the solution. Polysaccharide concentration dependence of the relationship between the temperature and viscosity shed light on the thermodynamic properties of κ-Car solution in the random coil state domain. Taking the dialyzed solution as reference (0 mM), we have shown that if the KCl salt concentration (5 and 10 mM) is increased, the temperature of characteristic critical concentration Cc (g/L) increases (Fig.1). Common consideration of Cc (g/L) in polymer physical chemistry supposes that bulk and excluded volume concentration should be the same at Cc (g/L). Therefore, considering that excluded volume (V_p) is spherical, i.e., $V_p = 4\pi R_p^3/3$, at Cc (g/L) the concentration equal m_p/V_p with m_p being the average mass of a single polymer in the V_p.With the polysaccharide molecular mass M_p known, the expression of Cc (g/L) is given as:

$$Cc = \frac{3M_p}{4\pi R_p^3 N_A} \qquad 4$$

where the inverse of Avogadro's number, $1/N_A$, is the mol quantity of a single polymer. Given that the polysaccharide average mass is constant, the variation of Cc (g/L) as shown in Fig.1 represents the polysaccharide volume in aqueous phase. The arrows in Fig.1 indicate the position of the minimum Cc (g/L) as well as the corresponding temperature named lower critical concentration temperature (LCCT).

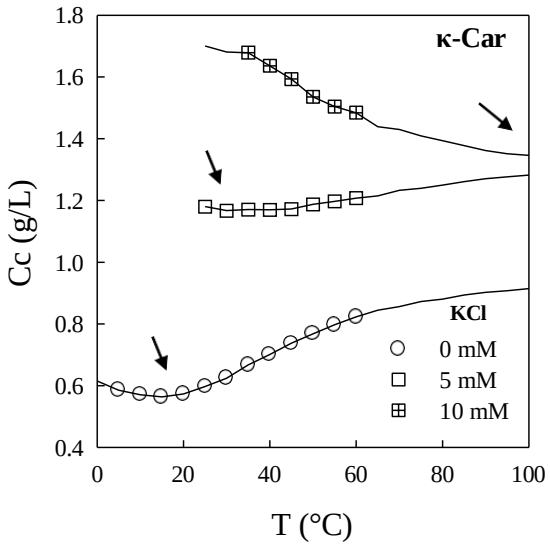


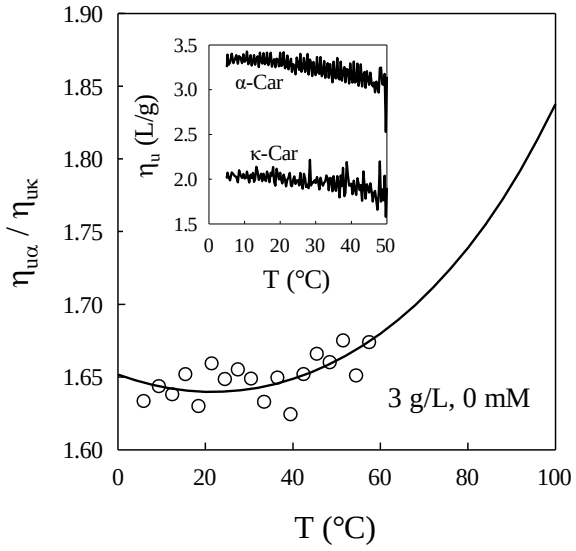
Figure 1: Temperature dependence of critical concentration of the κ-Car solution for different KCl salt concentrations. The arrows indicate the lower Cc with its temperature. The Cc was determined with $R^2 \geq 0.85$. The solid lines are the results from the temperature-viscosity function (Eq.2).

The minimum Cc (g/L) increases with the LCCT when the KCl salt concentration is increased. KCl salt concentration above 5 mM seems not applicable for determining the LCCT of κ-Car, therefore the results of 10 mM and above are simply indicative of the LCCT tendency. The particularly weak value of the KCl concentration stems from the ionic

selectivity property of κ-Car, which is characterized by a strong reaction in the presence of KCl salt more than NaCl salt. The consequence of this selectivity is that gels of κ-Car exhibit severe syneresis phenomenon in the presence of KCl rather than other food grade monovalent salts. The thermodynamic origin of this phenomenon could be explained by the contraction of the polysaccharide excluded volume, and accordingly, an increase of the solution Cc (g/L). The polysaccharide excluded volume is the pervaded volume of chemically linear links and physically cross links between the monosaccharide's molecules, and thus, is similar to a physical and chemical nanohydrogel (nHG) particles. Considering the bulk as a compact collection of such particles, viscosity, therefore, becomes a bulk flow issue of condensed suspension of nHGs near the Cc (g/L). Correlation of the rheological properties of this independently estimated volume is not illusory. Small and big nHG will lead to high and weak Cc (g/L) respectively. Therefore, shrinking and swelling properties of the nHG are derived from bulk flow in the polysaccharide concentration ranging below the entangled concentration regime (Colby, 2010). In dilute solution regime, these properties are derived from the polysaccharide intrachain interactions namely, hydrodynamic, excluded volume effect, water-monosaccharide and carrabiose-carrabiose interactions. These intrachain interactions determine the polysaccharide expansion and its conformational characteristics, which is strongly dependent on the polysaccharide affinity for the liquid media in terms of poor-, theta- and good-solvent. For the dialyzed solution, the κ-Car concentration dependence of the viscosity was shown to increase following a scaling law, with a scaling power equal to 1.15. According to the scaling law theory of viscosity, a full repulsion between the carrabiose should lead to a scaling power of 0.5 where neutral polymer in a good solvent yields 1.3. This result supposes that intrachain interactions between the carrabioses are not fully repulsive and the aqueous media is not good enough to promote full stretching of the polysaccharide. The length scale of the polysaccharide chain that resists stretching or swelling is considered as the

unperturbed size (R_0) of the polysaccharide (Colby, 2010). Thus, we think that water molecules should be out of this size domain due to excluded volume effect, because the free energy or elastic energy cost to swell the nHG on length scales smaller than R_0 is huge compared with Boltzmann energy (kT) and greater than the entropic energy. Conversely, monosaccharide-monosaccharide attractive interactions should sharply decrease on length scales larger than R_0 giving way for water-monosaccharide interactions to take place.

When κ-Car is converted to α-Car by the transfer of the sulfate group from the C4 of D-galactose to the C2 of 3,6-anhydro-D-galactose, we can see a significant increase of the viscosity of the dialyzed solution (Elmarhoum & Ako, 2022b). The specific viscosity per unit of concentration, noted η_u (in L/g), is the property of polymer that is derived from conformational characteristic as a result of excluded volume and water-polymer interactions. These interactions respectively determine the Cc (g/L) and intrinsic viscosity of the system as the limit of η_u when the polymer concentration decreased to 0 g/L (Ako, Elmarhoum & Munialo, 2022). Therefore, the ratio between η_u of α-Car and κ-Car represents better the conformational contribution to the viscosity difference between the two polysaccharides. The temperature dependence of η_u of α-Car and κ-Car are displayed in the insert of Fig.2 as $\eta_{u\alpha}$ and $\eta_{u\kappa}$ respectively for the concentration of 3 g/L in dialyzed solution (0 mM). The Fig.2 shows the temperature dependence of $\eta_{u\alpha}$ / $\eta_{u\kappa}$. These experimental measurement data are shown in open circles and are fitted using the temperature-viscosity function reported in our previous work (Eq.2) (Ako, Elmarhoum & Munialo, 2022).



250

251 *Figure 2: Temperature dependence of the ratio between α-Car reduced viscosity and κ-Car*
252 *reduced viscosity. The insert shows the reduced viscosity of the two polysaccharides and the*
253 *solid line is derived from the temperature-viscosity model (Eq.2).*

254 Though the specific viscosity per concentration shows a decreasing tendency with increasing
255 temperature for both α-Car and κ-Car polysaccharides, it can see from the fit function in Fig.2
256 that both the swelling and water molecules binding properties of κ-Car are more impaired by
257 heat. Considering $f = \eta_{u\alpha} / \eta_{u\kappa}$, the fluctuation of f between 5 °C and 60 °C led to f being taken
258 as an average value with a standard deviation of 1.65 ± 0.05. In presence of 10 mM KCl and
259 in the T-range [5 °C, 60 °C], the average quotient f was 1.70 ± 0.05, which supposes that 10
260 mM KCl salt impairs the swelling and binding water molecules properties of κ-Car compared
261 with α-Car. The thermal expansion of κ-Car and α-Car nHGs fit together with the coefficient
262 $B_2 \times T_c = 1.5 \times 10^{-2}$ /°C. However, the T_c are different as their values depend on the bulk
263 concentration. We found for the concentration of 3 g/L, 141 °C for α-Car and 153 °C for κ-
264 Car. Actually, the interaction potential between the nHG partially overlaps and this increases
265 in intensity and density with the increase of the concentration above the Cc (g/L) (Elmarhoum
266 & Ako, 2021). The regime of the concentration 3 g/L is semidilute for both systems and the

difference between their T_c may explain a difference in term of intensity and density of monomer-monomer interchain interactions including the interaction between water and the polysaccharides. With T_c of α-Car below the T_c of κ-Car, it implies that monomer-monomer attractive interactions are greater for κ-Car than for α-Car in dialyzed solution. When 10 mM KCl was added to the dialyzed solution and the solution was heated above 30 °C to melt κ-Car aggregates and unfold the helices, the temperature dependence of the viscosity fits well with the expansion coefficient $B_2 \times T_c$ and T_c of 1.4×10^{-2} /°C and 183 °C respectively. The α-Car solution with 10 mM KCl fits well with 1.55×10^{-2} /°C and 139 °C respectively and did not exhibit any features similar to the helix-coil conformational transition above 5 °C. The addition of salt to the solutions shed light on the polyelectrolyte effect on the thermal expansion properties of the polysaccharide including the repulsion interaction between the monomers in both inter and intrachain interactions. Water and polysaccharide interactions are influenced by salt accordingly. The 10 mM KCl salt has decreased the thermal expansion coefficient of κ-Car nHGs and increased the monomer-monomer attractive interaction due to screening electrostatic repulsion effect. The α-Car nHGs seems to not be sensitive as much as κ-Car nHGs to 10 mM KCl, but a weak increase of the thermal expansion coefficient was observed for α-Car. In addition to these results, we report a quotient *f* of 1.7 at 45 °C between the η_u of α-Car and κ-Car at 3 g/L, the quotient *f* was found to increase from 1.5 to 1.8, if the polysaccharide concentration is increased from 1 g/L to 5 g/L. We confirm by these results the relative lower ability of κ-Car to expand and swell in monovalent aqueous phase, which we have attributed to the sulfate group position effect in the carrabiose. The ionic selectivity feature of these results was reported previously (Elmarhoum & Ako, 2022b; Elmarhoum, Mathieu, Ako & Helbert, 2023).

3.2 The solution viscosity and self-assembly dynamic

The formation of helices is reported for the conformational properties of hydrogel-forming carrageenan (Bui, Nguyen, Renou & Nicolai, 2019; Ciancia, Milas & Rinaudo, 1997; Morris, Rees & Robinson, 1980). The κ-Car polysaccharide in solution has shown prompt aggregation and gelation properties particularly in the presence of KCl salt. The kinetics of both mechanisms in competition influence the viscoelasticity of the samples. Therefore, when a fresh solution of κ-Car in the presence of KCl is cooled to temperature range (T-range) below the coil-helix temperature (T_{ch}), the viscosity of the fresh sample sharply increases, likewise, the slope increases with the polysaccharide and salt concentrations (Meunier, Nicolai & Durand, 2000; Meunier, Nicolai, Durand & Parker, 1999). Though aggregation promotes gelation with increasing salt concentration at constant polysaccharide concentration, it could be fatal for the gelation of the samples in the presence of a relatively high concentration of KCl salt (100 mM) for weak polysaccharide concentrations (< 3 g/L).

Immediately, after cooling the fresh sample, if the sample was heated, the elasticity or alternatively viscosity of the samples decreases to reach the viscosity of the solution above the helix-coil temperature (T_{hc}) (Fig. 3a). The temperature hysteresis or gap between T_{ch} and T_{hc} is reported as a result of the self-assembly property of the hydrocolloids. Therefore, the viscosity stability of such hydrocolloid samples at a given temperature (T) depends not only on their self-assembling kinetics (long-time viscosity property) but also on the real-time property of the interactions between the hydrocolloids. Fresh and aged samples at $T > T_{hc}$, i.e., in random coil state, yield the same viscosity properties (insert of Fig. 3a). For example, the sample with 5 mM are fresh at storage temperature of 20 °C even after 60 days (square symbols in the insert of Fig. 3a), because their T_{hc} = 15 ± 2 °C (square symbols in the Fig. 3a). Hence, no distinction was made between the samples conserved at temperature above T_{hc} and the fresh or reset samples. However, the aged samples in T-range below T_{ch} have shown

distinctly two exciting viscosity properties: The real-time viscosity decreases with increasing the steady shear rate (open circles in the insert of Fig.3a) and the long-time viscosity increases with time at variable steady shear rate (Fig.3b).

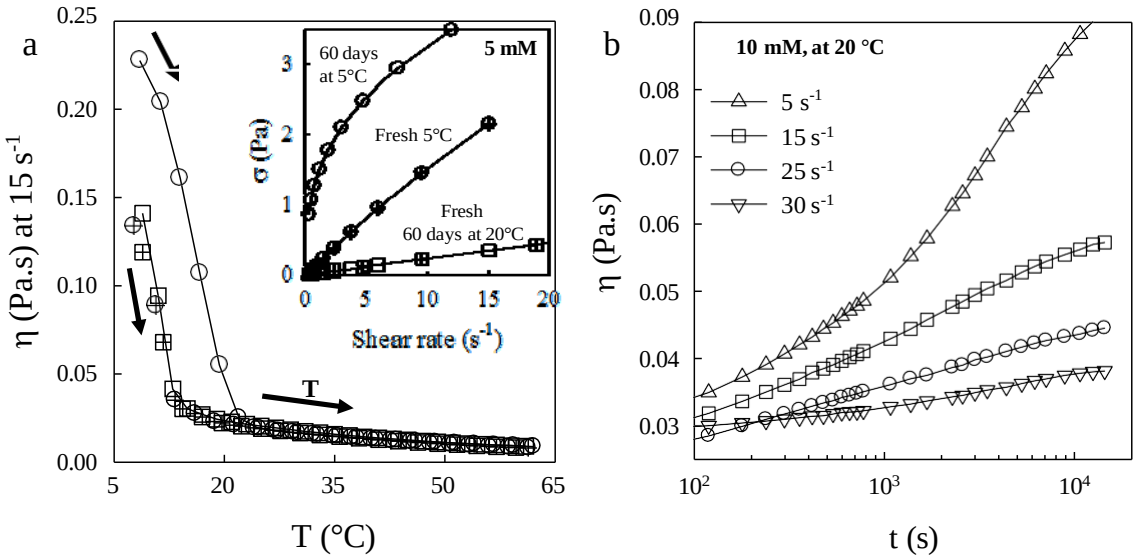


Figure 3:a) Temperature dependence of the viscosity of k-Car samples. The samples were loaded at 5 °C then heated to 60 °C. The open symbols are samples aged-at-rest for 60 days at 5 °C (circles) and at temperature above the helix-coil, 20 °C (squares). The crossed symbols are freshly prepared samples (circles) and reset to fresh from the aging samples (squares). The insert shows the shear rate dependence of the shear stress for the different tested samples. b) Kinetics of the viscosity of 3 g/L, κ-Car in presence of 10 mM KCl at 20 °C for different shear rates.

The mechanisms underlying these observations stem from the polysaccharide-water and polysaccharide-polysaccharide interaction dynamics. The interaction dynamics and links between the helices part of the polysaccharide chains are determined by temperature and salt

concentrations. Some of them are broken and/or their formation impeded by the shear rate. We have surprisingly observed that unlike κ-Car, the viscosity of α-Car solution decreases with time under shear. We show the results in the Fig.4 for 3 g/L in the presence of 100 mM KCl and 20 mM $CaCl_2$ at 5 °C. Alpha-carrageenan shows less sensitivity to KCl salt as its solution in the presence of 100 mM KCl did not gel at time scale >12 h. Conversely, the $CaCl_2$ salt induces gelation of the polysaccharide and this was observed for 6 g/L but not for the duplicated 3 g/L samples at rest during the rheological tests. The coil-helix transitions of the polysaccharide were observed for 100 mM KCl and 20 mM CaCl2 at ≈ 12 °C and 23 °C respectively. Comparatively, the gelation of α-Car is slower than the gelation of κ-Car and needs a considerable addition of a monovalent salt such as the gelation of the samples in presence of 400 mM KCl.

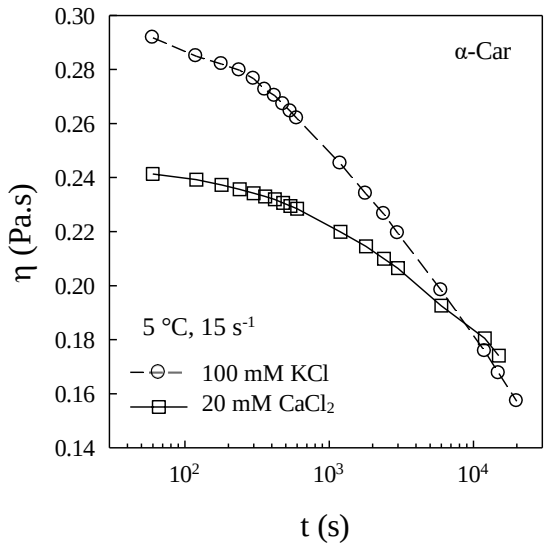


Figure 4: Kinetics of the viscosity under shear rate 15 s^{-1} of 3 g/L, α-Car in the presence of salt at 5 °C. Solid lines are guidelines for eyes

The ionic sensitivity and viscosity property of both κ-Car and α-Car have evidenced the chemical origin of their interactions with water. In actual sense, the two polysaccharides are similar at a molecular level with one sulfate group being carried by their carrabiose, but the position of the sulfate group is different. The transfer of the sulfate position from the C4 of D-galactose (κ-Car) to the C2 of 3,6-anhydro-D-galactose (α-Car), turns the positive rate of the viscosity kinetic (Fig. 3b) to negative (Fig. 4) with a significant impact on the ionic sensitivity and selectivity. Hence, the carrageenan properties in aqueous phase are clearly determined by the position of charges. Comparative analysis between the properties of κ-Car and α-Car with other similar polysaccharides in aqueous phase has shown the role of the anhydro cycle, charge, and the charge number carried by the polysaccharide monomers in their ionic sensitivity, selectivity, and affinity with water (Elmarhoum & Ako, 2022a; Elmarhoum, Mathieu, Ako & Helbert, 2023; Feke & Prins, 1974; Fujii, Yano, Kumagai & Miyawaki, 2000; Lindman, Karlström & Stigsson, 2010; Matsuo, Tanaka & Ma, 2002). For the selected similar polysaccharide, there are neutral polysaccharides such as agarose which have an anhydro cycle and cellulose without anhydro cycle. Agarose is a water-soluble polysaccharide but cellulose is insoluble in water, though cellulose carries several -OH groups, and thus has a good hydrogen bonding ability. We hypothesize that the anhydro cycle prevents a close contact between the monomers carrying this functional group. Thus, the anhydro cycle promotes the interaction between water and the monomers rather than interchain monomer interactions. On one hand, we have the λ-Car without an anhydro cycle but its carrabiose carries 3 anionic sulfate groups. The λ-Car does not gel in the presence of either mono or divalent cations, it displays only viscous behavior (Running, Falshaw & Janaswamy, 2012). Therefore, the charges prevent the contact between the monomers by electrostatic repulsion, and thus promote water and monomers interactions better than the anhydro cycle. On the other hand, we have the ι-Car with two anionic charges and anhydro cycle which is more

sensitive to divalent than monovalent counter ions, in the presence of which gelation of the ι-Car solutions could happen (Bui, Nguyen, Renou & Nicolai, 2019; Elfaruk, Wen, Chi, Li & Janaswamy, 2021). The κ-, ι- and λ-carrabiose with one, two and three anionic charges respectively react selectively with mono, di and trivalent cations. Contrastingly α-carrabiose carries one anionic charge but reacts preferentially with divalent cations rather than monovalent cations. Finally, given that two or three monovalent cations do not work as one divalent or trivalent respectively to reduce the intermolecular electrostatic repulsion, we propose that salt valence should equal the carrabioses valence for a better electrostatic reduction of their net charge(s). However, the anionic group on the 3,6-anhydro-D-galactose is hindered by the anhydro cycle and this implies screening instead of binding ionic sites mechanism (Ciancia, Milas & Rinaudo, 1997). Therefore, monovalent cations (K^+) are less efficient to screen α-Car charge than divalent cation (Ca^{2+}). The screening of the mono anionic charge on the α-Carrabiose by Ca^{2+} may turn the carrabiose net charge, $[carrabiose]^-$, to partial positive charge, $[carrabiose, Ca^{2+}]^{+\delta}$ in accordance with obseravtion (Elmarhoum & Ako, 2022a). However, the anionic group carried by κ-carrabiose is better exposed to the binding with cations. When screening and/or binding sulfate ions promotes attractive interactions between the monomers, intramolecular interactions take place preferentially.

The decrease of α-Car samples viscosities under 15 s^{-1} demonstrates that cross-links and interhelical interaction dynamics respectively yield at applied steady shear stress and rate. Furthermore, the initial viscosity of the tested α-Car samples are 10 times greater than the viscosity of tested κ-Car samples with same polysaccharide concentration. Therefore, we think that the α-Car helices self assembles into open aggregates which are less dense than those formed in the κ-Car systems. As soon as the helices are nucleated, they form a bigger size of swollen aggregate domains, thus they become more unstable under shear.

The shear rate (s^{-1}) defines the gradient of velocity between the inner rotor (bob) and outer stator (cup) cylinder on gap of ΔR = 1 mm. It interferes with the physical links properties in the sample to determine the length scale δr, below which the sample remains thick and unperturbed like a solid by the shearing. Under shear, the sample is arranged in layers number N as ($\Delta R/\delta r$). The thickness δr increases to bulk with decreasing shear rate toward 0 s^{-1}, which corresponds to the sample at rest. It is worth noting that, there is no real information on viscosity below δr and that the samples viscosity should result from the interfaces (shear band) between the stratum (δr). The cross-linking dynamics within the unperturbed layer should lead to sizes of clusters no larger than δr. In this case, the time dependence of the sample's viscosity at constant shear rate characterizes the dynamic and strength of polysaccharide-polysaccharide interactions in balance with the interaction between polysaccharide and water in the shear bands. If the links strength and frequencies of the cross-linking and the helices stacking mechanisms are greater enough to overcome the shear stress and rate, the viscosity of the sample should increase with time to stability when one of both linking dynamic and strength fail against the shear rate or stress respectively. Actually, three distinct points result from the viscosity properties of κ-Car show in Fig.3b.

In point (1) the viscosity increases with time because of connection kinetics between the helices, indicating that the connections frequencies and strength are greater than the applied shear rate and stress respectively.

In point (2) the viscosity stability is threshold by the shear rate and the decrease of the viscosity with shear rate threshold indicates that the connections are characterized by multi-frequencies cross-linking dynamic. Hence, all polysaccharide-polysaccharide linking dynamics lower than the applied shear frequency no longer contribute to the gelation mechanisms driven by the coil-helix conformational transition.

In point (3) the viscosity amplitude decreases if the shear rate, alternatively shear stress, is increased. But, given that the samples were loaded in solution state prior to the links formation, there is no information about the links strength in relation to the shear rate or stress.

A strong liaison could be formed between the helices after several weeks and months in both under shear and 0 shear conditions. We observed that the viscosities of aged-at-rest samples decrease under increasing shear rate toward stability above the viscosity of fresh samples. This observation is shown in the insert of Fig.3a for the 60 days aged sample at 5 °C as shear rate dependence of the shear stress. Likewise, the links between helices are characterized by multilevel of strength, therefore the shear stress acts like a shear stress threshold applicable on mesoscopic scale (few number of stacking helices) (Meunier, Nicolai & Durand, 2000). For this purpose, we define the stress threshold viscosity (η^S) and frequency threshold viscosity (η^F) as the viscosity of aged-at-rest and fresh samples respectively when a steady shear stress σ or rate $\dot{\gamma}$ is applied on the samples. The links in the aged-at-rest samples that may resist the applied shear stress will remain in the unperturbed layer of thickness δr and the links that did not sustain the applied shear stress will be progressively broken or disentangled in the shear band to the yielded viscosity η^S. The stress threshold viscosity η^S and frequency threshold viscosity η^F from aged-at-rest and fresh samples respectively are used to characterize the gelation mechanisms driven by the helix-helix interactions. The thermodynamic properties of these interchain connections strength determine the melting temperature, viscoelasticity of the unperturbed layer's δr and viscosity properties of the aggregate suspensions and fluid gel.

3.3 Time dependence of viscosity of aggregating / gelling samples

Interchain interactions and further formation of connection between the polysaccharides may lead to diverse sample characteristics. The solutions, aggregates suspensions, partially cross linked aggregate suspensions (fluid gel), fine stranded or coarser gels are most of the sample's characteristics reported in the literature (Bui, Nguyen, Renou & Nicolai, 2019; Garrec, Guthrie & Norton, 2013; Hermansson, Eriksson & Jordansson, 1991; Rochas, Rinaudo & Vincendon, 1980). However, these characteristics are determined by the kinetic and thermodynamic properties of the polysaccharide conformational transition (coil-helix) and interchain connections (Brunchi, Morariu & Bercea, 2014; Croguennoc, Meunier, Durand & Nicolai, 2000). In the presence of KCl the kinetics of the coil-helix transition are very fast, and nuclei are formed immediately at T_{ch} such that their contribution individually in the sample's viscosity and kinetics are a complex issue. The viscosity kinetics are analyzed through consideration of helices aggregation and/or percolation. Meunier et al., (2000) have assumed two distinctly populations of the polysaccharides in solution for determining the aggregation / gelation kinetics using light scattering measurements; one in fully helical conformation and the other in coil conformation, though for the authors one may consider that real polysaccharide would be partially in helical or coil state (Meunier, Nicolai & Durand, 2000; Tanaka & Wada, 1973). But the later coil state leads to non-aggregating samples regarding the sample's viscosity properties and thus are stable. We have compared the η^F and η^S at shear rate 5, 15 and 30 s^{-1} of different polysaccharide concentrations in the presence of 5, 10 and 15 mM KCl for 5 °C and 20 °C corresponding to the storage temperature of the samples. The behaviors of the viscosity data are the same between the two-storage temperatures. Moreover, not only does 5 °C work for the three KCl salt concentrations, but also allows a better contrast between η^F and η^S. The results are shown in table 1 for the storage samples at 5 °C. For these measurements, it is worth recalling that fresh samples are

reset from the aged ones and the η^F measurements were done in the same run after the η^S measurements by introducing in the measurement programs a heating and cooling temperature sweep of the initially aged samples. By doing this, we maintain the same concentrations as well as the instrument setting.

Table 1: Viscosity at 5 ± 0.5 °C of κ-Car samples in various measurement conditions aged and fresh (reset) samples at 5, 15 and 30 s^{-1} for 0.2 g/L to 9 g/L in presence of 5, 10, 15 mM KCl. A difference of ± 0.1 mPa.s between the viscosity values indicates significant difference between them in each row and column. After 60 days at 5 °C the solutions of 5 and 9 g/L in the presence of 10 and 15 mM KCl have gelled.

		0.2 g/L	1 g/L	5.0 g/L	9.0 g/L	0.2 g/L	1.0 g/L	0.2 g/L	1.0 g/L
		5 mM KCl η (mPa.s)				**10 mM KCl** η (mPa.s)		**15 mM KCl** η (mPa.s)	
η^F, reset	5 s^{-1}	1.9	4.0	40.5	165.0	1.9	4.1	1.9	8.7
η^S, 60 days after		2.0	4.5	127.7	522.4	2.7	22.7	3.6	59.2
η^F, reset	15 s^{-1}	1.9	3.9	38.7	145.6	1.9	4.1	2.0	7.7
η^S, 60 days after		1.9	4.4	84.7	253.3	2.5	14.7	3.1	28.8
η^F, reset	30 s^{-1}	1.9	3.8	36.3	125.2	1.9	4.0	2.0	6.8
η^S, 60 days after		1.9	4.3	63.6	166.6	2.4	11.2	2.8	19.4

The samples of 0.2 g/L in the presence of 5 mM at 5 °C were already stable above the shear rate of 5 s^{-1} and interactions strength between the aggregates in suspension likely yield below a steady shear stress of 10 mPa. If the KCl concentration was increased to 10 mM, then to 15 mM, the increment of viscosity between the fresh samples and aged-at-rest samples characterizes the interaction relaxation frequencies (shear rate in Hz) and the upper yield stress or link strength between aggregates in the suspension. In the presence of 10 mM and 15 mM KCl, the interaction relaxation frequencies are likely a threshold < 5Hz for both salt concentration. However, when they are formed at rest (0 /s shear rate) in the presence of 10

mM and 15 mM KCl, some of the resulting links sustain applied steady shear stress of 73 mPa and 85 mPa respectively. If now the concentration is increased to 1 g/L in the presence of 5 mM KCl, interaction frequencies above 30 Hz appear. Some of the links which are formed at rest could resist shear stress above 129 mPa, however these links may not emerge under the shear rate below 30 s^{-1}. The more the polysaccharide concentration and KCl salt, the faster the formation of links. However, increasing KCl concentration to 15 mM did not accelerate the links formation between nuclei for 0.2 g/L. We think that diffusion coefficient likely limited the nuclei aggregation. Therefore, a critical concentration may exist between 0.2 g/L and 1 g/L at which, the nuclei cross linking dependence on diffusion phenomenon is negligible. This concentration could be close to the critical concentration Cc (g/L) of the polysaccharide in coil state and may characterize the concentration of true gel formation (Meunier, Nicolai & Durand, 2000). For instance, the samples of 0.5 g/L, κ-Car did not form a true gel (simply by tilting the container) after 60 days at 5 °C in the presence of KCl concentration ranging below 50 mM, while the samples of 1 g/L gelled in the presence of 25 mM, KCl but collapsed to liquid samples in the presence of 100 mM KCl.

The polysaccharide needs to overlap their potential of interactions in solutions at least partially prior to finding the salt conditions for the sample gelation. In this case, the polysaccharide conformational states are rather relevant than their random motions (Lin, Lindsay, Weitz, Ball, Klein & Meakin, 1989; Savel'ev, Marchesoni, Taloni & Nori, 2006; Tanaka & Wada, 1973). Hence, the viscosity kinetics would therefore result from conformational transition evolution from fine stranded filaments to stiff segments. However, interconnections speed and strength between the filaments and stacking domains of the stiff segments could be weakened by decreasing either the salt, polysaccharide concentration or both. For these types of systems, the concentration of solid matter is not sufficient to determine the thermodynamic chemical potential. Because of its critical role, we may

consider or define the conformational transition as a potential of activity of the polysaccharide in solution, which activity influence the viscosity. However, a number of questions emerge such as (i) what is the optimum degree of the helix conformation in a given aqueous phase which characterizes the thermodynamic stability of the polysaccharide system? and (ii) how does the polysaccharide concentration regimes (dilute, semidilute, entangled) influence the helix optimum degree? The optical rotation of κ-Car reaches with time different degrees in function of combined ionic strength and temperature effects. Its rate is also influenced and equilibrium was not clearly demonstrated by the system after 12 h in log time scale (Meunier, Nicolai & Durand, 2000). It is well known from earlier reports on salt effects that KCl particularly promotes stiffness and stacking domains for κ-Car in solution (Ako, 2015; Hermansson, 1989; Hermansson, Eriksson & Jordansson, 1991). But these local phase separation processes could be fatal to the sample water holding capacity and macroscopic stability. Understanding their kinetics will contribute to resolving the origin of the resulting instabilities.

3.4 Influence of concentration on sample's viscosity kinetic

For all the samples exhibiting a coil-helix transition, kinetics of the sample's viscosity was done at constant temperature below the coil-helix temperature (T_{ch}). The time delay to cool the solution from T_{ch} to the kinetics measurement temperatures depends on the cooling rate. For an example, the solutions in the presence of 10 mM KCl show a T_{ch} = 25 °C, hence it takes 10 min to cool the solutions from 25 °C to 5 °C with a cooling rate of 2 °C/min during which the nucleation and nuclei characteristics of the solutions change following the temperature variation. The kinetics started immediately, which means 5 s for the first viscosity value at the measurement temperature. Because instantaneously connected helices

occur in the sample as soon as the temperature has reached T_{ch}, the viscosity dependently may result from the presence of nuclei in the sample between 0 and 5 s (Fig.5). In terms of the sample viscosities, 0 and 5 s correspond to the viscosity of the samples without salt and the first viscosity value at the measurement temperature respectively. The cooling rate was 2 °C/min and shear rate by default was 15 s^{-1} for all samples tested unless otherwise specified.

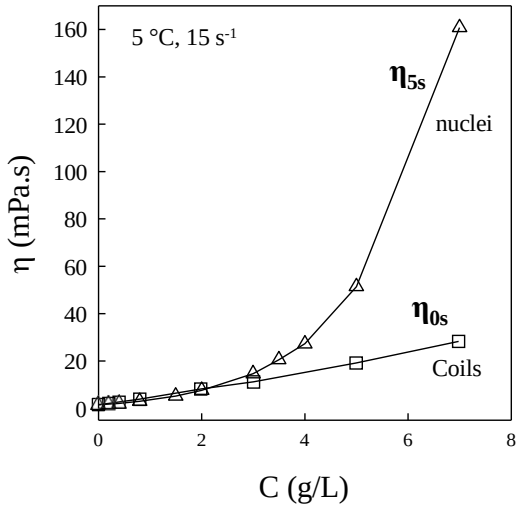


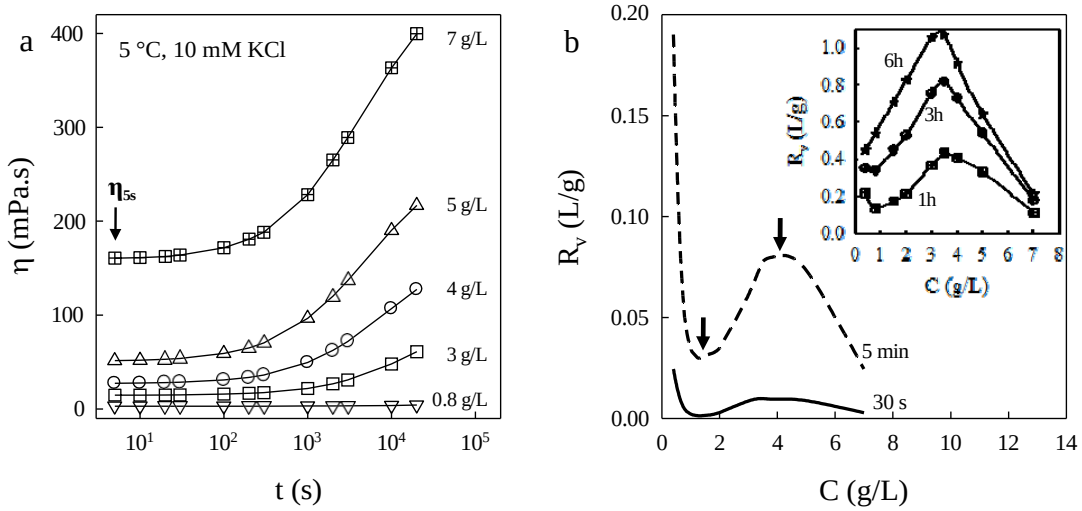
Figure 5: Concentration dependence of viscosity of κ-Car solution in the presence of 0 and 10 mM KCl salt at 5 °C under shear rate of 15 s^{-1}. The η_{5s} is the viscosity at 5 s after the temperature is set at 5 °C and η_{0s} is the viscosity without added salt. The solid lines are guidelines for eyes.

The helices start to aggregate as soon as they are nucleated and because of time imprecision on the nucleation kinetic we may consider the measurements beginning time step (δt = 5s), hence η_{5s} is considered as a threshold, and anything that may happen further in the sample is therefore due to only interactions and physical bounds formation between the nuclei prevailing in the samples within δt. Hence, the viscosity at any time t, η_t, minus η_{5s} is divided by η_{5s} and polysaccharide concentration to give the reduced relative increment viscosity, R_v

555 (L/g). The reduced viscosities R_v (L/g) are determined for all tested samples (Eq.5) as a
556 function of time and concentration. Taking variable time window from the viscosity kinetics
557 in Fig.6a, for examples 30 s, 5 min, 1 h, 3 h and 6h, the concentration dependence of R_v were
558 determined (Fig.6b) to analyze the effects of interactions through the polysaccharide
559 concentrations influence on the viscosity kinetics. Indirectly, the analysis may provide some
560 relevant conclusions on the conformational dynamics dependence on polysaccharide
561 concentration for a given condition of temperature, salt concentration and shear rate.

562 $$R_v = \frac{\eta_t - \eta_{5s}}{\eta_{5s} \cdot C} \qquad 5$$

563



564

565 *Figure 1: a) Kinetics of the viscosity under 15 s^{-1} for different κ-Car concentrations in*
566 *presence of 10 mM KCl. b) Concentration dependence of the relative increment viscosity per*
567 *unit of concentration (R_v) for different selected time steps, 30 s, 5 min and 1h, 3h, 6h (insert).*
568 *Solid lines are guidelines for eyes.*

569 The time dependence of viscosity of higher polysaccharide concentration evolves above lower
570 polysaccharide concentration samples, because the viscosity of the samples at the beginning

η_{5s} increases with increasing polysaccharide concentration. The logarithmic scale of time shows that the viscosity of the samples still increases and is not about to reach equilibrium. However, the reduced viscosities in Fig.6b show an interesting behavior with concentration, which is characterized by two characteristic concentrations in C-range between 0.2 g/L and 7 g/L. If we take the viscosity values of the samples in time step of 30 s, we observe that the relative viscosity starts to increase and to decrease from roughly the concentration of 1 ± 0.5 g/L and 3.5 ± 0.5 g/L respectively. Time steps above 30 s show the same extreme of concentration, but the increase and decrease of the reduced viscosity after 3 h are stiff within the C-range from 1 g/L to 3.5 g/L and from 3.5 g/L to 7 g/L with respectively a slop of 0.23 and 0.24 (insert of Fig.6b). Given that the viscosity increase with time is observed for all the samples, the decrease of reduced viscosity in C-range from 3.5 g/L to 7 g/L is not in agreement with both concentration and viscosity increase (Garrec, Guthrie & Norton, 2013). Therefore, although helix degree and number of interchain connections increase with time, this is likely not to happen in agreement with the increase of polysaccharide concentration above a characteristic maximum polysaccharide concentration (3.5 ± 0.5 g/L in this case). In any given aqueous phase and above the critical concentration from which the conformational transitions control the aggregation/gelation mechanisms rather than the polysaccharide diffusion coefficient, the increase of polysaccharide concentration beyond the characteristic maximum polysaccharide concentration will hinder or freeze the conformational kinetic to a certain degree. The effect may either delay the interactions and connection degrees in time, strengthen or result in both a delay and a strengthening. The delay in strength means the same kinetics, but with weak reduced viscosity. The delay in time means the same reduced viscosity will be found for higher concentration, but at a later time. Vertical and horizontal shift of the reduced viscosities dependence on time could help to at least conclude on whether lower and higher concentration follow the same kinetics and connection strength development

or not. To shift the reduced threshold viscosity, we simply divided the time dependence of the reduced threshold viscosities by their values at time step of 6h. The time axis is displayed in logarithmic scale of $t/\delta t$ with $\delta t = 5$ s. As we can see in Fig.7, all the samples of concentration above the characteristic minimum concentration of 1 ± 0.5 g/L, fall on the same master curve acceptably (see the guideline for eye in Fig.7). The result, we think is an indication that the samples of concentration above ≈ 1 g/L follow roughly same kinetics but with different links strength between the polysaccharide in either filaments, helices, or aggregate forms.

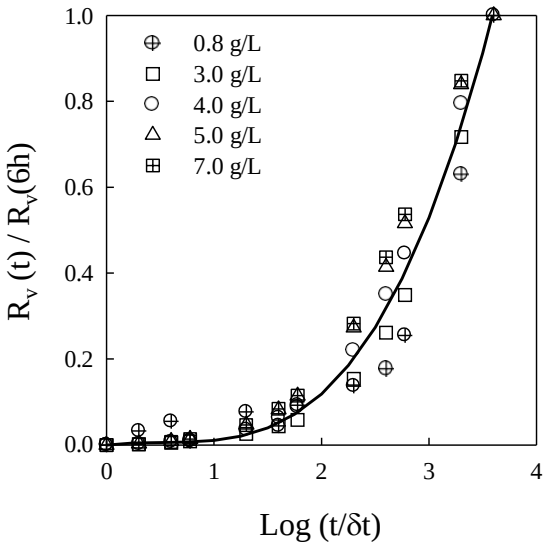


Figure 2: Master curve of the reduced viscosity kinetics for all the samples concentration displayed in Fig.6a. The $\delta t = 5$ s is the time for the measurement beginning and 6 h is the total time of the kinetics measurement. Solid line is a guideline for eyes.

The samples below 1 g/L, for instance 0.4 g/L and 0.2 g/L tested, are not on the master kinetics curve because their concentrations fall in dilute concentration regime, accordingly interchain liaisons are likely not dominant. Alternatively, the viscosity kinetic properties of these samples likely result from intrachain rather than interchain interactions and aggregations

612 are controlled by diffusion. However, coil-helix transition without aggregation (lower
613 concentration) seems to have lower impact on viscosity kinetics. Two months later, the
614 samples of concentration above 4 g/L in the presence of 10 mM had gelled at rest.
615 Considering this result with the fact that viscosity of the samples in C-range between ≈ 1 g/L
616 and 7 g/L (tested) follow roughly the same kinetics, we may hypothesize that after months
617 and years under 15 s^{-1} shear rate at 5 °C, the concentration dependence of reduced threshold
618 viscosity of the samples between ≈ 1 g/L and 3.5 g/L with ≈ 3.5 g/L and 7 g/L may finally fall
619 on the same negative slope. Hence, may be the samples ranging below 1 g/L could show an
620 increase of reduced threshold viscosity as a function of concentration. Accordingly, the
621 characteristic maximum concentration initially found at ≈ 3.5 g/L could after several hours,
622 days or months shift to ≈ 1 g/L and the characteristic minimum concentration initially at ≈ 1
623 g/L will be shifted to let's say the Cc (g/L) or may be lower after years. The study of the
624 polysaccharide in solution demonstrates that affinity between polysaccharide and water is
625 better when the polysaccharide is in coil than helix conformational state. Therefore, according
626 to our analysis, it results that the affinity of polysaccharide to water property is not a
627 colligative but a conformational issue. For κ-Car, the affinity would increase because helix
628 degree decreases with increasing the polysaccharide concentration in even potassium aqueous
629 phase.

630 # 4 Conclusion

631 Polysaccharide properties in aqueous phase strongly influence the process, texture, and
632 stability of polysaccharide-based mixtures. Given that conformational changes influence
633 directly the viscosity of many polysaccharides in aqueous mixtures, the degree of helicity that
634 controls kappa-carrageenan (κ-Car) interaction with water was derived from the viscosity
635 properties of κ-Car in aqueous phase. The coil and helix conformation of κ-Car correlates

with stable viscosity over a time scale of 60 days and with a continuous increase in the viscosity over undetermined time scale. So far, this evolution of the viscosity is attributed to the aggregation of the helices toward the system gelation considering that the helicity degree of the polysaccharide is stabilized. We conclude from this current study that the κ-Car helicity is stabilized by increasing the polysaccharide concentration at a degree more and more lower than the thermodynamic degree of the polysaccharide helicity, which is determined by the ionic strength, type of counter ions, and temperature. Therefore, the concentration regime of the suspensions is crucial for determining the κ-Car helicity degree from which the interactions with water and other type of components in the systems as well as aggregation and gelation occur. Comparing the properties of κ-Car with α-Car and other similar polysaccharides, we have emphasized the role of the chemical structure on their interactions with water, e.g., the anhydro function of the saccharide moieties with sulfate groups promotes better polysaccharide-water interactions than the saccharide moieties with only sulfate groups and much more better than neutral saccharide moieties.

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Declaration of competing interest

The authors declare no conflicts of interest.

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