

Thermoreversible gelation and viscoelasticity of κ -carrageenan hydrogels

Liu, Sijun; Huang, Sheng; Li, Lin

2016

Liu, S., Huang, S., & Li, L. (2016). Thermoreversible gelation and viscoelasticity of κ -carrageenan hydrogels. *Journal of Rheology*, 60(2), 203-214.

<https://hdl.handle.net/10356/85016>

<https://doi.org/10.1122/1.4938525>

© 2016 AIP Publishing. This paper was published in *Journal of Rheology* and is made available as an electronic reprint (preprint) with permission of AIP Publishing. The published version is available at: [<http://dx.doi.org/10.1122/1.4938525>]. One print or electronic copy may be made for personal use only. Systematic or multiple reproduction, distribution to multiple locations via electronic or other means, duplication of any material in this paper for a fee or for commercial purposes, or modification of the content of the paper is prohibited and is subject to penalties under law.

Downloaded on 13 Mar 2024 15:40:12 SGT

Thermoreversible gelation and viscoelasticity of κ -carrageenan hydrogels

Sijun Liu, Sheng Huang, and Lin Li^{a)}



School of Mechanical and Aerospace Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798, Singapore

(Received 25 July 2015; final revision received 5 December 2015; published 14 January 2016)

Abstract

The gelation behavior of κ -carrageenan in aqueous solution was studied by microdifferential scanning calorimetry and rheology. It was found that the formation and melting of κ -carrageenan hydrogels were thermally reversible and extremely sensitive to κ -carrageenan concentration. In comparison with the crossover of G' and G'' , the extrapolation method based on multiwave oscillation and Winter–Chambon criterion were able to give more accurate critical gel temperature T_c . At the gel point, the critical relaxation exponent n was almost constant whereas the critical gel strength S_g increased with κ -carrageenan concentration. In the stable gel state, the plateau modulus G_e depended on temperature according to a power-law scaling, $G_e \propto \varepsilon^{2.2}$, where ε is the relative distance ($=|T - T_c|/T_c$) and independent of κ -carrageenan concentration. The presence of potassium ions shifted the formation and melting temperatures of κ -carrageenan hydrogel to higher temperatures, and the temperatures for gel formation and melting increased with increasing potassium ions' content. © 2016 The Society of Rheology. [<http://dx.doi.org/10.1122/1.4938525>]

I. INTRODUCTION

Carrageenans belong to the family of hydrophilic linear sulphated galactans extracted from various species of marine red algae. κ -carrageenan, distinguished by its primary structure, is one type of carrageenans and is composed of alternating $\alpha(1-3)$ -D-galactose-4-sulfated and $\beta(1-4)$ -3,6-anhydro-D-galactose. It contains one sulphate group per disaccharide unit at carbon 2 of the 1,3 linked galactose unit. The ${}_4C^1$ conformation of 3,6-anhydro-D-galactose unit allows a helicoidal secondary structure, which is essential for the gel formation [1,2].

The gelation process of κ -carrageenan is complex even though the gelation of κ -carrageenan has been known for more than 30 years. There is a continuous revision and improvement in understanding of its gelation mechanism and gel properties based on new experimental results. It is generally accepted in the literature that the gelation of κ -carrageenan is achieved by two-steps. At high temperatures, κ -carrageenan molecules are present in water. Upon cooling, helices are formed, followed by the aggregation of these helices, which results in the gelation. Hermansson [3] studied the viscoelasticity of a 1 wt. % κ -carrageenan solution and found that there was an initial maximum in storage modulus G' upon cooling. With further decreasing temperature, the aggregation of helices led to the decrease in G' . However, Richardson and Goycoolea [4] did not observe this phenomenon when a perforated geometry was used to prevent the slippage of the sample. Cardoso and Sabadini [5] investigated the gelation of κ -carrageenan in light and heavy water and found that the formation and melting of κ -carrageenan

gels were thermoreversible, but the melting temperature in heavy water was about 3 °C higher than that in light water due to the high energy barrier of the helix-coil transition induced by the high cohesive energy of heavy water. Ueda *et al.* [6] found that the formation and dissociation of double helical aggregates resulted in a hysteresis between the cooling and heating curves. The similar experimental results have also been reported by Takemasa *et al.* [7], Tanaka [8], and Daniel-da-Silva *et al.* [9].

On the other hand, κ -carrageenan is a polyion and very sensitive to the presence of salts because the cations affect its coil-helix transition and aggregation behavior. Many studies have demonstrated that monovalent cations can bind to the helices, which will promote the aggregation of helices. For example, Michel *et al.* [10] confirmed the interaction between potassium ions and κ -carrageenan as potassium ions bind to the κ -carrageenan helices reducing considerable the charge density of helices. The studies by Watase and Nishinari [11] also showed potassium, rubidium, and cesium ions are more effective in inducing gelation than lithium and sodium ions because the shielding effect, inducing the decrease in the charge density of helices, increases with increasing the radius of alkali metal ions. Recently, MacArtain *et al.* [12] found that the addition of divalent calcium ions with low concentration can also promote the aggregation of helices where G' increases with increasing calcium ions' concentration. But, a high concentration of calcium ions may lead to the decrease of G' significantly. Morris *et al.* [13] thought that the decrease of G' in the presence of excessive cations should be attributed to the formation of an inhomogeneous network structure. During adding a mixture of potassium chloride and calcium chloride into an aqueous solution of κ -carrageenan, a synergistic effect was observed [14]. On the other hand, a number of studies indicated that the addition of inorganic particles also affect the gelation behavior of κ -carrageenan. For

^{a)}Author to whom correspondence should be addressed; electronic mail: mlli@ntu.edu.sg

example, Daniel-da-Silva *et al.* [15] found that the presence of Fe_3O_4 nanoparticles promoted the gelation of κ -carrageenan because the adsorption of potassium ions on the surface of Fe_3O_4 nanoparticles led to an increase in the potassium ion concentration within the “ κ -carrageenan cages.” All of these works indicated that there is a complicated process for the transition of coil to helix and the aggregation of helices. Therefore, understanding of κ -carrageenan's gelation behavior is important for further investigation of the effects of salts on the gelation behavior and viscoelastic properties of κ -carrageenan gels for various applications.

κ -carrageenan is one of the most important polysaccharides with a wide range of applications. For instance, κ -carrageenan has been used to improve the texture of cottage cheese and control the viscosity of dairy desserts, and as binders and stabilizers in the meat processing industry for the manufacturing of sausages and hamburgers [16–18]. κ -carrageenan is also used in various nonfood products such as cosmetics. κ -carrageenan can stabilize toothpaste preparations, and interacts with human carotene to give soft skin and silky hair in hand lotions and shampoos [19]. Recently, the importance of κ -carrageenan in pharmaceutical development has been shown. For example, κ -carrageenan is used to reduce the amount of polymorphic transformation in tabletting, or to fabricate controlled release delivery systems [20]. The wide application of κ -carrageenan owes to its physicochemical properties that depend on not only its chemical composition but also its microstructure formed in a hydrogel. However, only a limited number of studies have been carried out to understand the relationship of macroscopic properties of κ -carrageenan hydrogels with the microstructures so far [21].

On the other hand, a number of studies have been performed to characterize rheological properties of κ -carrageenan gels. At high temperatures, κ -carrageenan molecules are present in water as coils, and a κ -carrageenan aqueous solution shows a Newtonian fluid behavior. When the transition from coil to helix, followed by the aggregation of helix, is started upon cooling, the κ -carrageenan solution will become a gel where the value of G' is much larger than that of G'' , which is a very different feature from other hydrogels, such as the poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO₆₅-PPO₁₀₀-PEO₆₅) hydrogel with 20 wt. % concentration, which was formed through the packing of micelles at a heating rate of 1 °C/min, where G'' of the gel can be as high as the corresponding G' [22,23]. Many experimental results have also shown that various solvents and cations have important influences on viscoelastic properties of κ -carrageenan hydrogels. Ramakrishnan and Prud'homme [24] reported that the gel-sol transition of κ -carrageenan in a glycerol/water solution shows a single peak in the complex modulus at low glycerol concentrations, and two peaks when the glycerol concentration is higher than 40 wt. %. Chen *et al.* [25] found the purified κ -carrageenan formed a weaker gel and has a significantly narrower linear viscoelastic region than the κ -carrageenan gel containing potassium ions.

A κ -carrageenan solution changes its state from a liquid to a solid (=gel) in the vicinity of a critical value of a gelling parameter (e.g., temperature), which is referred to as the gel point. The occurrence of the gel point depends on the

characteristics of coils and the formation of helices and their aggregates upon cooling. Unlike chemical gels, the transition from a Newtonian fluid to a viscoelastic solid through the transition of coil to helix, followed by aggregation of helices, in a κ -carrageenan gelling system is thermoreversible. However, rheological studies of κ -carrageenan in aqueous solution did not explore the region of sol-gel transition so far.

In this paper, we report our systematical studies of the thermal and rheological properties of κ -carrageenan in aqueous solution by means of microdifferential scanning calorimetry (micro-DSC) and rheology. The thermoreversible gelation behavior of the κ -carrageenan hydrogels were clarified, and the rheological scaling laws were examined and discussed for understanding of the gelation and the relationship of the viscoelastic properties with the gel microstructures.

II. MATERIALS AND METHODS

A. Materials and solution preparation

κ -carrageenan in powder form was purchased from Sigma-Aldrich (Singapore). According to the supplier, the molecular weight of κ -carrageenan is about 3.0×10^5 g/mol. Before use, the κ -carrageenan powder was dried and kept in a desiccator to avoid the absorption of moisture at room temperature. Aqueous solutions of κ -carrageenan with various concentrations ranging from 2 to 4 wt. % were prepared by dissolving the powder in an appropriate volume of deionized water with continuous magnetic stirring at 80 °C for 2 h. The resulting aqueous solution was placed at room temperature and a transparent gel was obtained. The weight percentages are used to represent all the κ -carrageenan concentrations in this study for convenience. To investigate the effect of potassium ions on the formation and melting of κ -carrageenan hydrogels, 2 wt. % κ -carrageenan hydrogels with 0.1 and 0.3 mM potassium ions were prepared as well.

B. Microdifferential scanning calorimetry

A micro-DSC (Microcalorimeter, Microcal, Inc.) was used to determine the thermal properties of κ -carrageenan solutions through a thermal cycle of cooling to heating. The reference cell was filled with deionized water. The sample was preheated and injected into the sample cell with a volume 0.516 ml. The cooling and heating rate of 1 °C/min was employed. After each experiment was completed, the sample cell was cleaned by a continuous flow of hot deionized water. A water–water baseline micro-DSC test was followed to ensure no contamination of the sample cell by the last sample. The enthalpies of formation and melting of κ -carrageenan gels were calculated from the area of the sol–gel transition exothermic peak and that of the gel–sol transition endothermic peak, respectively.

C. Rheological measurement

The rheological measurement was performed on a rotational rheometer (DHR, TA Instruments, USA) with a parallel plate geometry of 40 mm in diameter and a gap of 0.55 mm. The sample for rheological measurement was transferred

directly from a glass bottle to the bottom plate of the rheometer using a spoon. After then, the sample was heated up to 70 °C and kept for 30 min at this temperature to destroy all possible helical structures. A thin film of low-viscosity silicone oil covering the sample perimeter was used to prevent solvent evaporation at long measurement times. Strain sweeps in the range of 0.1%–100% at frequencies of 0.1–2 Hz were carried out to determine the linear viscoelastic range of the solution in both sol and gel states. The stability of the sample was checked through the measurement of G' and G'' at 0.1 Hz in the linear viscoelastic region. Little changes in G' and G'' were found over a long period (120 min) at 70 °C with a strain amplitude of 1%, which indicated that the effect of water loss can be ignored. The following four rheological experiments were adopted for monitoring rheological evolution of κ -carrageenan solutions. (1) A temperature ramp with a cooling and heating rate of 1 °C/min at a fixed frequency of 1 Hz and strain of 1% was conducted over the temperature range of 70–20–70 °C, which covered both the sol–gel transition and the gel–sol transition. (2) The rheological temperature ramps with various cooling rates (0.5, 1, 1.5, and 2 °C/min) were performed from 70 to 20 °C at a fixed frequency of 1 Hz and strain of 1%. (3) The multiwave oscillatory temperature ramps with various cooling rates (0.5, 1, 1.5, and 2 °C/min) were carried out from 70 to 20 °C, where the basic frequency adopted was 1 rad/s and the harmonics ranged from 2 to 3. The ramp was conducted at a strain of 1% for the basic frequency (1 rad/s) and relative amplitude 50% for other frequencies with the final total peak strain of 1.75%, which was in the region of linear viscoelasticity. (4) A frequency sweep was performed at different temperatures in the angular frequency range of 0.1–100 rad/s and at a constant strain of 1%.

III. RESULTS AND DISCUSSION

A. Formation and melting of κ -carrageenan hydrogels

Figure 1 shows the micro-DSC curves of κ -carrageenan solutions with various concentrations. An exothermic peak was clearly observed during cooling, which is considered to be due to the sol–gel transition. With increasing κ -carrageenan concentration, the exothermic peak temperature (T_{fd}) and height increase. This is because the association of the neighboring κ -carrageenan chains becomes easier at high κ -carrageenan concentration, which leads to the increase in the number and/or size of the helical aggregates. In the subsequent micro-DSC heating curves, an endothermic peak is observed, which is due to the breaking up of aggregates, leading to the gel–sol transition. As observed, the endothermic peaks (T_{md}) shift to higher temperatures than the corresponding exothermic peaks under cooling, indicating the hysteresis of thermoreversibility. It is interesting to find that a small shoulder appears at the low temperature side of endothermic peak for the samples with low κ -carrageenan concentrations. The shoulder becomes narrower with increasing κ -carrageenan concentration and then almost disappears at the highest κ -carrageenan concentration (4.0 wt. %). Our observation is not consistent with what was observed by Iijima *et al.* [26]. Iijima *et al.* reported that as

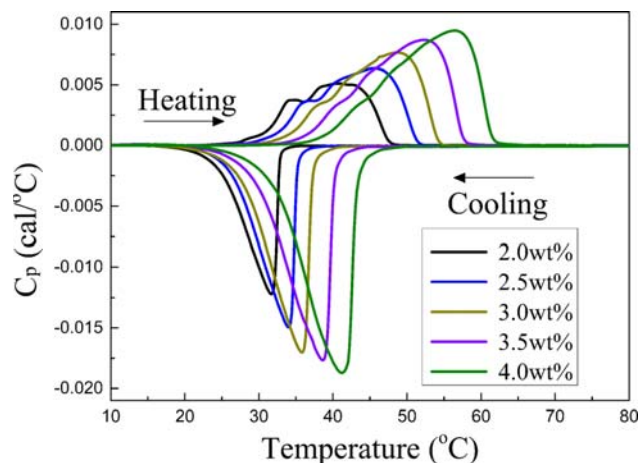


FIG. 1. Calorimetric thermograms for aqueous solutions of various κ -carrageenan concentrations in a cooling to heating process at a rate of 1 °C/min.

κ -carrageenan concentration exceeded 3.0 wt. %, the exothermic peak became broad and a shoulder appeared at the low temperature side of exothermic peak. A shoulder was also observed at the low temperature side of endothermic peak for 4.0 and 5.0 wt. % samples. Iijima *et al.* explained the appearance of a shoulder to be due to the formation of helices with various sizes and the presence of different kinds of defects in the junction zone. However, they did not provide any experimental evidence to support their viewpoint. In Fig. 1, we did not observe any subexothermic peaks during cooling, which would imply the formation of a homogeneous κ -carrageenan gel during cooling. That is to say, the mechanism for the appearance of a shoulder at the low temperature side of endothermic peak during heating is still unknown, which needs to be further studied in future.

Figure 2(a) shows the variation of gel formation and melting temperatures with κ -carrageenan concentration at a fixed scanning rate of 1 °C/min. It is apparent that T_{fd} and T_{md} shift to higher temperatures with increasing κ -carrageenan concentration. Meanwhile, a thermal hysteresis is clearly observed. Morris *et al.* [27] reported that the thermal hysteresis was a characteristic property of the double helices, which can not be observed for the single helices. If the temperature of thermal hysteresis is defined as $\Delta T = T_{md} - T_{fd}$, it can be found that ΔT increases with κ -carrageenan concentration. Here, it should be emphasized that the exothermic peak temperature decreases and the endothermic peak temperature increases with increasing scanning rate, which leads to that the thermal hysteresis temperature (ΔT) strongly depends on the scanning rate. Therefore, the discussion on the temperatures of gel formation and melting as well as ΔT is just based on the fixed scanning rate of 1 °C/min. The gel formation temperature (T_{fr}) and gel melting temperature (T_{mr}) obtained from the crossover of G' and G'' (Fig. 3) are higher than those obtained by micro-DSC due to the different definitions (the former was defined by the crossover of G' and G'' while the latter was defined by the exothermic and endothermic peaks), but they show the similar trends under the same scanning rate. Michel *et al.* [10] investigated the effect of cations

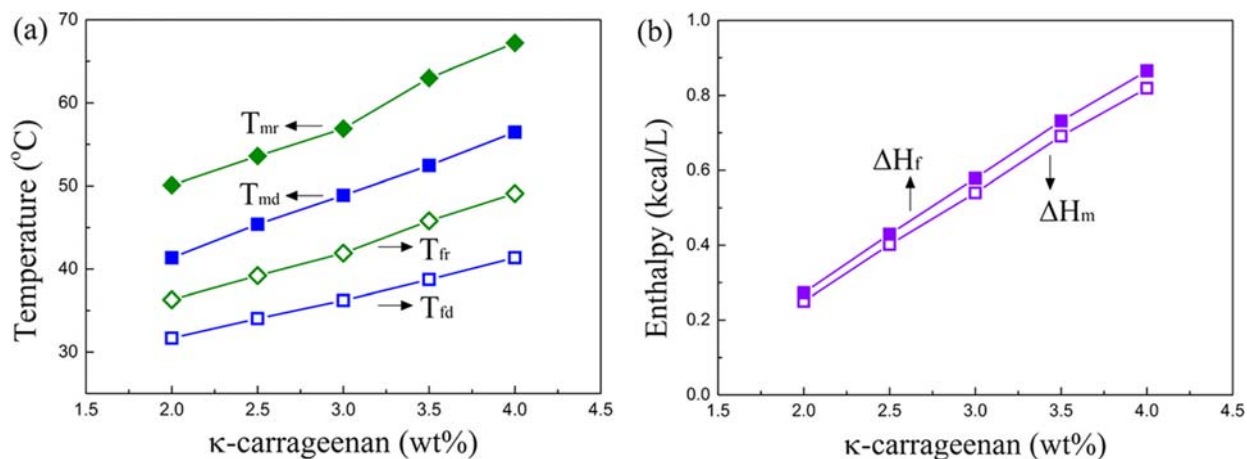


FIG. 2. (a) Dependence of gel formation and melting temperatures on κ -carrageenan concentration at a fixed scanning rate of $1^{\circ}\text{C}/\text{min}$; (b) enthalpies of the sol-gel transition and the gel-sol transition as a function of κ -carrageenan concentration.

on physicochemical properties of κ -carrageenan gel and found that the hysteresis increased with increasing potassium ion concentration due to the formation of more aggregates induced by cations. Wang *et al.* [28] performed ultrasound measurements and obtained the similar results. It is considered that the aggregates formed at low κ -carrageenan concentrations are small and less so that less energy is needed

for melting than the bigger and more aggregates formed at higher κ -carrageenan concentrations.

The dependences of the gel formation enthalpy (ΔH_f) and the gel melting enthalpy (ΔH_m) on κ -carrageenan concentration are shown in Fig. 2(b). The enthalpy was obtained by integrating a thermal capacity curve as a function of temperature. Therefore, the values of ΔH_f and ΔH_m greatly depend

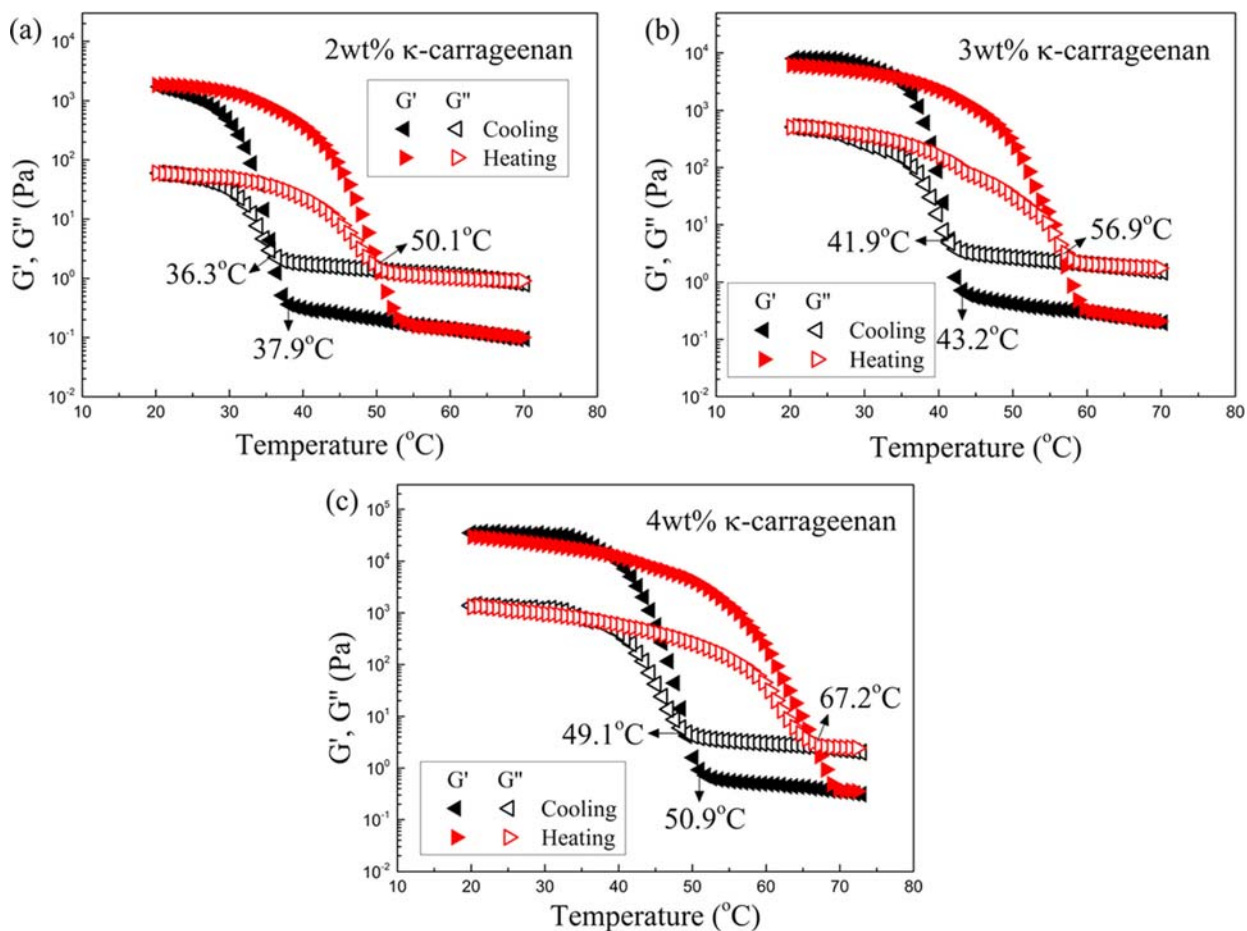


FIG. 3. Dependence of G' and G'' on temperature during rheological temperature ramp at a cooling and heating rate of $1^{\circ}\text{C}/\text{min}$ for (a) 2 wt. %, (b) 3 wt. %, and (c) 4 wt. % κ -carrageenan hydrogels. A frequency of 1 Hz and a strain of 1% were applied.

on the onset and offset temperatures of the endothermic and exothermic peaks. In order to minimize errors induced by the selection of a temperature window, we define the temperatures at which the thermal capacity increases or decreases by 0.1% in contrast with the baseline as the onset or offset temperatures. The increases in ΔH_f and ΔH_m with κ -carrageenan concentration are attributed mainly to the increasing aggregates. Meanwhile, ΔH_f is larger than ΔH_m , indicating that the formation of the aggregates released more energy than that required to melt the aggregates. This result sounds unreasonable because in a complete thermal cycle for a thermally reversible system, the total energy released during cooling should be the same as that absorbed during heating. The contradiction was due to the fact that the endothermic enthalpy [shown in Fig. 2(b)] did not include the energy used to heat the sample to the region of the gel–sol transition. From Fig. 1, it is noted that the gel–sol transitions or melting occurred at higher temperatures than the sol–gel transitions.

The transitions of sol–gel and gel–sol can also be detected by measuring the dynamic moduli during cyclic cooling and heating at a constant frequency. Temperature ramp was performed at the small strain amplitude of 1% to ensure that the gel structure was not significantly disturbed by the strain. Figure 3 shows the effect of temperature on the formation and melting of κ -carrageenan gels. Upon cooling, G' and G'' increase slightly with decreasing temperature due to the decrease in molecular chain mobility. When the temperature was decreased to a certain value (37.9, 43.2 and 50.9 °C for 2, 3, and 4 wt. % κ -carrageenan, respectively), G' and G'' increase sharply, and then G' exceeds G'' at 36.3, 41.9, and 49.1 °C for 2, 3, and 4 wt. % κ -carrageenan due, respectively, to the formation of a gel network. In the subsequent heating process, the aggregates of polymer chains dissociate and the network is broken up, as indicated by the decrease in G' and G'' . With further increasing temperature, the system becomes a liquid state ($G'' > G'$). If the temperature at the crossover of G' and G'' during cooling or heating is denoted as the gel formation temperature (T_{fr}) or the gel melting temperature (T_{mr}), respectively, both T_{fr} and T_{mr} increase as the κ -carrageenan concentration increases, indicating that more thermal energy is needed to disrupt the much denser or stronger gel structures formed at higher κ -carrageenan concentrations, which is in agreement with the micro-DSC results. It is observed that there is a pronounced hysteresis between the cooling and heating curves. The temperature of thermal hysteresis, ΔT ($= T_{mr} - T_{fr} = 67.2 - 49.1 > 56.9 - 41.9 > 50.1 - 36.3$), increases with increasing κ -carrageenan concentration.

We further examined the thermal reversibility of the sol–gel transition and gel–sol transition of κ -carrageenan by conducting three complete cycles at a fixed heating and cooling rate of 1 °C/min for the 2 wt. % κ -carrageenan solution. Each thermal cycle was programmed from 80 to 10 °C for cooling and 10 to 80 °C for heating. The same thermal cycle was repeated for the next two cycles. The three heating thermograms and the three cooling ones are plotted together in Fig. 4, and the cycle orders are indicated with the numerals. For ease of visualization, the thermogram curves for the second and third runs were vertically shifted by arbitrary amounts to prevent the curves from overlapping. It is apparent that all

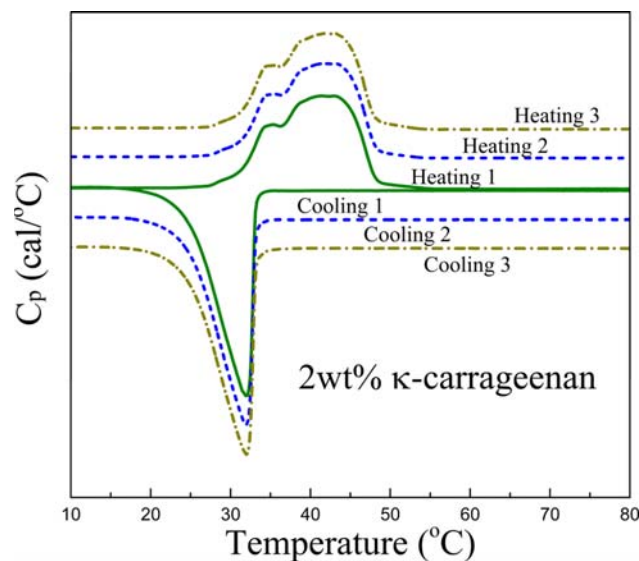


FIG. 4. Thermal capacity, C_p , as a function of temperature for the 2 wt. % κ -carrageenan hydrogel during three thermal cycles 10–80–10 °C at the heating and cooling rate of 1 °C/min.

three thermal cycles produced the same thermograms. If the calorimetric curves 2 and 3 in Fig. 4 are to be superposed on the curve 1, they overlap perfectly. In the cyclic rheological temperature ramp experiments, the same thermal reversibility is also shown for the corresponding κ -carrageenan solution (results not shown). The conclusion here is that the formation and melting of κ -carrageenan hydrogels are completely thermoreversible. In the studies of a κ/ι -hybrid carrageenan solution, Souza *et al.* [29] also found the thermal transitions are fully reversible in five successive heating and cooling cycles.

B. Determination of critical gel temperature

The critical temperature for gelation at a given κ -carrageenan concentration is important in practical applications, such as food and dairy industries, controlled drug delivery and pharmaceutical industries [30,31]. The gel formation during cooling can be readily observed by rheological temperature ramp, and then, the gel temperature can be determined by the temperature at the crossover of G' and G'' . However, the gel temperature determined by this procedure may vary with the adopted cooling rate or frequency as well as the kinetics of gel formation. A brief discussion on the determination of a critical gel point can be found from Lau *et al.* [32]. Here, we adopted three methods to determine the critical gel temperature even if each produces different results based on the definition of the critical gel. First of all, we adopted the extrapolation method based on power law analysis. An example is shown in Fig. 5(a) where the 2 wt. % κ -carrageenan solution was cooled at 1 °C/min. The complex viscosity, η^* , was measured as a function of temperature or cooling time. Here, η^* was plotted against cooling time t on a double logarithmic scale, from which the linearly increasing region of viscosity was identified as indicated by red rhombuses. The initial viscosity, which was independent of cooling time, was used as a baseline to determine the time of

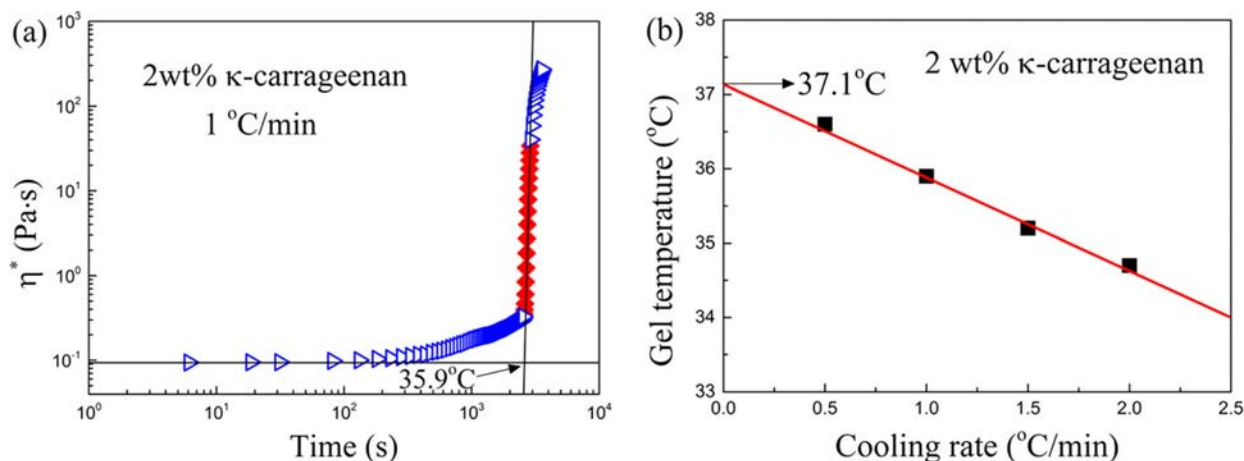


FIG. 5. (a) Power law analysis applied to complex viscosity of 2 wt. % κ -carrageenan at a cooling rate of 1 °C/min and a fixed frequency of 1 Hz. The red rhombuses indicate the region in which the data were used to fit the trendline, which was extrapolated backward and intercepted with the initial viscosity baseline. The temperature at this time is defined as the gel temperature at the cooling rate of 1 °C/min. (b) Relationship of the gel temperature obtained by power law analysis with cooling rate for the solution of 2 wt. % κ -carrageenan.

gelation from its intersection with the sharply increasing viscosity line (the red rhombuses). Finally, the time of gelation was converted to the corresponding temperature based on the experimental data of the temperature ramp. The power law analysis for finding the intersection is explained in the caption of Fig. 5. In this way, we obtained the gel temperature of 35.9 °C for the 2 wt. % κ -carrageenan solution at the cooling rate 1 °C/min. In comparison with the crossover of G' and G'' , the gel temperature obtained by power law analysis is much correct because the time of gelation is determined by the intersection of the sharply increasing viscosity and the initial viscosity. But, this temperature is not the equilibrium temperature because the slope of the sharply increasing viscosity depends on the cooling rate. In order to determine the equilibrium temperature, the temperature ramps with various cooling rates (0.5, 1.5, and 2 °C/min) were carried out, and then, the gel temperature obtained by the power law analysis was plotted against cooling rate as shown in Fig. 5(b). By extrapolating cooling rate to zero, we obtained the equilibrium temperature of 37.1 °C for the 2 wt. % κ -carrageenan solution.

Using the extrapolation method based on power law analysis, the equilibrium temperature was obtained at a fixed frequency of 1 Hz. However, a number of studies showed that the complex viscosity at a single frequency is not sufficient in most cases since the structural evolution usually shows a strong dependence on frequency [33]. Therefore, multiwave oscillation under a temperature ramp was performed to get the frequency dependence of modulus as well as the temperature dependence during the sol–gel transition. In the so-called multiwave oscillation, a nonsinusoidal periodic strain, which is a linear superposition of several sinusoidal waves with different frequencies and strain amplitudes, is applied to the sample. The total stress response can also be the superposition of the stress responses to the individual sinusoidal strains according to the Boltzmann superposition principles if the total strain is small enough and lies in the linear regime. In this work, the multiwave oscillatory temperature ramp at the cooling rate of 1 °C/min was carried out, and the variation of phase angle (δ) with temperature was obtained for the 2 wt. % κ -carrageenan solution as shown in Fig. 6(a). The value of $\tan \delta$ at high temperatures decreased slightly, indicating that the κ -carrageenan

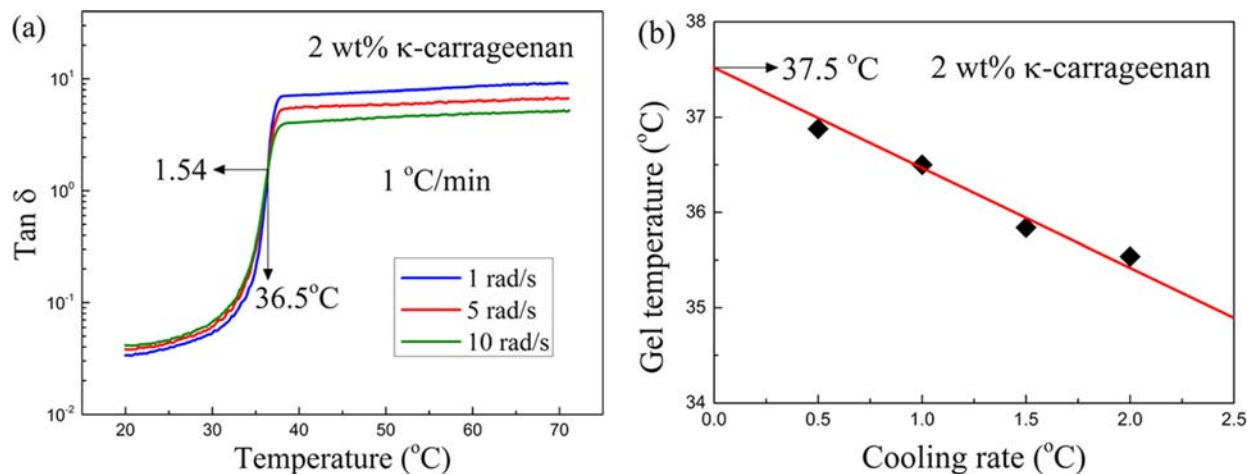


FIG. 6. (a) Temperature dependence of loss tangent, $\tan \delta$, during a multiwave oscillatory temperature ramp for the 2 wt. % κ -carrageenan solution at a cooling rate of 1 °C/min. (b) Relationship of the gel temperature obtained by multiwave oscillation with cooling rate for 2 wt. % κ -carrageenan solution.

solution was in a liquid state. As the temperature decreased further, $\tan \delta$ dramatically decreased and became independent of frequency at 36.5 °C, which was denoted as the gel temperature because a gelation variable loses its dependency on frequency and converges at the same point. However, the gel temperature obtained by independence of frequency shows a strong dependence of cooling rate because the multiwave oscillatory temperature ramp was carried out at a fixed cooling rate 1 °C/min. In order to determine the equilibrium gel temperature, the multiwave oscillatory temperature ramps with different cooling rates (0.5, 1.5, and 2 °C/min) were carried, and then, the gel temperature obtained by independence of frequency as a function of cooling rate was shown in Fig. 6(b). By extrapolating cooling rate to zero, the equilibrium gel temperature 37.5 °C was obtained for the solution of 2 wt. % κ -carrageenan. It is believed that 37.5 °C is very close to the critical gel temperature because the extrapolation method based on multiwave oscillation is independent of adopted cooling rate and frequency.

As is well-known, a physical gel is a three-dimensional network formed from flexible chains through physical cross-linking. The critical change from the liquidlike state to the solidlike gel is known as the sol–gel transition at which the zero shear viscosity and relaxation time of the system diverge. In the nonequilibrium state, the sol–gel transition temperature can be determined by a single frequency temperature ramp like the crossover of G' and G'' (Fig. 3) or power law analysis [Fig. 5(a)] as well as by a multiwave oscillatory temperature ramp like Fig. 6(a). However, many researchers are more interested in the thermodynamic properties of gels in the equilibrium state, and the equilibrium gel temperature can be obtained by the extrapolation method like Fig. 6(b), which is close to the critical gel temperature. As a matter of fact, the equilibrium gel temperature is also able to be obtained by a frequency sweep at various temperatures for a thermal sensitive gel. An example is the frequency dependency of G' and G'' at different temperatures for the 2 wt. % κ -carrageenan solution as shown in Fig. 7, which demonstrates how a κ -carrageenan solution changes from a liquid to a solidlike gel by passing through the sol–gel transition temperature. In Figs. 7(a) and 7(b), G' and G'' are presented

as a function of angular frequency ω . The measuring temperature was varied from 50 to 20 °C. At high temperatures such as 50 and 40 °C, the terminal behaviors of $G' \propto \omega^2$ and $G'' \propto \omega$ are observed at low frequencies, showing a Newtonian fluid behavior. With decreasing temperature, G' and G'' at low frequencies increase and deviate from the terminal behavior even if both G' and G'' still show the dependence on frequency. When the temperature decreases to 37 °C, a plateau is formed and the slope of G' is approaching to 0 at $\omega \rightarrow 0$. With further decreasing temperature, G' and G'' become independent of frequency over the whole frequency range, indicating the formation of a solidlike gel.

According to the Winter–Chambon criterion [34], the dynamic mechanical behavior at the critical gel point obeys a scaling relationship between dynamic shear moduli and frequency, $G'(\omega) \sim G''(\omega) \propto \omega^n$. This scaling law allows for facile identification of the gel point with a series of temperature or frequency sweeps. Based on Winter–Chambon criterion, the gel point can also be determined from a multifrequency plot of $\tan \delta$ vs a gelling variable such as time, temperature, or concentration, depending on which parameter governs the gelation process. The experimental results have shown that this method is reliable and valid for determination of the critical gel point for many gels such as PVC/DOP systems [35,36], where PVC and DOP stand for poly(vinyl chloride) and bis(2-ethylhexyl) phthalate, respectively. The Winter–Chambon criterion has been applied widely to determine the critical gel point for various gel mechanisms, such as the gel formation by self-assembly [37], electrostatic interaction [38], ion association [39], hydrogen bonding [40], packing of micelles [22], etc. From the frequency sweep for the 2 wt. % κ -carrageenan solution shown in Fig. 7, a liquid state was observed at temperatures above 38 °C and a solidlike behavior was observed at 37 °C and below. Thus, the sol–gel transition should take place in between 37 and 38 °C. Figure 8 shows a multifrequency plot of $\tan \delta$ vs temperature. All curves pass through the common point at 37.4 °C. The other κ -carrageenan solutions show the similar behavior (data not shown here), but the difference is that the gel temperature increases with increasing κ -carrageenan concentration.

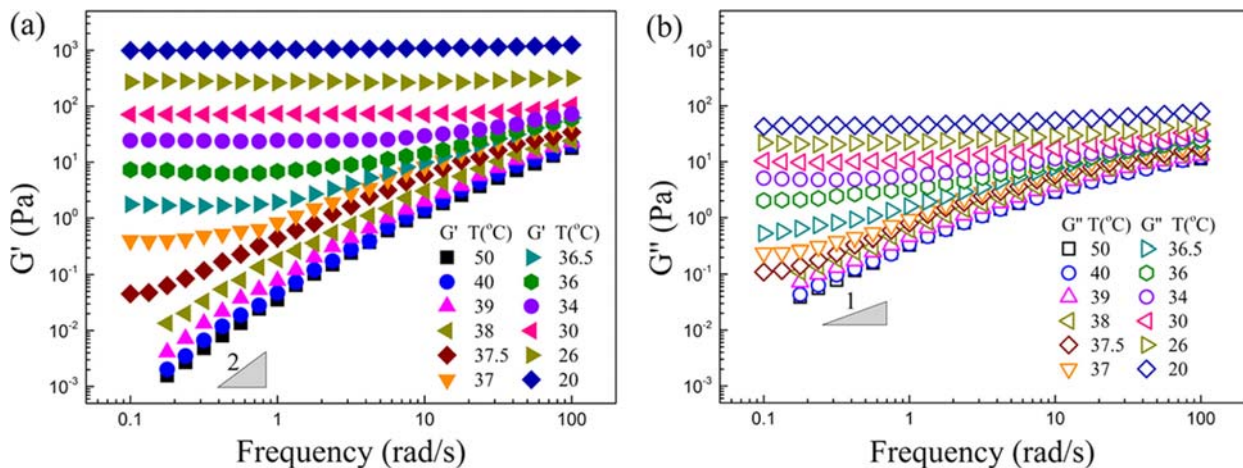


FIG. 7. G' and G'' as a function of angular frequency for the 2 wt. % κ -carrageenan solution at different temperatures.

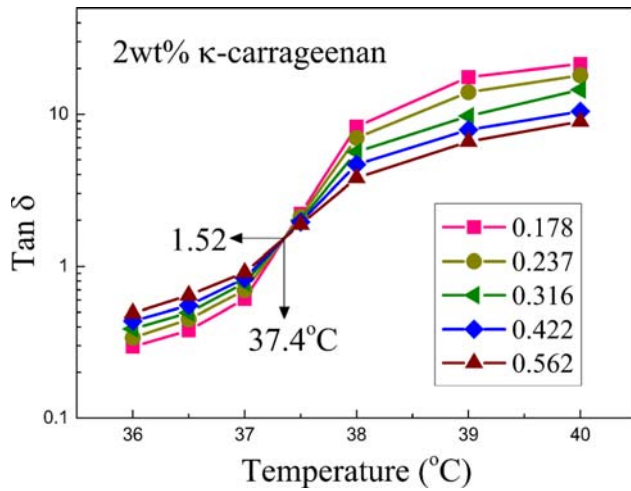


FIG. 8. Dependence of loss tangent, $\tan \delta$, on temperature at different angular frequencies in rad/s as indicated for the 2 wt. % κ -carrageenan solution.

Table I lists the gel temperatures with various κ -carrageenan concentrations obtained by crossover of G' and G'' , extrapolation methods and Winter–Chambon criterion, respectively. The gel temperature obtained by the crossover of G' and G'' is lower than that obtained by extrapolation methods and Winter–Chambon criterion. This may be because the crossover of G' and G'' obtained from the rheological temperature ramp is a nonequilibrium process, which was carried out at a given frequency (1 Hz) and a fixed cooling rate (1 °C/min). Because the linear increasing region of complex viscosity in power law analysis strongly depends on the adopted frequency, the gel temperature obtained by the extrapolation method based on multiwave oscillation is close to that obtained by the Winter–Chambon criterion. That is, to say, the gel temperatures obtained by the extrapolation method based on multiwave oscillation and the Winter–Chambon criterion are more close to the real critical gel temperature.

C. Scaling law

Gelation can be described using the scaling laws. Various scaling laws have been developed and used to explain or predict rheological features of gelling systems, especially for the sol–gel transition. The widely used scaling law for the rheological behavior of a gelling system in the vicinity of the gel point is [35,41, 42]

$$G''(\omega)/G'(\omega) = \tan \delta = \tan (n\pi/2), \quad (1)$$

which is based on the power law behavior of the shear relaxation modulus $G(t)$ at the gel point

$$G(t) = S_g t^{-n}, \quad (2)$$

where n is named as the critical relaxation exponent, which determines the stress relaxation rate at the gel point. S_g is the critical gel strength, which can be simply understood as the relaxation modulus at the gel point when the relaxation time $t = 1$ s. A similar expression can also be applied for G' and G'' at the critical gel point

$$G'(\omega) = G''(\omega) / \tan (n\pi/2) = S_g \omega^n \Gamma(1-n) \cos(n\pi/2). \quad (3)$$

Here, $\Gamma(1-n)$ is the Gamma function. By knowing n , one can calculate S_g from $G'(\omega)$ or $G''(\omega)$ at the gel point using Eq. (3).

A number of researches have shown that n is not universal and varies with the gelling systems. For example, the sol–gel transition induced by electrostatic interaction in the mixture consisting of oppositely charged polyelectrolyte (quaternized hydroxyethylcellulose ethoxylate, QHEC) and nanocrystalline cellulose (NCC), n changes from 0.785 to 0.773 at different concentrations of QHEC [38]. For the gelation of alginate which was induced by Cu^{2+} ions and *in situ* released Ca^{2+} ions, Lu *et al.* [43,44] found that n decreased with increasing alginate concentration in the former case, while n was independent of alginate concentration in the latter case. Many studies have also demonstrated that n is a constant [45–47]. Figure 9(a) shows the dependence of n on κ -carrageenan concentration. It is apparent that n is independent of κ -carrageenan concentration. The universal value of n means that the sol–gel transition takes place at the same viscoelastic distance from a completely viscous liquid ($n = 1$) or from a completely elastic solid ($n = 0$). From Table I, we know that the critical gel temperature increases with increasing κ -carrageenan concentration, which implies that there are more junctions formed at a higher sol–gel transition temperature to maintain the same viscoelasticity.

The physical nature of a gelling system at the gel point can also be described by the critical gel strength S_g defined by Eq. (3). The studies of chemical gels have shown that the decrease in n leads to the increase in S_g . S_g is related to the physical strength of a gel network at the gel point. Figure 9(b) shows the dependence of S_g on κ -carrageenan concentration. Although n is a constant, S_g increases with increasing κ -carrageenan concentration. This indicates that S_g is related

TABLE I. The gel temperatures obtained from the crossover of G' and G'' , extrapolation methods and Winter–Chambon criterion.

κ -concentration (wt. %)	Crossover of G' and G'' (°C)	Extrapolation from power law analysis (°C)	Extrapolation from multiwave oscillation (°C)	Winter–Chambon criterion (°C)
2.0	36.3	37.1	37.5	37.4
2.5	39.2	40.0	40.6	40.4
3.0	41.9	42.6	42.9	43.0
3.5	44.8	45.8	46.2	46.2
4.0	49.1	49.7	50.2	50.1

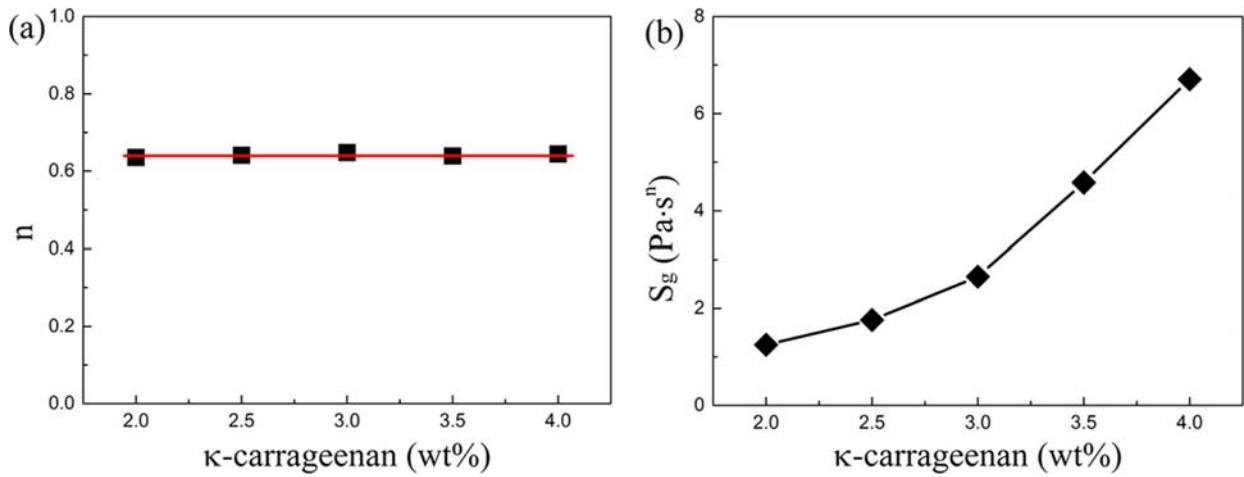


FIG. 9. Relationships of (a) the critical relaxation exponent n and (b) the critical gel strength S_g with κ -carrageenan concentration.

to the total mass of κ -carrageenan in the solution. The increase of S_g with κ -carrageenan concentration may be considered to be due to the increase in the aggregate density at the gel point. The similar experimental phenomenon has also been reported in the PVC/DOP gel system that S_g increases with increasing critical gel concentration [36].

From Fig. 7, we know that as the temperature decreases, G' gradually becomes independent of frequency, showing a plateau at each temperature. Let the plateau value of G' at low frequencies be denoted as the quasiequilibrium modulus G_e and then one scaling law has been established for G_e

$$G_e = k\varepsilon^z, \quad (4)$$

where k is the front constant, $\varepsilon = |p - p_g|/p_g$ is the relative distance of a gel variable (p) from the sol-gel transition point (p_g), and z is the scaling exponent [48]. If we defined the gel temperature obtained through the Winter-Chambon criterion as the critical gel temperature, it is easy to calculate the relative distance ε . The dependence of G_e on ε at various κ -carrageenan concentrations is shown in Fig. 10(a). It is observed that G_e increases with decreasing temperature at a constant κ -carrageenan concentration due to the formation of

much more aggregates. On the other hand, the relative distance at a fixed temperature increases with increasing κ -carrageenan due to the increase in the critical gel temperature, which leads to that G_e increases with increasing κ -carrageenan concentration at a certain temperature. To examine the validity of the scaling law for G_e in the stable gel state, a linear fitting to the data was performed, which gave the following relation:

$$G_e = k\varepsilon^{2.2}, \quad (5)$$

where k equals to 3.83, 4.04, 4.19, 4.38, and 4.60 for the solutions of 2, 2.5, 3, 3.5, and 4 wt. % κ -carrageenan, respectively. The correlation coefficients, which were used to evaluate the goodness of fitting, were higher than 0.98. The theories based on the different assumptions have predicted different values of z . For example, using the percolation model, de Gennes [49] predicted $z = 1.9$. Based on the Bethe lattice, the mean-field theory predicted $z = 3.0$ [50]. Different values of z also were experimentally obtained from various gel systems. For example, Li and Aoki [51] reported $z = 2.6$ for PVC gels induced by the formation of microcrystallites. Liu and Li [22] obtained $z = 3.0$ for PEO₆₅-PPO₁₀₀-

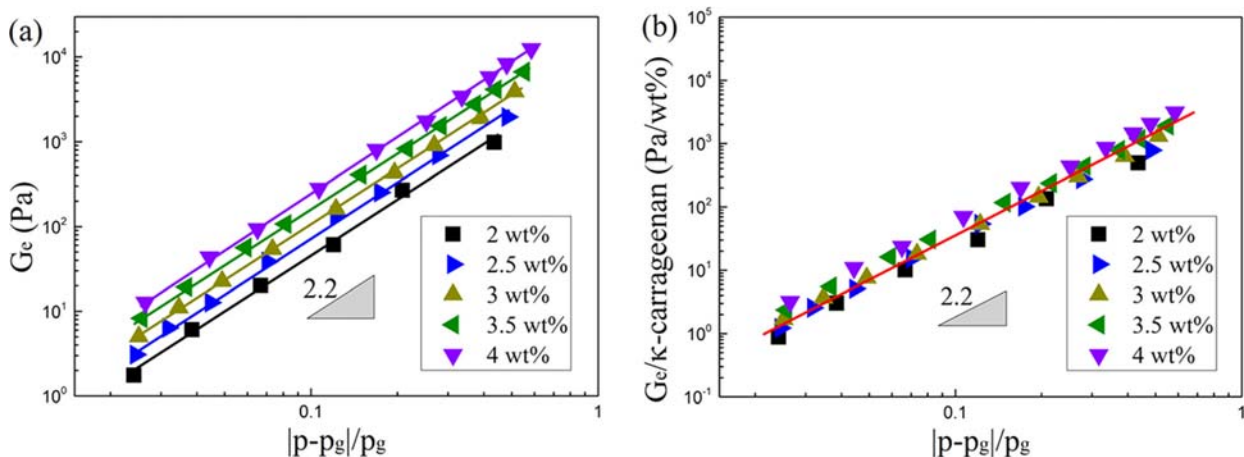


FIG. 10. Dependences of (a) the plateau modulus G_e and (b) the normalized plateau modulus G_e/κ -carrageenan concentration on the relative distance ε .

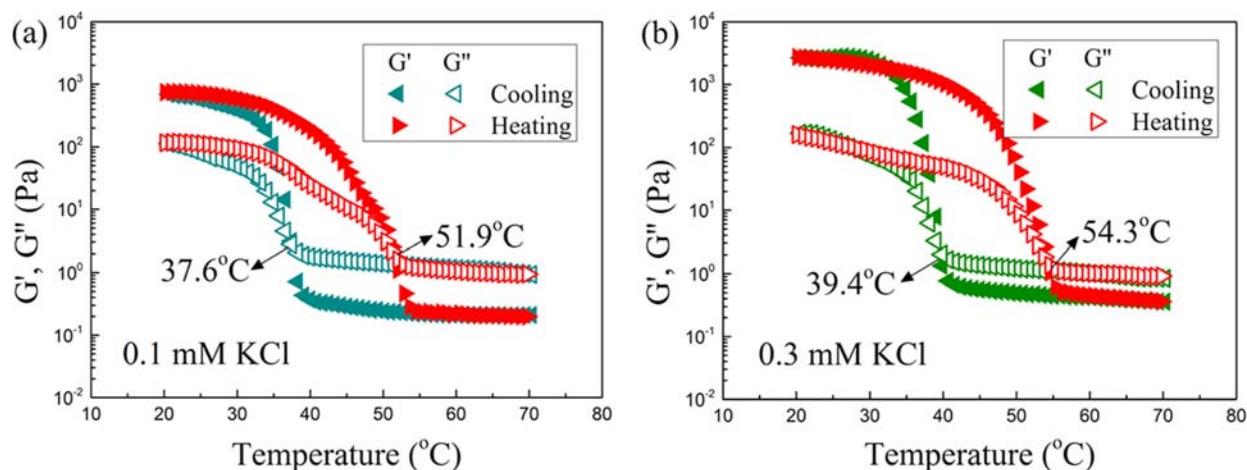


FIG. 11. Effects of KCl content (a) 0.1 mM and (b) 0.3 mM on the formation and melting of 2 wt. % κ -carrageenan.

PEO₆₅ hydrogels induced by the packing of micelles. For gels formed by hydrogen bonding, Kjoniksen and Nystrom [40] obtained $z = 4.7$ for poly(vinyl alcohol) (PVA) gel and Hsu and Jamieson [52] obtained $z = 1.9$ for gelatin gel. However, for the methylcellulose gels induced by hydrophobic association, Li *et al.* [53] found that a single scaling law could not be used to express the entire G_e . The variation of z at various gel systems suggests that z should be related to the different gelation mechanisms and structures. In this study, we obtained $z = 2.2$ for κ -carrageenan hydrogels, which is within the range of z values reported in the literature. On the other hand, when G_e was normalized by the corresponding κ -carrageenan concentration, it is surprising to find that the normalized plateau moduli almost overlap at different κ -carrageenan concentrations and increase with increasing the relative distance as shown in Fig. 10(b). This indicates that the gel strength in the stable gel state relates the relative κ -carrageenan mass. At a constant relative distance, the plateau modulus per unit of relative mass is almost constant even if the critical gel temperature increases with increasing κ -carrageenan concentration.

D. Effect of KCl on κ -carrageenan gel

Figure 11 demonstrates the effect of potassium ions on T_{fr} and T_{mr} of 2 wt. % κ -carrageenan. During adding 0.1 mM potassium ions into the solution of 2 wt. % κ -carrageenan as shown in Fig. 11(a), T_{fr} and T_{mr} increase from 36.3 and 50.1 °C to 37.6 and 51.9 °C, respectively, indicating that the presence of potassium ions promotes the aggregation of helices to lead to the sol-gel transition and gel-sol transition to occur at higher temperatures. When the content of potassium ions was increased to 0.3 mM as shown in Fig. 11(b), T_{fr} and T_{mr} increase to 39.4 and 54.3 °C, which further indicates that the temperatures of the sol-gel transition and the gel-sol transition increase with increasing potassium ions' content. Meanwhile, the temperature of thermal hysteresis, $\Delta T (= T_{mr} - T_{fr} = 54.3 - 39.4 > 51.9 - 37.6 > 50.1 - 36.3)$, increases with increasing potassium ions' content. That is, to say, the formation of more aggregates induced by the presence of potassium ions increases the thermal hysteresis, which is

consistent with the experimental results by Michel *et al.* [10] and Wang *et al.* [28].

On the other hand, it is easy to observe the effect of KCl on G' at 20 °C for 2 wt. % κ -carrageenan hydrogel from Figs. 3(a) and 11. It is obvious that G' decreases from 1875 Pa in the absence of KCl to 867 Pa in the presence of 0.1 mM KCl. When KCl concentration was increased to 0.3 mM, G' increases to 2519 Pa, which shows a peculiar dependence on KCl concentration. Compared to the experimental results of Morris *et al.* [13] and Nguyen *et al.* [14], at which the former found that a maximum modulus value appears at KCl concentrations of ~ 0.1 – 0.15 M and the latter found that G' increases with increasing KCl concentration, we can conclude here that the effect of KCl on the κ -carrageenan gel strength is complex, which may depend on the sulfate content and molecular weight of κ -carrageenan. Furthermore, for 2 wt. % κ -carrageenan hydrogel in the presence of 0.3 mM KCl, it is surprising that a maximum value was observed during cooling. The same rheological temperature ramp was performed using a hatched plate instead of a parallel plate and the similar experimental result was observed, which indicates that the decrease in G' was not induced by the slippage of sample like what was reported by Richardson and Goycoolea [4].

IV. CONCLUSIONS

In this paper, we studied the thermal and rheological properties of κ -carrageenan in aqueous solution, focusing on the sol-gel transition and scaling laws. The experimental results indicated that the formation and melting of κ -carrageenan hydrogels were completely thermoreversible, but the gel formation temperature T_f and the gel melting temperature T_m increased with increasing κ -carrageenan concentration. Meanwhile, increasing κ -carrageenan concentration also led to the increase in the thermal hysteresis temperature $\Delta T = T_m - T_f$. The gel temperatures were obtained by the crossover of G' and G'' , extrapolation methods of power law analysis and multiwave oscillatory temperature ramp, and Winter-Chambon criterion, respectively. The gel

temperatures obtained using the Winter–Chambon criterion approached to the equilibrium gel temperature obtained using the extrapolation method of multiwave oscillatory temperature ramp and defined as the critical gel temperature and used for the scaling analysis. At the gel point, the critical relaxation exponent n kept constant whereas the critical gel strength S_g increased with increasing κ -carrageenan concentration, indicating that the sol–gel transition took place at the same viscoelastic distance from a completely viscous liquid or from a completely elastic solid even though S_g increased with κ -carrageenan concentration. In the stable gel state, the plateau modulus G_e obeyed a scaling law, $G_e = k\varepsilon^{2.2}$, which is close to the prediction by the entanglement theory. The presence of potassium ions shifted the formation and melting temperatures of the κ -carrageenan hydrogel to higher temperatures, and the temperatures of formation and melting increased with increasing potassium ions' content.

ACKNOWLEDGMENT

This work was supported by the Academic Research Fund Tier 1 (RG100/13) from the Ministry of Education, Singapore.

References

- [1] Chronakis, I. S., L. Piculell, and J. Borgstrom, "Rheology of kappa-carrageenan in mixtures of sodium and cesium iodide: Two types of gels," *Carbohydr. Polym.* **31**, 215–225 (1996).
- [2] Watase, M., and K. Nishinari, "Rheology, DSC and volume or weight change induced by immersion in solvents for agarose and kappa-carrageenan gels," *Polym. J.* **18**, 1017–1025 (1986).
- [3] Hermansson, A. M., "Rheological and microstructural evidence for transient states during gelation of kappa-carrageenan in the presence of potassium," *Carbohydr. Polym.* **10**, 163–181 (1989).
- [4] Richardson, R. K., and F. M. Goycoolea, "Rheological measurement of κ -carrageenan during gelation," *Carbohydr. Polym.* **24**, 223–225 (1994).
- [5] Cardoso, M. V. C., and E. Sabadini, "The gelling of κ -carrageenan in light and heavy water," *Carbohydr. Res.* **345**, 2368–2373 (2010).
- [6] Ueda, K., M. Itoh, Y. Matsuzaki, H. Ochiai, and A. Imamura, "Observation of the molecular weight change during the helix-coil transition of κ -carrageenan measured by the SEC-LALLS method," *Macromolecules* **31**, 675–680 (1998).
- [7] Takemasa, M., A. Chiba, and M. Date, "Gelation mechanism of κ - and γ -carrageenan investigated by correlation between the strain-optical coefficient and the dynamic shear modulus," *Macromolecules* **34**, 7427–7434 (2001).
- [8] Tanaka F., "Thermoreversible gelation driven by coil-to-helix transition of polymers," *Macromolecules* **36**, 5392–5405 (2003).
- [9] Daniel-da-Silva, A. L., F. Pinto, J. A. Lopes-da-Silva, T. Trindade, B. J. Goodfellow, and A. M. Gil, "Rheological behavior of thermoreversible κ -carrageenan/nanosilica gels," *J. Colloid Interface Sci.* **320**, 575–581 (2008).
- [10] Michel, A. S., M. M. Mestdag, and M. A. V. Axelos, "Physico-chemical properties of carrageenan gels in presence of various cations," *Int. J. Biol. Macromol.* **21**, 195–200 (1997).
- [11] Watase, M., and K. Nishinari, "Effect of alkali metal ions on the viscoelasticity of concentrated kappa-carrageenan and agarose gels," *Rheol. Acta* **21**, 318–324 (1982).
- [12] MacArtain, P., J. C. Jacquier, and K. A. Dawson, "Physical characteristics of calcium induced κ -carrageenan networks," *Carbohydr. Polym.* **53**, 395–400 (2003).
- [13] Morris, V. J., A. Tsiami, and G. J. Brownsey, "Work-hardening effects in gellan gum gels," *J. Carbohydr. Chem.* **14**, 667–675 (1995).
- [14] Nguyen, B. T., T. Nicolai, L. Benyahia, and C. Chassenieux, "Synergistic effects of mixed salt on the gelation of κ -carrageenan," *Carbohydr. Polym.* **112**, 10–15 (2014).
- [15] Daniel-da-Silva, A. L., R. Loio, J. A. Lopes-da-Silva, T. Trindade, B. J. Goodfellow, and A. M. Gil, "Effects of magnetite nanoparticles on the thermorheological properties of carrageenan hydrogels," *J. Colloid Interface Sci.* **324**, 205–211 (2008).
- [16] Millan, A. J., M. I. Nieto, C. Baudin, and R. Moreno, "Thermogelling polysaccharides for aqueous gelcasting. II. Influence of gelling additives on rheological properties and gelcasting of alumina," *J. Eur. Ceram. Soc.* **22**, 2217–2222 (2002).
- [17] Modi, V. K., K. P. Yashoda, and S. K. Naveen, "Effect of carrageenan and oat flour on quality characteristics of meat kofta," *Int. J. Food Prop.* **12**, 228–242 (2009).
- [18] Sanchez-Herencia, A. J., A. J. Millan, M. I. Nieto, and R. Moreno, "Gel-forming of nickel powders from aqueous slurries," *Adv. Mater.* **12**, 1192–1195 (2000).
- [19] Valenta, C., and K. Schultz, "Influence of carrageenan on the rheology and skin permeation of microemulsion formulations," *J. Controlled Release* **95**, 257–265 (2004).
- [20] Aliste, A. J., and N. L. Del Mastro, "Anomalous rheological behaviour of gelatin-carrageenan-water system induced by ionizing radiation," *Mol. Cryst. Liq. Cryst.* **448**, 781–787 (2006).
- [21] Funami T., "Atomic force microscopy imaging of food polysaccharides in relation to rheological properties," *Food Sci. Technol. Res.* **16**, 13–22 (2010).
- [22] Liu, S., and L. Li, "Multiple phase transition and scaling law for poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) triblock copolymer in aqueous solution," *ACS Appl. Mater. Interfaces* **7**, 2688–2697 (2015).
- [23] Liu, S., H. Q. Bao, and L. Li, "Role of PPO-PEO-PPO triblock copolymers in phase transitions of a PEO-PPO-PEO triblock copolymer in aqueous solution," *Eur. Polym. J.* **71**, 423–439 (2015).
- [24] Ramakrishnan, S., and R. K. Prud'homme, "Effect of solvent quality and ions on the rheology and gelation of κ -carrageenan," *J. Rheol.* **44**, 885–896 (2000).
- [25] Chen, Y., M. L. Liao, and D. E. Dunstan, "The rheology of K^+ - κ -carrageenan as a weak gel," *Carbohydr. Polym.* **50**, 109–116 (2002).
- [26] Iijima, M., T. Hatakeyama, M. Takahashi, and H. Hatakeyama, "Effect of thermal history on kappa-carrageenan hydrogelation by differential scanning calorimetry," *Thermochim. Acta* **452**, 53–58 (2007).
- [27] Morris, E. R., D. A. Rees, I. T. Norton, and D. M. Goodall, "Calorimetric and chiroptical evidence of aggregate-driven helix formation in carrageenan systems," *Carbohydr. Res.* **80**, 317–323 (1980).
- [28] Wang, Q., B. Rademacher, F. Sedlmeyer, and U. Kulozik, "Gelation behaviour of aqueous solutions of different types of carrageenan investigated by low-intensity-ultrasound measurements and comparison to rheological measurements," *Innovative Food Sci. Emerging Technol.* **6**, 465–472 (2005).
- [29] Souza, H. K. S., L. Hilliou, M. Bastos, and M. P. Goncalves, "Effect of molecular weight and chemical structure on thermal and rheological properties of gelling κ /I-hybrid carrageenan solutions," *Carbohydr. Polym.* **85**, 429–438 (2011).
- [30] Li, L., R. Ni, Y. Shao, and S. Mao, "Carrageenan and its applications in drug delivery," *Carbohydr. Polym.* **103**, 1–11 (2014).

- [31] Prajapati, V. D., P. M. Maheriya, G. K. Jani, and H. K. Solanki, "Carrageenan: A natural seaweed polysaccharide and its applications," *Carbohydr. Polym.* **105**, 97–112 (2014).
- [32] Lau, B. K., Q. Q. Wang, W. Sun, and L. Li, "Micellization to gelation of a triblock copolymer in water: Thermoreversibility and scaling," *J. Polym. Sci., Part B: Polym. Phys.* **42**, 2014–2025 (2004).
- [33] Liu, S., C. X. Zhou, and W. Yu, "Phase separation and structure control in ultra-high molecular weight polyethylene microporous membrane," *J. Membr. Sci.* **379**, 268–278 (2011).
- [34] Winter, H. H., and F. Chambon, "Analysis of linear viscoelasticity of a crosslinking polymer at the gel point," *J. Rheol.* **30**, 367–382 (1986).
- [35] te Nijenhuis, K., and H. H. Winter, "Mechanical properties at the gel point of a crystallizing poly(vinyl chloride) solution," *Macromolecules* **22**, 411–414 (1989).
- [36] Li, L., and Y. Aoki, "Rheological images of poly(vinyl chloride) gels. 1. The dependence of sol-gel transition on concentration," *Macromolecules* **30**, 7835–7841 (1997).
- [37] Liu, S., W. Yu, and C. X. Zhou, "Solvents effects in the formation and viscoelasticity of DBS organogels," *Soft Matter* **9**, 864–874 (2013).
- [38] Lu, A., Y. X. Wang, and Y. Boluk, "Investigation of the scaling law on gelation of oppositely charged nanocrystalline cellulose and polyelectrolyte," *Carbohydr. Polym.* **105**, 214–221 (2014).
- [39] Liu, X. X., L. Y. Qian, T. Shu, and Z. Tong, "Rheology characterization of sol-gel transition in aqueous alginate solutions induced by calcium cations through in situ release," *Polymer* **44**, 407–412 (2003).
- [40] Kjoniksen, A. L., and B. Nystrom, "Effects of polymer concentration and cross-linking density on rheology of chemically cross-linked poly(vinyl alcohol) near the gelation threshold," *Macromolecules* **29**, 5215–5222 (1996).
- [41] Martin, J. E., and D. Adolf, "The sol-gel transition in chemical gels," *Annu. Rev. Phys. Chem.* **42**, 311–339 (1991).
- [42] Martin, J. E., D. Adolf, and J. P. Wilcoxon, "Viscoelasticity of near-critical gels," *Phys. Rev. Lett.* **61**, 2620–2623 (1988).
- [43] Lu, L., X. X. Liu, and Z. Tong, "Critical exponents for sol-gel transition in aqueous alginate solutions induced by cupric cations," *Carbohydr. Polym.* **65**, 544–551 (2006).
- [44] Lu, L., X. X. Liu, Z. Tong, and Q. X. Gao, "Critical exponents and self-similarity for sol-gel transition in aqueous alginate systems induced by in situ release of calcium cations," *J. Phys. Chem. B* **110**, 25013–25020 (2006).
- [45] Li, L., H. Uchida, Y. Aoki, and M. L. Yao, "Rheological images of poly(vinyl chloride) gels. 2. Divergence of viscosity and the Scaling law before the sol-gel transition," *Macromolecules* **30**, 7842–7848 (1997).
- [46] Aoki, Y., L. Li, and M. Kakiuchi, "Rheological images of poly(vinyl chloride) Gels. 6. Effect of temperature," *Macromolecules* **31**, 8117–8123 (1998).
- [47] Aoki, Y., L. Li, H. Uchida, M. Kakiuchi, and H. Watanabe, "Rheological images of poly(vinyl chloride) gels. 5. Effect of molecular weight distribution," *Macromolecules* **31**, 7472–7478 (1998).
- [48] Mours, M., and H. H. Winter, "Relaxation patterns of nearly critical gels," *Macromolecules* **29**, 7221–7229 (1996).
- [49] de Gennes, P. G., *Scaling Concepts in Polymer Physics* (Cornell University, New York, 1979).
- [50] Flory, P. J., "Molecular size distribution in three dimensional polymers. I. Gelation," *J. Am. Chem. Soc.* **63**, 3083–3090 (1941).
- [51] Li, L., and Y. Aoki, "Rheological images of poly(vinyl chloride) gels. 3. Elasticity evolution and the scaling law beyond the sol-gel transition," *Macromolecules* **31**, 740–745 (1998).
- [52] Hsu, S. h., and A. M. Jamieson, "Viscoelastic behaviour at the thermal sol-gel transition of gelatin," *Polymer* **34**, 2602–2608 (1993).
- [53] Li, L., P. M. Thangamathesvaran, C. Y. Yue, K. C. Tam, X. Hu, and Y. C. Lam, "Gel network structure of methylcellulose in water," *Langmuir* **17**, 8062–8068 (2001).