

High-Concentration Hydrogels of κ -Carrageenan

Prepared Using Subcritical Water

Jun-ichi Horinaka^{a,*} and Koshiro Hara^a

^a Department of Material Chemistry, Graduate School of Engineering, Kyoto University,

Nishikyo, Kyoto 615-8510, Japan

* Corresponding author.

E-mail: horinaka.junichi.5c@kyoto-u.ac.jp

Tel: +81-75-383-2454

Fax: +81-75-383-2458

ABSTRACT

κ -Carrageenan hydrogels have been prepared at very high concentrations beyond the previous limit of conventional κ -carrageenan hydrogels. By dissolving κ -carrageenan using subcritical water at 150 °C, homogeneous translucent hydrogels have been obtained from 15 wt% to 40 wt%. The high-concentration hydrogels have exceptionally high Young's modulus (E_0) ranging 10^6 – 10^7 Pa and exhibit an unknown concentration (c)-dependence of $E_0 \propto c^{1.0-1.1}$. The distinctive mechanical properties of the high-concentration hydrogels are also demonstrated by modifying the stress (σ)-strain (ε) curves. Curves of σ / E_0 versus ε for the high-concentration hydrogels seem to fall on a single curve that is remarkably different from that for the conventional 3 wt% hydrogel. The gel-to-sol transition temperature (T_m) proves high thermal resistance of the high-concentration hydrogels; T_m increases with c and exceeds 100 °C at 30 wt% and 40 wt%. It has been confirmed that separated pieces of high-concentration hydrogels are unified into a single disk above T_m . The endothermic enthalpy of the gel-to-sol transition per unit weight of κ -carrageenan is almost constant regardless of c among the high-concentration hydrogels, which is consistent with the claim that the high-concentration hydrogels have similar distinctive network structure.

Keywords

κ -carrageenan hydrogel, high concentration, subcritical water, mechanical strength, thermal resistance

1. Introduction

κ -Carrageenan is a species of anionic polysaccharides extracted from red algae. It is well known that κ -carrageenan is soluble in hot water to some extent and the aqueous solutions form physical gels in the vicinity of room temperature during subsequent cooling (Morris et al., 1980; Viebke et al., 1994; Ikeda et al, 2001). κ -Carrageenan hydrogels have so far been applied to a variety of commercial products making good use of their eco- and bio-compatibility as natural materials (Qureshi et al., 2019; Valenta & Schultz, 2004; Yang et al., 2020). Accordingly, modification of the mechanical properties of κ -carrageenan hydrogels has been extensively investigated to deliver the textures required in the practical applications (Watase & Nishinari, 1986; Rochas et al., 1990; Sason & Nussinovitch, 2018; Roque et al., 2022). Even now, the establishment of new methods to expand the mechanical properties has been eagerly sought after in order to utilize the advantages of κ -carrageenan hydrogels as green soft materials more widely.

The primary method for altering the mechanical properties of κ -carrageenan hydrogels is to change the concentration of κ -carrageenan (Watase & Nishinari, 1982; Watase & Nishinari, 1985; Watase & Nishinari, 1987). The obtained hydrogels become stiffer monotonously as the pre-gel solution concentration increases. On the molecular level, this trend is simply attributed to the increase in the number density of network chains in the hydrogels. Actually, however, because of fairly low solubility of κ -carrageenan in water, the concentration of κ -carrageenan is likely to be

limited even when hot water is used near the boiling point under atmospheric pressure. So far, a κ -carrageenan hydrogel of 12 wt% has been reported in literature (Watase & Nishinari, 1987), while our first-hand experience shows that the maximum concentration of κ -carrageenan hydrogel is around 5 wt%. These facts imply that preparation of κ -carrageenan hydrogels having concentrations significantly higher than 12 wt% is almost impossible in the conventional manner. Consequently, the mechanical properties of κ -carrageenan hydrogels have not ranged widely; for example, Young's modulus for uniaxial stretching is usually in the order of 10^4 – 10^5 Pa (Watase & Nishinari, 1982; Watase & Nishinari, 1987). Therefore, a technological breakthrough in obtaining high-concentration hydrogels of κ -carrageenan has been highly desired.

The mechanical properties of κ -carrageenan hydrogels can be modified by two other methods, namely using additives such as inorganic salts and changing thermal procedure of gelation. The effects of both methods are often explained based on the network structure specific to κ -carrageenan hydrogels, where each crosslink in the gel network structure consists of aggregated κ -carrageenan chains existing in helical conformations (Watase & Nishinari, 1982; Horinaka et al., 2022). Because of the anionic nature of κ -carrageenan, the aggregation is stabilized by the electrostatic interaction between κ -carrageenan and the counter cations (Watase & Nishinari, 1982; Watase & Nishinari, 1985). Therefore, additional cation species derived from an inorganic salt, for example, would contribute to further stabilization of the aggregates and

strengthen the network structure of κ -carrageenan hydrogels to some extent, although the effects of additives on the network structure may vary with their ionic nature or hydrophilicity. Thermal procedure for gelation also affects the stability of the crosslinks of κ -carrageenan hydrogels. It has been reported that the number of chains involved in a crosslink depends on the cooling rate, so that larger and more stable crosslinks tend to be formed as the cooling rate becomes slow (Iijima et al., 2007). These previous studies have shown that the thermal stability of κ -carrageenan hydrogels is improved, that is, the gel-to-sol transition temperature increases, substantially by adding salts and/or slowing the cooling rate. However, it is very likely that both of them have subsidiary effects on the mechanical properties of κ -carrageenan hydrogels.

Subcritical water conditions generally refer to a high-temperature and high-pressure region existing below the critical temperature of water (374 °C) where water still exists in the liquid state above 100 °C. Subcritical water is worthy of note because of its particular interactions with organic compounds. Since the dissociation constant is much higher compared with liquid water under atmospheric pressure, subcritical water shows acid- or base-catalyzed hydrolysis of organic compounds. In addition, subcritical water tends to resemble organic solvents regarding solubility as the relative permittivity becomes lower with increasing temperature and exhibits better solubility against hydrophobic substances accordingly. The dual nature of subcritical water as a solvent, namely the anomalous high activity mentioned above and the original safety of water,

has been interested in the framework of the green chemistry; for example, subcritical water is nowadays utilized for detoxification of hazardous substances as well as for extraction of useful components from food wastes (Adschiri et al., 1993; Rogalinski et al., 2008; Meillisa et al., 2015; Basak & Annapure, 2022). So far, however, there have been no investigations of subcritical water aiming to prepare κ -carrageenan hydrogels. There is a possibility that κ -carrageenan dissolves in subcritical water beyond the previous limit of concentration, so that high-concentration hydrogels could be formed under subcritical water conditions.

In this study, a new procedure where high-concentration hydrogels of κ -carrageenan can be prepared using subcritical water has been reported; in fact, κ -carrageenan hydrogels having the concentration from 15 wt% to 40 wt% have been successfully obtained. It has been revealed that the mechanical properties of the hydrogels such as Young's modulus and the toughness are definitely superior to those of the conventional low-concentration κ -carrageenan hydrogels. In addition, as expected, the high-concentration hydrogels exhibit exceptional thermal resistance; actually, the melting point of the hydrogels increases with concentration and exceeds 100 °C. It is certain that the preparation method proposed in this study has made a breakthrough in the mechanical as well as the thermal properties of κ -carrageenan hydrogels. Moreover, this study could be the beginning of creating high-concentration hydrogels of various polysaccharides, which leads to wider application of the green polymers.

2. Experimental Section

2.1 Preparation of Hydrogels

κ -Carrageenan (TCI, Japan; Lot. KI620-UN), which have a chemical structure of alternating α -(1-3)-D-galactose-4-sulfate and β -(1-4)-3,6-anhydro-D-galactose units (Le Questel, 1995), was used without further purification. The weight average and the number average molecular weights for this sample were determined to be 2.14×10^6 and 3.09×10^5 respectively by the gel permeation chromatography with pullulan standards. The metal content (%) regarding K^+ , Na^+ , Ca^{2+} , and Mg^{2+} species were evaluated to be 6.5, 1.0, 0.41, 0.19 respectively by the atomic absorption spectroscopy (for K^+ and Na^+) and the inductively coupled plasma atomic emission spectroscopy (for Ca^{2+} and Mg^{2+}). High-concentration hydrogels were prepared as follows. Powdery κ -carrageenan was mixed with water at weight fractions ranging from about 15 wt% to 40 wt%, taking the reported upper limit of 12 wt% into consideration. Since κ -carrageenan was not dissolved in water completely at high concentrations under atmospheric pressure, as mentioned above, the crumbly mixtures were first kneaded at room temperature to be seemingly uniform as far as possible. As described later, the weight fraction of the mixture roughly corresponded to the concentration (c) of the resultant hydrogel. Then each mixture was put into cylindrical or rectangular molds made of stainless steel and was hermetically sealed with a rubber sheet. Using

a hydraulic hot press machine, the sealed mold was heated up to 150 °C as fast as possible—actually it took about 5 min. The pressure inside the mold at 150 °C was estimated to be about 0.6 MPa, indicating that the high-concentration mixtures experienced subcritical water conditions. Soon after that, the mold was cooled slowly down below 40 °C over 90 min. Low-concentration κ -carrageenan hydrogels of $c = 3$ wt% were prepared likewise for comparison. In addition, 3 wt% κ -carrageenan hydrogels were prepared in the conventional manner as follows in order to assess the influence of subcritical water conditions. The same κ -carrageenan was put into water and was stirred well at 85 °C for an hour until a clear aqueous solution was obtained. Then the solution was cooled down to 25 °C over 30 min, so that the cooling rate in this temperature range was roughly comparable with that for the high-concentration hydrogels.

2.2 Mechanical measurement

Uniaxial stretching measurements were conducted with a tensile machine TENSILON RTM-500 (Orientec Corp., Japan). The hydrogels were cut out with a dumbbell-shaped punching blade (#7, JIS K 6251) to have the length and the width of the narrow parallel section of 12 mm and 2 mm respectively. The initial distance between markers in the parallel section of the specimens was fixed at 10 mm and the distance was monitored with a camera during tensile tests to calculate the nominal strain (ε). The uniaxial stretching was performed at a constant speed where the

distance between the clamps of the tensile machine increased 2.2 mm min^{-1} until the specimen broke. The dumbbell-shape was employed in order to avoid breaks of the specimen near the clamps; in practice, the results in which breaks occurred between the markers were adopted and three successful results were at least collected for each c to secure the reproducibility. The nominal tensile stress (σ) was estimated from the obtained force and the initial cross section of each specimen—approximately the width of 2 mm and the thickness of 1 mm. To confirm complete gelation at high concentrations, the stress relaxation behavior was also measured for the 30 wt% κ -carrageenan hydrogels under a constant strain of 5 %. A rectangle-shaped specimen was used for the stress relaxation measurement. All the mechanical measurements were carried out at 25 °C and the tested specimens were coated with silicone oil to avoid concentration change by water evaporation.

2.3 Thermal measurement

Differential scanning calorimetry (DSC) measurements were carried out using a DSC7020 calorimeter (Hitachi High-Tech Sciences, Japan). 5-10 mg of each hydrogel was weighed exactly and was set in an aluminum pressure-proof pan. The heating process for the high-concentration hydrogels started from 30 °C towards a temperature more than 40 °C higher than the melting point (T_m) at 10 °C min^{-1} , while the cooling process was conducted from 150 °C at the same rate.

In addition, T_m of the 30 wt% hydrogel was determined by observing the melting behavior to ensure that the DSC peak on the heating process corresponds to the gel-to-sol transition. First a disk-shaped hydrogel made with the cylindrical mold was cut into two pieces and put them back shifting one of the pieces from the original position. Then the sample was hermetically sealed again and was heated up to a target temperature with the hot press machine. After keeping it at the target temperature for 30 min, the sample was cooled down to room temperature. It was checked whether or not the separated pieces are unified into a single disk along the mold via melting at the target temperature above T_m . This observation procedure was repeated with raising the target temperature by 1 °C until exceeding T_m ; in practice, T_m was determined as the lowest target temperature at which a single disk is formed. It should be mentioned that a new hydrogel specimen was used for every new target temperature.

3. Results and discussion

3.1 Formation of high-concentration hydrogels

Figure 1 shows photos of the 15 wt% mixture of κ -carrageenan with water (a) before and (b) after being processed under subcritical water conditions. The mixture before the process appears opaque and crumbly even at 15 wt% due to fairly low solubility of κ -carrageenan in water. In contrast, after experiencing subcritical water conditions, pieces of the mixture have been gathered

to form a homogeneous translucent object. Less transparency probably arises from a great number of aggregations of κ -carrageenan chains forming crosslinks of the network structure. Similar drastic changes in appearance through experiencing subcritical water conditions have been obtained for the mixtures of higher weight fractions up to 40 wt%. By measuring the weights of the samples between before and after the process, it has been found that the weight of each sample reduces about 5 % during the process due to unavoidable water evaporation and diffusion inside the mold. At the same time, the slight weight loss indicates that the translucent objects obtained after experiencing subcritical water conditions undoubtedly keep water inside. From the appearance and the weight, it has been concluded that high-concentration hydrogels are formed successfully through the procedure where κ -carrageenan is dissolved in subcritical water at high concentrations. To strengthen this conclusion, it has been additionally checked whether the high-concentration hydrogels exhibit equilibrium stresses under constant small strains. Figure 2 shows the stress relaxation behavior for the 30 wt% κ -carrageenan hydrogel. It is obvious that the stress attains an equilibrium value of 2.1×10^5 Pa at around 100 s, which satisfies the rheological requirement that complete gels must behave as solids. Now that formation of hydrogels has been confirmed as described above, it is clearly stated here that the concentration of κ -carrageenan hydrogel ($= c$) is calculated based on the weight after the gelation process, that is, after the slight weight loss, throughout this study. It is worth noting that the concentration of κ -carrageenan

hydrogels has reached a record high of 40 wt% beyond the previous limit of 12 wt% by utilizing subcritical water.

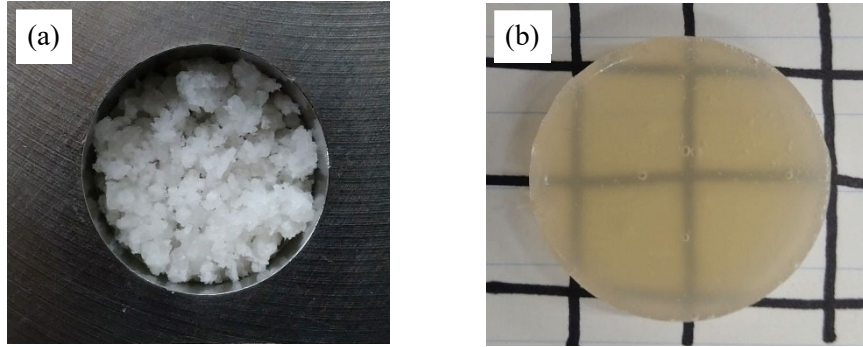


Figure 1. Photos of 15 wt% mixture of κ -carrageenan with water (a) before and (b) after being processed under subcritical water conditions. Mixture appears opaque and crumbly in (a) due to fairly low solubility of κ -carrageenan in water. In contrast, product looks homogeneous and translucent in (b).

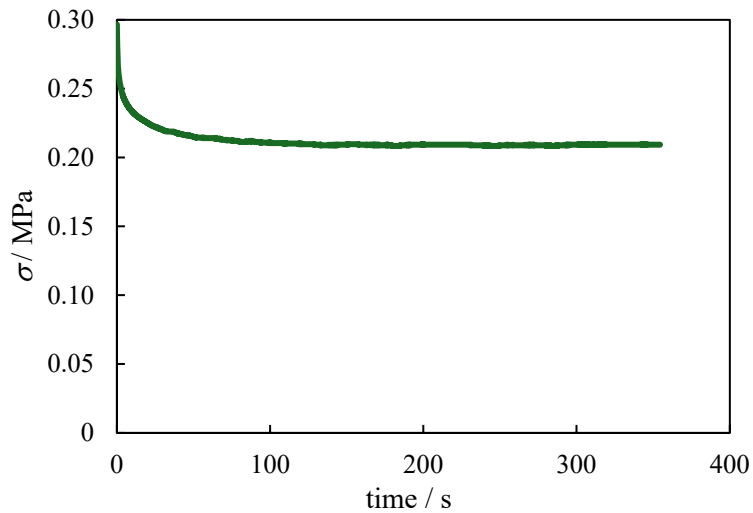


Figure 2. Stress relaxation behavior of 30 wt % κ -carrageenan hydrogel prepared using subcritical water. The stress reduces with time after a constant strain of 5 % applied at 0 s and reaches 2.1×10^5 Pa at around 100 s. It is clearly seen that the stress remains almost constant after 100 s, which is consistent with the rheological requirement that a complete gel exhibits an equilibrium stress under a constant strain.

There have been some earlier studies in which κ -carrageenan hydrogels were prepared at relatively high concentrations by the conventional method; that is, aqueous solutions of κ -carrageenan were first obtained at near 100 °C under atmospheric pressure and then the solutions poured into molds were cooled until hydrogels were formed (Watase & Nishinari, 1982; Watase & Nishinari, 1985; Watase & Nishinari, 1987). So far κ -carrageenan hydrogels up to 12 wt% have been prepared, although the 12 wt% hydrogels have been used only for DSC analysis and mechanical properties of κ -carrageenan hydrogels have been reported for $c \leq 10$ wt% because of difficulties in molding for mechanical measurements (Watase & Nishinari, 1987). The same research group have reported preparation of high-concentration hydrogels of agarose, a neutral polysaccharide, using low-molecular-weight fractions having better solubility. Thermal properties of agarose hydrogels have been shown up to 40 wt% using the lowest fraction, while the mechanical behavior has been examined up to 30 wt% (Watase et al., 1989). Although the pre-gel solutions of agarose were prepared using an autoclave at 105 °C, the method was basically a conventional one where the pre-gel solutions were poured into molds for subsequent gelation under atmospheric pressure. High-concentration hydrogels of agar up to 23 wt% have been prepared in a similar manner by another research group; agar was mixed with water in a hermetically-sealed glass bottle and the pre-gel solutions obtained at 140–145 °C were poured into molds after being cooled down to about 70 °C (Ayyad et al, 2010). Other than polysaccharides,

preparation of poly(vinyl alcohol) hydrogels with saline has been achieved at high polymer concentrations by using repeated freeze-thaw cycles (Stammen, 2001).

3.2 Mechanical properties

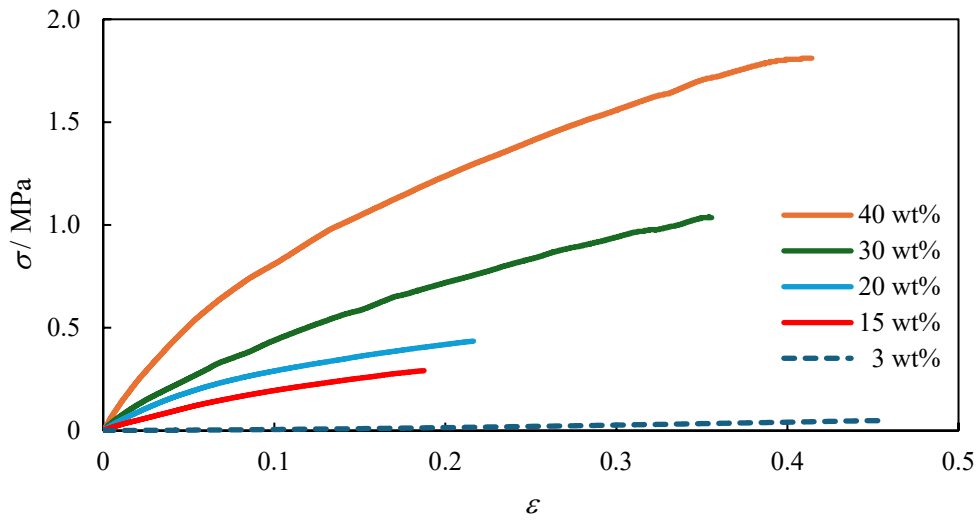


Figure 3. Stress-strain curves for high-concentration hydrogels of κ -carrageenan and for 3 wt% hydrogel prepared in the conventional manner. High-concentration hydrogels exhibit $\sigma > 10^5$ Pa at most of the ϵ range and σ for 40 wt % hydrogel exceeds 10^6 Pa. Result for 3 wt% hydrogel prepared using subcritical water is not shown due to fragility of the specimen.

Figure 3 shows the stress-strain curves for the high-concentration hydrogels of κ -carrageenan. It is seen that σ at a given ϵ increases with c monotonously; in fact, σ for the high-concentration hydrogels exhibit $\sigma > 10^5$ Pa over most of the ϵ range examined. It is disclosed here that 3 wt% κ -carrageenan hydrogels prepared using subcritical water are too fragile to be available

for the tensile tests. Instead, the stress-strain curve for the 3 wt% hydrogel prepared in the conventional manner has been shown in the figure with a dotted line. The 3 wt% hydrogel exhibits σ below 10^5 Pa throughout ε range examined. Young's modulus (E_0) estimated from the initial slope of the stress-strain curve for each high-concentration hydrogel is double-logarithmically plotted against c in Figure 4 with the same color as in Figure 3. Note that the data points give the average values of E_0 of all adopted results including results in Figure 3 and the vertical error bars represent those standard deviations. The high-concentration hydrogels of κ -carrageenan exhibit E_0 ranging 10^6 – 10^7 Pa, being roughly two orders of magnitude greater than 5.8×10^4 Pa of the conventional 3 wt% κ -carrageenan hydrogel shown in Figure 3. Young's modulus of the 40 wt% κ -carrageenan hydrogel over 10^7 Pa is greater than that of commercial natural rubber in bulk by a factor of about ten (Shibayama, 2009; Shibayama, 2012). As far as we know, the 40 wt% hydrogel is the firmest as-prepared hydrogel ever made in terms of E_0 . Furthermore, it seems that the c -dependence of E_0 for the high-concentration hydrogels exhibits $E_0 \propto c^{1.1}$ relationship as indicated by a solid line in the figure. Note that the solid line has been drawn analyzing the four data points by the linear least-squares method. In earlier studies of conventional κ -carrageenan hydrogels, it has been proposed that the elastic modulus is proportional to about the fourth power in a low-concentration region and turns to the second-power dependence above a critical concentration of ca. 4 wt%. Although the critical concentration varies with the metal content of

κ -carrageenan hydrogels, the two concentration regions having the fourth- and the second-power dependences have been reported for a large number of κ -carrageenan hydrogels (Watase & Nishinari, 1982; Nishinari & Horiuchi, 1977). On the contrary, the c -dependence of E_0 for $c = 15\text{--}40$ wt% obtained in this study cannot be represented neither by the fourth- nor second-power dependence, revealing the existence of unknown region where $E_0 \propto c^{1.1}$ holds at higher concentrations of $c \geq 15$ wt%. This result suggests that the high-concentration hydrogels of κ -carrageenan are considerably different in the network structure from conventional κ -carrageenan hydrogels, although it is beyond the scope of our understanding to propose the specific structure at the present stage. The added chains with increasing c generally contribute to the increase in the elastically active chains to some extent, but such increase can be considered less effective at higher concentration range where chains exist sufficiently, which possibly provides a qualitative explanation for the low exponent of the high-concentration hydrogels.

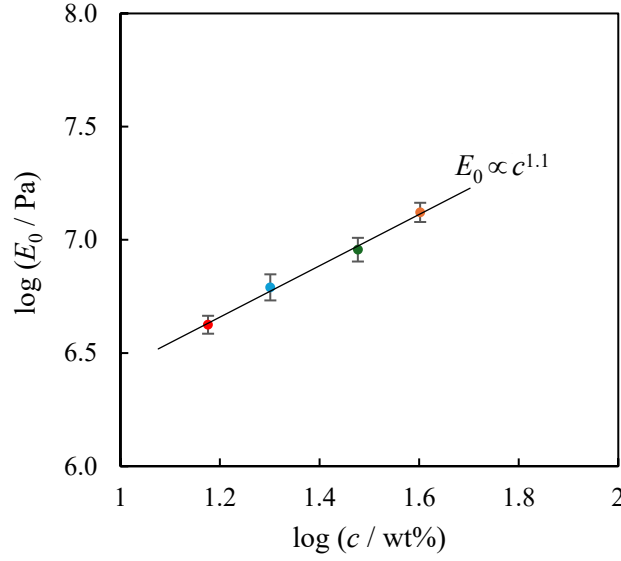


Figure 4. Double-logarithmic plot of E_0 against c estimated from the initial slope of the stress-strain curve. Data points give the average values of E_0 of all adopted results and the vertical error bars give standard deviations. The solid line represents the linear least-squares fit. It has been found that high-concentration hydrogels of κ -carrageenan have outstanding E_0 ranging 10^6 – 10^7 Pa and exhibit a relationship of $E_0 \propto c^{1.1}$.

Back in Figure 3, it is observed that σ at break (σ_B), which is σ at the end of the stress-strain curve, increases with c and exceeds 10^6 Pa at $c = 40$ wt%. Figure 5 provides the average values of σ_B at each c with the standard deviations calculated from all adopted results, where σ_B substantially increases with c . The mechanical superiority of the high-concentration κ -carrageenan hydrogels is also acknowledged regarding the toughness (U_T), which is defined as the area between the stress-strain curve and the abscissa. As expected from the curves in Figure 3, the 40 wt% hydrogel has U_T of $3.2 \times 10^5 \text{ J m}^{-3}$ that is 44 times as large as that for the conventional 3 wt% hydrogel. Concerning ε at break (ε_B) for the κ -carrageenan hydrogel, ε_B

increases with increasing c , but all the high-concentration hydrogels take lower ε_B values than the conventional 3 wt% hydrogel, as plotted in Figure 6. Recalling that 3 wt% κ -carrageenan hydrogels prepared using subcritical water are fragile, the relatively small ε_B for the high-concentration hydrogels might be partly attributed to use of subcritical water. There is a possibility that subcritical water in the gelation process somehow damages κ -carrageenan chains in the network structure; in other words, ε_B could be improved in the future by using subcritical water in more sophisticated manner. The effect of using subcritical water is discussed later again regarding thermal properties.

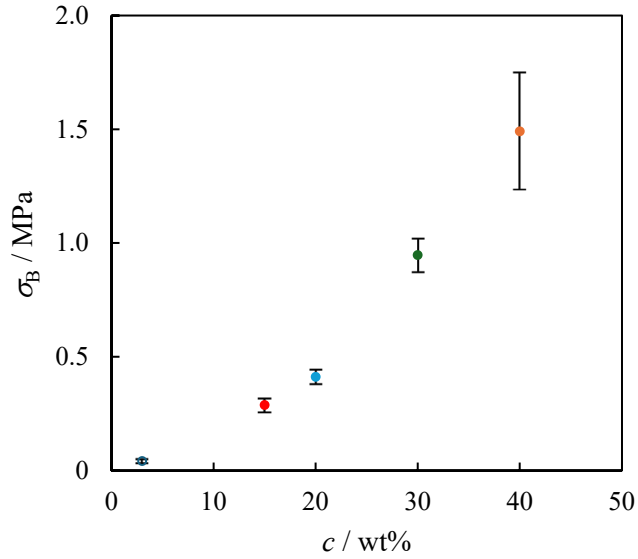


Figure 5. Average values of σ_B for high-concentration hydrogels of κ -carrageenan at each c with the standard deviations calculated from all adopted results. σ_B increase monotonously with c including that for the 3 wt% hydrogel prepared in the conventional manner (unfilled circle).

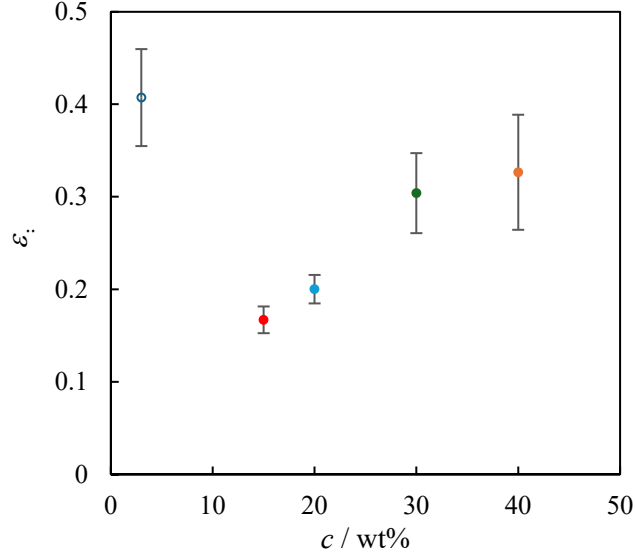


Figure 6. Average values of ε_B for high-concentration hydrogels of κ -carrageenan at each c with the standard deviations calculated from all adopted results. ε_B increases monotonously with c but remains lower than that for the 3 wt% hydrogel prepared in the conventional manner (unfilled circle). Use of subcritical water might be responsible for the relatively small values of ε_B .

Now we compared the mechanical properties of the firmest κ -carrageenan hydrogel, namely the 40 wt% hydrogel, with those of synthetic polymer gels specially designed as tough gels—double network gels (Gong et al., 2003; Yu et al., 2009), slide ring gels (Okumura & Ito, 2001; Ito, 2007), nanocomposite gels (Haraguchi & Takehisa, 2002; Haraguchi et al., 2003) and tetra-PEG gels (Sakai et al., 2008; Sakai et al., 2010). The tough gels generally show σ_B of the order of 10^5 Pa at large extensions around $\varepsilon = 10$; in contrast, the 40 wt% κ -carrageenan hydrogel has greater σ_B more than 10^6 Pa at about $\varepsilon = 0.3$. Therefore, it is admitted that our high-concentration κ -carrageenan hydrogels have an advantage of the mechanical strength over the tough gels, while

the tough gels have superior toughness to the κ -carrageenan hydrogel due to excellent extensibility.

It makes sense to modify the stress-strain curves in Figure 3 as σ / E_0 versus ε curves. As shown in Figure 7, the four curves for the high-concentration hydrogels are very likely to fall on a single curve and the trend is remarkably different from that of the curve for the conventional 3 wt% hydrogel. It is realized that the high-concentration hydrogels commonly give upward convex curves. In contrast, the curve for the 3 wt% hydrogel shows a downward convex behavior throughout the stretching. Downward convex behavior has been generally reported for κ -carrageenan hydrogels prepared in the conventional manner in the previous study (Watase & Nishinari, 1985). This discrepancy in the curves strongly supports our above-mentioned claim that the network structure of the high-concentration hydrogels of κ -carrageenan is considerably different from that of the conventional hydrogels. Moreover, the coincidence of σ / E_0 versus ε curves for the high-concentration hydrogels—in other words, the fact that the difference in σ of these hydrogels can be reduced commonly by $c^{1.1}$ —demonstrates that the network structures and their deformations are mutually similar among the high-concentration hydrogels.

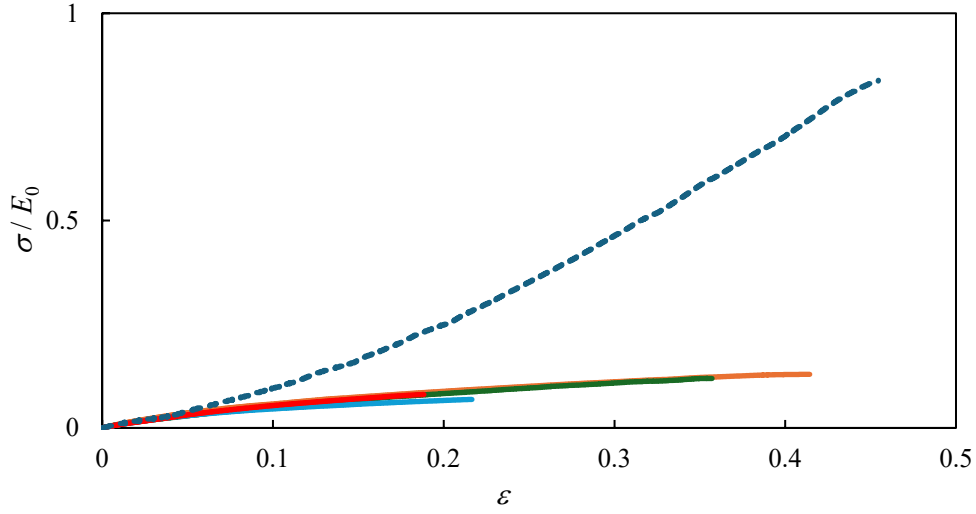


Figure 7. σ / E_0 vs. ε curves for high-concentration and 3 wt% hydrogels of κ -carrageenan remade from those in Figure 3. Four curves for high-concentration hydrogels seems to fall on a single curve, while the curve for the 3 wt% hydrogel exhibits a remarkably different trend from that. This result suggests that network structures of high-concentration hydrogels are mutually similar and are different from that of conventional hydrogel.

3.3 Thermal properties

Figure 8 shows T_m (gel-to-sol) and the gelation (sol-to-gel) temperature (T_{gel}) for the high-concentration hydrogels of κ -carrageenan determined from each DSC curve as the onset of a peak appearing on the heating and cooling processes respectively. It is seen that T_m as well as T_{gel} monotonously increases with c , so that T_m exceeds 100 °C at very high concentrations; namely, 107 °C at 30 wt% and 131 °C at 40 wt%. Following a similar trend, T_{gel} also attains 109 °C at 40 wt% in spite of a hysteresis that T_{gel} is lower than T_m . It is emphasized that the gel-to-sol and the sol-to-gel transitions of hydrogels above 100 °C, exceeding the boiling point of water under

atmospheric pressure, can be realized by using subcritical water. In Figure 8, T_m for the 3 wt% κ -carrageenan hydrogels prepared by different conditions are also shown; using subcritical water (filled circle) and in the conventional manner (unfilled circle). Although the mechanical properties of the 3 wt% hydrogels are totally different depending on the preparation conditions, as described above, their T_m values are the same within the experimental error. Since the detected heat flow originates from the disaggregation of helical κ -carrageenan chains forming crosslinks, the damage to κ -carrageenan chains under subcritical water conditions might be the chain scission with preserving the chemical structure of the repeating unit which affects the crosslinking.

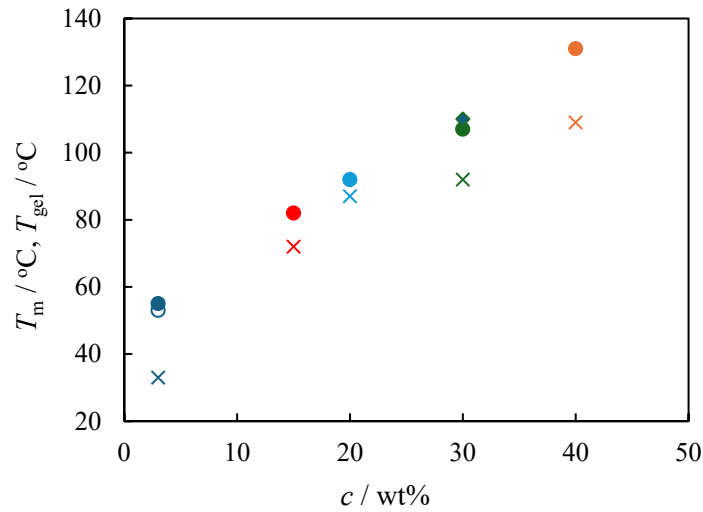


Figure 8. T_m (circle) and T_{gel} (cross) for the high-concentration hydrogels of κ -carrageenan determined from DSC. T_m as well as T_{gel} monotonously increases with c and exceeds 100 °C at very high concentrations. T_m for 3 wt% hydrogels seem the same within the experimental error regardless of the preparation conditions. T_m estimated from the appearance for 30 wt% hydrogel (diamond) agrees well with that from DSC.

Figure 9 illustrates photos of the 30 wt% hydrogel in the cylindrical mold; (a) before heating, (b) after heating to the target temperature of 109 °C, and (c) after heating to 110 °C. Two pieces of semicircular hydrogels are put in the mold before heating so that they are overlapped as illustrated. While the appearance of the separated pieces put overlapped is almost the same in (a) and (b), a single disk is formed along the mold in (c). It should be emphasized that the appearance of the κ -carrageenan hydrogels alters drastically by increasing the target temperature by 1 °C. Accordingly, T_m of the 30 wt% hydrogel can be determined to be 110 °C within the error less than 1 °C. This observation can be expressed in other words that the hydrogel shows self-healing above T_m where the cut pieces are unified again. The value of T_m estimated on the appearance basis agrees well with that from DSC (107 °C), as plotted in Figure 8 with a diamond symbol. By observing the appearance of the hydrogels, it has been directly confirmed that the DSC peak observed on the heating process corresponds to the gel-to-sol transition.

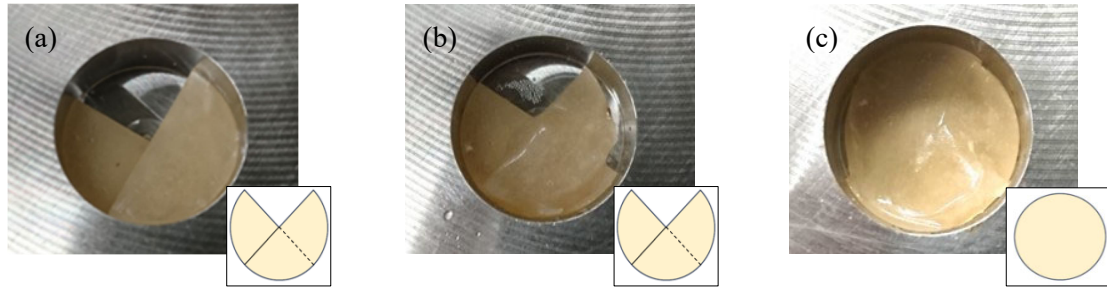


Figure 9. Photos of 30 wt% hydrogel in the cylindrical mold; (a) before heating, (b) after heating to 109 °C, and (c) after heating to 110 °C. While the appearance of separated specimens is almost the same in (a) and (b), a single disk is formed along the mold in (c). T_m of 30 wt% hydrogel can be accordingly determined to be 110 °C within the error less than 1 °C and coincides with that from DSC. Illustrations are placed for better understanding.

The endothermic enthalpy (ΔH) of the gel-to-sol transition of the κ -carrageenan hydrogels can be estimated from the area of DSC peak observed on the heating process. Previous study on conventional κ -carrageenan hydrogels up to 12 wt% has pointed out that ΔH increases with c until 4 wt% and then decreases (Watase & Nishinari, 1987). However, our result shows that ΔH tends to increase with c , so that ΔH for the high-concentration hydrogels have significantly larger ΔH than the 3 wt% hydrogels. In Figure 10, ΔH per unit weight of κ -carrageenan ($\Delta H_w; = 100\Delta H / c$) for the high-concentration hydrogels of κ -carrageenan is plotted against c together with those of the 3 wt% hydrogels. Although ΔH_w for the 20 wt% hydrogel might look exceptionally high, it is very interesting that values of ΔH_w are almost independent of c over a wide range from 3 to 40 wt %. Within the high concentration region of $c \geq 15$ wt%, comparable values of ΔH_w seem consistent with the claim that the high-concentration hydrogels have similar network structures

with each other and therefore result in the distinctive mechanical properties shown in Figures 4 and 7. To take it a step further, the relationship $\Delta H \propto c$ expected from Figure 10 could quantitatively corresponds to the c -dependence of E_0 , because the relationship in Figure 4 can be interpreted as $E_0 \propto c$ within the experimental error. On the other hand, the closeness of ΔH_w between the high-concentration hydrogels and the 3 wt% hydrogels shown in Figure 10 is difficult to explain at the present stage. If ΔH_w for the 20 wt% hydrogel is assumed significantly high, that is, ΔH_w increases and then decreases with increasing c , such trend might be qualitatively explained by two contrasting factors, namely an increasing effect due to the number of crosslinks per chain and a decreasing effect due to the sulfate groups which hinder the formation of crosslinks, as suggested in the earlier study (Watase & Nishinari, 1987).

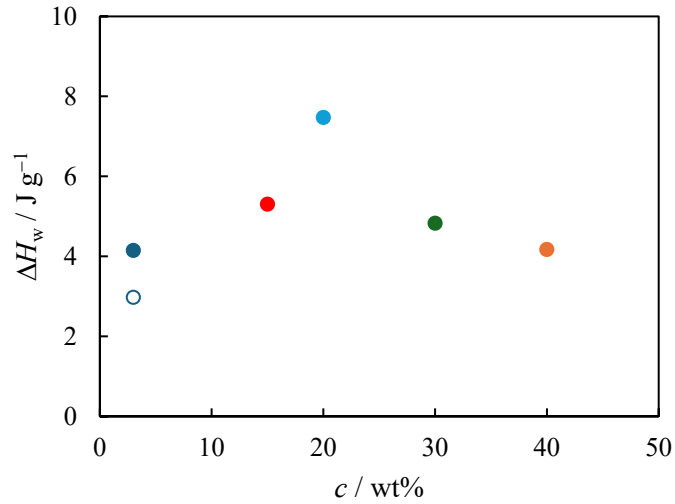


Figure 10. Endothermic enthalpy per unit weight of κ -carrageenan (ΔH_w) for high-concentration hydrogels. ΔH_w for 3 wt% κ -carrageenan hydrogels prepared using subcritical water (filled circle) and in the conventional manner (unfilled circle) are also shown. Although ΔH_w for the 20 wt% hydrogel might be exceptionally high, ΔH_w seems almost independent of c over a wide range from 3 to 40 wt %.

4. Conclusion

High-concentration hydrogels of κ -carrageenan have been prepared successfully at 40 wt% beyond the previous concentration limit by heating mixtures of κ -carrageenan and water in a hermetic mold to 150 °C. It is likely that κ -carrageenan is dissolved in subcritical water at higher concentrations than in ordinary water and so homogeneous gelation occurs in the subsequent cooling. The obtained high-concentration hydrogels exhibit extremely high Young's modulus and mechanical strength compared to known network materials. The mechanical properties including the toughness would be even better by changing the temperature of subcritical water and the heating and cooling procedures, which is under investigation. In this study, it has been found from the mechanical properties that the network structures of the high-concentration hydrogels of κ -carrageenan prepared using subcritical water are mutually similar and are totally different from that of the conventional hydrogel. On the other hand, it has been suggested regarding the thermal properties that there is a similarity in the melting behavior of κ -carrageenan hydrogels regardless of the preparation methods. These currently unaccountable findings are planned to be examined in the near future using techniques for studying structural features such as small angle X-ray scattering. On application aspects, the superior mechanical properties of the high-concentration hydrogels of κ -carrageenan would be most suitable for firm biomaterials like cartilage replacements in combination with the biocompatibility.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability

The data that support the findings of this study will be available on request.

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