# Locust Bean Gum Hydrogels Formed by Freezing and Thawing

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**Summary:** Locust bean gum (LBG) hydrogels were prepared by freezing and thawing. It was found that the junction zone of LBG hydrogels is tightly formed by repeating freezing and thawing. During this process, LBG molecules not connected with the junction zone are excluded from the gel portion and the remaining molecules gradually form densely packed hydrogels. Molecular conformation in the sol state affects the rate of the junction formation. Obtained LBG hydrogels are thermally stable and no gel-sol transition was observed in temperatures from 40 to 100 °C by the observation of differential scanning calorimetry (DSC). Non-freezing water content calculated from the DSC melting peak of water in the gel indicates that the junction zone became dense with increasing freezing and thawing.

**Keywords:** bound water; freezing; hydrogels; locust bean gum; thawing

## Introduction

Among various types of polymers having gel forming capabilities, poly(vinyl alcohol) (PVA) has been reported to form hydrogels by freezing and thawing.[1-6] Recently, we found that locust bean gum (LBG) also forms hydrogels by freezing and thawing.[7] Polysaccharide physical gels have a wide range of applications, such as medical, food and agricultural, due to their high water holding capability. If the formation and dissociation of polysaccharide gels are controllable, water holding and releasing functions can be programmed. On this account, gels prepared by freezing and thawing are considered to be a target material whose dissociation of cross-linking can be manipulated. LBG is a polysaccharide whose chemical structure is shown in Figure 1. A 1,6 $\alpha$-D- galactose side chain is attached to each 4 repeating units of 1, 4- $\beta$-D-mannose main chain.[8,9] When the side chain is attached to either three (tara gum)[10] or two repeating units (guar gum), [9, 11-13] gelation is not observed.[7] It is thought that an appropriate chemical structure for the formation of hydrogen bonding between the hydroxyl groups is crucial in order to form a

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gel structure. At the same time, it was found that gelation by freezing and thawing is affected by cooling rate, which affects the size of ice formed in the LBG solution.[7] It is thought that the size of ice necessarily corresponds to the size of the junction zone, which is known as the cross-linking point of gels.[14, 15]

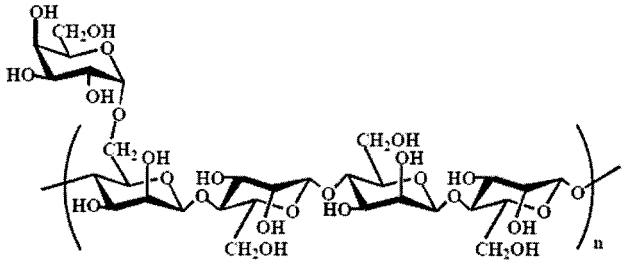

Figure 1. Chemical structure of LBG.

In our previous studies, it was found that the gelation behaviour and physical properties of polysaccharide hydrogels are markedly affected by the non-equilibrium nature of the sol state.[16, 17] When an aqueous solution is annealed in a sol state, the structure of the junction zone varies according to the degree of equilibration.[18] The above fact was confirmed for physical gels prepared by ordinal gelation, such as gellan gum.[14,16] Moreover, if aqueous solutions of polysaccharides which have been considered as non gel forming saccharides, such as xanthan gum[17, 19, 20] and hyaluronan,[21] are annealed at an appropriate temperature, flexible gels are formed. In the case of hydrogels formed by freezing and thawing, the junction zone is formed when ice grows, LBG chain molecules are pushed out by ice growth and the junction zone is formed by the stacking of the LBG molecules. This situation suggests that the structure of the junction zone is necessarily affected by the conformational structure of molecular chains in the sol state and the size of ice formed during freezing.

PVA hydrogels formed by freezing and thawing are thermally reversible and the gel-sol transition is observed at around 60 °C[1]. In contrast, thermal reversibility of LBG gels has not been reported. In this study, the effect of annealing in the sol state on gelation, thermal stability and the structural change of water in the LBG hydrogels is investigated.

There are various methods to analyze the gelation process of polysaccharides, such as rheological measurement,[15, 22] thermal measurements,[23-26] small x-ray scattering,[27-29]

and neutron scattering,[29] etc. In this study, differential scanning calorimetry (DSC) was used in order to investigate LGB gels, since bound water content in polymers has been quantitatively obtained by DSC.

## Experimental

**Gelation.** LBG extracted from carob seeds (Caratonia silique) was commercially obtained from Sigma Chemical Co., USA. The molecular weight was 3.1 x $10^5$, according to the manufacturer.

The LBG aqueous solution was prepared as follows. (1) 10 mL of 0.5, 1, 2 and 3 weight % of aqueous solutions were prepared in a glass vessel with a polyethylene inner lid and a plastic outer lid. (2) Each vessel was maintained at 105 °C for 2 hours. No mass loss was observed during heating at 105 °C. (3) After annealing at 105 °C, the first series of samples were directly transferred to a freezer whose temperature was -15 °C and maintained for 24 hours. The second series of samples were cooled to 25 °C, maintained for 2 hours and then frozen at -15 °C. The third series of samples were annealed at 60, 70 and 80 °C for 2 hours then frozen at -15 °C. (4) Frozen samples were unfrozen to 25 °C. This process took more than 6 hours. (5) The above freezing and thawing was repeated between 1 and 10 times. In this study, the number of freezing and thawing cycles is stated as "*n*". After freezing and thawing, the samples were stored at 25 °C.

Glassware and used tools were boiled in hot water in order to sterilize them before sample handling. Samples were carefully kept in glass vessels. Fungi were not observed after three months of storage, even if the glass vessels had been opened several times. The shape of the gels was recorded using a digital camera (Sony, MOVIE ).

The gels were removed from the glass vessel and weighed quickly. The gel ratio (Rg) is defined as:

$$\text{gel ratio (Rg )} = (\text{mass of gel }) / (\text{total mass of solution }),\ \text{g g}^{-1} \quad (1)$$

The residual amount is treated as a mass of sol. The gel portion was taken out from the vessel and dried in an oven at 105 °C for 2 hours. The mass of dry LBG was weighed. The amount of LBG in the gel is defined according to the following equation:

$$\text{LBG content} = (\text{mass of dried gel}) / (\text{mass of gel}),\ \text{g g}^{-1} \quad (2)$$

The mass of samples was measured using either a Sartorius microbalance MC-210S (±1 x $10^{-5}$) or a Sartorius ultramicro-balance (± 1 x $10^{-7}$).

**Water content determination.** The water content of the gel component was measured by

the tea bag method. A poly(ethylene terephthalate) (PET) nonwoven fabric sheet (thickness 0.2 mm) was supplied from Daiki Co., Osaka, Japan (commercially named Omuro No 102). The tea bag (5 x 7 cm) was made by heat sealing at 250 °C using the PET sheet. The water content was measured as follows. (1) A weighed tea bag was immersed in distilled water for 5 minutes at 25 °C. (2) A pinhole was made in the bottom of both corners of the tea bag in order to drain excess water and the bag was maintained in air for 5 min. Afterwards the tea bag was weighed. (3) The gel was put in the tea bag and quickly weighed. (4) The tea bag with the gel sample was immersed in a water bath for 30 min., maintained in air for 5 min. and weighed. (5) The temperature of the water bath was varied at 30, 40, 50 and 60°C, respectively. (6) Used samples were dried at 105 °C for 2 hours and weighed.

The water content (*Wc*) of gel samples was calculated.

$$W_c = (\text{mass of water})/(\text{mass of dried gel}) \text{ , g g}^{-1} \quad (3)$$

A completely dried sample was again immersed in water at 25 °C. The *Wc* of once-dried samples was also calculated.

**Gel-sol transition temperature.** Both visual observation and differential scanning calorimetry (DSC) were carried out in order to confirm the gel-sol transition temperature. A Seiko differential scanning calorimeter DSC 2000 C equipped with a cooling apparatus was used. Gel was sealed in an aluminum sealing type pan. The sample mass was 5-7 mg, and the heating rate was 10- 20 °C/min in order to increase apparent sensitivity. The temperature was varied from 0 to 100 °C.

Visual observation was carried out as follows. (1) Ca. 2 g of gel sample was put in a test tube and into which a thermometer was inserted. (2) The test tube was immersed in a water bath whose temperature was heated at a rate of ca. 3 °C /min and heated to 90 °C.

**Non-freezing water content.** The same DSC as shown in the experimental section was used. Temperature was varied as follows: (1) the sample was cooled from 40 to -100 °C at a cooling rate of 10 °C/min., (2) the sample was maintained at -100 °C for 5 minutes and heated at 10 °C/min to 40 °C, and (3) processes (1) and (2) were repeated. Non-freezing water content ($W_{nf}$) was calculated using the following equation.[30, 31] Enthalpy of the melting of water (334 J $g^{-1}$) was used for the calculation. In this study, values obtained from heating curves were used for calculation.

$$W_{nf} = [1 - (\Delta H_m)/334]/ (m_{dry\ gel}) \text{ g g}^{-1} \quad (4)$$

where $\Delta H_m$ is enthalpy calculated from the melting peak of the gels and $m_{dry\ gel}$ is the mass of the dry gel.

**Morphological observation.** Gels were observed at 25 °C using a polarizing light microscope (Leitz, Orthplan POL) equipped with a Leica digital camera DC100.

## Results and Discussion

By visual observation, the solution (1 wt%) changed into a homogeneous gel by one cycle of freezing and thawing. When *n* exceeded 2, the gel and sol portion could clearly be separated. It was clearly seen that the gel portion densely co-aggregates from the sol portion at $n = 3$. The size of the gel decreases with increasing *n*, although the exact volume could not be determined due to the irregular shape. When the concentration of the solution is 2 and 3 wt%, all samples form 100% gel and no syneresis was observed.

Figure 2 shows relationships between gel ratios (Rg) defined in Equation (1), the concentration of solutions, and freezing and thawing cycles (*n*). With increasing concentration, the *R*g shows 100% gelation regardless of *n*. On the other hand, when the concentration is less than 1 wt%, the Rg decreases with increasing *n*, suggesting that the separated sol portion increases. Figure 3 shows the relationships between LBG content in the gel, *n* and the concentration of the solution. The dotted line shown in Figure 3 indicates the LBG content in the gel when the whole solution transforms to gel and no syneresis is observed. As clearly seen, LBG concentration in the gel increases with increasing *n*. At *n* =

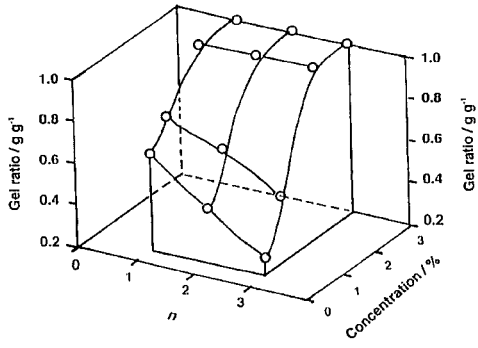


Figure 2. Relationships between gel ratio (*R*g), numbers of freezing and thawing (*n*) and concentration (wt%).

3, syneresis can be observed and LBG molecules in the gel portion become dense. The above observation indicates that the structure of the LBG junction zone seems to be quite different from that of PVA hydrogels formed by freezing and thawing.[1] In the case of PVA, the whole solution homogeneously changes into gels when the concentration exceeds ca. 5 wt%. During freezing and thawing, LBG molecules tightly bond with each other and the water molecules are excluded from the gel portion. The junction zone is enlarged gradually by the increase of *n*. The difference between PVA and LBG may come from the complex chemical structure of LBG, which makes it difficult to homogenize the solution.

In our previous studies, it was found that polysaccharide solutions are homogenized with the cooperative exchange of restrained water molecules in an oscillatory manner during the annealing process.[17-19] On this account, as stated in Experimental section, LBG solutions having various thermal histories were prepared to form hydrogels by freezing and thawing. Figure 4 shows the relationships between LBG content in the gel, *n* and the annealing temperature of the sample in the sol state. The LBG solution was 1%. The line observed at 25 °C shows the results without any annealing. It is confirmed that LBG content in the gel portion increases in annealing temperatures, as shown in Figure 4.

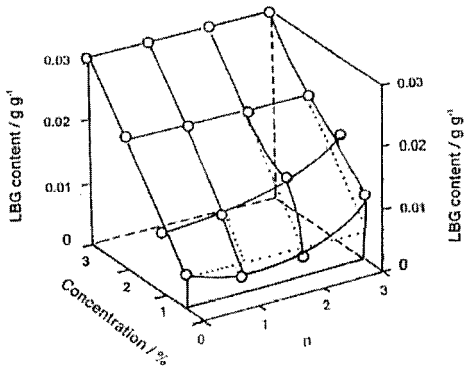


Figure 3. Relationships between LBG content in the gel, *n* and concentration of solution.

The broken line shown in Figure 4 indicates where the LBG content reaches ca. 4% by an *n* increase. This suggests that gelation of LBG is accelerated when the sol is annealed at a high temperature. This phenomenon is similarly observed in ordinal physical gels, such as gellan gum,[16, 18] xanthan gum[17, 19] and hyaluronate.[21]

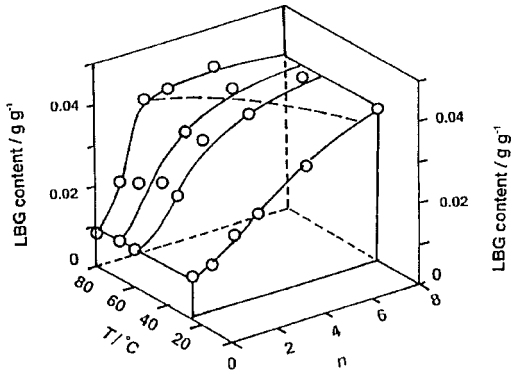


Figure 4. Relationships between LBG content in the gel portion, *n* and annealing temperatures of the sample in the sol state. Original concentration of sol=1 wt%.

Since LBG molecules co-aggregate with each other via hydrogen bonding, the junction zone should decompose when the gel is treated at a high temperature, if the gel is thermo-reversible. In DSC heating curves, no endothermic peak due to gel sol transition was found. Studies of gel-sol transition using DSC have been reported by several investigators concerning polysaccharide hydrogels, such as gellan gum,[23] agarose,[25] *k*-carragenann[25] and methyl cellulose.[26] Reported enthalpy values of gel-sol transitions are in a range from 0.5 to 6 mj $mg^{-1}$. In the case of PVA hydrogels prepared by freezing and thawing, the enthalpy of transition measured by DSC was 0.5 mJ $mg^{-1}$. Based on the above facts, it is clear that LBG hydrogels formed by freezing and thawing are thermo-irreversible and no gel-sol transition was observed.

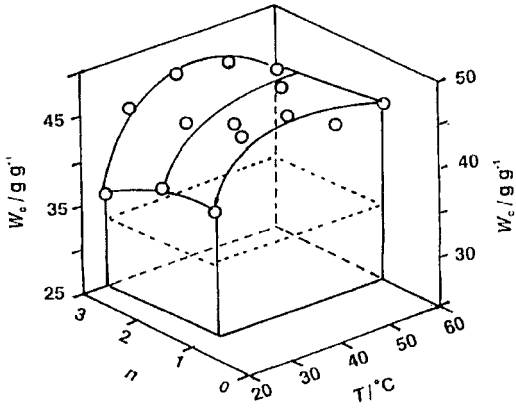


Figure 5. Three dimensional presentation between Wc, temperature of swelling and *n*. Concentration = 3 wt%.

Furthermore, in order to confirm this fact, gels were immersed in boiling water and maintained for 5 to 10 min. The gel portion remained according to the visual observation and mass loss was found to be less than 10 to 15%, according to the weighing method as described in the Experimental section.

In order to investigate the thermal stability of LBG hydrogels at a high temperature, the *W*c of LBG gel samples was measured by the tea bag method, as stated in the Experimental section. Figure 5 shows the three dimensional relationships between *W*c, swelling temperature and *n*. When the temperature increases, *W*c increases, showing the highest *W*c's (ca. 46 g $g^{-1}$) at around a swelling temperature of 45 °C. At a high swelling temperature, *W*c decreases slightly. It is thought that loosely linked molecular chains are separated from the junction zone due to the enhanced movement of network chains. Figure 5 indicates that LBG hydrogel is thermally stable, i.e., the junction zone is stable, once it is established. It is clearly seen from the facts that *W*c levels off at the temperature range over 50 °C. LBG hydrogel is different from PVA gel, although both samples are formed by freezing and thawing. The swollen samples were dried and again immersed in distilled water at 25 °C. The *W*c of once-dried samples was 15 to 20 g $g^{-1}$. This fact indicates that strong intermolecular bonding of LBG molecules is established during the drying process and once-dried LBG is water insoluble. Similar results can be observed when cellulose single fibers are dispersed in an aqueous solution and dried.[32]

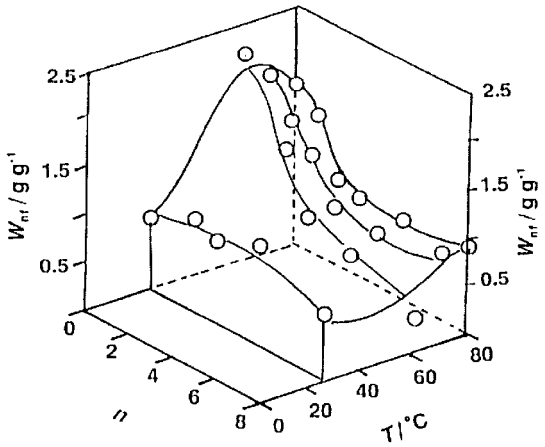


Figure 6. Relationship between non-freezing content (*W*nf), *n* and annealing temperature at sol state concentration 1 wt %.

Polarizing light micrographs of wet and dry LBG gels were taken. Using a polarizer and a photosensitive plate, a color change from blue to yellow was observed. It is evident that molecular chains are fabricated in a cross-linked mesh structure and that each thread aligns along the fiber axis direction.

The amount of water tightly restrained by the junction zone of LBG hydrogels was determined by DSC. It is known that water molecules which are strongly restrained by matrix polysaccharides show no first order phase transition.[30] This kind of water is defined as non-freezing water (*W*nf). *W*nf values evaluated from the DSC melting peak of LBG hydrogels as functions of *n* and concentration are shown in Figure 6. As shown in a three dimensional diagram, the *W*nf value decreases with increasing *n*. This fact suggests that LBG molecules in the junction zone become regularly aligned, and water molecules directly bonded to hydroxyl groups of LBG are excluded when an inter-molecular hydrogen bonding is established.

## Conclusion

From the above results, it is concluded that the junction zone of LBG hydrogels is tightly formed by freezing and thawing. During this process, LBG molecules not connected with the junction zone are excluded from the gel portion and the remaining molecules gradually form densely packed hydrogels. Molecular conformation in the sol state affects the rate of the above junction formation. This fact suggests that LBG solution is not in a thermal equilibrium. Obtained LBG hydrogels are thermally stable and no gel-sol transition was observed in a temperature range of 40 to 100 °C. Non-freezing water content calculated from the DSC melting peak of water in the gel indicates that the junction zone became dense with increasing freezing and thawing by excluding water restrained in the junction zone.

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